Alteration in Intestinal Microbial Flora of Rats with Tail Cups to Prevent Coprophagy. (25821)

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(Introduced by I. Zipkin)
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There is indirect evidence from studies of animals fed diets containing sulfonamides or antibiotics that intestinal microorganisms synthesize vitamins of importance to host. This concept has been strengthened lately by the fact that germfree rats develop a severe Vit. K deficiency on a Vit. K-free diet whereas conventional animals on the same diet do not show any symptoms(1). Signs of folic acid deficiency have been found in germfree rats on a deficient diet, but animals with normal microbial flora on the same diet show no signs of folic acid deficiency(2). However, the question still remains whether the vitamins synthesized in intestinal tract of laboratory animals are utilized by direct absorption or only after coprophagy. Barnes and Fiala(3) have demonstrated that rats showed signs of Vit. K deficiency when coprophagy was prevented by means of a plastic tail cup. Control rats on the same Vit. K-free diet had no symptoms. These findings suggest that the vitamins synthesized by the microbial flora of rats are utilized mainly through recirculation of the feces. The possibility exists, however, that use of the tail cups produces qualitative or quantitative changes in the microbial flora thus diminishing synthesis of the vitamin. One way of investigating this possibility is to evaluate intestinal flora of animals with tail cups through direct bacterial counts. The aim of the present investigation has therefore been to compare intestinal microbial floras in groups of rats with and without tail cups. The lactobacilli were chosen for study because they constitute an important sector of the microbial flora of intestinal tracts of rodents(4, 5), and because a satisfactory medium was available for their selective isolation and enumeration(6,7).

Materials and methods. Two series of rats were used in the study. The animals of *Series A* were part of a study on influence of a pyridoxine deficient diet on frequency of urinary calculi in rats with and without tail cups. In *Series A* there were 4 groups of weanling Sprague-Dawley rats. Group No. 1 had a nondeficient diet without tail cups; group 2 had the same diet and tail cups. Groups No. 3 and 4 had a pyridoxine-deficient diet without and with tail cups respectively. Numbers of lactobacilli were determined on the cecal contents of these animals at the end of the experimental period of 8 weeks. In *Series B*, 36 rats of both sexes from the Wistar strain used at Notre Dame Univ. for germfree research were weaned on the 21st day to a diet low in Vit. K(1). Bacterial counts of the fresh feces were made on the same day making up the baseline value for both groups. At 22 days of age tail cups were put on half of the rats and lactobacillus counts of the feces were then recorded once a week in both groups to the age of 9 weeks when all rats were sacrificed. Numbers of lactobacilli were then also determined in the contents of the ceca. All animals were kept in individual cages with screen bottoms. Food and water were available *ad libitum.* For lactobacillus counts, the SL medium of Rogosa(6) was modified by reducing glucose content to 1.0% and adding 0.5% L-arabinose and 0.5% sucrose. Weighed amounts of fresh feces or cecal contents were serially diluted in 0.03% sterile yeast extract water and plates were poured at final dilutions of 10^2, 10^4, and 10^6. In *Series B* plates were also poured at a dilution of 10^8. Plates were counted after 4 days incubation in air at 37°C. The only organisms other than lactobacilli found to grow on the SL medium in the present study were occasional colonies of filamentous yeasts of the genera *Geotrichum* or *Candida*. These were easily distinguished by their large size and

TABLE I. Lactobacillus Counts of Cecum Contents in *Series A*.

Group	Pyridoxine	Tail cups	No. of animals	Mean No. of lactobacilli/g*	Distribution of animals according to No. lactobacilli/g* $<10^2$	10^2-10^6	$>10^6$
1	+	—	12	$2.8 \cdot 10^7$	0	2	10
2	+	+	9	$4.5 \cdot 10^6$	1	4	4
3	—	—	11	$1.6 \cdot 10^8$	0	0	11
4	—	+	11	$3.1 \cdot 10^7$	2	6	3
1, 3	+ and —	—	23	$9.1 \cdot 10^7$	0	2	21
2, 4	+ and —	+	20	$1.9 \cdot 10^7$	3	10	7
1, 2	+	+ and —	21	$1.8 \cdot 10^7$	1	6	14
3, 4	—	+ and —	22	$9.5 \cdot 10^7$	2	6	14

* Based on wet wt.

typical growth on the surface of the medium. The statistical analyses were performed by the Chi square method.

