Impaired Water Metabolism in Germfree Rats[1] (35124)

MEIR LEV, RAYMOND H. ALEXANDER, AND STANLEY M. LEVENSON
(Introduced by E. J. Hehre)

Departments of Surgery and of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461

From the earliest studies, germfree (GF) animals have been shown to differ from conventional (CONV) animals in several important respects. GF animals have relatively odorless feces which are wetter than those of CONV animals. In addition, particularly in GF rodents, an enlarged cecum is found which together with its contents may reach 30% or more of the animal's body weight. The cecal contents of GF rodents are much more fluid than those of CONV animals. Increased fluid contents are also found elsewhere in the intestines of GF animals. This excessive amount of water in the gastrointestinal tract of the GF animal suggested to us that water metabolism may be impaired in germfree rodents.

Materials and Methods. GF and CONV male Fischer rats obtained from the Charles River Laboratory, Wilmington, Mass., were used. These were 3–4 months old and weighed approximately 200 g at the beginning of the experimentation. Routine microbiological culture techniques were employed to confirm their germfree status (1). The CONV rats were placed 2/cage in plastic isolators, unsterilized but otherwise identical to those housing the GF rats, for at least 1 week before experimentation to acclimate them to the experimental surroundings. Conventionalized rats were GF animals which had, on one occasion, cecal contents from a CONV rat placed in their drinking water and on their food about 3 weeks prior to experimentation. They, too, were housed in plastic isolators. All rats were fed diet 4101C obtained from the Purina Co., St. Louis, Mo., sterilized by steam 30 min 260°F in a high vacuum autoclave.

Water gavages were performed by the method of Hirata and Asano (2). Solid food was withdrawn at about 4–5 p.m. and withheld for 16 hr but water was available *ad libitum*. Tap water or 0.9% NaCl at room temperature was then delivered in an amount corresponding to 5% of body weight from a syringe via an infant feeding tube placed in the stomach of the animal. One hr later this procedure was repeated. The rats were placed in metabolism cages and, following the second gavage (zero time in the figures), urine samples were collected and measured at 10-min intervals for the first hour (beginning at zero time), then hourly for 4–5 hr, and at 24 hr. Rats were lifted and their lower abdomens were tickled to obtain samples of urine. Urine excreted during the hour between gavages was variable and usually small in amount; it was not measured and is not included in the figures.

Saline gavages and urine collections followed the same protocol.

Usually the animals were rested for 2 weeks between experiments, as in the water, saline, and water gavage experiments performed (see Table I). It was found that when animals were retested at shorter intervals, *e.g.*, intervals of 3–4 days, the differences between GF and CONV animals diminished and, in addition, many instances of red colored urine were found mainly in the GF animals but also in CONV rats. These urines showed a "moderate" to "large" reading for the presence of blood when examined by the Labstix reagent test (Ames Co., Elkhart, Ind.).

To permit study of urinary excretion following absorption of water from tissues other

[1] Supported by U. S. Public Health Research Grant No. AMGM-5664 and Research Career Award to S. M. Levenson No. GM 14,208.

TABLE I. Concentration of Na^+, K^+, and Cl^- in Urine of GF, CONV, and Conventionalized Rats.

Expt. gavage[a]	No. of rats	Av values and range[b] (mEq/liter) Na^+	K^+	Cl^-	3-hr urine vol (ml)
A Water	6 GF	5.6 (3.0– 8.7)	13.0 (6.7–25.6)	11 (4.9– 32)	52.8
	6 Conventionalized	5.2 (1.6– 10.9)	7.9 (3.6–12.3)	6.6 (2.4– 9.2)	74.9
	6 CONV	3.0 (0.9– 6.1)	10.5 (4.6–22.1)	4.0 (0.9– 13)	80.1
B Saline	6 GF	167 (107 –215)	41.4 (33.1–34.1)	213 (180 –235)	46.3
	7 Conventionalized	155 (129 –186)	33.8 (20 –54.9)	217 (155 –318)	68.9
	8 CONV	150 (119 –179)	46.0 (34.1–56.9)	190 (171 –241)	56.0
C Water	7 GF	3.9 (1.7– 8.7)	7.4 (2.4–16.7)	11 (6.7– 19)	99.3
	7 Conventionalized	11.3 (2.1– 23.5)	7.4 (4.6–19.2)	19 (3.4– 42)	87.9
	8 CONV	3.0 (1.5– 6.5)	6.1 (4.1– 8.7)	6.6 (3.0– 14)	135.4

