Effect of the Normal Microbial Flora on Gastrointestinal Motility.* (32430)

GERALD D. ABRAMS AND JANE E. BISHOP (Introduced by R. H. Kahn)
Department of Pathology, The University of Michigan Medical School, Ann Arbor, Mich.

Comparison of germfree with conventional animals has shown that the development of many "normal" traits of the host depends upon the presence of the resident microbial flora. This is particularly true within the gastrointestinal tract where such varied parameters as mucosal surface area and morphology, epithelial renewal, cecal size and enzyme content, xylose absorption, and resistance to infection are all strikingly influenced by the presence or absence of a flora(1,2,3,4,5). In a recent study of germfree and conventional mice(6), quantitative microbiological data and the results of carmine dye studies suggested that the "normally" rapid rate of emptying of the small intestine might also be determined, to a significant degree, by the conventional flora. The purpose of the present study was to assess this point precisely by following the passage of a non-absorbable, radioactive tracer through the gastrointestinal tract of germfree and conventional animals.

Materials and methods. Animals. CD-1 Swiss mice (Charles River Breeding Laboratories) weighing 25-35 g were used. The germfree animals were reared in isolators using standard gnotobiotic techniques(7), while their conventional controls were reared in the open laboratory. All animals were fed an autoclaved L-356 diet (General Biochemicals Corp.). Approximately equal numbers of males and females were used.

Administration of test meals. Before test-feeding, all animals were fasted overnight with free access to water. Each animal received 1 microcurie of yttrium-91, as YCl_3, in 0.5 cc of brain heart infusion broth, administered intragastrically, *via* a polyethylene catheter passed perorally. Immediately after being fed, the animals were placed in individual cages with coarse mesh bottoms to facilitate collection of feces, and were allowed free access to food and water. Groups of germfree and conventional mice, 6 animals per group, were sacrificed at 2, 4, 6, 16, and 24 hours after administration of the test meal. For the 16 and 24 hour experiments, the germfree mice were fed within their isolators, using a filter sterilized Y-91 solution taken into the isolators in glass ampoules, and were brought into the open laboratory just prior to sacrifice. For the 2, 4, and 6 hour experiments, the germfree animals were removed from their isolators and fed in the open laboratory along with their conventional counterparts.

Quantitative methods. At the end of each experimental interval, the animals were sacrificed by decapitation, and several portions of the gastrointestinal tract were isolated by ligatures. The stomach, small intestine, cecum, colon, and collected feces of each animal were placed in individual tubes, and the radioactivity present in each sample was determined using a well scintillation counter. For a given animal, the radioactivity present in each sample was expressed as a per cent of the total radioactivity recovered in the 5 samples, according to the method of Marcus and Lengemann(8). Since Y-91 is not absorbed, this fractional distribution serves as a measure of the rate of propulsion of the test meal through the gastrointestinal tract. Mean values for each segment and interval were calculated for germfree and conventional mice.

Results. The data from all of the experiments are summarized graphically in Fig. 1-5. In each instance, the mean radioactivity present in the particular segment is plotted as a function of time, for both germfree and conventional mice. Standard errors of the means are indicated by the vertical bars.

Gastric emptying (Fig. 1) was found to be more rapid in conventional animals than in their germfree counterparts. Although in-

