THE ROLE OF THE MICROBIAL FLORA IN UREMIA

I. Survival Times of Germfree, Limited-Flora, and Conventionalized Rats after Bilateral Nephrectomy and Fasting

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For many decades, investigators have intensively studied the responses of animals rendered uremic by a variety of experimental means, and have extensively speculated on the importance to these responses of the uremic animal's so called "normal" indigenous flora (1, 2). Much of the discussion has centered on the retention by the uremic host of "toxic" substances which are known or postulated to derive from microbial activity in its intestine and to be normally excreted by the kidneys. However, despite the reiteration of these concepts, we are unaware of any systematic exploration of the extent and nature of the contribution of the indigenous flora and its various components to the pathogenesis of renoprival states. As a first step in this direction, we removed both kidneys from germ free rats and from such rats previously contaminated intentionally with one or more intestinal microorganisms and compared their responses. In this paper, we show that the microbial status of the rat significantly influences its ability to endure the lethal sequelae of acute anuria and of food and water deprivation. We will present the histopathology of these and other rats as well as some biochemical alterations in the communication that follows (3).

Materials and Methods

General.—To determine whether microbial status influences survival time in uremia, three separate series of experiments (Experiments I, II, and III) were conducted in which bilateral nephrectomy was performed on healthy rats. The groups in each experiment were studied concurrently to enable direct comparison of performance.

Fischer rats, of either sex, were received germfree from the Charles River Breeding Laboratories, North Wilmington, Massachusetts, at 4 to 5 wk of age and used as described below. All rats were continuously maintained in isolators from birth to death. The standard procedures used in this laboratory for maintaining and monitoring these animals have been detailed (4–6).

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The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

The rats were automatically subjected to 12-hr alternating periods of illumination (6:00 a.m. to 6:00 p.m.) and darkness. Temperatures within the isolators stayed between 23 and 26°C.

All rats, at death or sacrifice, were autopsied and tissues were preserved in 10% buffered formalin; the histopathology is reported in the accompanying communication (3).

Statistical significance of the differences between mean values was assessed by the "t" test (7) and was set at a probability level of 1% or less.

Experiments I and II.—The rats were 4 to 5 months old at the time of surgery. Approximately 4 wk before surgery, the germfree rats were divided into two matched groups. One group remained germfree (GF). The other was transferred to another identical isolator and conventionalized (CONV) by only once adding, to their food, water and bedding, a 48-hour thioglycollate broth culture (50 ml) of cecal contents (ca. 0.5 g) from rats of the "closed" conventional animal colony of the Walter Reed Army Institute of Research. This group (CONV), having been contaminated with a mixed microbial flora, was then maintained in all respects as though it were GF.

All rats were fed steam-sterilized, semisynthetic L-356 diet (prepared by General Biochemicals, Chagrin Falls, Ohio) and water *ad libitum* until the day before surgery when they were allowed only 5% dextrose in 0.9% saline *ad libitum*, which they drank avidly. After surgery, the rats were deprived of food and water until they died to insure control of their nutritional status which would not be possible were they allowed to eat and drink *ad libitum*, and to avoid the handling necessary for a forced-feeding regimen. It is well known that rats, in any event, develop anorexia within 48 hr after bilateral nephrectomy. Moreover, the fasting regimen we imposed by design enabled us to compare the tolerance for the lethal effects of starvation exhibited by the sham-nephrectomized GF and CONV rats in Experiment II.

The day before surgery, the abdomen was depilated. Surgery on all rats was performed within a steel surgical isolator (Model ROPU 500, Reyniers and Sons, Chicago). Aseptic precautions were observed for surgery on contaminated rats. After anesthesia had been induced by intramuscular administration of 40 mg/kg of sodium pentobarbital, an upper midline laparotomy was made, and the kidneys were isolated from the adrenals and excised. For control purposes (Experiment II), sham nephrectomy was done on a group of rats matched in body weight as closely as possible with their nephrectomized partners; this consisted of laparotomy, manipulation of the intestines, and allowing the intestines to remain out of the abdomen for the time needed to remove the kidneys of its experimental partner. All wounds were closed in two layers with two continuous 4-0 silk sutures. The rats were then returned to their respective isolators, where they were housed individually in cages with raised wire bottoms. The time of death of the nephrectomized and sham-nephrectomized rats was noted; death of the latter was due to starvation.

