Effect of normal microbial flora on intestinal surface area[1]

HELMUT A. GORDON AND EDITH BRUCKNER-KARDOSS
Lobund Germfree Life Research Laboratories, Department of Biology, University of Notre Dame, Notre Dame, Indiana

GORDON, HELMUT A., AND EDITH BRUCKNER-KARDOSS. *Effect of normal microbial flora on intestinal surface area.* Am. J. Physiol. 201(1): 175–178. 1961.—The mucosal surface area of the small intestine was determined in young adult, germfree and conventional rats. On the average, the germfree values were found to be 30% lower than those of conventional animals. This reduction was pronounced in the mid- and lower parts of the small intestine and relatively slight in the upper segment. It is assumed that the higher surface area values of conventional rats are due to the "physiologic" stimulation by the normal microbial flora.

COMPARATIVE STUDIES in germfree and conventional animals have indicated that the absence of stimulation by the normal microbial flora imparts distinct characteristics to the host (1). It has been shown that, in germfree animals, the wall of the small intestine weighs less per unit body weight, harbors fewer elements of the reticuloendothelial system, and contains less water, percentagewise, than in conventional controls. Among the various layers of the intestinal wall, the lamina propria showed the greatest reduction in the germfree state. The presence of these "germfree characteristics" has been demonstrated in the rat (2–6), and the chicken (3, 6–8), and have been corroborated by concomitant observations in the guinea pig (9, 10) and the mouse (3).

In the course of this work the impression was gained that in germfree animals the surface area of the intestinal mucosa may also be affected. The possibility of a flora effect of this nature is of interest in itself. In addition, knowledge of the gut's mucosal surface in germfree animals is needed to quantitate properly the transport across the intestinal membrane as it may be affected by by the microbial flora, a field of investigation for which the germfree "tool" animal is eminently suited. The purpose of this paper is to supply some of this information.

Received for publication 3 January 1961.

[1] This work received general support from the Office of Naval Research and the Army Surgeon General (Contract NONR-1623); from the National Institutes of Health, grants A-2658(C1) and A-566; and from the University of Notre Dame.

METHODS

The animals used in this series were inbred Wistar male rats, aged 115–123 days, and weighing 280–360 gm. The germfree rats, representing the 10th–11th generation from the original, Caesarian-born ancestors, were maintained throughout their lives in steel isolators of Reyniers (11). The germfree status was established according to the method of Wagner (12). The conventional animals were reared in the open colony. In both experimental groups, three to four animals were housed per square foot of floor space. A semisynthetic-type diet, L-356 (13, 14), steam sterilized (at 120 C, 20 min) was uniformly fed after the animals were weaned from the dam; both ration and sterilized tap water were offered ad libitum. In all details of germfree and conventional rearing, routine procedures were followed (15). This insured good and comparable growth and clinical health both in the germfree and conventional group. Germfree characteristics previously described (5) have been observed also in the present germfree rats. However, there was no indication of adrenal enlargement and reduced weight of the thymus in these animals.

At termination of the experiment the rats were transferred from their housing units to the conventional laboratory environment and killed in the early afternoon hours without prior fasting. As an anesthetic, pentobarbital sodium was given intraperitoneally, 40 mg/kg body wt. When surgical anesthesia was attained, the abdominal cavity was opened and the small intestine, cut at both ends, was separated from the mesentery and passed directly into Bouin fixative (fixation time 24 hr at room temperature). The time of exposure of the germfree rats to the conventional environment in this operation was a maximum of 15 min. Under these circumstances, uniform and rather scanty amounts of intestinal contents could be found in the small intestines of both germfree and conventional rats. Technical difficulties prevented us from maintaining constant hydrostatic pressure in the gut; however, in all animals the intraluminal pressure appeared slight on visual observation. The length of the total, fixed small intestine

