

## Cecal Enlargement, Cardiac Output, and O<sub>2</sub> Consumption in Germfree Rats\* (32962)

BERNARD S. WOSTMANN, EDITH BRUCKNER-KARDOSS, AND P. L. KNIGHT, JR.  
*Lobund Laboratory, University of Notre Dame, Notre Dame, Indiana 46556*

A comparison of the germfree and conventional rodent indicates generally similar gross morphology, with elements of the reticuloendothelial system tending towards underdevelopment. The germfree rodent, however, does possess a most conspicuous anomaly in the enlarged cecum (1). Apparently this enlargement does not interfere with the animal's health as depicted by normal growth, reproduction, and life span (2).

Recent observations indicate, however, physiological differences between the germfree and conventional rat. Cardiac output (CO) in the germfree animal is approximately  $\frac{2}{3}$  of that in its conventional counterpart (3). The data of Desplaces *et al.* (4) show a lower basal metabolic rate (BMR) and lower <sup>131</sup>I binding in the germfree rat, suggesting a decrease in thyroid activity. Wostmann *et al.* in studies on cholesterol metabolism, found smaller amounts of total CO<sub>2</sub> exhaled by the germfree rat (5). Levenson *et al.* also reported lower BMR values in germfree rats (6).

One obvious explanation of these differences could be the metabolic demands placed on the host by the activity of its intestinal microflora, whether this is due to the metabolism of the flora itself, or due to the activity (e.g., detoxification) forced on the host by the presence of its microflora. If this hypothesis were true, then removal of the cecum might depress the levels of BMR, CO, and thyroid activity in the conventional animal, while leaving them unaltered in the germfree animal. Preliminary experiments showed, however, that cecectomy had exactly the opposite effect, increasing the germfree level of CO to that of the conventional animal, and leaving the conventional CO unaltered. Thus it would appear that the cecal contents of the germfree rat may affect its CO. Gordon has reported the presence of a toxic factor(s)

in the cecum of germfree rats and mice (7). These possibly related findings led to a further investigation of the effect of cecectomy on CO and to a study of O<sub>2</sub> consumption in cecectomized rats. The results of these studies not only confirm that cecectomy of germfree rats results in "normal" CO values, but also indicate that this procedure nearly abolishes the reduction of O<sub>2</sub> consumption found in the intact germfree rat.

*Methods.* All rats were males of either Wistar or Sprague-Dawley origin. Germfree animals were obtained from production colonies maintained at the Lobund Laboratory. Conventional rats were genetically closely related to the germfree stock.

*Cececctomy.* Cececctomy was performed under pentobarbital anesthesia at the age of 6 weeks. Aseptic surgical techniques were used for the conventional rats while the germfree animals were operated on inside the isolater. Through a small incision in the lower abdomen the cecum was lifted out carefully and the main part of the organ ligated with nylon thread and excised. The small remaining part of the cecum served as connection between the small and the large intestine. Survival after operation was 100% in both experimental groups. Further details of this technique have been reported elsewhere (8).

*Cardiac output.* Animals used in these studies were of Wistar origin, maintained on steam sterilized, fortified L-462 diet (9). The age range was 3-4 months. The animals were selected according to body weights (250-300 gm) to obtain better similarity between the experimental groups. The CO was determined in germfree, germfree cececctomized, conventional, and conventional cececctomized animals by the method of Sapirstein (10) using the indirect Fick principle. <sup>86</sup>Rb in isotonic saline was injected into the saphenous vein. Blood samples of approx. 0.03 ml were collected from the cannulated carotid artery over a period of 30 sec. Ali-

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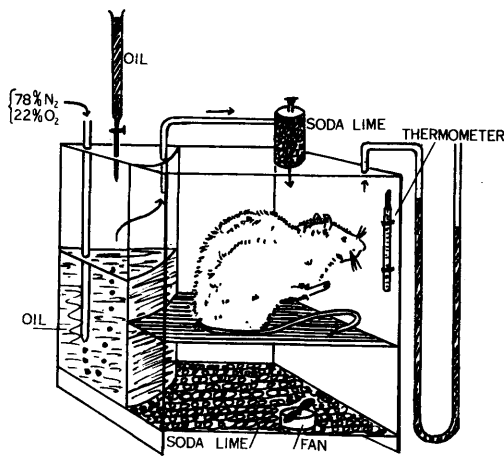


FIG. 1. Schematic representation of oxygen consumption chamber (for details see text).

quots of these samples were used to determine the concentration curve of the isotope. The ensuing CO value was expressed in ml per kg of body weight per min (ml/kg per min). Details of anesthesia, technique and values obtained in intact germfree and conventional rats have been reported earlier (3).

