JOURNAL OF BACTERIOLOGY, July, 1966
Vol. 92, No. 1

Stability of the Lactobacillus Population in Feces and Stomach Contents of Rats Prevented from Coprophagy

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Received for publication 29 January 1966

ABSTRACT

LEV, MEIR (Albert Einstein College of Medicine, New York, N.Y.), RAYMOND H. ALEXANDER, AND STANLEY M. LEVENSON. Stability of the *Lactobacillus* population in feces and stomach contents of rats prevented from coprophagy. J. Bacteriol. **92:** 13–16. 1966.—Lactobacilli were enumerated in the feces of rats prevented from coprophagy by tail-cupping. No differences were found when numbers of these organisms were compared with lactobacilli in feces of control rats, without tail-cups. High and similar numbers of lactobacilli were found in the stomachs of rats with and without tail-cups. The effect of coprophagy on fecal lactobacilli was therefore negligible.

Coprophagy is a mechanism by which nutrients (particularly vitamins) synthesized in the lower portion of the gut but poorly absorbed at this site are made available when eaten and absorbed in the upper portion of the gut. Besides nutrients, feces also contain large numbers of microorganisms which are ingested when coprophagy is practiced. The effect of these ingested organisms is of some interest.

Gustafsson and Fitzgerald (2) observed that the numbers of lactobacilli in the feces of rats prevented from coprophagy by tail-cups were lower than the numbers of lactobacilli in the feces of control rats without tail-cups. This effect was greatest in rats fed a vitamin K-deficient diet; in this case, counts of lactobacilli fell from 10^9 to 10^5 per gram (wet weight) of feces in cupped rats over a period of 4 weeks, whereas the counts fell from 10^9 to 10^7 in the uncupped control rats. These authors pointed out that coprophagy may be an essential mechanism for the maintenance of the gut flora.

Powell and Lev (*in press*) repeated the above work using a complete diet and Gustafsson's K-deficient diet. There was no difference in numbers of lactobacilli in the feces of control rats and those which had been prevented from coprophagy for 6 weeks. In addition, the numbers of streptococci and coliforms were also found to be unaffected by tail-cupping. Similarly, lactobacillus counts of stomach contents of control rats and of rats with tail-cups all were within the range of 10^6 to 10^7 per gram (wet weight). Lactobacilli in the feces decreased from 10^8 to 5×10^6 in the uncupped rats on the K-deficient diet, a finding similar to that of Gustafsson and Fitzgerald (2).

Recently, Fitzgerald, Gustafsson, and McDaniel (*in press*) repeated their original work and again found that numbers of lactobacilli decreased in the feces of cupped male rats on several different diets, but that coliforms and streptococci increased significantly. Because of the discrepancy of our results from those quoted above, it was decided to extend these experiments.

MATERIALS AND METHODS

Animals. The rats used in the first experiment were females of the Fischer strain which were 46 days old at the beginning of the experiment. In the second experiment, Sprague Dawley female rats, 22 days old at the start of experiment, were used. All rats were housed individually in 2-mesh stainless-steel cages. Water was given *ad libitum*, and the animals were fed the fortified regular lab chow (Purina) *ad libitum*. The tail-cupping procedure was a modification of that of Barnes, Fiala, and Kwong (1).

Bacteriology. Feces were collected in the mid-morning on aluminum foil and processed within 0.5 hr after passage. With tail-cupped rats, the cup was cleaned out and replaced, and fresh feces were removed 0.5 hr later. The fecal pellets were dispersed by shaking in a weighed bottle containing glass beads.

Decimal dilutions were made in Trypticase Soy Broth (BBL). Organisms were counted by the method of Miles and Misra (4), and four counts were made of each dilution. Lactobacilli were enumerated on L B S Medium (BBL), and the plates were incubated for 48 hr at 37 C, after which colony counts were made.

Experiment 1. Plastic tail-cups were placed on 18 animals and the same number of animals were used as uncupped controls. The animals were watched constantly; feces on the trays below a cage with a cupped animal indicated that the tail-cup was not completely effective, and, if a single pellet was found, the animal concerned was discarded. Ten additional animals had sham cups placed on them. These cups were placed in the same relative position and were the same as the true cups except for a large hole enabling the rat to get at its feces; the piece of plastic cut out from the bottom was screwed to the top of the cup so that its weight was the same. Counts were made of feces from individual rats at the start and after cupping for 3 and 4 weeks. Lactobacilli in stomach contents were counted at the termination of the experiment.

Experiment 2. In the second experiment, true tail-cups were placed on twenty 22-day-old rats, sham cups on ten, and ten uncupped control rats were used. Counts were made of feces from individual animals each week for 3 weeks. In this experiment, feces collected during 0.5 hr and during a 24-hr period after voiding were used.