Experiment III.—Five groups (Groups A to E) of 6- to 7-month-old rats, of both sexes were studied. As before, each group of rats was obtained germfree and maintained in isolators throughout the experiment. Cultures of bacteria that were to be used to contaminate the GF rats (groups B, C, and D) in their respective isolators were grown separately in 50 ml of thioglycollate broth. The appropriate culture(s) or sterile broth were introduced into the isolators and added to the food, water, and bedding twice a week for 4 wk; 5 days were allowed to elapse between the last addition of broth and bilateral nephrectomy. The contents of the broth and the particular microbial status we desired to establish preoperatively by this procedure in these 5 groups were as follows:

Group	Broth	Desired microbial status
A	Sterile	Germfree
B	48 hr culture of: *Staphylococcus albus*	Monocontaminated
C	48 hr culture of: *S. albus* *Proteus mirabilis*	Dicontaminated
D	48 hr culture of: *S. albus* *P. mirabilis* *Escherichia coli* *Streptococcus faecalis*	Tetracontaminated
E	Sterile	Conventionalized (see text below)

The rats in group E were germfree until 2 months before surgery. At that time, they were conventionalized as described above. The microorganisms present in group E included the four facultative anaerobes present in group D, and strict anaerobes that were not identified. Fecal material was obtained at weekly intervals and at the termination of the experiment. Specimens were cultured on sheep blood, MacConkey's and Sabouraud's agars, and thioglycollate broth to insure that the bacterial make-up of the groups was as desired. The bacteria used to contaminate rats of groups B, C, and D were originally isolated from the same source used for conventionalization of the rats of group E.

Experiment III was performed as were Experiments I and II except as described above and, in addition: halothane anesthesia was used for surgery as previously described (8); the 5 corresponding groups of control rats were deprived of food and water, but had no surgery; and controls were sacrificed by exposure to carbon dioxide after 1 wk of fasting.

The four types of bacteria used to contaminate groups B to D were selected because they are representative of fecal flora most commonly found in our conventional rats, and because previous experience in this laboratory (9) gave no evidence that these bacteria, singly or in combination, would significantly alter the morphology of the bowel from that characteristic of the germfree animal.

RESULTS

Experiments I and II.—The survival time of every GF rat was greater than that of any CONV rat after bilateral nephrectomy (Table I). The difference between the average postnephrectomy survival times of the GF and CONV rats was statistically significant in both experiments and no sex difference was noted.

As shown in Table II, the reverse was true for the survival times of the sham-nephrectomized rats dying of starvation: the CONV rats outlived their GF counterparts by about a week and, in addition, a sex difference was noted. The *male* sham-nephrectomized fasting rats outlived the corresponding *females*, and all animals of both sexes considerably outlived their nephrectomized counterparts, irrespective of microbial status.

Even though the females were consistently smaller than the males, there was no correlation between initial body weight and survival time within any of the groups, nor was a difference found in the rate of weight loss between

groups. The nephrectomized rats were not weighed during life to avoid excessive handling. The sham-nephrectomized rats were weighed at weekly intervals during their fast to obtain an estimate of catabolic rate with starvation.

Except for the differences in survival time, few other differences were noted among the groups. All rats became less active with time, becoming totally inactive and tachypneic shortly before death, which usually followed a period of coma lasting several hours. No convulsions were seen. However, nephrectomized rats became hyperreactive to auditory and tactile stimulation, responding abnormally to slight provocation with a transient side-to-side motion of the body and prolonged trembling.

TABLE I

Comparison of Preoperative Body Weights and Postoperative Survival Times of Fasting GF and CONV Rats Dying of Uremia After Bilateral Nephrectomy

Experiment	Sex	Body wt		Survival time		
		GF	CONV	GF	CONV	*P* value
		g	*g*	*hr*	*hr*	
I	M	204±8.4 (4 rats)	172±1.8 (4 rats)	122±3.7 (113–130)*	89±5.2 (75–100)	<0.01
II	M	207±6.4 (7 rats)	194±8.4 (6 rats)	132±5.8 (108–151)	68±6.2 (48–84)	<0.01
	F	143±3.3 (4 rats)	156±3.0 (3 rats)	131±9.1 (119–147)	68±14.3 (41–90)	<0.01

Mean ± SE of mean.
* Observed range.

