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Received Nov. 27, 1968. P.S.E.B.M., 1969, Vol. 131

Tensions of O_2 and CO_2 in Gas Pockets of Germfree Rats* (33824)

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Animals devoid of bacteria provide a means for finding out whether the usual bacterial flora have influence on "normal" physiological functions. The effectiveness of two of the primary physiological systems, the pulmonary and cardiovascular systems, can be evaluated by the end result of their function, that is, the tissue O_2 and CO_2 tensions. Cardiac output (1) and metabolic rate (1, 2) have been reported to be 20–30% below normal in germfree rats. However, these changes are not necessarily associated with changes of tissue gases, for tissue pO_2 and pCO_2 depend on the balance between local blood flow and local metabolism.

A simple direct technique which can be utilized to measure tissue gases in unanesthetized animals is the subcutaneous gas pocket. Gas is introduced into contact with tissue and left until the O_2 and CO_2 in the pocket come near equilibrium with their dissolved counterparts in the tissue (3, 4).

Materials and Methods. Rats were kept in three plastic film isolators at the Lobund germfree laboratories and maintained according to established procedures. In each isolator were 6 males and 6 females, 60–70 days of age. Twelve control rats in one of the isolators had been born as germfree but were removed from the germfree environment at 35 days of age and kept under ordinary laboratory conditions until the first day of the experiment. Thus they were "conventional" rats with normal bacterial flora, but during the experiment were kept in the same kind of environment as the germfree rats in the other two isolators.

A pocket of 30 ml of air was formed on the back of each rat with hypodermic needle and syringe. A sample for analysis on the Scholander 0.5-ml apparatus (5) was taken from the pocket of each rat 2 days later and additional air was injected into each pocket. Then the 12 germfree rats in one of the isolators were monocontaminated with a normal rat intestinal bacterium, *Clostridium difficile,* by giving the culture with a stomach tube and mixing the culture in the drinking water. Gas samples were taken from these monoinocculated rats the following 2 days, and from each rat in the other two isolators 4 days after forming the pockets. Routine bac-

* Supported in part by USPHS Research Grant AM-09293 Natl. Inst. Arthritis and Metab. Dis., NIH, and by the Office of Naval Research, Contract No. N00014-68-A-0216.

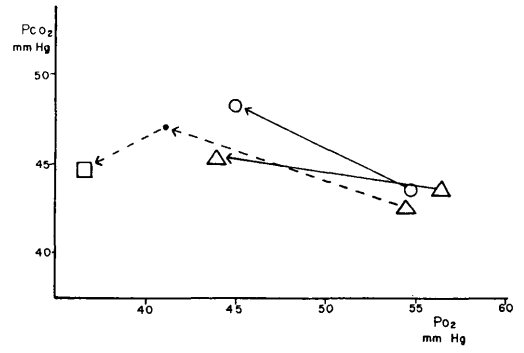

FIG. 1. Oxygen and carbon dioxide tensions in gas pockets of germfree rats (Δ); conventional rats (○), and germfree rats which have been newly infected (□): each point is mean of samples from 11 or 12 rats. The three points to the right show pocket gases 2 days after formation of the pocket, and the arrows show points to the left obtained 4 days after formation. Note that the newly-infected rats were germfree on the earlier analysis and that an additional point for this group, taken between the two other samplings, is included as a dot.

teriological tests were performed to ascertain germfree status and monocontaminated status according to the established method (6). The inner surface of the gas pocket of monocontaminated rats were examined bacteriologically and no *Clostridium difficile* was found.

In each isolator, males and females were kept in separate cages except for 30–90 min while samples were being taken. During the sampling period all the rats were socially active and excited.

Results. Figure 1 shows pocket pCO_2 and pocket pO_2, plotted against each other, for the three different groups of rats, conventional controls (○) germfree (Δ) and germfree rats that had been purposely infected (□). On the early sample (three points at the right) the conventional group is very close to the two germfree groups (Δ). On the last day, the germfree and conventional points are very little different for pO_2, but the germfree point is lower than conventional for pCO_2 (SE for both pCO_2 and pO_2 of the two groups is 1 mm Hg).

