subjects do not lead to confusion when the test is applied clinically. In patients with enzyme deficiencies oleic acid gives higher serum concentrations of radioactivity than does fat(8) which is the reverse of the case with normals. Furthermore, the serum levels observed in abnormals are usually so much lower than those in normals that interpretation is not difficult.

Summary. A test has been proposed for evaluation of intestinal malabsorption states. It involves administration of isotopically tagged whole fat on one day and fatty acid on a separate occasion. By comparison of resultant radioactivity in blood and urine one can judge whether an abnormality found is due primarily to lack of proper digestive enzymes or to intrinsic difficulty with the absorption process. Normal figures with standard deviations are given for serum levels of radioactivity and for urinary and fecal loss. It was found that in normal subjects serum concentrations of radioactivity were lower following ingestion of tagged fatty acid than whole fat and the possible significance of this difference is discussed.

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Effects of Orally Inoculated Bacteria on Gastrointestinal Flora of Newborn Mice. (22516)

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This study was undertaken to ascertain if overwhelming feedings of both normal and abnormal microorganisms at birth would produce any obvious changes in the ecology of the intestinal bacterial flora and in the health and development of white mice. Since part of the normal flora is already established by the fifth day after birth(1), the organisms were fed to newborn mice, in order to eliminate the possibility that the preexisting flora would prevent the establishment of these organisms. Because of the recent interest in *Pseudomonas aeruginosa* and *Proteus vulgaris* as agents of infantile diarrhea, they were chosen as 2 of the test organisms.

Most of the attempts to produce experimental shigellosis have been with young or adult laboratory animals. Since the antagonistic action of the gastrointestinal flora of

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these animals has been postulated as one explanation to account for the negative results obtained(2,3), Shigella species were also included in this study.

Methods. Pregnant Swiss albino mice were isolated in separate cages and observed. During the experiments, their diet consisted of Purina mouse pellets. Four to 6 hours after birth, all the newborn mice of a given litter were fed 0.05 ml of a 24 hour nutrient broth culture (app. 5 x 10^7 cells) of one of the following organisms: *Shigella dysenteriae, Shigella paradysenteriae, Proteus vulgaris* and *Pseudomonas aeruginosa* freshly isolated from human infectious processes. *Escherichia coli* and a lactobacillus strain previously isolated from normal adult mice were also used. The organisms were deposited into the oral cavity of each newborn mouse using a tuberculin syringe and an 18 gauge needle from which the bevel had been ground away. The mice were then returned to the cage containing their mother. During the course of the experiments, 13 days, the litters were suckled by their mothers. Each day the mice were weighed and their general health observed. At 2 day intervals through the thirteenth day, one mouse from each litter was sacrificed. Its gastrointestinal tract from the level of the diaphragm to the rectum was aseptically removed, weighed, added to 2.0 ml of sterile saline and homogenized using a Virtis homogenizer. Tenfold serial dilutions of this material were prepared and 0.1 ml of each dilution plated in duplicate on the appropriate media as noted below. Colony counts were done after 48 hours incubation at 37°C. The spread-plate method was used throughout to permit the subculture and identification of all the colony types which appeared. V-8 juice agar, azide blood agar base and MacConkey agar were used routinely to determine the numbers of lactobacilli, Gram-positive cocci and Gram-negative enteric organisms present. In those experiments where the mice were fed with *P. vulgaris,* the agar content of the MacConkey medium was increased to 4% in order to inhibit the spreading of these organisms. Thus, more accurate colony counts were obtained. *Control experiments* on nor-

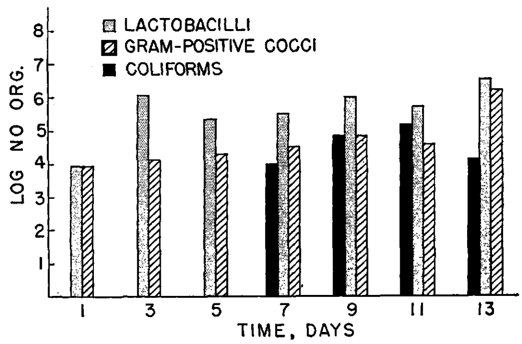

FIG. 1. Emergence of gastrointestinal flora of normal mice.

mal mice were conducted as described above except that the newborn mice were fed sterile nutrient broth instead of a bacterial culture.