TABLE II

Comparison of Preoperative Body Weights and Postoperative Survival Times of Fasting GF and CONV Rats Dying of Starvation After Sham Nephrectomy (Experiment II)

Sex	Body wt		Survival time		
	GF	CONV	GF	CONV	P value
	g	*g*	*hr*	*hr*	
M	212±6.9 (6 rats)	192±7.8 (7 rats)	355±9.8 (317–386)*	526±25.6 (468–552)	<0.01
F	146±3.0 (4 rats)	153±6.7 (4 rats)	251±11.1 (230–278)	413±41.8 (295–492)	<0.01

Mean ± SE of mean.
* Observed range.

Gross examination of the CONV rats at autopsy revealed no evidence of bacterial invasion. The cecum was enlarged and thin-walled in all the GF rats, both at surgery and at death. The sham-nephrectomized rats at death appeared markedly emaciated from starvation. No other gross abnormality was seen at autopsy.

In Table III, the weight losses of male rats are presented. The females were too few in number for a meaningful statistical evaluation of results, but the calculated values were similar. The average daily weight loss of the starving,

TABLE III

Comparison of Body Weight Losses of Fasting GF and CONV Male Rats Dying of Starvation or Uremia (Experiment II)

Variable	Sham-nephrectomized		Nephrectomized	
	Germfree	CONV	Germfree	CONV
Life span, days.....	14.7±0.4 (6)	21.9±1.1 (7)	5.5±0.2 (7)	2.8±0.3 (6)
Daily wt loss per rat:				
During 1st wk, %*	4.0±0.3 (6)	4.8±0.3 (7)		
During 2nd wk, %*	3.3±0.1 (5)	3.6±0.5 (7)		
During 3rd wk, %*		2.6±0.2 (5)		
Total wt loss/rat over life span, %‡	45.0±0.1 (6)	61.0±0.5 (7)	23.0±1.5 (7)	16.0±1.1 (6)
Daily wt loss/rat over life span, %‡	3.1±0.1 (6)	2.8±0.4 (7)	4.2±0.1 (7)	5.9±0.5 (6)

Mean ± SE of mean; number of animals in parentheses.

* As a per cent of body weight at the beginning of the week.

‡ As a per cent of initial body weight.

sham-nephrectomized rats during the first 2 wk may be compared and are essentially the same for the GF and CONV rats. The differing survival times of the GF and CONV rats precludes comparison of their average total weight loss, and the rate of weight loss cannot be compared because it decreased with increasing duration of starvation. While the comparison is less than precise, the GF males during their postnephrectomy survival of 5.5 days manifested an average daily weight loss of 4.2% which is similar to the daily weight loss of 4.0% suffered by the corresponding sham-nephrectomized rats during their first 7 days of starvation.

Experiment III.—Having demonstrated in Experiments I and II that CONV rats die more rapidly than GF rats after bilateral nephrectomy, Experiment III was performed to interpolate, between these extremes of microbial status,

three groups of GF rats that had been contaminated in advance of nephrectomy with one (group B), two (group C), or four (group D) known intestinal bacteria, as described, and to again compare survival times.

The animals in groups B to D during their 4-wk course of controlled contami-

TABLE IV

Comparison of Survival Times of Fasting Germfree, Limited-Flora, and Conventionalized Rats After Bilateral Nephrectomy (Experiment III)

Group	Microbial status	No. of rats	Body wt	Survival time	Observed range
			g	*hr*	*hr*
A	Germfree	6	269±7	122±6.2	90–139
B	Monocontaminated	10	188±10	112±8.9	60–135
C	Dicontaminated	9	189±14	109±3.4	89–119
D	Tetracontaminated	8	188±11	91±8.1*	57–128
E	Conventionalized	11	195±17	76±4.3‡	41–95

Mean ± SE of mean.
See text for specific bacterial contaminants present.
* Significantly less than germfree, $P < 1\%$.
‡ Significantly less than germfree, $P < 0.1\%$.