The germfree rats that were purposely infected differ from both conventional and germfree groups in that pO_2 is lower and at the same time the pCO_2 does not rise along with the fall of pO_2.

Figures 2 and 3 show the same data as Fig. 1, but with the analyses for males and females averaged separately. On Fig. 2 most of the points lie on a trend line which makes an angle of about 25° with the horizontal axis. Only the second point for the germfree females (▲ at lower left) is away from the general trend. It is apparent that most of the difference between the conventional and germfree points in Fig. 1 is due to the germfree females. Triangles without lines indicate data obtained with the group of germfree rats which were later monocontaminated; the separation of the two open triangles at the right illustrates that considerable variability can occur between apparently identical groups of animals.

Figure 3 shows data from the newly-infected rats; males and females follow a similar course over the 3 days of sampling but females consistently have higher pO_2 and lower pCO_2.

Discussion. The results obtained with these experiments are overlaid on the reorganization of the tissue which occurs in the first

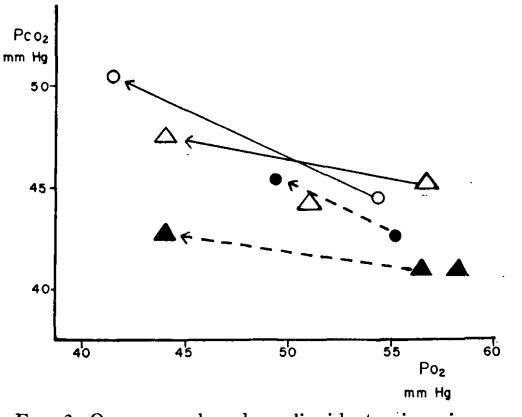

FIG. 2. Oxygen and carbon dioxide tensions in gas pockets of male rats (open symbols and solid lines) and female rats (closed symbols and broken lines). Each point is mean of 5 or 6 analyses. As in Fig. 1, germfree rats are indicated as triangles and conventionals as circles, and points to the right and left of the line show samples taken 2 and 4 days after pocket formation, respectively. Included are two extra points (triangles without lines) for the germfree rats which were later purposely infected.

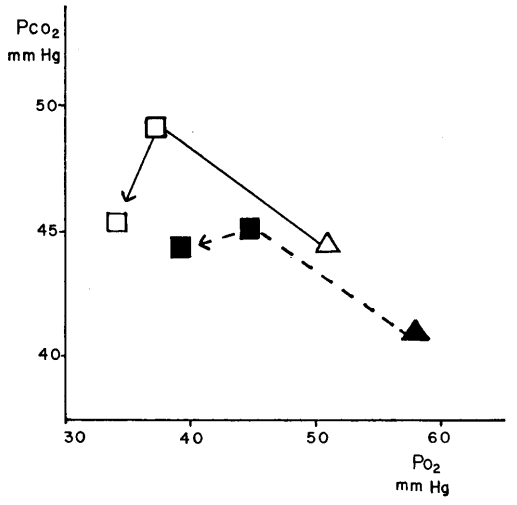

FIG. 3. Newly-infected rats, differences between males (open symbols and solid lines) and females (closed symbols and broken lines). The rats were maintained germfree until samples were obtained 2 days after pocket formation (triangles at the right); then they were innoculated and samples taken the following 2 days (squares, center and left).

week after formation of a gas pocket (7). The consistent movement to the left and upward on the pO_2–pCO_2 diagram is interpreted as a decrease of blood flow as the tissue's reaction to the presence of gas subsides.

It appears that there are only minor differences in tissue gas tensions, measured by the subcutaneous gas pocket technique, between germfree and conventional rats. The differences between the second and fourth day after pocket formation are larger, especially for pO_2, than differences due to the germfree condition, sex, or infection. Figure 2 shows that the main factor in the divergence of the germfree and conventionals at the left of Fig. 1 is that the germfree females have a low pCO_2, about 5 to 8 mm Hg below that of the conventional and germfree males, and 2.5 mm Hg below that of the conventional females. The female conventional rats (Fig. 2) seem to be on the same oblique trend line as the males but lower and to the right, consistent with the idea that the females have higher local blood flow relative to the local metabolic rate of the tissue (4). However, the germfree females (Fig. 2) deviate from the trend line of the other groups, or perhaps are on a separate trend line of their own.