**O<sub>2</sub> consumption.** The O<sub>2</sub> consumption was determined in germfree conventional, and germfree and conventional cecectomized Sprague-Dawley rats by a modification of the method of Schwartz and Nodell (23). In one group of animals (series 1) data were first obtained in the germfree state, while subsequent measurements were made after a 2-month conventionalization period in the conventional animal quarters. Age and range of body weight are indicated in Table II. All animals had been maintained on a commercial type diet<sup>1</sup> and had been fasted 16–20 hours before O<sub>2</sub> consumption was determined. Each rat was then introduced into the 8 × 8 × 8-inch plastic metabolism chamber containing soda lime as a CO<sub>2</sub> absorbent (Fig. 1), for a period of 1 hour prior to the first determination. During this time a mixture of 22% O<sub>2</sub> and 78% N<sub>2</sub> was flushed through the chamber. Subsequently the inlet and outlet were closed and the change in pressure in the chamber indicated by the

manometer was counteracted by buretting mineral oil into the system. The milliliters of mineral oil thus corresponded to the amount of O<sub>2</sub> consumed by the animal at a constant pressure within the chamber. Five or more 5 min determinations were made on each animal and the median value expressed as ml per kg of body weight per min (ml/kg per min). All readings were corrected to standard conditions (0°C, 760 mm Hg).

**Body temperatures.** These were obtained from 3–4 months old Wistar rats maintained on diet L 462. A standard clinical thermometer was inserted rectally 1 inch deep for 2 min. All animals were kept in the same air-conditioned room. Animals designated as fasted were deprived of food overnight. Half of the conventional rats were kept for 3 days prior to the determination under germfree-like conditions within a plastic isolator next to the germfree animals kept in a similar isolator.

**Results.** Table I indicates that although the CO value of the Wistar germfree rat is about 32% lower than that of its conventional counterpart, surgical removal of the cecum brings CO back to the level of the conventional animal. The data obtained from conventional animals agree well with those reported by others (12, 13), but reflect the use of pentobarbital as indicated by Popovic and Kent (14). The data in Table I represent a selection from a larger material on the basis of body weight (range 250–300 gm) in order to obtain more comparable data. However,

TABLE I. Cardiac Output of Intact and Cecectomized Germfree and Conventional Male Wistar Rats.

Group	Body wt.	CO (ml/kg per min)
Germfree	270 (10)	137 ± 7
Germfree cecectomized	272 (10)	201 ± 11
Conventional	277 (10)	203 ± 11
Conventional cecectomized	263 (8)	218 ± 5

Age 3–4 months; body wt. range 250–300 gm; animal numbers in parentheses; SE values given. Germfree vs germfree cecectomized  $p < 0.01$ ; germfree vs conventional  $p < 0.01$ ; and germfree vs conventional cecectomized  $p < 0.01$ .

<sup>1</sup> Diet L-485 (11) manufactured to Lobund Laboratory specifications by Teklad Corp., Monmouth, Illinois.

TABLE II. O<sub>2</sub> Consumption in Intact and Cecectomized Germfree and Conventional Male Sprague-Dawley Rats.<sup>a</sup>

Group	Body wt.	O <sub>2</sub> (ml/kg per min)
Germfree series I	371 (9)	11.2 ± 0.3
Germfree all	374 (15)	11.5 ± 0.4
Germfree cecectomized	400 (13)	13.5 ± 0.3
Conventionalized series I	472 (8)	13.6 ± 0.5
Conventional	417 (8)	15.2 ± 0.4
Conventional cecectomized	350 (4)	15.3 ± 0.5

<sup>a</sup> Age 3–4 months except for series I from which germfree data were obtained at 3 months and “conventionalized” values 2 months later. Body weight range 350–450 gm except for “conventionalized” and “conventionalized cecectomized.” Animal numbers in parentheses; SE values given. Germfree vs germfree cecectomized  $p < 0.01$ ; germfree vs conventionalized (series I)  $p < 0.01$ ; germfree vs conventional  $p < 0.01$ ; germfree cecectomized vs conventional  $p = 0.01$ ; and conventionalized vs conventional  $p = 0.08$ .

evaluation of all material shows essentially the same results.