Results. The sequence of appearance of the normal gastrointestinal flora in the newborn mice was as follows (Fig. 1): Gram-positive cocci appeared within 3 hours after birth. Lactobacilli were observed after approximately 8 hours. Coliform organisms made their appearance about the 7th day after birth. Once established, all the above organisms persisted in quantity during the 13 days of the control experiments. Also, it was found that all the above organisms were part of the normal gastrointestinal flora of adult mice. Lactobacilli and *E. coli* when introduced at birth had no effect on the emergence and persistence of the normal flora and on the growth of the mice. Although superinfection with lactobacilli resulted in initially higher counts of this organism than those with the control animals, by the third day the number of lactobacilli in the superinfected and control animals is the same. Although coliform organisms did not usually appear until the seventh day in the normal animals, premature infections with *E. coli* at birth did establish the organisms at that time. The organisms persisted but did not exceed at any time the number found in the uninoculated mice.

When freshly isolated strains of both S. *dysenteriae* and *S. paradysenteriae* were fed to newborn mice, viable organisms were shown to be present in the G. I. tract 24 hours after oral inoculation. The quantity of organisms recovered at that time indicates that some of

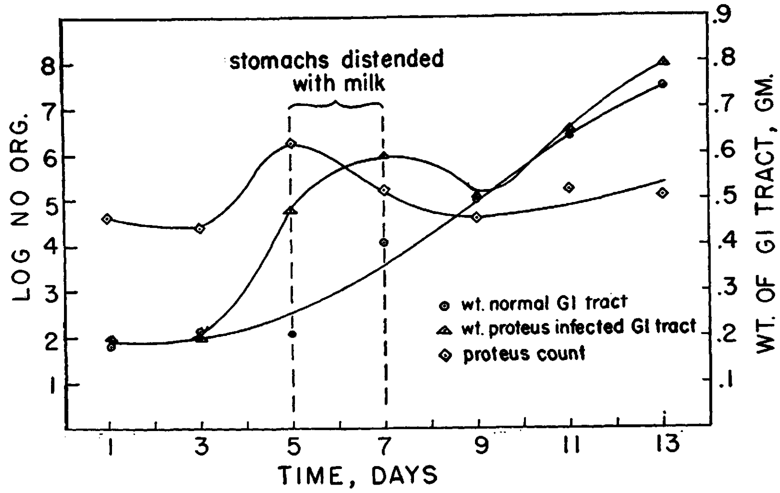

FIG. 2. Weights of gastrointestinal tracts of mice infected with *Proteus vulgaris* in relation to No. of these organisms recovered. Distention of the stomachs in infected animals occurred as indicated.

the organisms were able to pass the gastric barrier and survive in the intestinal tract. However, the number of organisms recovered (2.5×10^3) and the fact that none could be recovered by the third day, suggests a lack of multiplication. The weights, general health and normal flora of these animals did not significantly vary from that of the controls.

Hammond *et al.*(4) found that *Ps. aeruginosa*, when orally inoculated into adult mice, disappeared by the third day in more than 50% of the animals. However, in the experiments described herein using newborn mice, this organism was found to persist in quantity. Thus, the age of the animal must be an important factor in the establishment of this organism in the intestinal tract of mice.

The most marked effect on both the animals and their normal intestinal flora was observed in those groups of mice which were orally infected with *P. vulgaris*. The feeding of overwhelming doses of this organism at birth delayed the emergence of lactobacilli for 4 days. Furthermore, a delay of 6 days in the onset of coliform organisms was also noted in these animals. A striking difference was noted in the weights of the entire G. I. tracts and general appearance of the stomachs. The stomachs of these mice were markedly distended and filled beyond capacity with coagulated milk on about the 5th day and for 2 days thereafter (Fig. 2). These findings coincide with the time of the maximum counts of *P. vulgaris*. On the basis of limited data, it would appear that the gains in weight of these mice occurred at greater rates than those of the control animals (Fig. 3). The weights given represent the total weights less those of the G. I. tracts.