TABLE V

Comparison of Body Weight Losses and Cecal Weights of Unoperated Germfree, Limited-Flora, and Conventionalized Rats After 7 Days of Fasting (Experiment III)

Group	Microbial status	No. of rats	Initial Body wt	Body wt loss at 7 days	Cecal wt % Final body wt
			g	%	
A	Germfree	2	268	31.1	5.7
B	Monocontaminated	6	192±18	30.1±1.2	9.0±1.6
C	Dicontaminated	6	182±13	27.3±1.2	6.3±0.6
D	Tetracontaminated	6	182±15	25.4±2.9	8.8±1.0
E	Conventionalized	6	197±22	26.3±1.1	0.6±0.1

Mean ± SE of mean.
See text for specific bacterial contaminants present.

nation either maintained their body weight or showed small gains therein. All rats appeared healthy at the time of surgery. After nephrectomy, the animals of all groups behaved no differently from those of Experiments I and II, described above, exhibiting no remarkable differences among them except in survival time (Table IV).

From Table IV, it is evident that the mean survival time after nephrectomy decreased with increasing order of complexity of the flora present. A statistically significant difference was shown between the GF (group A) and tetracontam-

inated rats (group D), and the significantly shorter survival time of CONV rats (group E) compared to GF rats (group A) observed in Experiments I and II was confirmed.

At surgery and at death postnephrectomy, the CONV rats (group E) had small, normal-sized ceca, whereas all limited-flora rats (groups B to D) had an enlarged, thin-walled cecum that was indistinguishable from that of the GF rats. The same held true for the control rats for groups A to E, when they were sacrificed and autopsied after 7 days of food and water deprivation, which they all survived (Table V). Signs of infection or other lesions were not seen in any of the rats.

As may be seen in Table V, the average total body weight loss by the 5 groups of intact, fasting controls was similar.

DISCUSSION

We would like to emphasize that all contaminated animals studied were germfree until they were associated, in randomly assigned groups, with one or more microbial species, and that they were subsequently maintained and handled in all respects as were the germfree animals. Thus, the observed experimental differences, to the best of our knowledge, reflect solely the known difference in microbial status of the animals that were studied.

The results show that fasting CONV rats, both male and female, die more rapidly after removal of their kidneys than corresponding GF rats. The survival times of a total of 21 GF and 24 CONV rats were compared after nephrectomy in 3 experiments; with the exception of 1 rat in Experiment III, all nephrectomized GF rats in each series outlived their CONV counterparts. Thus, there was virtually no overlap in postnephrectomy survival times. The over-all average survival times after bilateral nephrectomy were 127 hr (range: 90 to 151 hr) for the 21 GF and 75 hr (range: 41 to 100 hr) for the 24 CONV rats. The latter value is in general agreement with the average postnephrectomy survival times reported by others for open-laboratory conventional rats studied under somewhat different experimental conditions; these averages range between 63 and 78 hr (10–13). To our minds, the over-all 2-day longer survival of the GF rat is significant not only statistically but biologically. Moreover, we were able to demonstrate that the postnephrectomy survival time of limited-flora animals decreases, in general, as the complexity of their flora increases. Tetracontaminated rats had an average postnephrectomy survival time that was statistically significantly shorter than that of the GF rats, and statistically no different from that of the CONV rats. Thus, our results specifically demonstrate that an indigenous flora adversely affects the rat's ability to endure renal ablation and, in general, reflect on the importance of microbial status in affecting the lethal course of uremia.

Because rats were denied food and water postoperatively (for reasons already

outlined), it is important to establish that the longer survival of the anuric GF rats cannot be ascribed to an underlying greater tolerance of GF rats for the lethal effects of starvation. The nondependence of differences in postnephrectomy survival time on tolerance to starvation is shown in several ways. (*a*) We observed that the sham-nephrectomized CONV rats lived considerably longer than their GF counterparts when deprived of food and water. This observation is concordant with that made previously in this laboratory that starved multicontaminated mice survive significantly longer than starved GF mice (14). This difference is not explicable on the basis of a differential loss of weight in mice (14) or in rats (Table III). Furthermore, the weight losses suffered by the 5 groups of unoperated controls (Experiment III) when sacrificed after one week of food and water deprivation were similar (Table V). Thus, the GF rats outlived their CONV partners after nephrectomy and starvation despite the fact that they proved less tolerant to the lethal effects of starvation after sham nephrectomy. The provision of food and water after nephrectomy might have been of greater benefit to the GF than to the CONV rats and might have resulted in a magnification of the observed difference in survival time in favor of the GF animal. (*b*) The CONV rats endured starvation approximately 2.5 wk longer than they did anuria and the GF rats 1 wk longer. Thus, the lethal effects of renal ablation were manifested long before the lethal limit of endurance for starvation was reached. (*c*) The fasting nephrectomized GF and CONV rats died apparently possessing a reserve of metabolizable tissue mass that was, respectively, 2 and 4 times that expended to the time of death from starvation (Table III). (*d*) While comparatively few female rats were studied in Experiment II, it appears that whether they are multicontaminated or free from microbes, they endure the lethal consequences of nephrectomy as well as the corresponding males. Bergman and Drury (13) also found that sex had little effect on the survival time of conventional rats deprived of food and water after bilateral nephrectomy. The females, however, were less resistant to starvation than the males. We do not have a ready explanation for this sex difference which persisted regardlesss of microbial status.