[a] Experiments A, B, and C were performed in sequence on the same groups of animals with an interval of 14 to 20 days between each experiment. When the water gavage experiments were combined, Na^+ concentration of urines from GF and CONV rats differed at the level of significance $0.02 > p > 0.01$. Cl^- values also differed at level of significance $0.02 > p > 0.01$. K^+ levels were not significantly different. In all experiments, urine samples analyzed were those excreted in the 3-hr period following the second gavage.

[b] Range values are shown in parentheses.

than the gut, a solution of 4% glucose in pyrogen-free distilled water was injected subcutaneously on both sides of the backs of the rats. The rats showed no discomfort from the subcutaneous administration of volume equivalent to 10% of the body weight. Zero time (Fig. 5) is the point of glucose administration.

Urine samples from each experimental period were examined for total solids using a T.S. American Optical refractometer (American Optical Corp., Buffalo, N.Y.). They were analyzed for sodium and potassium content by atomic absorption spectrophotometry (Perkin-Elmer model 303, Perkin-Elmer Corp., Norwalk, Conn.), and for chloride with a Buchler-Cotlove chloridometer (Buchler Instruments Inc., Fort Lee, N. J.).

Results. The result of an experiment showing diuresis following gastric water gavage is shown in Fig. 1. Two effects are shown: (i) the germfree animals show a delay of approximately 1 hr in onset of urine output; and (ii) the cumulative amount of urine excreted in the first few hours by the GF rats is generally less than that found in the CONV animals. A further experiment using a different group of 4 germfree and 4 CONV animals confirmed these effects.

In further experiments, three groups of animals were compared: a GF group, a CONV group, and a group of conventionalized rats. These last animals were litter mates of the GF group, which 3 weeks prior to the experiment were deliberately contaminated by feeding them with the cecal contents of a CONV rat, then maintained in an isolator. As shown in Fig. 2, the GF animals, as found

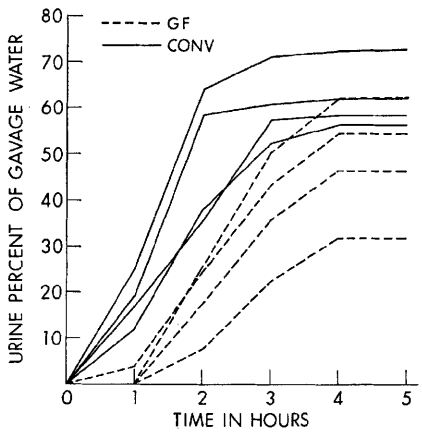


FIG. 1. Urine outputs of 4 individual germfree and 4 conventional rats following gastric gavage of water equivalent to 10% body weight, one-half having been given at zero time and one-half an hour earlier. A variable but usually small volume of urine was excreted between gavages; it was discarded and not included in the measurement.

