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Production and Inhibition of Gas in Various Regions in the Intestine of the Dog.* (31194)

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The production and composition of gases in the lumen of the intestine have been ascribed to a fermentation process of various types of foods consumed(1,2). Although it has been accepted that this process takes place in the colon, there has been little attempt to investigate the relationship between the colon and various levels of the small intestine as gas-producing areas following injections of a gas-producing food into these areas(3,4,5).

To study this relationship, experiments were so designed that known amounts of gas-producing and non-gas-producing foods could be introduced into surgically prepared intestinal segments of normal and antibiotically pretreated dogs. The assumption was that the fermentation, or gas-producing process, is associated with the intestinal flora which are susceptible to various types and concentrations of antibiotics.

Material and methods. Female mongrel dogs having an average weight of 12.8 kg were dewormed with Tenarids‡ worm tablets one week prior to their use. The animals were fasted 24 hours before anesthetization with nembutal, after which time a 10 cm midline incision was made on the abdomen. Isolated intestinal segments 50 cm in length were prepared in the duodenum, upper jejunum, and lower ileum by placing ligatures about the intestine. The colonic segment measured only 40 cm in length due to the shortness of this region in the dog. Cannulae 3 inches in length, made from one-half inch diameter flexible plastic tubing, were placed at each end of the 4 segments. Plastic adapters were also fitted to each cannula so that a segment could be closed off, or coupled to a gas collection apparatus when desired.

To record the degree of gas production and the inhibition that occurred due to antibiotics in various segments of the intestine and colon, 3 different series of experiments were performed using the following diets: (1) a non-gas-producing control-methyl cellulose homogenate, (2) a gas-producing navy bean homogenate, and (3) a navy bean homogenate after pretreating the animals with various antibiotics.

The methyl cellulose homogenate was prepared by homogenizing 50 g of methyl cellulose in enough distilled water to make a 200 ml volume; 50 ml of this homogenate were injected into each of the 4 segments. The material was inserted through the cannula in

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TABLE I. Factors Influencing Volume and Composition of Gas Production in Various Areas of the Intestine of the Dog.

Group*	Treatment	Intestinal segments	Mean gas vol, cc/3 hr	Mean gas composition			
				% CO_2	% O_2	% N_2	% H_2
1	Methyl cellulose	Duodenum	.00				
		Jejunum	1.50	9.30	13.90	76.80	.00
		Ileum	"	"	"	"	"
		Colon	"	"	"	"	"
2	Navy bean homogenate	Duodenum	5.70	21.26	6.47	40.37	33.90
		Jejunum	4.90	21.00	4.66	51.50	27.88
		Ileum	15.00	21.67	6.00	54.65	27.74
		Colon	31.90	34.26	3.80	28.81	33.16
3A	Neomycin Sulfate + Sulfathalidine + navy bean homogenate	Duodenum	1.50	14.00	10.50	85.00	.00
		Jejunum	1.20	10.30	9.30	81.50	.00
		Ileum	1.40	9.30	9.80	82.60	.00
		Colon	1.00	9.00	12.70	77.70	.00
3B	Mexaform + navy bean homogenate	Duodenum	.00				
		Jejunum	.50†				
		Ileum	1.50	9.20	12.80	78.00	.00
		Colon	.00				
3C	Vioform + navy bean homogenate	Duodenum	.50†				
		Jejunum	2.50	10.30	4.90	65.60	20.50
		Ileum	3.50	14.60	6.50	58.50	20.40
		Colon	3.20	20.90	5.70	56.70	19.50

* Five dogs per group.

† Nonsufficient gas for analysis.

the upper end of each segment, thus flushing any air or intestinal contents present in the segment out through the lower cannula. The navy bean homogenate was prepared by homogenizing a 1 lb can of commercial pork and beans with 100 ml of distilled water. Fifty ml of this homogenate was injected into each segment. Five dogs were used in each of the above 2 groups.

