Effect of Exposure to Hyperoxic, Hypobaric, and Hyperbaric Environments on Concentrations of Selected Aerobic and Anaerobic Fecal Flora of Mice

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Alterations in selected aerobic and anaerobic fecal microflora of the mouse were determined during exposure to hyperoxic and normoxic hypo- and hyperbaric environments. Examination of fecal cultures obtained during exposure for 6 weeks to either 60 or 77% oxygen concentration at 1 atmosphere absolute revealed little alteration in the aerobic or anaerobic flora. There appeared to be only a retardation in the reduction of the Klebsiella-Enterobacter flora which normally occurs after weaning. During exposure to hypobaric environments (100% O₂, 0.2 atmosphere absolute), significant alterations in concentrations of Escherichia coli, slow lactose fermenters, Klebsiella-Enterobacter, and enterococci were found in some instances. All alterations were toward increased concentrations. Variations in concentrations of different colony types of obligately anaerobic gram-positive (anGPR) and gram-negative (anGNR) rods cultured during the same experiments also occurred. One colony type of anGPR appeared to decrease while a second type increased in numbers. Concentrations of three colony types of anGNR were generally, but not always, increased. During hyperbaric exposure (2.8% O₂, 7.5 atmospheres absolute), increased concentrations of Klebsiella-Enterobacter, E. coli, slow lactose fermenters and enterococci were also noted. Changes in numbers of both colony types of anGPR, when occurring, were in the direction of lower numbers. Alteration in numbers of anGNR were in both directions but were more frequent in the direction of higher numbers. After return to normal air for 4 weeks of either hypo- or hyperbaric exposure, fecal concentrations of all organisms tended to revert toward control values with the exception of the anGPR which remained in lower concentrations after termination of the hyperbaric exposure. These observations indicate that, despite the great variation in the fecal flora among individual mice, it is possible to discover the effects induced by altered gaseous environments.

Man’s exposure to the altered atmosphere environments required for habituation of undersea shelters and journeys into space pose both recognized and unknown potential health hazards, few of which have been examined in detail. Observations are required on the effects of these environments on the interaction between the host and its normal microflora and the potentially pathogenic microorganisms which he harbors. The indigenous microflora of the human body (3, 17, 18) have been investigated under normal conditions as well as under conditions of simulated altitude. Similar data (1, 2) for the effect of exposure to hyperbaric atmospheres, simulating depth in the sea or in habitats in the sea, are fragmentary and are even more difficult to obtain. This difficulty is due to the fact that the number of samples that can be obtained at intervals during exposure is insufficient to compensate for the usual biological variations. An example of the complexity encountered in attempting to assay the fecal flora of 20 normal individuals is given by the results of Moore and Holdeman (16) who were able to distinguish 113 different kinds of organisms from 1,147 isolates.

Dubos et al. (4-6) have shown that there is an interrelation between the fecal flora of mice and their growth rate and susceptibility to experimental infection under altered conditions of maintenance. It appeared feasible, therefore, to utilize the mouse as an experimental model in investigations on the effect of altered environments on the normal fecal microflora, since it represents a uniform host maintained on an identical diet. This report describes our obser-
vations on changes in incidence and numbers of selected types, or species, of bacteria in the feces of laboratory mice, as affected by various intervals of exposure to hyperoxic, hyperbaric, or hypobaric environments.

**MATERIALS AND METHODS**

**Experimental animals.** All experiments were performed with female mice from the NMRI white Swiss mouse colony raised under conventional conditions. Animals were obtained at 21 to 23 days old (immediately following weaning), coded individually for inclusion in experimental groups of 10 animals and then housed together for 1 to 2 weeks before exposure to parabiosis (any alteration of the normal pressure or composition of the gaseous environment [11]). They were fed a uniform diet of specific-pathogen-free, baked laboratory mouse diet (Detrick and Gambrell, Frederick, Md.) and tap water ad lib. Preliminary determinations of concentrations of the various organisms present in the fecal flora of individual mice received from the breeding colony indicated great variability. More uniform levels of the various aerobic and anaerobic microflora were cultured after the mice had been housed together for 1 to 2 weeks. This stabilization of the indigenous flora can be attributed to aging of the animal and to the environmental conditions of colony maintenance, as described by Dubos et al. (6).