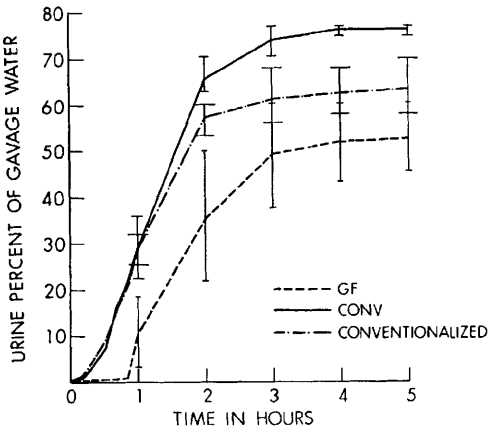


FIG. 2. Average urine outputs of 4 germfree, 4 conventional, and 4 conventionalized rats following gastric gavage of water; Expt. A.

previously, show an initial delay of urine output and a lower proportion of water excreted as urine compared with the CONV group. The conventionalized animals show an effect intermediate between the GF and CONV in that there was no initial delay but the cumulative total urine excreted was less than in the CONV group. A repeated experiment using different groups of 4 animals/group showed essentially the same result.

It then seemed of interest to us to follow excretion of urine subsequent to a gavage of 0.9% sodium chloride. We found that the time of onset of urinary excretion and total output of GF, conventionalized, and CONV groups are similar (Fig. 3). When a comparison is made in CONV animals of urine output following saline gavage with that following a water gavage, as shown in Fig. 4a, the urine volume following administration of saline is only about one half as great (after the first 0.5 hr) as following water. In contrast, in the GF group (Fig. 4b) in the early stage of the experiment, *i.e.*, the first 100 min, output of urine is actually greater following saline administration by gavage than that following water gavage. The time course of urine excretion following saline gavage is the same in GF and CONV rats (Fig. 4a and b).

Attempts were made to measure urine output following intravenous loading of GF and CONV rats with 4% glucose in water. However, the experiments were hampered by the debilitating effect on the rats of keeping them in plastic restraining cages for the 1–2 hr required for intravenous loading. The fur of the animals became very damp and only a small proportion of the dose administered was excreted as urine. Therefore, this approach was discontinued.

In other experiments, 10% body weight of 4% glucose was injected subcutaneously. The results of these experiments (Fig. 5) show a pattern similar to those following gastric gavage of water; there is initial delay in urine excretion and in total output by the GF as compared to the CONV group. There is one important difference—the onset of excretion was delayed 0.5–1 hr in the following gavage, whereas following subcutaneous loading a delay of 2 hr was found in the GF group.

Table I shows the results of analyses of urines from water and saline gavage experiments. Experiments A, B, and C were performed in sequence using the same groups of animals with an interval of 14 to 20 days

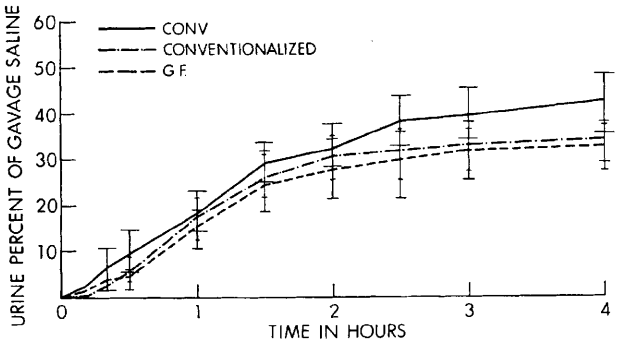


FIG. 3. Average urine outputs of 4 germfree, 4 conventional, and 4 conventionalized rats following 0.9% NaCl gavage.

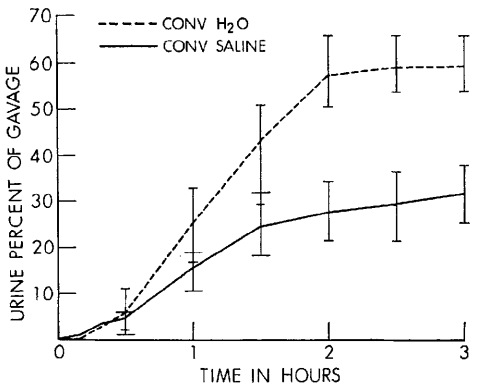
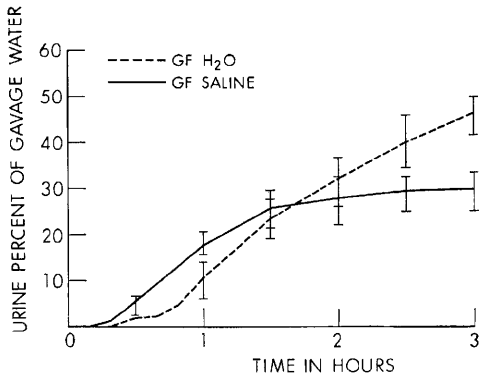


