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Effects of Microbial Flora on Cardiac Output and Other Elements of Blood Circulation.* (28658)

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It has been observed that the microbial flora imparts elements of mild ("physiological") inflammation to the wall of the intestinal canal which are portrayed by an increment in weight, hydration and reticulo-endothelial cell population of the tissue(2). These are accompanied by elevated levels of histamine, though observations on this issue are not always consistent(3,4). It has also been observed that the germfree‡ gut appears paler in the living animal(5).

Observations of this nature drew attention to effects which the microbial flora might exert on elements of blood circulation and it was decided to explore the possible effects more systematically. As an initial step, it was endeavored to obtain expressions on composition, volume, pressure and flow of blood in germfree and conventional control rats.

Methods. 1. *Animal material.* The rats used were of Wistar origin. The germfree animals represented the 12th and 13th generation offspring of the original cesarean born ancestors. The conventional controls were genetically closely related to the germfree stock. All were males, normally born and suckled. Age at term was 3-4½ months. The diet was steam sterilized, fortified practical type L-462(6). The germfree rats were housed in the steel isolators, the conventional controls in the open colony room. In all details of rearing, routine procedures were followed(7).

One day before sacrifice the germfree rats were transferred into plastic isolators(8). All animals were starved overnight but were given free access to water. At term they were anesthetized inside the isolator (35 ± 5 mg steam sterilized pentobarbital sodium/kg body weight i.p.), aiming at identical depth of narcosis. Thus, the needed exposure and cannulation of blood vessels was started at the time of disappearance of the corneal reflex and pain response to tail-pinching. A uniform time schedule was maintained in

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‡ At this writing the germfree animal may be defined as a host reared in absence of demonstrable living bacterial mycological, protozoan and macroparasitic associates, according to the test procedures routinely employed(1). The possible presence of pleuropneumonia (PPLO) like organisms, rickettsiae and virus has been investigated, but so far results are negative.

TABLE I. Comparison of Data in Germfree and Conventional Rats.
All males. Age: 3-4½ mo. Diet: L-462 sterilized. n = No. of observations. M = arithmetic mean. S.D. = standard deviation. p = significance of difference.

				n	M	S.D.	p
Rat Group 1	Body wt, g (corrected for cecal contents)		germfree	10	271	14	
			conventional	12	271	22	1.00
	Heart wt (mg/100 g body wt)		germfree	10	325	36	
			conventional	12	349	31	.14
	Cardiac output (ml/min/kg body wt)		germfree	10	137	21	
			conventional	12	201	27	.01
		"isolator"	conventional*	2	196-198	—	
Rat Group 2	Body wt, g		germfree	8	297	21	
			conventional	9	303	18	.50
	Mean arterial pressure (carotid, mmHg)		germfree	8	124	15	
			conventional	8	121	13	.70
	Hematocrit (% packed red cells)		germfree	8	47.2	2.7	
			conventional	9	44.4	2.7	.03
	Hemoglobin, g %†		germfree	12	16.0	.6	
			conventional	14	15.1	.9	.03
	Blood, dry %†		germfree	12	21.8	1.5	
			conventional	14	20.2	.5	.01
	Blood vol (ml/100 g body wt)	plasma	germfree	8	2.57	.37	
			conventional	9	3.43	.23	.01
		cell	germfree	8	2.28	.30	
			conventional	9	2.75	.25	.01
		total	germfree	8	4.85	.60	
			conventional	9	6.18	.41	.01

* Comparable conventional male rats from open colony transferred into sterile steel isolator at age of 30 days. For next 10 wk maintained under the same environmental conditions as germfree rats (in diet, air flow at atmospheric pressure, temperature, cage space and cleanup operations).

† Data derived from comparable germfree and conventional male rats, fed steam-sterilized semi-synthetic diet L-356.

various steps of the procedures. Terminal evaluation of the rats took place outside of the isolator; in the case of the germfree animals the time elapsed from removal from the isolator to sacrifice did not exceed 10 minutes.

2. *Cardiac output and weight of heart.* Using the indirect Fick principle, the method described by Sapirstein(9) was employed to determine cardiac output. The indicator was Rb^{86} in a dose of approximately 1 μC, given as 0.05 ml of an $Rb^{86}Cl$ solution in isotonic saline injected within 0.5 seconds into the saphenous vein. Samples of blood were taken from the cannulated carotid artery over a period of approximately 30 seconds after injection of the isotope. The frequency of sample collection was 100/min with a sample volume of approximately 0.03 ml. From these, 0.02 ml was used to determine the level of radioactivity. This allowed ample observations for drawing arterial concentration curves of the isotope. After termination of the experiment, the heart was weighed.

3. *Ancillary observations.* In another group of comparable rats, the following observations were made: (a) Mean arterial blood pressure (carotid artery by mercury manometer); (b) Hematocrit; (c) Total plasma volume (conventional Evans blue method adapted for small animals; the sterile dye was injected into the saphenous vein and sampled in the carotid after the lapse of 5 minutes). From these, total red cell volume and blood volume per 100 g body weight were calculated.