**Collection and preparation of specimens.** For each experiment, feces from individually coded mice in each group were cultured on day of placement in altered environments (0 week), and cultured regularly at 2-week intervals during the period of exposure to parabiosis, and in some cases, as shown, after return to ambient air.

Fecal pellets were obtained from each individual mouse by holding it in a clean, ventilated one-half pint (0.24 liter) ice-cram carton until sufficient pellets were expelled to provide a sample of at least 0.1 g. Each sample was transferred to a sterile, tared, 37-mm plastic petri dish with lock-tight lid and weighed. An appropriate volume of phosphate-buffered saline, pH 7.4, was added to give a 1:10 dilution and the pipette tip was used initially to macerate the pellets. The tight-fitting petri dish lid permitted shaking on a Vortex, Jr. mixer (Scientific Products, Inc.) until a homogenous suspension was available for transfer. Serial 10-fold dilutions were prepared using individual 1-ml pipettes, and 0.1-ml portions were pipetted to the surface of the various selective culture media. Four to five 10-fold dilutions, plated in duplicate, were usually used to provide a countable range of colonies on each particular selective medium. A uniform surface distribution was obtained by use of a petri dish turntable (Fisher Scientific Co.) and sterile bent glass rod spreaders. The numbers of colony-forming units per gram (wet stool) were converted to logarithms and the geometric means and standard errors of the means were compared, using Student's t test for unpaired values, to determine the significance of differences between the various experimental groups at each time interval.
chambers with doors sealing externally. Reduced pressure, 0.2 atmosphere absolute (ATA), approximating a simulated altitude of 11,400 m (37,000 ft), was obtained through the use of a duo-seal Hi-Vac pump, model 1402 (Welch Scientific Co., Skokie, Ill.), manufactured under oil-free conditions and utilizing a tri-cresyl phosphate lubricant to provide explosion-proof handling of 100% oxygen atmospheres. Hypobaric environments were maintained with a flow rate of 2.5 liters/min by connecting the gas source to a two-stage regulator valve with a model 49 vacuum regulator (Mathieson Co., Inc., N.J.) attached and monitoring the flow directly through a flowmeter attached to the chamber. The flow rate from chamber to vacuum source was also adjustable through a model no. 8 Cartesian Manostat (Manostat Corp., New York) to provide a precise and dependable control of the reduced chamber pressures.

Hypobaric atmospheres, 7.5 ATA (95 lb/in² gauge), were obtained by direct connection of the chambers (internally sealing doors) to the double-stage reducing valve on the desired gas mixture at high pressure. A controlled flow rate of 2.5 liters/min was maintained by direct exhaust from the chamber through a flowmeter. Since the flow rate exceeded by almost 10 times the respiratory volume of the 10 test animals in a given chamber (15), the O₂ concentration remained constant and the PCO₂ was only slightly increased.

RESULTS

In general, measurements of numbers of the aerobic fecal microflora excrated were limited to members of Klebsiella-Enterobacter, enterococci and SLF coliforms, since typical E. coli were found in significant numbers only sporadically in experimental groups from the NMRI mouse colony. While measurements of aerobic and facultative anaerobic lactobacilli in preliminary experiments indicated the presence of high concentrations, their numbers were found to be uniform and stable under the altered experimental conditions and measurements were omitted after the first experiments. The anaerobic media and cultural methods utilized permitted measurement of concentrations of two colony types of anGPR and three colony types of anGNR. Preparation of serial dilutions for plating in phosphate-buffered saline in an air atmosphere precluded measurement of the more fastidious or highly oxygen-sensitive anaerobic microflora present, and the selection of suitable decimal dilutions for plating to estimate the usual microbial populations may have masked the presence of other anaerobes present in small numbers.