FIG. 4a. Comparison of urine outputs following gastric gavage of water (4 animals per group) with that following 0.9% NaCl gavage into conventional rats. (b) Comparison of urine outputs following gastric gavage of water (4 animals per group) with that of 0.9% NaCl into germfree rats.



between each experiment. All analyses were made on urines excreted in the 3-hr period following the second gavage. It was found that the total amounts of Na^+, Cl^-, and K^+ excreted in 3 hr following water gavage are similar in GF and CONV groups. When the water gavage experiments were combined (experiments A and C), Na^+ concentrations in the urines from GF and CONV rats differed at a level of significance $0.02 > p > 0.01$; Cl^- values also differed at this level of significance but K^+ values were not significantly different. The results from conventionalized animals were variable.

In the saline loading experiments, the concentrations and total amounts of Na^+, Cl^-, and K^+ excreted in 3-hr urine volume were generally much higher than those found following water loading and no significant differences were found among the various groups of rats.

Discussion. Our results show that following water gavage or the subcutaneous injection of 4% glucose in water, GF rats show a delay of 0.5–1 hr in onset of urination and the total volume of urine excreted by the GF during the first several hours is less (about 10%) than is found with CONV animals. In the steady state, Crowley and Gruber in this laboratory have shown that GF rats drink more water than the CONV group, the difference

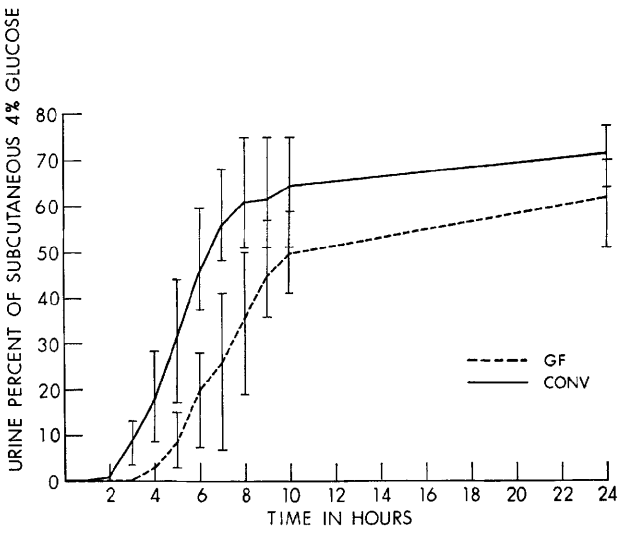


FIG. 5. Average urine outputs of 6 germfree and 6 conventional rats following subcutaneous injection (all at zero time) of 4% glucose in an amount equivalent to 10% body weight.

representing the greater fecal water excretion by the GF.

The role of the microbial flora in these processes is obscure. Animals which had been conventionalized for 3 weeks are still somewhat abnormal with respect to diuresis following gavage of water, although not to the same degree as the GF. This result was surprising to us because a balanced bacterial flora is already established at this time, the cecum has assumed a normal size, and the gut contents of the ileum and cecum have the appearance of the gut contents of CONV rats. The full effects of the microbial flora may depend on specific microorganisms not established in the conventionalized group or may require a time longer than 3 weeks to become evident.