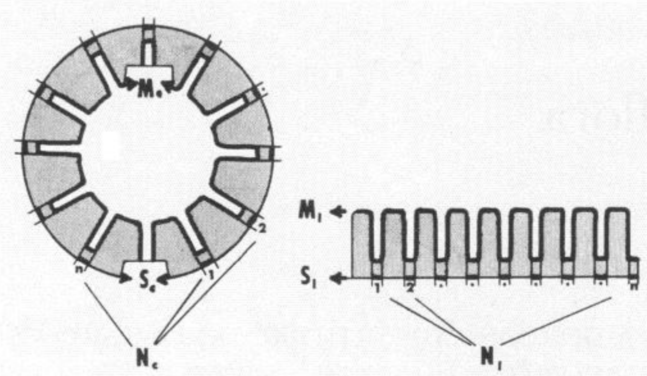

FIG. 1. M_c and M_l = mucosal surface in cross- and longitudinal sections. S_c and S_l = serosal surface in same. N_c and N_l (1 + 2 + ... + n) = length of mucosa not occupied by villi (sum of "intervillar spaces").

was next established (16). Approximately 2-cm samples of the intestine were taken: *a*) next to the pyloric end of the stomach ("upper"), *b*) at the midpoint between pyloric end and ileocecal valve ("mid"), and *c*) adjacent to the ileocecal valve ("lower"), and embedded in celloidin-paraffin according to Apáthy (17).

The estimation of the total mucosal surface area of the small intestine was made, following the procedure of Warren (18) as modified by Fisher and Parsons (19). This method of geometrical reconstruction, based on taking linear measurements of surface in transverse and longitudinal sections of the intestine, provides an expression for the area of the mucosal and serosal surface.

One complete cross- and one longitudinal section (cut at 4μ and separated by not more than 1 cm of gut length) was taken from the upper, mid-, and lower small intestine samples in each animal. No intestinal specimens containing lymph follicle tissue were used. The stain employed was Mayer's hemalum-eosin Y.

The tracing of surfaces was made with a microprojector (linear magnification 53 ×) on paper. One tracing was made from each slide; this included the total cross section and an approximately 0.3-cm length of the longitudinal section. The surface length of the tracings was established with a map measurer calibrated in centimeters. The measurements made were designated as follows (Fig. 1): M_c and M_l, length of mucosal surface in cross- ($_c$) and longitudinal ($_l$) sections of intestine; S_c and S_l, length of serosal surface in the same specimens; N_c and N_l represent the length of the mucosal surface not occupied by villi (sum of intervillar spaces). From these linear measurements, the length of mucosal surface per unit serosal surface (R) and the cumulative length of intervillar spaces per unit serosal surface (F) were calculated both for the cross- and longitudinal sections for the intestine ($R_c = M_c/S_c$; $F_c = N_c/S_c$ and $R_l = M_l/S_l$; $F_l = N_l/S_l$). The mucosal surface area per unit serosal surface (R_a) was established with the Warren formula as modified by Fisher and Parsons (19): $R_a = (R_l - 1)(1 - F_c) + (R_c - 1)(1 - F_l) + 1$; the mucosal surface area per centimeter gut length is: $M_a = gS_cR_a$ (where g = magnification factor). The expression M_a, calculated from the three tissue samples of the small intestine multiplied by one-third of the gut length, gave the value of the mucosal surface area for the upper, mid-, and lower segments; their sum is the total mucosal surface area of the small intestine.

No attempt was made to establish the measurements of individual villi.

RESULTS

The main results of the present observations are given in Table 1. The total mucosal surface area of the small intestine in the germfree rats was approximately 30% lower than in the conventional animals. In terms of various segments of the small intestine, the germfree deficit was demonstrable in the entire length of the gut; however, this difference in surface area from the conventional controls was most prominent in the mid- and lower portions. As indicated in this table, the mean body weight of both animal groups was essentially the same.

Table 2, an addendum to Table 1, indicates that the smaller mucosal surface of the germfree group was caused mainly by a reduction in "mucosal contour." Thus, the ratio of mucosal and serosal surface length in cross- and longitudinal sections (R_c and R_l) was generally lower, while the ratio of intervillar spaces and serosal surface length (F_c and F_l) was higher in the germfree group. Two additional determinants of intestinal surface area, the intestinal length and serosal circumference, were essentially similar in the opposing categories. As a consequence of these characteristics the mucosal surface area per unit serosal surface (R_a) and the mucosal surface area per centimeter of gut length (M_a) were reduced in the germfree group.

The differences portrayed in Table 1 and 2 were significant for most elements measured in the mid- and lower small intestine. In the upper gut, the deficit in surface of the germfree small intestine resulted from the slight reduction of various intercurrent elements. Only on compounding these to mucosal surface area, did the difference from the conventional gut become statistically significant.