Chamber environmental temperatures (23 to 25 °C) of the various gas phases utilized for hyperoxic-normobaric and hypobaric-normoxic exposures caused little, if any, environmental stress due to body heat loss (8). Mice exposed to either O₂-N₂ or O₂-He hyperbaric atmospheres, however, were stressed by chilling. These results were the basis for our studies (8) in which we attempted to correct for conditions leading to heat loss. Evidence of one of the combined effects of pressure and body heat loss is presented in Fig. 1 showing the decreased average weight of mice during O₂-He and O₂-N₂ exposure as compared to similar groups during hypoxic-normobaric exposures with normoxic P0₂. The greater weight loss observed with 7.5 ATA O₂-He as compared to an O₂-N₂ atmosphere is indicative of the higher thermal conductivity of helium. Although there appears to be some adaptation to the environment and weight recovery after the first 2 weeks of exposure, the growth rates never approach those of the hypoxic-normobaric mice. On return to air from the pressurized O₂-He environment, a rapid recovery in weight occurred, but the weight did not equal control mouse weights during the 4 weeks of recovery period.

Effect of increased O₂ concentrations at normal pressure. Determination of concentrations of aerobic and anaerobic fecal microflora during exposure to 90, 77, or 40% O₂ environments at normal pressure revealed only minimal differences between these and air control mice groups. Whereas mice exposed to a 90% O₂ environment survived only the first 2 weeks of exposure, similar groups readily tolerated either 60 or 77% O₂ concentrations for 6 weeks. Numbers of SLF (10⁴ to 10⁹/g) and enterococci (10⁷ to 10⁹/g) present before exposure to increased O₂ concentrations were found to decrease slightly during the 6 weeks of exposure, but similar decreases were also observed in the air control mice. The alterations appear to follow the usual changes in population dynamics that occur during aging of the mouse colony (5). In contrast, the normal incidence of mice excreting detectable numbers (10⁹/g) of Klebsiella-Enterobacter varied from 65 to 90% 1 week after weaning (4 weeks of age) and then decreased steadily to 10% or less by 12 weeks of age. During exposure to the hyperoxic environments, the incidence of mice excreting Klebsiella-Enterobacter tended to remain elevated for at least 4 weeks (Fig. 2). The probability of random distribution, analyzed by Chi square, of the incidence of Klebsiella-Enterobacter in mice maintained in air or under hyperoxic conditions for 4 weeks is 0.05 to 0.02 for the 77% O₂ and 0.02 to 0.01 for the 60% environments. It appears that hyperoxic exposure at ambient pressure tends to retard the usual disappearance of these particular organisms from the intestinal microflora.
Fig. 1. Effect of normoxic exposure at reduced pressure (100% O₂, 0.2 ATA) and normoxic exposure at increased pressure (2.8% O₂, 7.5 ATA) in O₂-N₂ or O₂-He atmosphere on average mouse weight as compared with ambient air normoxic controls. Each symbol represents the mean of 10 mice.

Fig. 2. Effect of hyperoxic and normoxic exposure at 1 ATA on the number of mice excreting detectable numbers (10⁵/g) of Klebsiella-Enterobacter organisms. Symbols: (Δ) 90% O₂, 10 mice; (●) 60% O₂, 10 mice; (○) 77% O₂, and (×) tank air, total of two experiments, 10 mice each.

Numbers of anGPR and anGNR, as measured by our cultural procedures, were found to be uniformly present in high concentrations (10⁴ to 10⁶/g) before exposure to hyperoxic atmospheres and to remain relatively unchanged throughout the 6 weeks of exposure to any of the hyperoxic environments.

Effect of normal oxygen environment at reduced pressure. Results of representative experiments showing the effect of a normoxic hypobaric environment (0.2 ATA, 100% O₂ atmosphere) on the fecal aerobic flora of mice are shown in Fig. 3 and 4. Differences in the numbers of SLF were small, but were obtained consistently throughout the 8-week period of exposure to the altered atmosphere. The numbers decreased after return of the mice to normal air (Fig. 3). Klebsiella-Enterobacter
recovered sporadically from control mice (Fig. 2) were often found in high numbers, but the concentration was reduced by the eighth week of exposure to the hypobaric environment and after return to normal air (Fig. 3).