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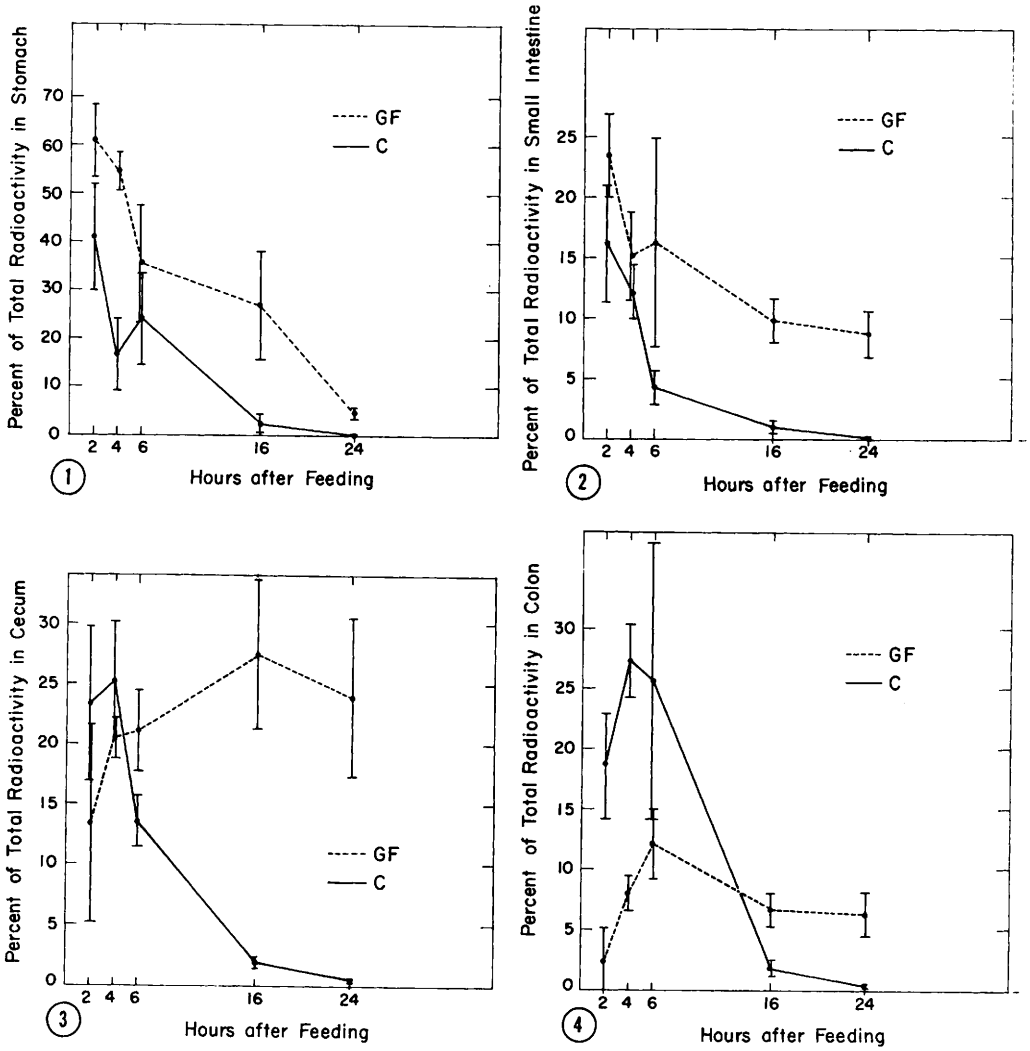

FIG. 1. Passage of yttrium 91 through stomach of germfree and conventional mice.
FIG. 2. Passage of yttrium 91 through small intestine of germfree and conventional mice.
FIG. 3. Passage of yttrium 91 through cecum of germfree and conventional mice.
FIG. 4. Passage of yttrium 91 through colon of germfree and conventional mice.

dividual values were quite variable during the early intervals, the mean amounts of retained radioactivity at 2, 4, and 6 hours were consistently lower in the conventional animals. At 16 hours, when the stomachs of conventional mice retained only traces of the test meal, those of germfree mice yet contained over one-fourth of the total. A difference in the two groups of animals was still apparent at 24 hours ($P<0.01$).

The data relative to the small intestine (Fig. 2) likewise indicate a more rapid passage of the test meal in conventional as compared to germfree animals. Although the values at 2, 4, and 6 hours reflect the variability of gastric emptying at these times, the mean radioactivity was again lower in conventional mice at every one of these early intervals. Transit through the conventional small intestine was more rapid than that through the germfree, as indicated by the fact that even though the gastric input into this segment was greater throughout the 6-hour interval in conventional animals, the

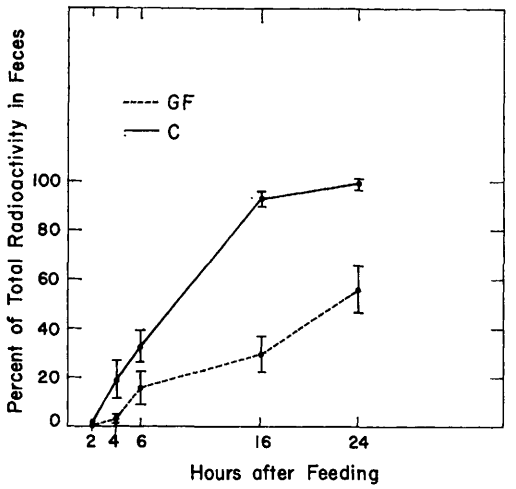

FIG. 5. Passage of yttrium 91 into feces of germfree and conventional mice.

amount retained in the small intestine during this period was less. This was more clearly borne out at 16 and 24 hours when the small intestines of conventional mice were found to contain less than 1% of the total radioactivity, as compared to almost 10% in the germfree animals ($P<0.01$).