The GF rats at all times were found to have the markedly enlarged cecum typical of the GF rodent (15–17). This was always in sharp contrast to the considerably smaller cecum noted by us at all times in the CONV rats. However, as anticipated (9), the ceca of the mono-, di-, and tetracontaminated rats at death postnephrectomy were grossly indistinguishable from those of their GF counterparts. The corresponding control rats (Experiment III) that were sacrificed after 1 wk of food and water deprivation had ceca which, relative to body weight, were at least 10 times heavier than those of their CONV partners (Table V). It would seem, therefore, that the large cecum was not advantageous to either the *sham-nephrectomized rats* since the GF died sooner from starvation than their CONV counterparts, or to the *nephrectomized rats*

since the tetracontaminated rats (with a "germfree-sized" cecum) manifested a survival time which was significantly shorter than that of the GF rats, and statistically no different from that of the CONV rats with a small cecum.

Although we have shown that the establishment of an indigenous flora in GF animals shortens their lives after bilateral nephrectomy, but prolongs their lives after food and water deprivation, we do not know precisely which microorganisms of the so called "normal" indigenous flora are most effective or importantly involved in these responses, nor precisely how these influence the host's response to either anuria or starvation. As it is conceivable that those microbes which promote prolongation of survival after starvation are not those which effect a reduced tolerance for anuria, the task of determining the responsible microbes may not be a simple one. The complexity of the problem is exemplified by the fact that whereas we have regularly succeeded in morphologically conventionalizing the GF rat's intestine and normalizing the size of its cecum with the partially characterized, mixed microbial flora of the ordinary rodent's cecal contents, we have, thus far, not been able to reproduce this by means of pure cultures of bacteria isolated from the same cecal contents. We put this fact to experimental use, however, by showing that *S. albus*, *P. mirabilis*, *S. faecalis*, and *E. coli* were clearly effective in reducing survival time postnephrectomy but were ineffective in conventionalizing the intestine (3). Though the limited-flora animals with one, two, or four bacteria showed a corresponding diminution in their ability to endure anuria, the CONV rats showed the shortest postnephrectomy survival times of all. Furthermore, as will be fully described (3), only the CONV rats dying of uremia showed cecal erosions histologically, which, interestingly, correlated with a shorter survival time.

Phenomena thought to result from the interaction of the host and its indigenous flora that are regularly seen in animals living in an "open" environment (or in CONV animals) may be difficult to reproduce in animals with a defined flora since many intestinal organisms cannot be identified, let alone enumerated, because of the ignorance of their biological characteristics and lack of adequate cultural techniques (18). Dubos and his coworkers (18) have discussed the probability ". . . that there exists in the normal flora certain microbial species that have not been obtained in culture." They cite the inability, by means of pure cultures so far tested, to restore the resistance of the mouse to experimental salmonellosis after streptomycin, or to accelerate the elimination of *E. coli* and enterococci which develop large populations in the mouse's intestinal tract after penicillin administration. They point out, however, that these antibiotic-induced alterations are corrected by feeding fresh fecal or intestinal contents to the mice, but that such material becomes inactive in this regard after heat treatment or the addition of germicides. The problem of the permanent morphologic conventionalization of the germfree gut by fully characterized micro-

organisms, and the histopathologic changes that we found in the ceca of only the CONV rats dying of uremia (3) may be related to certain intestinal microorganisms which are present in the conventional or conventionalized rat but which are still unidentified or difficult to cultivate in vitro (19–21). However, a variety of intestinal microorganisms, both aerobes and strict anaerobes, that can be cultured await testing, either singly or in combination, in GF animals for their possible modifying influence on the course of uremia or starvation. These include some anaerobic bacteria that have only recently been selectively cultured in vitro and quantitated (19, 21). It especially remains to be determined what effect *E. coli* and *S. faecalis*, singly or together, may have on postnephrectomy survival time in view of their significant effect in the present study when acting in concert with *S. albus* and *P. mirabilis*.