*E. coli*, recovered irregularly from normal control mice, reached high levels in several instances in mice exposed to the hypobaric atmosphere or returned to normal air (Fig. 4). Gram-positive enterococi (fecal streptococci), recovered in moderate but constant numbers in control mice, were greatly increased in numbers during the entire period of exposure of the mice to the altered atmosphere. The level returned to the control levels, after return of the mice to normal air (Fig. 4).
A summary of the alterations observed in concentrations of aerobic fecal microflora during 4 weeks of exposure in six experiments and an additional 6 to 8 weeks of exposure in four of the same experiments are tabulated in Table 1. Also included are two experiments in which observations were made after return of experimental mouse groups to ambient air for 4 weeks. Although the results are not always the same as those shown in Fig. 3 and 4, the changes were always in the direction of larger numbers of organisms.

When changes in the number of anaerobes were determined under the same environmental condition even greater variation was encountered. Representative results of enumerations of one colony type of anGPR and one colony type of anGNR are shown in Fig. 5. There was a decrease in the number of anGNR at 4 and 6 weeks of exposure to the altered environment and a return to comparable numbers in test and control groups at 8 weeks and upon return to normal air.

A summary of the alterations in the obligately anaerobic microflora observed in four experiments is given in Table 2. The hypobaric environment appeared to affect the two anGPR colony types differently, causing a decrease in one type and an increase in the other type. The numbers of all three colony types of anGNR were generally increased, but this was not always the case.

**Effect of normoxic environment at increased pressure.** The hyperbaric environments were achieved by maintaining a 2.8% O₂ concentration in N₂ at 7.5 ATA in three experiments and in He at 7.5 ATA in an additional two experiments. Since it was not possible to recognize different patterns in relative numbers of the various bacteria in the two environments, the results of the five experiments were combined for convenience of presentation. The mice maintained in the hyperbaric O₂-He environment were sampled twice after return to normal air.

The alterations in concentration of aerobic fecal microflora are shown in Table 3. With the exception of one decrease noted in numbers of *Klebsiella-Enterobacter* organisms after 2 weeks of hyperbaric exposure, any changes in concentration were toward increased values. Changes occurred least commonly with *E. coli* and most frequently with enterococci. One example of the concentrations of enterococci is given in Fig. 6. Differences in numbers between normal and test mice were small but consistently obtained.

Alterations in concentration of the cultivable obligately anaerobic microflora occurring during exposure to the hyperbaric environments are shown in Table 4. Changes in the numbers of anGPR, when occurring, were in the direction of lower numbers of both colony types during the period of exposure to the hyperbaric environment as well as upon return to normal air. With anGNR changes occurred in both directions, but were somewhat more frequent in the direction of higher numbers. Changes encountered

### Table 1. Effect of exposure to 100% O₂ environment at 0.2 ATA on concentrations of intestinal aerobic microflora

| Environment | No. of weeks | Aerobic microflora |  |
|-------------|--------------|--------------------|---|
|             |              | E. coli | SLF | Klebsiella-Enterobacter | Enterococci |
|             |              | >*     | <*  | >     | <     | >     | <     |
| 100% O₂     | 0            | 0*     | 6*  | 0     | 5     | 0     | 6     |
|             | 2            | 0      | 6    | 0     | 3     | 0     | 2     |
|             | 4            | 3      | 3    | 5     | 1     | 1     | 3     |
|             | 6            | 1      | 3    | 2     | 2     | 0     | 3     |
|             | 8            | 1      | 3    | 2     | 2     | 0     | 2     |
| Return to normal air | 10 | 1    | 1    | 0     | 1     | 1     | 1     |
|             | 12           | 0      | 2    | 0     | 1     | 1     | 0     |

* Simulated 11,430 m (37,000 ft) altitude, normoxic P0₂.

* Observations in which numbers of microorganisms were found to be significantly greater, equal to or significantly smaller than those of air control mice. Significance determined by Student's t, using the logarithms of the numbers of organisms isolated. Degrees of freedom were usually 18. Probability of random distribution: P < 0.05.

* Total of three equals number of experiments performed at each interval of exposure.
FIG. 5. Effect of normoxic exposure at reduced pressure (100% $O_2$, 0.2 ATA) on fecal concentrations of single colony types of anGPR and anGNR nonsporing rods. Symbols: (O) anGPR, (●) anGPR control, (□) anGNR, (■) anGNR control.