Results. The results are given in Table I. Average cardiac output in the germfree group was approximately 30% lower than in the conventional controls, with no overlap between individual values in the two groups. The conventional rats showed essentially the

same cardiac output values as reported in the literature for comparable animals(9).

Arterial blood pressure was normal and essentially the same in both groups. The cell component in the hematocrit value was slightly elevated, but blood plasma volume was substantially reduced in the germfree group. The calculated blood cell volume was slightly reduced. Accordingly, total blood volume was appreciably lower in germfree rats in comparison to conventional controls. Body weight was the same in the animals forming the basis of this comparison.

Discussion. The results of this series indicate that the absence of the normal microbial flora is associated with marked changes in the circulation. While showing normal values of arterial blood pressure, signs of hemoconcentration are clearly indicated in the germfree rat. The reduction of cardiac output in this group is obvious. In this detail the maintenance of similar depth of anesthesia during the experiment is an essential prerequisite(10). The conditions of anesthesia used here appear to be an acceptable compromise between this stipulation and the condition imposed by the germfree experiment.

The question arises whether these and other characteristics of the germfree rat can be linked directly to the absence of microbial flora, or are they possibly the result of nonspecific stress phenomena caused by the artificial conditions under which these animals are kept? Absence of microbial stimulation in germfree animals has been demonstrated. In addition to the above mentioned absence of a mild inflammatory response in some organs, the humoral defenses are considerably underdeveloped(11,12,13). On the other hand, signs of pronounced stress could not be demonstrated in germfree rats(14). It is postulated that germfree animals display primarily a lack of specific effects which the viable flora impart to the host under normal conditions. In the study reported here, this conclusion is fortified by the fact that conventional rats reared in germfree-type isolators and maintained on sterile diets showed cardiac output values entirely within the range of normal values (Table I).

Thus it may be assumed that the reduction in cardiac output and total blood volume in the germfree group is related, in some way, to the absence of a "defensive burden" in these animals. Supporting this are preliminary findings which indicate a marked reduction in regional blood flow (method of Sapirstein(9)) in such organs of the germfree rat which normally are in close relationship with the microbial flora (*e.g.*, liver(15), small intestine and skin(16)). From this viewpoint, organs remote from the flora (*e.g.*, kidneys) show no difference between the opposing animal categories. A flora-conditioned increase of regional blood flow may stem from effects on vascular musculature. Bacterial products entering the tissue in exposed areas could induce locally higher levels of active histidine decarboxylase(17). Histamine produced by this enzyme system could then act as a local regulator of circulatory responses to changes in microbial environment(18).

The hemoconcentration in the germfree group, as indicated in Table I, may be linked to the substantially enlarged cecum (approximately 2 $\times$ for the cecal sack, 5 $\times$ for the contents) which is commonly observed in germfree rodents(7,14,19,20,21). Cecal enlargement, based mainly on reduced muscle tonus, has been related to the lack of a viable flora in the cecal contents(3). Dry matter percentages of intestinal contents in the upper reaches of the digestive tube are essentially the same in germfree and conventional rats, while they are considerably more liquid in the germfree lower bowel, mainly in the cecum. Recent observations indicate that conventional rats are able to maintain hypertonicity in their cecal contents while in germfree animals the contents are essentially isotonic with the blood(16). The feces voided by the germfree animal are more moist than in conventional controls. The mechanism of these changes in water balance is unknown. Anomalies of this nature might suggest altered adrenal corticoid output-degradation in these animals. The slight but consistent adrenal enlargement(14) together with total urinary corticoid elimination at the upper range of normal values found in germfree rats(16) favor this speculation.

Notwithstanding the local reductions in

regional blood flow, the total metabolic rate of germfree rats appears to be approximately similar to that of conventional controls(16). An unchanged metabolic rate and a reduction in cardiac output imply increased absolute oxygen transport by the blood(23). In part, this may be achieved by the blood's increased oxygen capacity in germfree conditions (*via* increased hemoglobin content, Table I). Furthermore, the virtual nonexistence of perivascular space and of cell collections around the minute blood vessels in the lungs of germfree animals(24,25), and also the reduction in hydration, connective tissue and RE elements in other organs(14,26), indicate that oxygen diffusion capacity, and therefore oxygen extraction, may regionally be enhanced in conditions of germfree life. The opposite process is known to take place in conditions of edema and fibrosis(27).

The absence of a microbial flora thus establishes a new set of conditions for the circulatory system of the rat. Originating mainly from reduced fluid flow, "germfreeness" results in a reduced work load for the heart of the animal.

Summary. Cardiac output in young adult germfree rats was found to be approximately 30% lower than in conventional controls. Concomitantly, signs of mild hemoconcentration were observed in the germfree group, which may be linked to changed water balance in these animals. The reduced work performance of the heart indicated in the germfree group is associated with the absence of a microbial flora.

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