Table II shows the effect of cecectomy on the O<sub>2</sub> consumption of Sprague-Dawley germfree rats. The average value of 15.2 ml of O<sub>2</sub>/kg per min determined for the intact conventional rat agrees with the value of 15.3 given by Adolph (15). The values obtained with germfree animals were on the average 25% lower. Conventionalization of the germfree rats increased O<sub>2</sub> consumption. The slightly lower than conventional values found in this group (series 1) would seem to be related to the older age and higher body weight of these animals which went through a 2-month conventionalization period after the “germfree” values were obtained. Removal of

the cecum of the conventional rat has little or no effect on the O<sub>2</sub> consumed by the animal, but removal of the cecum of the germfree rat increases the average amount of O<sub>2</sub> consumed to a value only slightly below the conventional level (11.5–13.5 ml/kg per min).

Table III indicates that body temperatures of germfree rats (100.4°F, nonfasted and 100.7°F, fasted) tended to be slightly higher than the temperatures of conventional rats (99.8°F, fasted and 99.9°F, nonfasted). In conventional rats no appreciable difference was found between the groups maintained inside and outside the plastic isolator.

*Discussion.* The lower O<sub>2</sub> consumption of the germfree rat reported here confirms data obtained by others (4, 6, 16). Together with the smaller size of the heart (1) and the lower CO (3), this appears to be a reflection of decreased metabolic requirements. Removal of the enlarged cecum restores these parameters to normal or near normal values.

The cecum of the germfree rat begins to increase abnormally approximately 2 weeks after birth (17). Accompanying the increase in size is the accumulation of a number of bioactive substances. Gordon has described toxic and musculo-active principles (7), and Lindstedt *et al.* hexosamine-containing materials (18) which because of their acidic poly-electrolyte characteristics may upset the electrolyte pattern in the cecum (19). The abnormal accumulation of such substances may result in absorption of larger than usual amounts. One or more of these materials then might, by some unknown mechanism, exert a depressant action on O<sub>2</sub> consumption and cardiac output. This concept seems to be supported by data given by Desplaces *et al.*

TABLE III. Body Temperature of Male Wistar Rats.<sup>a</sup>

	Fasted (16 hours)	Nonfasted
Germfree (10)	100.7 ± 0.4	100.4 ± 0.2
Conventional series I (5)	—	99.9 ± 0.1
Conventional (isolator) series I (5)	100.2 ± 0.3	—
Conventional (isolator) total (9)	99.8 ± 0.3	—

<sup>a</sup> Age 3–4 months; animal number in parentheses; SE values given. Conventional (isolator) animals were housed within a plastic isolator next to the germfree isolator for 3 days prior to temperature reading (see text). Germfree vs conventional (fasted)  $p = 0.06$ .

which indicate a lower basal metabolic rate and a lower  $^{131}\text{I}$  binding in the germfree rat (4). Removal of the enlarged cecum might not prevent the formation of the factor(s) responsible for these phenomena, but could strongly reduce its absorption by eliminating its residency in the cecum (20). The fact that cecectomized germfree rats show  $\text{O}_2$  consumption values which are still slightly, but significantly lower than those found in conventional animals could indicate some residual effect of these toxic metabolites. However, the absence of a viable, metabolizing microflora might offer another explanation for the persistently slightly lower values in these animals.

Many facets of metabolism seem to be influenced by the germfree state. Wostmann *et al.* (21) not only found lower vitamin  $\text{B}_1$  levels but also lower regional blood flow values in the liver of the germfree rat as compared to the conventional animal. It appears probable that the smaller amount of  $\text{O}_2$  consumed by the germfree rat is directly related to the lower energy metabolism of the liver indicated by these data. Certainly these details must be considered when metabolic studies of the germfree rat are made.

The present data do not confirm the lower rectal temperatures reported by Levenson *et al.* in germfree rats. In their studies with germfree, mono-associated and conventional rats these authors state, however, that no apparent relationship existed between  $\text{O}_2$  consumption and  $\text{CO}_2$  production on the one hand, and body temperatures on the other hand (6). The present data appear to confirm this point of view. The data also indicate that, although the confinement of the plastic isolator generally results in higher than ambient temperature and relative humidity within the unit (22), housing within an isolator per se does not lead to appreciable higher body temperature.