TABLE 2. Effect of exposure to 100% $O_2$ environment at 0.2 ATA on concentrations of intestinal anaerobic microflora

| Environment          | No. of weeks | Anaerobic GPR | Anaerobic GNR |
|----------------------|--------------|---------------|---------------|
|                      |              | Type 1 | Type 2 | Type 1 | Type 2 | Type 3 |
| 100% $O_2$           |              | >*     | -*     | <*     | >     | <     |
| 0                    | 0*           | 0      | 4      | 0      | 4     | 0     |
| 2                    | 0            | 4      | 0      | 2      | 2     | 0     |
| 4                    | 0            | 2      | 2      | 2      | 2     | 0     |
| 6                    | 0            | 1      | 1      | 1      | 1     | 1     |
| 8                    | 0            | 3      | 0      | 2      | 1     | 1     |
| Return to normal air | 10           | 1      | 0      | 0      | 1     | 0     |
|                      | 12           | 0      | 1      | 0      | 1     | 0     |

* Simulated 11,430 m (37,500 ft) altitude, normoxic $PO_2$.
* See Table 1.
* See Table 1.

with one colony type of anGPR are shown in Fig. 6. At most intervals with this type there was a pronounced reduction in their numbers.

DISCUSSION

The data shown in this paper represent some of the more relevant observations made in the course of an extensive study of the fecal flora of mice exposed to a variety of parabaric environments. This study complemented the investigation of the effect of the same environmental conditions on aerosol infections of mice with influenza virus and the chlamydial agent of mouse pneumonitis and intraperitoneal infection with coxsackievirus (9–12). The importance of including the "normal" fecal flora in such a program of investigation was made clear by the studies of Dubos and Schaedler (4–6, 20–23). These authors have traced the evolution of some of the fecal microflora in the mouse and have elucidated the role that they play, beneficial or
TABLE 3. Effect of exposure to 2.8% O<sub>2</sub> environment at 7.5 ATA on concentrations of intestinal aerobic microflora*  

| Environment       | No. of weeks | Aerobic microflora |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|-------------------|--------------|--------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|                   |              | E. coli            | SLF<sup>a</sup> | Klebsiella-Enterobacter | Enterococci |  |  |  |  |  |  |  |  |  |  |  |
| 2.8% O<sub>2</sub> | 0            | 0<sup>a</sup> | 5<sup>c</sup> | 0<sup>e</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> |
|                   | 2            | 0<sup>e</sup> | 5<sup>c</sup> | 0<sup>e</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> |
|                   | 4            | 1<sup>e</sup> | 3<sup>c</sup> | 0<sup>e</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> |
|                   | 6            | 0<sup>e</sup> | 2<sup>c</sup> | 0<sup>e</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> |
| 8                 | 8            | 0<sup>e</sup> | 2<sup>c</sup> | 0<sup>e</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> |
| Return to normal air | 10           | 0<sup>e</sup> | 1<sup>c</sup> | 0<sup>e</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> |
|                   | 12           | 0<sup>e</sup> | 1<sup>c</sup> | 0<sup>e</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> | 0<sup>c</sup> |

* Simulated 65 m (213 ft) depth sea water, normoxic PO<sub>2</sub>.  
* See Table 1.  
* See Table 1.

Fig. 6. Effect of normoxic exposure at increased pressure (2.8% O<sub>2</sub>-He, 7.5 ATA) on fecal concentrations of enterococci and one strain of anGPR. (○) enterococcus, (●) enterococcus control, (□) anGPR, (■) anGPR control.