In the series of experiments in which the effects of navy bean homogenates were tested for gas production in animals pretreated with antibiotics, 3 different experimental procedures were followed. In the first group of 5 animals, one g of Neomycin Sulfate§ was given orally every hour for 4 hours, and then every 4 hours, for a total of 10 treatments. In this group 0.5 g of Sulfathalidine¶ was also given 3 times per day for 6 treatments prior to administering the bean homogenate. To the next group Mexaform‖ (Entobox 20 mg and Vioform 200 mg and Antrenyl 2 mg) was given orally at a dosage of 4 tablets per day for 2 days prior to the experiment. The last group of 5 animals received Vioform (iodochlorhydroxyquin‖) orally at a dosage of 500 mg per dog 4 times per day for 2 days prior to administration of the navy bean homogenate(6).

Gas volumes were recorded from each intestinal segment 3 hours after injection of the homogenates by allowing the gas to displace an acid solution in the gas collecting apparatus. The gas composition was determined with a Fisher-Hamilton Clinical Gas Partitioner.

Results. A comparison of the mean intestinal gas volumes and compositions 3 hours after giving an inert or gas-producing homogenate is given in Table I. Repeated gas analyses from dogs used in this investigation failed to show the presence of methane and, therefore, no mention will be made of this gas component. When an inert methyl cellulose homogenate was introduced into the isolated intestinal segments, it was usually found that 1 to 2 cc of air inadvertently became trapped in the segment. A determination of the composition of this small gas sample found in the intestinal segments of dogs proved it to be identical in composition to

§ Neomycin Sulphate: Ely Lilly & Co., Indianapolis, Ind.

¶ Sulfathalidine: Merck Sharp & Dohme, Rahway, N. J.

‖ Mexaform and Vioform: CIBA, Summit, N. J.

that observed when room air samples are introduced into the intestinal segments and allowed to equilibrate for similar periods of time. It has been observed that this type of equilibrium with the CO_2 and O_2 in the blood can occur in the human colon in 45 and 180 minutes, respectively(7). When the navy bean homogenate was tested, it was observed that the largest volumes of intestinal gas occurred in the colon and next in the ileal segments. Significant volumes of gas, however, were also produced in the duodenal and jejunal segments. Analysis of these gas samples showed a particularly high carbon dioxide and hydrogen concentration.

In group 3A, when animals were pretreated with a combination of antibiotics, Neomycin Sulfate, and Sulfathalidine, the gas volumes were significantly reduced in all the intestinal segments to a level similar to those in the methyl cellulose series. The analysis of the composition of these gas samples also showed a gaseous concentration similar to that found in animals given an inert methyl cellulose homogenate. The pretreatment of dogs in group 3B with Mexaform also inhibited gas formation, to an even more dramatic degree than with Neomycin and Sulfathalidine. The composition of the gas sample obtained from the ileum proved to be practically identical with the room air injections. After treatment of animals with Vioform (3C), a significantly reduced gas volume was also observed in all the intestinal segments following administration of the navy bean homogenate. However, analysis of these gas samples showed that the carbon dioxide and hydrogen concentrations tended to be higher, especially in the ileum and colon, than those from dogs given the other antibiotic treatment. This suggests an incomplete bactericidal effect of Vioform upon the intestinal flora.

Discussion. Although the increased volume of intestinal gas, and also the high concentrations of carbon dioxide and hydrogen found in the gas, have been attributed to bacterial fermentation in the colon, others have held the opinion that this is highly unlikely. In the present study it has been shown that in the dog the intestinal bacteria residing in the duodenum and jejunum may add significantly to that gas produced in the lower ileum and colon. The results also show that certain specific antibiotics can effectively sterilize both the small intestine and colon segments so that subsequent administration of a gas-producing navy bean homogenate into these segments fails to elicit gas production. These facts suggest that the same type of gas-producing organism is present in the upper small intestine as in the lower intestine and colon, the only difference being the number of organisms present in the different areas. The results also suggest that the organism responsible for the gas production is of the anaerobic type, and it is the action of these bacteria on the navy bean homogenates that produces large volumes of intestinal gas which has a high carbon dioxide and hydrogen concentration. Eisman *et al*(6) have shown that the anaerobic flora of the gastrointestinal tract are significantly reduced or totally destroyed by Mexaform and Vioform, while the normal aerobic and coliform flora increased during treatment. Thus, from these and previous investigations in this laboratory with Mexaform and Vioform on the inhibiting effect on gas formation in humans, it is deduced that the increased gas volume and high carbon dioxide and hydrogen concentrations resulting from eating a gas-producing food are due to the anaerobic spore-forming intestinal flora.