DISCUSSION

Germfree and conventional animals which are used in portraying the effect of the normal flora on the animal

TABLE 1. *Mucosal surface area of small intestine and mean body weight*

Segment	Germfree	Conventional	*P*
	Mucosal surface area of small intestine, cm^2		
Upper ⅓	249±29	296±20	<0.01
Mid ⅓	144±23	263±24	<0.01
Lower ⅓	73±14	112±16	<0.01
Total	466±37	671±35	<0.01
	Body weight, gm		
	312±22	320±22	0.57

No. of rats: 7 germfree, 7 conventional (all males). Values are means ±SD. Body wt corrected for cecal contents.

TABLE 2. *Intercurrent measurements of mucosal surface area of small intestine*

	Segment	Germfree	Conventional	*P*
	Upper	4.12±0.77	4.85±0.41	0.07
R_c	Mid	2.53±0.34	3.88±0.69	<0.01
	Lower	1.97±0.48	2.35±0.52	0.20
	Upper	8.58±1.25	8.02±1.19	0.45
R_l	Mid	5.07±0.73	7.91±0.68	<0.01
	Lower	2.79±0.86	3.55±0.55	0.01
	Upper	0.10±0.05	0.06±0.06	0.24
F_c	Mid	0.22±0.08	0.10±0.04	0.01
	Lower	0.29±0.07	0.15±0.01	<0.01
	Upper	0.11±0.09	0.09±0.06	0.75
F_l	Mid	0.17±0.10	0.09±0.08	0.17
	Lower	0.28±0.09	0.15±0.06	0.02
	Upper	10.52±1.59	11.34±1.65	0.37
R_a	Mid	5.52±1.14	9.80±1.06	<0.01
	Lower	2.94±0.57	4.29±0.83	<0.01
	Upper	8.73±1.21	9.91±0.91	0.08
M_a	Mid	5.04±0.82	8.50±0.85	<0.01
	Lower	2.55±0.60	3.71±0.65	0.01
	Upper	0.83±0.05	0.90±0.13	0.25
S_c	Mid	0.93±0.13	0.87±0.07	0.35
	Lower	0.86±0.07	0.87±0.06	1.00
L.I.		86.4±9.3	89.5±4.3	0.48

Values are means ±SD. R_c and R_l = length of mucosal surface per unit length serosal surface in cross- and longitudinal sections. F_c and F_l = cumulative length of intervillar spaces per unit length serosal surface in cross- and longitudinal sections. R_a = mucosal surface area per unit serosal area. M_a = mucosal surface area per cm gut length. S_c = serosal circumference, cm. L.I. = length, small intestine, cm.

TABLE 3. *Mucosal surface area and dry matter per centimeter of gut length*

	Germfree		Conventional	*P*	Mean Germfree Deficit, %
		Mucosal surface area, cm²			
n = 7	3.4±0.5	n = 7	5.4±0.6	<0.01	−37
		Dry matter, mg			
n = 15	5.7±1.4	n = 14	8.7±1.3	<0.01	−34

Values are means ±SD. Both mucosal surface area and dry matter content refer to lower 50% length of small intestine. Mucosal surface area has been recalculated from present data. Dry matter values were obtained from rats comparable to those of the present series in terms of strain, age, sex, diet, body wt, and bacteriological status.

host must meet various requirements. Thus, it is stipulated that the germfree animal should differ from its conventional counterpart only in terms of an absent flora and in the absence of host responses to such microbial associates. It is further expected that other, nonbacterial variables, such as the effects of confinement or of sterilization of the diet, should not enter into the picture. The presently available germfree rats, such as the ones used in this series, essentially satisfy these criteria (1).

The method used for determination of mucosal surface area gave reproducible results in our hands. As the aim of this work was a relative comparison of the intestinal surface of germfree and conventional rats, systematic errors of the procedure, such as caused by shrinkage following fixation of the tissue, had little effect on the results. The source of error in this sense was further reduced by the fact that, in addition to similar age and sex, all rats of this series displayed essentially the same mean body weight, intestinal length, and serosal circumference of the various intestinal segments tested. Working with low intraluminal pressure, the possibility of unfolding Lieberkühn crypts was negligible, as this will not take place at pressures lower than 35 cm of water (19).