We cannot be sure that a significant alteration of the intestinal flora did not take place during the relatively brief survival period after bilateral nephrectomy, or, as is more likely, during the longer survival period after food and water deprivation in the sham-nephrectomized CONV rats, since ". . . lasting alterations in the flora, both qualitative and quantitative can result from environmental and physiological disturbances" (18). Schreiner and Maher (reference 2, p. 70), in reviewing the biochemistry, pathogenesis, and treatment of uremia, have also pointed to this problem in a biochemical vein: "There is a real need for more complete studies on the question of urea reentry in the acute and chronic uremic organism and the possibly significant role that gastrointestinal tract bacteria may play in making ammonia nitrogen available. In this situation, one cannot extrapolate from the normal since the bacterial flora in uremic patients may be different and since we know that there are marked elevations in the urea concentration of the blood, the luminal fluid in, and the secretions flowing into the gastrointestinal tract."

The significance of intestinal ureolysis, which does not occur in GF rats (22), and its possible relationship to the levels of urea in blood and cecal contents, and the cecal erosions of dying uremic CONV rats will be discussed in the paper that follows (3).

Our results may be interpreted as adding new life to the long entertained tenet that microbial activity in the intestine plays a deleterious role in uremia, especially by contributing factors which are noxious to the anuric animal, both locally and systemically. However, in view of the perplexing array of bodily disturbances that follows nephrectomy and the need for a more comprehensive general accounting of similarities and dissimilarities in physiology, metabolism, and body composition of *intact* germfree and bacteria-laden animals, a full understanding of the manner in which the indigenous microbial flora influences the fatal course of uremia requires further study, but we think our present findings and those in the following paper reveal that its role is a significant one.

SUMMARY

Germfree rats were used in 3 experiments to study the effects of the microbial flora on survival time after acute uremia produced by a one-stage bilateral nephrectomy. Germfree rats, limited-flora rats, and conventionalized rats (all maintained continuously in isolators) were subjected to nephrectomy or to sham nephrectomy, deprived of food and water until they died, respectively, of uremia or of starvation, and their survival times compared. To establish a limited defined flora in advance of nephrectomy, germfree rats were either monocontaminated (*Staphylococcus albus*), dicontaminated (*S. albus* and *Proteus mirabilis*) or tetracontaminated (*S. albus*, *S. faecalis*, *P. mirabilis*, and *E. coli*); to conventionalize germfree rats, they were exposed to the mixed microbial flora contained in the cecal contents of ordinary rats, which was the source of the aforementioned bacteria and which included other uncharacterized microorganisms as well.

The intestine of all rats with a limited flora persisted in a morphologic state that was virtually no different from that of the germfree rat, including the presence of an enlarged, thin-walled cecum; by contrast, the intestine of the conventionalized rats permanently assumed the morphological characteristics of ordinary, open-laboratory rats with the cecum reduced to normal size.

After nephrectomy and food and water deprivation (death from anuria):

(*a*) All germfree rats but one outlived their conventionalized counterparts in each of the 3 experiments; the 21 germfree rats (127 hr) lived, on the average, 2 days longer than did the 24 conventionalized rats (75 hr). No sex difference was demonstrated.

(*b*) The rats with a limited flora died correspondingly sooner as the complexity of their flora increased; survival time of the tetracontaminated rats was significantly shorter than that of the germfree rats, and statistically no different from that of the conventionalized rats.

After sham nephrectomy and food and water deprivation (delayed death from starvation):

(*a*) All rats, irrespective of microbial status or sex, outlived their fasting nephrectomized partners. The conventionalized rats endured starvation approximately 2.5 wk longer than they did anuria and the germfree rats 1 wk longer.

(*b*) All conventionalized rats, both male and female, outlived their respective germfree counterparts by about 1 wk.

(*c*) All males, irrespective of microbial status, survived longer than did the females; the average difference was 4 days.

The differences in tolerance to anuria or starvation did not correlate with initial body weight or rate of weight loss.

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