Summary. The relationship between the small intestinal and colonic flora and the gas volumes and composition resulting from the introduction of navy bean homogenates into surgically prepared intestinal segments of normal and antibiotically pretreated dogs was investigated. Intestinal gas production was effectively inhibited or greatly reduced in animals pretreated with Neomycin Sulfate and Sulfathalidine, Mexaform, and Vioform, following the administration of navy bean homogenates. Mexaform and Vioform effectively destroyed the anaerobic bacteria of the intestinal tract while the normal aerobic and coliform bacteria increased in total numbers, indicating that the increased gas production from a navy bean homogenate was due to the anaerobic intestinal flora. Contrary to some current belief, it has been shown that bac-

terial action in the duodenum, jejunum, and ileum of the dog may add significantly to the total intestinal gas volume of animals fed navy bean homogenates.

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Radioautographic Localization of Hydralazine-1-C_{14} in Arterial Walls.* (31195)

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Hydralazine, a potent antihypertensive agent first described in 1950(1), decreases peripheral resistance and thereby reduces arterial pressure, despite increased cardiac output(2). In contrast, most other currently available antihypertensive agents produce at least part of their effect by decreasing cardiac output. While the precise mechanism of its action is not known, there is evidence to suggest that hydralazine acts peripherally(3). Activity has been shown to depend on a pyridazine ring with an attached hydrazine side chain(4). The fate of hydralazine has been studied by using isotopically labeled compound with a radiocarbon atom between the pair of nitrogen atoms in the pyridazine ring and the pair in the hydrazine side chain. Two hours after receiving hydralazine-1-C_{14} by intramuscular injection, normal mice had higher levels of radiocarbon in aorta than in any other tissue tested, and the rate of disappearance of radioactivity was slower from aorta than from other tissues(5). The work reported here further emphasizes the unusual affinity of hydralazine for vascular tissue by demonstrating the striking radioautographic localization of radiocarbon from hydralazine-1-C_{14} in arteries throughout the body.

Methods. Adult female Tumblebrook mice weighing between 15 and 20 g were given single intramuscular injections of 0.4 mg of hydralazine-1-C_{14} containing 4.8 μc of C_{14}. The animals were sacrificed by a blow on the head from 2 to 240 hours later. Sections from tissues fixed in formalin for 24 hours and frozen sections from unfixed tissues were cut to thicknesses of 7-10 μ. Sections from brain, lung, heart, spleen, liver, kidney, and thigh muscle were placed upon glass slides which were dipped into 1% celloidin in alcohol-ether and allowed to dry. In a darkroom, the edge of a cover slip was dipped into Kodak Nuclear Track Emulsion (NTB3), which had been warmed to 40°C, and lightly drawn over each slide. The slides were placed in plastic light-tight boxes and exposed at 4°C for from 15 to 240 days. After exposure, the slides were developed in Kodak D19 developer, washed, fixed, and rewashed.

To determine whether fixation in formalin leached radiocarbon from tissue, the fixing solution was tested and found to have accumulated as much as 15% of the radioactivity from tissues of animals sacrificed 2 hours after injection of hydralazine-1-C_{14} but less than 3% from tissues of animals sacrificed 24 hours after injection. At all intervals after hydralazine injection, radioautographs of fixed tissues looked both quantitatively and qualitatively similar to those obtained from frozen unfixed tissues. Underlying structures

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