Most workers have regarded the gut as the organ showing the greatest differences between GF and CONV animals. The gut is the main area for the absorption of nutrients and products of bacterial synthesis and is also in direct contact with a large microbial population. However, compared to the CONV group, there was a longer lag in urinary excretion in the GF group following subcutaneously injected water (4% glucose) than that found following gavage of water. This may suggest that the absorption of water from the subcutaneous tissues also is abnormal in the GF animals.

The mechanism of the impairment of these aspects of water metabolism is unknown. The high water content in the intestine and feces of the GF animals would suggest that net absorption of water from the intestine is a prime cause. The importance of inorganic ions in the transport of water is well known. Asano (3, 4) in studies of the enlarged cecum of GF rats has shown that there is a paucity of Cl^- in their cecal contents and that conditions which reduce cecum size also increase levels of Cl^-. Our experiments have shown that the GF animals following saline administration excreted urine equal to the CONV group and because of this fact, at an early period (first 80 min) GF rats excrete more urine following a saline gavage than following a gavage of water. Goldner *et al.* (5) have shown that divalent cations are important in a nonspecific manner in the restoration of normal intestinal permeability after depletion by chelation. The importance of Ca^{2+} and Mg^{2+} in metabolism of water by GF animals is as yet unknown, although it has been shown that GF rats excrete more Ca^{2+} in their urine and less in their feces than do CONV rats (6).

Our results show that concentrations of Na^+ and Cl^- in urines GF animals following gavage of H_2O are significantly higher than the concentrations of these ions in urines of CONV animals. K^+ concentrations were similar in both groups. The cumulative amounts of Na^+, Cl^-, and K^+ excreted were the same. This shows that whereas the excretion of Na^+ and Cl^- by GF animals under these conditions is normal, the GF animal's ability to excrete water is impaired.

Several factors remain to be determined such as the comparative renal and certain hormonal functions in GF and CONV animals, *e.g.*, the plasma levels of ADH and the comparative response of GF and CONV animals to injected ADH.

A further possibility is that the gut of the GF animals is abnormal in that a greater exchange of water between the gut and the blood and vice versa exists, *e.g.*, there is a malfunction in the ability to concentrate water from the gut due to an excessive rate flow of water from the blood. This could also result in the excessive hydration of gut contents which is characteristic of the GF animal.

It is therefore apparent that the impaired ability to metabolize water in the germfree animal could be due to many factors. The elucidation of the factors involved is of fundamental importance to understanding the physiology of the germfree animal and the effects of microorganisms on mammalian metabolism and physiology.

Summary. Diuresis following gastric gavage of water was examined in germfree and conventional rats. Germfree rats began to excrete urine 1 hr later than the conventional group and the volume of urine excreted by the germfree animals was less than excreted by the conventional group. Saline excretion patterns were the same for the two groups of

animals. When 4% glucose was administered subcutaneously, excretion patterns were similar to that obtained following gastric gavage of water except that the onset of excretion was delayed 2 hr in the germfree as compared with the conventional group.

We thank Dr. Henry D. Lauson for his valuable criticism and advice concerning this manuscript.

1. Lev. M., *in* "Animals for Research. Principles of Breeding and Management" (W. Lane-Petter, ed.), p. 139. Academic Press, New York (1963).

2. Hirata, K., and Asano, S., Keio, J. Med. **7**, 107 (1958).

3. Asano, T., Proc. Soc. Exp. Biol. Med. **124**, 424 (1967).

4. Asano, T., Amer. J. Physiol. **217**, 911 (1969).

5. Goldner, A. M., Cassidy, M. M., and Tidball, C. S., Proc. Soc. Exp. Biol. Med. **124**, 884 (1967).

6. Gustafsson, B. R., *in* "Nutrition and Metabolism Minerals," p. 40. NATO Advanced Study Inst., The Germ-Free Animal in Research, Hampshire, England (1965).

Received June 8, 1970. P.S.E.B.M., 1970, Vol. 135.