The values of mucosal surface area of the small intestine for comparable conventional rats as given by Verzár and McDougall (20), Wood (21), and Fisher and Parsons (19) are essentially in unison with those of our conventional animals. Slight discrepancy between some linear measurements of Fisher and Parsons and those of the present series may be attributed to differences in the intraluminal pressures used. In view of the reduction in weight, in reticuloendothelial cell content, and in lamina propria tissue of the germfree small intestine, which were previously mentioned, the concomitant reduction of the mucosal surface area in these animals appears as a plausible finding. The fact that this reduction was greatest in the mid- and lower portions of the small intestine, i.e., in areas which are normally rich in flora elements, is also in accord with this anticipation.

It is of some interest to compare the present findings with other characteristics of the gut, which in addition to the unit mucosal surface area have been used for standardization of intestinal absorption. Among these, e.g., the dry weight of the gut wall is commonly assumed to be proportional to intestinal surface. Table 3 portrays in germfree and conventional rats the mucosal surface area along with the weight of dry matter of the small intestine, both calculated per centimeter of gut length. As seen from these data, the reduction of the mucosal surface in the germfree animal, compared with the conventional, was fairly well paralleled by a reduction in dry matter,[2] which, considering the scattering of data, was essentially of the same order.

It appears that the area of the small intestine's mucosal surface is a characteristic of the animal host, which is appreciably affected by the microbial flora. Experiments dealing with the transfer of materials across the intestinal wall, using animals exposed to known or absent microbial stimulation, must take this variable into consideration. On first approximation, the amount of dry matter per unit length of gut may be regarded as a usable substitute for the surface area value.

REFERENCES

1. GORDON, H. A. *Am. J. Diges. Diseases* 5: 841, 1960.
2. GUSTAFSSON, B. *Acta Pathol. Microbiol. Scand. Suppl.* 73: 1, 1948.
3. GORDON, H. A. *Ann. N. Y. Acad. Sci.* 78: 208, 1959.
4. WOSTMANN, B. S., AND E. BRUCKNER-KARDOSS. *Am. J. Physiol.* 197: 1345, 1959.
5. GORDON, H. A., AND B. S. WOSTMANN. *Anat. Record* 137: 65, 1960.

[2] The markedly reduced wet weight and slightly elevated dry matter percentage of the germfree gut wall in comparison to the conventional (5) results in reduced total dry matter content.

6. GORDON, H. A., AND E. BRUCKNER-KARDOSS. *Acta Anat.* 44: 210, 1961.
7. REYNIERS, J. A., M. WAGNER, T. D. LUCKEY, AND H. A. GORDON. *Lobund Reports* No. 3, p. 7, 1960.
8. GORDON, H. A., AND E. BRUCKNER-KARDOSS. *Antibiotics Ann. 1958/59* 1012, 1959.
9. GLIMSTEDT, G. *Acta pathol. Microbiol. Scand. Suppl.* 30: 1, 1936.
10. PHILLIPS, B. P., P. A. WOLFE, AND H. A. GORDON. *Ann. N. Y. Acad Sci.* 78: 183, 1959.
11. REYNIERS, J. A. *Ann. N. Y. Acad. Sci.* 78: 47, 1959.
12. WAGNER, M. *Ann. N. Y. Acad. Sci.* 78: 89, 1959.
13. LARNER, J., AND R. E. GILLESPIE. *J. Biol. Chem.* 225: 279, 1957.
14. WOSTMANN, B. S. *Ann. N. Y. Acad. Sci.* 78: 175, 1959.
15. REYNIERS, J. A., P. C. TREXLER, AND R. F. ERVIN. *Lobund Reports* No. 1, p. 1, 1946.
16. HYMAN, M. A. *Am. J. Clin. Pathol.* 23: 517, 1953.
17. ROMEIS, B. *Mikroskopische Technik.* München: Leibnitz, 1948, p. 106.
18. WARREN, R. *Anat. Record* 75: 427, 1939.
19. FISHER, R. B., AND D. S. PARSONS. *J. Anat.* 84: 272, 1950.
20. VERZÁR, F., AND E. J. MCDOUGALL. *Absorption from the Intestine.* London: Longmans, Green & Co., Ltd. 1936.
21. WOOD, H. O. *J. Anat.* 78: 103, 1944.