Results. Series A. There was a significant difference in distribution of lactobacillus counts in cecal contents of the rats with and without tail cups in Series A (Tables I, II). This difference was also present when comparison was made between the cupped and the noncupped rats on the pyridoxine containing diet. Pyridoxine deficiency resulted in a marked growth retardation but had no statistically significant influence on number of lactobacilli, although mean values seem to be higher in the deficient groups.

When the means of number of bacteria for noncupped and cupped rats are compared, the former have about 5 times more lactobacilli than the latter. The value of comparison of the means of bacterial counts is debatable, however, in view of the disproportionate influence of single high or low values. For instance, in the group of rats on the pyridoxine free diet and without tail cups there were 2 rats with such high values that counting was impossible even in the highest dilution.

TABLE II. Statistical Analysis of Distribution of Lactobacillus Counts in *Series A*.

Pyridoxine	Tail cups	Probability
+ —	— —	$p > .5$
+ —	+ +	"
+ +	+ —	$.01 > p > .001$
— —	+ —	$p < .001$
+ and — + and —	+ —	"
+ —	+ and — + and —	$p > .5$

Series B. At the start of the study and the day before the tail cups were applied, there was no difference between the 2 study groups in lactobacillus counts of the feces (Table III and Fig. 1). During the following 3 weeks the groups with tail cups showed a considerable decrease in lactobacilli. This decrease is statistically significant both by *intra-* group and *inter*-group comparison. The decrease amounts to about 5 log values in the means by *intra*-group and 3 log values by *inter*-group comparison. This lower difference by *inter*-group comparison in turn depends on a down-

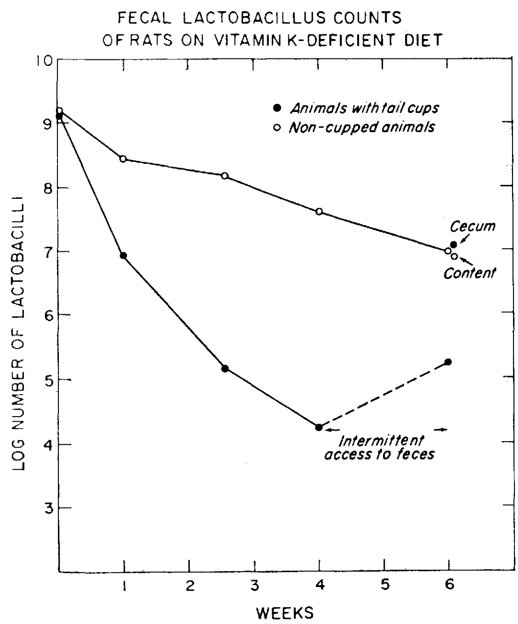

FIG. 1.

TABLE III. Lactobacillus Counts in Series B.

Time in wk after cupping	Material	Tail cups	No. of animals	Mean No. of lactobacilli/g*	Distribution of animals according to No. of lactobacilli/g* $<10^2$	10^2-10^6	$>10^6$	Probability
0	Feces	+	18	$1.1 \cdot 10^9$	0	2	16	p >.5
		—	”	$1.6 \cdot 10^9$	0	0	18	
1	”	+	”	$9.5 \cdot 10^6$	0	10	8	.05 > p >.01
		—	”	$4.6 \cdot 10^8$	0	4	14	
2.5	”	+	”	$1.7 \cdot 10^5$	1	16	1	p <.001
		—	”	$1.8 \cdot 10^8$	0	7	11	
4	”	+	”	$2.6 \cdot 10^4$	2	16	0	”
		—	”	$5.9 \cdot 10^7$	0	9	9	
6	”	+	”	$2.5 \cdot 10^5$	0	17	1	”
		—	17†	$9.9 \cdot 10^6$	1	8	8	.01 > p >.001
6	Cecum content	+	18	$1.6 \cdot 10^7$	0	14	4	p >.5
		—	17	$8.6 \cdot 10^6$	0	11	6	

* Based on wet wt; initial counts performed before cups were applied.
† One animal died of pneumonia. No sign of hemorrhage.

ward trend also in the rats without tail cups. When initial values of this latter group are compared with those in the same group at 3 weeks, this downward trend is just on the borderline of being significant ($0.10>p>0.05$).