Movement to a separate trend line may have occurred in the newly-infected rats. Figures 1 and 3 show that the first day after innoculation the points seem to have moved along the oblique trend line, but more strongly than for the other groups, suggesting that the infection causes a greater decrease of blood flow than occurred in the others. The next day, however, the points move to a position below the general trend line.

These displacements in the newly-infected rats and in the germfree females cannot be interpreted without further information: They could be due to hyperventilation, to metabolic acidosis which would decrease the pCO_2 of the entire body due to compensatory hyperventilation, to change of the quality of metabolism of the tissue, or to changes in the blood's gas-carrying characteristics (4). The low pCO_2 found in female germfree rats could be explained if many of them chanced to be in the same stage of their estrous cycle. We have data (unpublished) showing rat gas pocket pCO_2 cycles that are synchronous with the sexual cycle.

Summary. Tissue O_2 and CO_2 tensions were estimated with subcutaneous gas pockets in germfree rats, conventional rats, and germfree rats that were newly-infected with a normal rat intestional bacterium, *Clostridium difficile*. Differences between groups were not large. Newly-infected rats appeared to have low local blood flow the first day and an additional unexplained low pCO_2 the second day after infection. Germfree female rats had lower pCO_2 than germfree or conventional males and conventional females.

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Received Dec. 2, 1968. P.S.E.B.M., 1969, Vol. 131.

Staining Technique in Disc Electrophoresis for Disclosing Absence of Normal Serum Proteins in Patients with Hematologic Neoplasms (33825)

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At least 25 protein bands can normally be identified by disc electrophoresis in human serum on polyacrylamide gels stained with aniline blue black. Other reagents are available to aid in the detection of suspect bands. Coomassie brilliant blue R-250 is such a reagent, and is therefore suitable for ascertaining the absence of normal serum proteins in patients with various diseases, such as multiple myeloma.

Materials and Methods. Fifty-five serum samples were collected from patients with various neoplaams. Included were 14 lymphosarcomas (LSA), 11 chronic lymphocytic leukemias (CLL), 12 chronic granulocytic leukemias (CGL), 6 undifferentiated lymphomas (L), 4 multiple myelomas (MM), 4 Hodgkin's disease (HD), 3 acute myeloblastic leukemias (AML), 2 giant follicular lymphomas (GFL).

Twenty-nine specimens obtained from blood donors served as controls. Sera were stored at −20° during the collection period (about 18 months) and clarified immediately before use by centrifugation at 20,000*g* for 20 min.

Disc electrophoresis was performed by the modified method of Ornstein and Davis (1). The anionic gel system provided a final concentration of 7.5% acrylamide with a running pH of 9.3.

[1] Supported by grants from the John Hartford Foundation, the Leukemia Research Foundation, American Cancer Society, and the Leukemia Society of America, Inc.

TABLE I. Normal Serum Proteins Absent from Patient Samples.

Patient group[a]	No band X	No band Y
LSA	5	1
CGL	4	3
CLL	3	2
L	2	1
GFL	1	1
MM		1
AML	0	2
HD	0	4

[a] LSA = lymphosarcoma; CLL = chronic lymphocytic leukemia; CGL = chronic granulocytic leukemia; L = undifferentiated lymphoma; MM = multiple myeloma; HD = Hodgkin's disease; AML = acute myeloblastic leukemia; GFL = giant follicular lymphoma.

Three reagents were used to stain protein bands: 0.02% aniline blue black in 3% acetic acid, 0.004% nigrosin in 3% acetic acid, and 0.02% Coomassie brilliant blue R-250 in 7% acetic acid. Gels immersed overnight in each reagent and destained daily with 7% acetic acid for 3 days.

Test preparations of partly purified elastase (20 mg/ml) demonstrate three bands with aniline blue black staining whereas Coomassie brilliant blue R-250 discloses five additional well defined bands without dye precipitation on the external gel surface.

Results. Table I carries the results of this study. One of two separate bands, present in all control sera, was absent in 30 of the 55 sera obtained from patients. Both bands were