RESULTS

Experiment 1. The experiment was started with 18 rats with true tail-cups and was finished with seven, i.e.; the tail-cups of 11 rats were not satisfactory (see above).

As can be seen in Table 1, the numbers of lactobacilli in the feces and stomach contents did not differ significantly among groups of rats with true, sham, or no tail-cups. There was also no trend in the increase or decrease of numbers of these microorganisms over the 4-week experimental period. The body weights of the control, tail-cupped, and sham tail-cupped animals are shown in Table 2. While the uncupped rats grew at an average rate of 5 g per week, the rats with true cups did not gain weight. The rats with sham cups gained about 9 g each during the 4-week period. The histology of the gastrointestinal tracts of these animals will be reported elsewhere.

Experiment 2. As in the previous experiment, no significant or consistent differences were found between the groups with true, sham, or no tail-cups (Table 3). In this experiment, the tail-cupping of only one animal of 20 was unsatisfactory during the 4-week experimental period. Collection of feces over a 24-hr period did not significantly alter counts. The weights of these animals are shown in Table 4. The average weight gain during the 4 weeks of the experiment for true, sham, and uncupped rats was 146, 180, and 194 g, respectively.

DISCUSSION

No reduction in numbers of lactobacilli was found in rats prevented from eating their own feces. This confirms the findings of Powell and Lev (*in press*). The present results were obtained with rats of a different strain and a different age, which were fed a different diet; the experiments were made with modified microbiological techniques and in a different laboratory. The fact

TABLE 1. *Viable counts of lactobacilli*[a]

Weeks after tail-cupping	Sample	Cups	Viable counts			No. of rats
			Avg	Median	Range	
0	Feces	None	8.3×10^7	6.3×10^7	7.6×10^6–2.3×10^8	10
		−Sham[b]	4.6×10^7	2.7×10^7	9.7×10^6–1.3×10^8	10
		−True[b]	6.2×10^7	4.8×10^7	3.7×10^6–1.8×10^8	18
3	Feces	None	6.4×10^7	4.6×10^7	2.0×10^7–2.0×10^8	8
		+Sham	6.0×10^7	4.8×10^7	3.8×10^6–3.4×10^8	10
		+True	8.1×10^7	2.2×10^7	3.6×10^6–5.4×10^8	9
4	Feces	None	4.4×10^7	3.3×10^7	1.8×10^7–7.6×10^7	8
		+Sham	6.5×10^7	4.5×10^7	2.3×10^5–2.8×10^8	10
		+True	6.2×10^7	1.9×10^7	4.1×10^6–2.4×10^8	7
4	Stomach Contents	None	5.9×10^8	4.1×10^7	4.7×10^6–4.3×10^9	8
		+Sham	2.0×10^8	6.2×10^7	1.4×10^6–6.2×10^8	10
		+True	2.1×10^8	1.1×10^8	1.6×10^4–6.6×10^8	7

[a] No. of organisms per gram (wet weight).

[b] These groups of rats were sampled before sham or true tail-cups were placed on them.

TABLE 2. *Average weights of rats with and without tail-cups and with sham tail-cups*[a]

Group	Cups	No. of rats	Weeks				
			0	1	2	3	4
I	True	9	136±6	133±7	134±7	132±11	137[b]±12
II	Sham	10	138±6	134±10	138±9	143±12	146 ±11
III	None	8	138±6	146±7	154±9	158±8.5	162 ±9

[a] Results are expressed as means ± SD; 1 vs. III, $P < 0.005$.
[b] Represents 7 rats.