Obviously the level of general metabolism of the germfree rat is lower than that of its conventional counterpart. But whatever its actual cause the animal appears to be in a healthy state as evidenced by normal growth, reproduction, and life span.

*Summary.* Germfree rats have cardiac out-

put and  $\text{O}_2$  consumption values respectively 30% and 24% lower than found in comparable conventional rats. Surgical removal of the enlarged cecum typical for these animals resulted in cardiac output values similar to those obtained with conventional rats, while  $\text{O}_2$  consumption increased significantly to levels only slightly lower than seen in the conventional control group. No significant difference in body temperature was found between comparable groups of germfree and conventional rats.

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## The Effect of Pregnancy on the Cytology of Mouse Peritoneal Fluid (32963)

LARRY MCGOWAN, ROBERT H. DAVIS, AND LESLIE S. KREISLER

*Department of Obstetrics and Gynecology, Thomas M. Fitzgerald Mercy Hospital, Darby, Pennsylvania 19023; and Department of Obstetrics and Gynecology, Hahnemann Medical College and Hospital, Philadelphia, Pennsylvania 19102*

Pregnancy profoundly influenced the composition of serous body cells in women by significantly lowering mesothelial cells and lymphocytes and elevating polymorphonuclear leukocytes when compared to nonpregnant and postpartum women (1-3). In order to make possible useful comparisons with women, the present study was undertaken to determine the effect of pregnancy on the cytology of mouse peritoneal fluid. To our knowledge, no similar studies have been reported.

**Materials and Methods.** We cohabited adult female mice (CF-1; 25-30 gm) with adult males (30 gm) of the same strain in a 3:1 ratio under standard animal room conditions. Every morning, we inspected the females for a vaginal plug to establish day 1 of pregnancy. The serous fluid from the abdominal cavity of pregnant females was aspirated by us on days 3, 9, and 17 of gestation as well as 1 week postpartum by means of a No. 27 gauge needle attached to a 1-ml tuberculin syringe. In order not to penetrate the viscera, we introduced the needle into the abdominal cavity with the animal's ventral surface facing upward. By holding the needle firmly in place, we rotated the mouse back to its normal position so that fluid would drain toward the needle. We spread the aspirated specimen on an albumin-coated slide and stained it by Papanicolaou's procedure (4). Two hundred consecutive cells were counted by us and were grouped as mesothelial cells, lymphocytes, polymorphonuclear leukocytes, histiocytes, mast cells, and "other cells". The last category was less than 1% of the total sample and contained nuclei without cyto-

plasm which could not be further identified. Abdominal fluid was aspirated from nonpregnant control mice on the same days as the pregnant females so that 60 normal control counts from 15 animals were averaged to obtain the mean normal control values.

The significance of difference between counts taken on different days of gestation was computed using the formula,  $SE = [\Sigma d^2/N(N-1)]^{1/2}$  and Student's *t* test. We calculated the standard error for each mean cell count and the probability values (*p*). To characterize the cells of the mouse peritoneal cavity, standard morphologic criteria were used by us (4).

**Results.** The influence of each stage of pregnancy and 1 week postpartum on mouse peritoneal fluid cytodifferential counts is seen in Table I. In normal control aspirations, the percentage of mesothelial cells, lymphocytes, polymorphonuclear leukocytes, histiocytes, mast cells, and "other cells" were 56.0, 33.7, 3.2, 4.1, 0.4, and 2.6%, respectively.

The number of mesothelial cells were significantly low ( $p = 0.02$ ) in cytologic specimens obtained on day 9 of gestation when compared to day 3. Lymphocytes decreased on day 17 ( $p < 0.05$ ) when compared to day 9, but returned to normal in the postpartum period.

As pregnancy progressed, polymorphonuclear leukocytes increased in a stepwise fashion. The control polymorphonuclear leukocyte count was  $6.5 \pm 0.7$  which rose to  $12.5 \pm 1.4$  on day 17 of pregnancy ( $p < 0.01$ ). This change in cell count represents a 92.3% increase in polymorphonuclear leu-