Once established, *Ps. aeruginosa* like *P.*

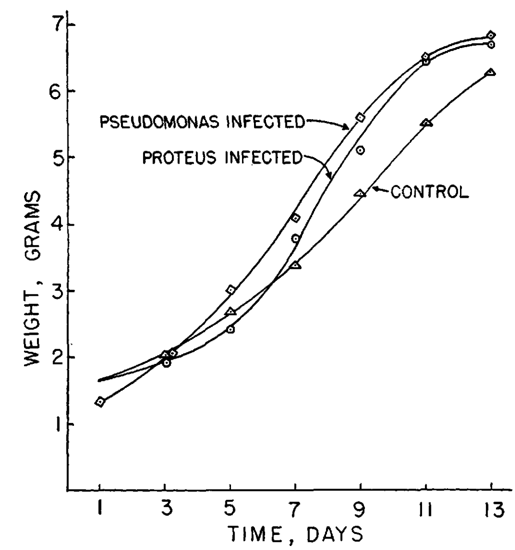

FIG. 3. Gains in wt of mice orally inoculated at birth with *Pseudomonas aeruginosa* and *Proteus vulgaris*. Figures given represent total weights of the animals less those of the G.I. tracts.

vulgaris persisted in the mice during the experiments. Changes observed in these animals were similar to but not as pronounced as those found in the experiments with *P. vulgaris*. Gains in weight greater than those of the control animals were also noted in these mice (Fig. 3).

Discussion. Since the studies on the normal intestinal flora were terminated at time of weaning (13 days), a variable due to change of feeding habits was eliminated. Lactobacilli normally appeared in quantity in 24 hours. Therefore, it was not surprising that superinfection with this organism at birth had no effect. As coliform organisms did not emerge as part of the normal flora till about the seventh day in the control mice, it is of interest that the oral feeding of *E. coli* at birth established this organism at that time, but did not otherwise affect the bacterial ecology. The numbers of each of these 2 organisms recovered from the homologously infected mice did not exceed those found in the control animals. Thus, it would appear that there is an ecological constant for each of these organisms. Inasmuch as *P. vulgaris* and to a lesser extent *Ps. aeruginosa* did inhibit the emergence of both lactobacilli and coliform organisms, it is noteworthy that no permanent adverse effects in these mice were observed. In fact, these animals gained weight at a higher rate than the controls. This observation warrants further investigation. The distension of the stomachs with coagulated milk, which occurred at the time when the viable counts of *P. vulgaris* in the intestines are highest, invites speculation.

Attempts to produce typical shigellosis by feeding the organisms to adult or young animals have been generally unsuccessful(5). Cooper and Keller(6) were unable to infect white mice by gavage with living *Shigella sonnei*. However, repeated feedings of this organism resulted in the development of immunity. In our experiments with *Shigella dysenteriae* and *Shigella paradysenteriae* the organisms gained entrance to the G. I. tract but did not persist even though coliform organisms did not make their appearance until 5 days later. Consequently, in this instance, the resistance of the animals to this infection must be explained by a mechanism other than antibiotic production by *E. coli* as suggested by some workers(2,7).

Summary. The emergence and persistence of Gram-positive cocci, lactobacilli and coliform organisms in the gastrointestinal tracts of Swiss mice from birth to weaning were investigated. Changes in normal bacterial ecology due to oral feedings with both normal and abnormal organisms were described. *Proteus vulgaris* and to a lesser extent *Pseudomonas aeruginosa* delayed the emergence and decreased the quantity of both lactobacilli and *Escherichia coli*. These mice had moderate increases in growth rates over those of the control groups. Recently isolated Shigella species when fed to newborn mice survived passage through the stomach. They did not persist in the G. I. tract nor cause changes in the mice.

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