TABLE 3. *Viable counts of lactobacilli*

Weeks after tail-cupping	Time feces samples	Cups	Viable counts[a]			No. of Rats
			Avg	Median	Range	
	hr					
0	0.5	None	9.5×10^7	3.9×10^7	2.0×10^6–3.3×10^8	10
		Sham	1.7×10^8	1.1×10^8	1.1×10^7–4.8×10^8	9
		True	1.2×10^8	3.1×10^7	1.0×10^6–8.9×10^8	18[b]
	24	None	6.6×10^7	1.9×10^7	5.3×10^6–2.9×10^8	10
		Sham	8.9×10^7	5.3×10^7	1.3×10^6–2.6×10^8	10
		True	7.3×10^7	4.0×10^7	2.8×10^5–3.0×10^8	20
1	0.5	None	3.0×10^8	2.7×10^8	1.5×10^7–9.2×10^8	10
		Sham	3.0×10^8	2.7×10^8	1.1×10^8–6.5×10^8	10
		True	2.5×10^8	2.0×10^8	3.5×10^5–8.4×10^8	16[c]
	24	None	2.3×10^8	2.0×10^8	4.8×10^7–5.3×10^8	10
		Sham	1.8×10^8	1.3×10^8	5.0×10^6–5.2×10^8	10
		True	4.2×10^8	3.8×10^8	2.1×10^7–1.2×10^9	20
2	0.5	None	9.1×10^7	6.0×10^7	7.5×10^6–2.5×10^8	10
		Sham	2.5×10^8	2.5×10^8	2.5×10^7–5.5×10^8	9
		True	1.4×10^8	9.0×10^7	3.3×10^5–4.0×10^8	20
	24	None	1.4×10^8	1.3×10^8	7.2×10^6–4.3×10^8	10
		Sham	1.6×10^8	8.0×10^7	6.5×10^6–4.0×10^8	9
		True	2.5×10^8	2.0×10^8	1.1×10^7–6.6×10^8	20
3	0.5	None	2.5×10^8	2.5×10^8	1.5×10^8–3.5×10^8	10
		Sham	2.1×10^8	1.9×10^8	1.0×10^7–4.0×10^8	8
		True	8.5×10^7	5.4×10^7	2.4×10^6–3.4×10^8	20
	24	None	2.1×10^8	1.7×10^8	5.0×10^7–5.7×10^8	10
		Sham	2.6×10^8	2.8×10^8	9.2×10^7–5.3×10^8	9
		True	2.2×10^8	1.8×10^8	1.5×10^5–5.7×10^8	19

[a] No. of organisms per gram (wet weight) of feces.
[b] Two not sampled.
[c] Four not sampled.

that the same result was found indicates that generally there is no reduction in numbers of lactobacilli due to tail-cupping procedures. Were coprophagy to have an effect on fecal lactobacilli, one would expect a magnified difference in stomach lactobacillus populations between cupped and noncupped rats. However, no differences in numbers of lactobacilli were found, and the stomachs of all three groups contained large and similar numbers of lactobacilli. Coprophagy, therefore, has a negligible effect.

The reasons for the divergence between our

TABLE 4. *Average body weight per animal per week*

Group	Cups	No. of rats	Weeks				
			0	1	2	3	4
I	True	22	48	64	99	145	194[a]
II	Sham	10	48	73	121	175	228
III	None	10	48	74	121	177	232

[a] Represents 21 rats.

results and those of Gustafsson and Fitzgerald (2) are obscure. Gustafsson and Fitzgerald (2) took their samples from tail-cupped rats over a period of 24 hr, whereas in experiment 1 only fresh feces collected over 0.5 hr were used. The difference in procedure would not be sufficient to explain why in the experiments of Gustafsson and Fitzgerald (2) a progressive fall in count occured over a period of 4 weeks. In experiment 2 with young rats, collection of feces over 24 hr had no significant effect. We do not think sex per se is a factor, since there were no differences between our findings with female rats and those of Powell and Lev (*in press*) with male rats. Although diet may have some influence on the intestinal bacteria, it does not seem to be the critical factor in explaining the differences among the results of Gustafsson and Fitzgerald (2), Powell and Lev (in press), and ourselves. Gustafsson and Fitzgerald (2) used younger animals than we used in our first experiment. Their results showing a fall in lactobacilli may have been the effect of a developing flora in a young animal. The age of animals harboring lactobacilli was not found to be a factor since, in our later experiment, no differences were found in lactobacilli from rats 22 days old at the beginning of the experiment.

If animals had had limited access to their feces, this could explain failure to find a reduction in numbers of lactobacilli in our experiments. However, rigorous care was taken to ensure that animals which had ineffective tail-cups were excluded from the experiment. These were judged by the rigid criterion of a single pellet on the tray beneath the cage. For this reason, in experiment 1, 9 of 18 rats were discarded in the first 3 weeks; 2 more were discarded in the last week of the experiment. This illustrates the great difficulty of absolute prevention of coprophagy by use of tail-cupping techniques. However, in experiment 2, with younger animals of a different strain, the losses due to unsatisfactory tail-cupping were negligible (1 rat of 20).

It is difficult to imagine why the rat or other animals would need a mechanism such as coprophagy for maintaining gut bacterial populations. Chicks, which do not practice coprophagy when raised on wire floors where ingestion of feces is presumably minimal, nevertheless maintain gut populations of lactobacilli which are as high as those found in the rat (3, 6). Human beings were also found to have high numbers of lactobacilli in their feces (5). Our results indicate that, in common with chicks and man, rats do not need coprophagy to maintain their lactobacillus flora.

ACKNOWLEDGMENTS

We thank Melissa Becsy, Catherine Hales, Emilio Alcarez, and Albert Milford for their dedicated technical assistance. We also thank R. Fitzgerald for allowing us to read his paper prior to publication.

This investigation was supported by grant DA-49-193-MD-2681 from the Research and Development Command of the U.S. Army Medical Service, and by grants A-5664 and K6-GM-14208 from the National Institutes of Health.

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