Prior to 5th week, the animals never had access to their feces whereas during the 5th and 6th weeks almost all were able to practice coprophagy for at least one day due to faulty operation of the tail cups. Eight of the animals succeeded at least once in freeing themselves completely of the cups. Of these 8 rats all had higher numbers of lactobacilli than during the preceding week. In 3 cases the increase was more than 2 log values. Among the remaining 10 animals in this group, 7 showed an increase while the counts of 3 animals declined. The fact that all the rats with the tail cups could practice coprophagy to a certain extent during the last 2 weeks could also be an explanation for the lack of difference between cupped and non-cupped rats as far as lactobacillus counts of the cecal contents are concerned. Another possibility is that the difference between counts in the feces of rats with and without tail cups is due to bacterial population changes in the tail cups after feces have been voided. If this were so, however, it is difficult to understand why lactobacillus counts increased in the feces of the cupped rats when these rats were able to practice coprophagy.

The present study demonstrates the importance of coprophagy for maintenance of the intestinal flora. Although these observations have been confined to the changes in the population of intestinal lactobacilli, it is obvious that the balance between other types of microorganisms in the intestinal environment might also be affected when coprophagy is prevented. Consequently in the study of specific vitamin deficiencies in animals with tail cups, one must consider not only the factor of reduced intake of the vitamins usually to be found in the feces but also the possibility of variations in intestinal levels of vitamins due to synthesis or depletion by the altered intestinal microflora.

Summary. Under conditions in which coprophagy was completely prevented by use of tail cups, the number of lactobacilli in cecal contents and feces of rats was reduced significantly. Thus, the possibility exists that studies of vitamin deficiencies observed under conditions of cupping may be complicated by changes in intestinal microflora.

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Adrenal Gland Weights of Hereford and Brahman Cattle. (25822)

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Information about weights of organs may be helpful in estimating functional status of the organ. This concept has been widely used with endocrine glands since some methods of assaying hormone output have been based upon gland weight. Hartman and Brownell (1), Bachmann(2) and Chester Jones(3) have recently reviewed the scattered data concerning adrenal weights of the rat, man, dog, rabbit, cat and mouse, but little or no information is presented on cattle adrenals. Sisson and Grollman(4) merely describe the shape of the adrenal glands and their normal position in the bovine body, without mentioning size or internal anatomy. In view of the paucity of information concerning weight of adrenals of cattle, the following data are presented.

Methods. Adrenal glands were obtained from 119 Hereford (*Bos taurus*) and Brahman (*Bos indicus*) beef cattle, slaughtered by standard abattoir procedures. Fifty-eight were steers, 5 were bulls and the remainder females. All animals except bulls were slaughter cattle, selected at random from groups of known Herefords or Brahmans. All animals had carcass grade either U.S. Standard or Good. Animals were transported to slaughterhouse holding pens on day previous to killing and given access to water only. On day of slaughter animals were weighed, then stunned, bled, skinned and dressed. To avoid damage to adrenal glands, they were removed before the carcass was split. This removal occurred approximately 15 minutes after death. Adrenal glands were cleaned of excess fat and the gland from each side of animal weighed separately. One gland taken at random from each of 13 Hereford heifers was dissected into cortical and medullary regions under dissecting microscope, then weighed.

Results. Fresh adrenal and body weights for each group of animals are presented in Table I. Data of weight ratios for the 2 groups of steers and Hereford heifers were subjected to analysis of variance since each group was sufficiently large and comparable in size. The Hereford heifers had a significantly larger ($P < .05$) adrenal to body weight ratio than either group of steers, while there was no significant difference in weight ratios of the 2 steer groups. Weights of cortex and medulla for the 13 dissected glands are presented in Table II.

Discussion. Adrenals of beef cattle are much smaller in relation to body weight than those of most other mammals examined. Expressed in mg, adrenal weight/100 g of body weight (mg %) the male rat, according to Korenchevsky(5), has a ratio varying from 42 to 14 decreasing with age, and the human decreases from over 100 g/100 kg body weight at birth to 30 g at puberty and 25 g at 70 years of age. Other instances of higher ratios of adrenal weight to body weight are cited by Chester Jones(3). Published data on adrenal weights of cattle are summarized in Table III, and would appear to confirm the low gland to body weight ratios observed in cattle by the authors. The data for the Angus and Jersey cows(6) differ from the remainder possibly because of the very high

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