Deterioration, in the health of the mouse. It can be assumed from their studies that any pronounced deviation from "normal" colonization of the intestinal tract will be associated with other changes in the physiology of the host, although causal relationships are difficult to establish. Some of the limitations imposed by the conditions of our experiments have already been discussed. Fecal specimens, although not reflecting accurately intestinal contents, had the advantage that they could be collected repeatedly from the same mice. They were probably representative of the microflora of the lower intestines, since in most cases they were collected within minutes after the mice had been removed from the chambers. The enumeration of bacteria was limited to those that were numerous and readily cultivable and, of necessity, identification within each genus was limited to colonial type. Variations among identically treated mice were very large and only the more divergent patterns could be shown to be of any significance. Because of the limitations discussed above...
any conclusions must be made with caution. It is apparent, however, that all three alterations of the normal gaseous environment, hyperoxic, low pressure, and high pressure under normoxic conditions, may or may not result in changes in aerobic microflora, but when changes occur they are almost invariably in the direction of higher numbers. Examples of these changes are delay in the reduction in numbers of Klebsiella-Enterobacter that occur in mice as they age (Fig. 2 and 3), small but consistent increases in the moderate numbers of SLF (Fig. 3), appearance on occasion of large numbers of E. coli, which, otherwise, are not conspicuous in mouse feces (Fig. 4), or pronounced increases in the numbers of enterococci (Fig. 4). Effects of the three altered environments on the anaerobic flora are less clear. Hyperoxia has no measurable effect on the anaerobic flora and the other two environmental conditions have resulted in either type of change (Tables 2 and 4). It is not possible to attribute the changes in microflora to the direct effect of the altered gaseous environment. It is more likely that the effect was of an indirect undetermined nature. If some of the changes are attributed to cold stress in the case of pressurized mice, this explanation is not applicable to mice that were exposed to reduced pressure.

The implications of altered microflora for the long-term state of health of the mouse can only be surmised. It is logical to assume that an organism emerging in large numbers, when it is only a minor component of the microflora under normal circumstances, may well be a potential pathogen. The importance of some of the microorganisms for providing proper nutritional balance, especially when there are dietary restrictions, was discussed by Schaedler et al. (23) and more recently by Luckey et al. (14). Increases in concentrations of certain of the gram-negative enteric flora may also cause an iron deficiency in the host due to the secretion of greater amounts of bacterial chelating agents as indicated by Luckey et al. (13). The importance of chelating agents in the competition between the mammalian host and microorganisms for available iron was discussed by Weinberg (25).

Data on the aerobic and anaerobic microflora of human feces were usually collected from persons who were subjected to the single variable of altered diet or the dual variable of altered diet and atmosphere. For example, Gall and Riely (7) subjected four individuals to “space-type” diet and noted that although the number of anaerobes remained constant there was a shift in the type of organisms isolated. The aerobic flora differed from that usually encountered by the frequent presence of Shigella and enteropathogenic E. coli. Cordaro et al. (3) studied the fecal flora of six subjects exposed to 0.35 ATA normoxic He atmosphere and receiving an experimental diet. The only change noted was a reduction in the numbers of enterococci. The values returned to normal when the subjects returned to normal atmospheric conditions and regular diet. The microbiological change was attributed to the diet.

In conclusion, the determination of changes in the microbiological flora or susceptibility to pathogens by individuals exposed to altered gaseous environments is beset by the difficulty

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**Table 4. Effect of exposure to 2.8% O₂ environment at 7.5 ATA on concentrations of intestinal anaerobic microflora**

| Environment          | No. of weeks | Anaerobic GPR | Anaerobic GNR |
|----------------------|--------------|---------------|---------------|
|                      |              | Type 1        | Type 2        | Type 1        | Type 2 | Type 3 |
|                      |              | >*            | >*            | >*            | >*     | >*    |
| 2.8% O₂              |              | 0             | 0*            | 0             | 0*     | 2*    |
|                      | 2            | 0 1           | 0             | 0             | 0      | 2     |
|                      | 4            | 0 3           | 0             | 0             | 0      | 2     |
|                      | 6            | 0 1           | 0             | 0             | 0      | 2     |
|                      | 8            | 0             | 0             | 0             | 0      | 2     |
| Return to normal air | 23,24        | 0             | 0             | 0             | 0      | 2     |

* Simulated 65 m (213 ft) depth sea water, normoxic PO₂.
* See Table 1.
* See Table 1.
of separating the numerous variables that commonly accompany the change in environment. The data presented in this paper furnish baseline information on the concentration of the kinds of organisms studied in the mouse as an experimental animal subjected to three altered environments under conditions that minimized concurrent variables. There is little doubt that the altered environments did produce stresses that elicited changes in the number of aerobic and, in some cases, of the anaerobic microorganisms. The clinical significance of these changes is not known.

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