At the 2 and 4 hour intervals, the cecum (Fig. 3) contained slightly more radioactivity in conventional as compared to germfree animals, reflecting the more rapid input from above. However, from 6 hours on, the cecum in the germfree animals retained a greater percentage of the radioactivity. This undoubtedly reflects the gross cecal distension characteristic of the germfree mouse. The data relative to the colon (Fig. 4) again reflected an earlier input from above in conventional animals.

The above differences in gastrointestinal transit in germfree and conventional mice resulted, finally, in strikingly different rates of appearance of radioactivity in the feces of the two groups (Fig. 5). Within 16 hours of feeding, the conventional animals had passed over 90% of the radioactivity into the feces, as compared to less than 30% in the germfree animals ($P<0.001$). The difference remained highly significant at 24 hours ($P<0.01$).

Discussion. The above values for the distribution of radioactivity are no doubt affected somewhat by the ingestion of non-radioactive food by the animals following the administration of Y-91. Germfree and conventional animals, however, ingest equal quantities of food. There is furthermore no reason to believe that mixing of radioactive and non-radioactive gastrointestinal contents differs in a systematic way in germfree and conventional mice. Therefore, the divergent values obtained in the two groups of animals can be accepted as representing actual differences in propulsive activity of the gastrointestinal tract.

The basic fact established by these experiments is that the normal microbial flora influences the rate at which luminal contents are propelled through the gastrointestinal tract. Transit times through various portions of the tract that would ordinarily be considered "normal" (*i.e.*, those in conventional animals) are seen actually to represent a degree of what might be termed "physiologic acceleration" in response to the flora. Although the cecum has attracted the attention of most investigators commenting upon the tone and/or motility of the germfree gastrointestinal tract, the present data demonstrate that propulsion along the entire tract is affected by the presence or absence of a flora.

The results of this study confirm the suggestion previously made(6), that the normal flora may contribute to the defense of the small intestine against microbial invasion by stimulating peristaltic emptying. In the conventional animal, this results in rapid transport of potential pathogens into the large bowel where the growth of these pathogens can be supressed by the rich colonic flora(9). Without its propulsive capability, the small intestine is relatively unprotected against microbial overgrowth, since neither the secretions nor the scant flora of the small intestine possess significant direct antimicrobial activity(10).

The differences in gastrointestinal transit observed in the two groups of animals may also have important implications with regard to digestion and absorption of various nutrients although this has not yet been documented.

The mechanisms by which the normal flora exerts its influence upon the gastrointestinal motility are obscure. Neither the identity of

the responsible organisms nor the site of their action is known. Comparison of germfree and conventional animals, however, has revealed differences in the metabolic activity of neurons of Auerbach's plexus(11), in the intestinal complement of pharmacologically active amines(12), in the luminal content of certain musculo-active substances(13), and most recently, in the threshold of response of cecal smooth muscle to chemical stimulation(14). At present, in our laboratory, the *in vitro* neuromuscular activity of various isolated segments of germfree and conventional gastrointestinal tract is being studied in an attempt to clarify the possible role of these factors.

Summary. The rate of propulsion of gastrointestinal contents was compared in germfree and conventional mice using a non-absorbable radioactive substance, yttrium 91. Following intragastric administration, the marker was propelled much more rapidly through the gastrointestinal tract of conventional as compared to germfree animals, demonstrating that "normal" propulsive activity is determined to a significant degree by the presence of the normal flora. This function of the flora has important implications for host defense. The precise mechanism of action of the flora is obscure.

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Starch and Iron Absorption. (32431)

FORREST D. GARRETSON AND MARCEL E. CONRAD
Department of Hematology, Walter Reed Army Institute of Research, Washington, D.C.

The habitual ingestion of large amounts of laundry starch is a common practice among Negro females in certain geographic areas of the United States(1). The amount ingested may exceed two pounds of starch daily and is frequently associated with marked iron deficiency(2). The severity of "starch eaters anemia" seems greater than expected from an iron deficient diet alone(3) and led us to postulate that starch interfered with iron absorption. The present investigation was performed to study this hypothesis and reports the acute and chronic effects of starch ingestion upon iron absorption in rats.

Materials and methods. Male albino rats, Walter Reed Carworth Farms strain, weighing 140 to 200 g were obtained and measurements of iron absorption and kinetics were performed when animals on a normal high protein diet weighed 170 to 200 g. The principles of laboratory animal care as promulgated by the National Society for Medical Research, were observed. The standard laboratory diet contained 25% protein and 9 mg of iron per 100 g dry weight. Special diets were made with starch (Argo), sucrose (USP), casein (USP) and corn oil (Mazola) and were supplemented with an iron-free commercial salt mixture and vitamins. Sufficient ferrous sulfate was added to certain of the prepared diets