THE ROLE OF THE MICROBIAL FLORA IN UREMIA

II. Uremic Colitis, Cardiovascular Lesions, and Biochemical Observations*

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In the preceding paper, the adverse influence of the indigenous microbial flora on rats rendered uremic by bilateral nephrectomy was shown by a comparison of the postnephrectomy survival times of germfree (GF), limited-flora, and conventionalized (CONV) rats (1). This report concerns the histopathology of these animals and the biochemical and morphologic observations on groups of GF and CONV rats sacrificed 48 and 72 hr after bilateral nephrectomy and of their controls. These studies have provided some correlations between the metabolic and structural alterations of the renoprival state. They reveal that uremic colitis is bacterially induced, that cardiovascular pathology is modified by the microbial state of the host, and provide tangible evidence of the effect of the indigenous microbial flora on the uremic host.

Materials and Methods

Chemistry.—Chemical determinations were made on a group of rats separate from those previously reported (1). 4- to 6-month-old germfree Fischer rats were used. Half were conventionalized a month before surgery as previously described (1). Bilateral nephrectomy or sham nephrectomy was performed as before (1) under halothane anesthesia (2). 48 or 72 hr after surgery, rats were removed from their isolators and anesthetized with halothane. 10 mg/kg body weight of sodium heparin (U.S.P., without preservative) in sterile isotonic saline were given intraperitoneally. Laparotomy was performed, the abdominal aorta was cannulated with PE 50 tubing, and the rats were exsanguinated; the variable volumes of blood collected

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account, in part, for the differences in the total number of animals on which the various chemical determinations were ultimately made. The blood so obtained, the cecal contents collected as quantitatively as possible, and thigh muscle were used for chemical determinations. Blood was drawn into capillary tubes and centrifuged for 5 min to determine hematocrit (Microcapillary Centrifuge, model MB, International Equipment Co., Boston).

Sodium and potassium concentrations were determined by flame photometry (Auto-Analyzer, Technicon Co., Chauncey, New York). Other procedures were modified for small samples when necessary.

Total protein was measured by the method of Lowry et al. (3), total inorganic phosphorus by the Fiske-Subbarow method (4), and urea nitrogen by the diacetylmonoxime method (5). Glucose was determined by the glucose oxidase method (Worthington Corp, Freehold, New Jersey, enzymes) (6). A modification of the method described by Diehl and Ellingboe (7) for

TABLE I

Urea Nitrogen Concentration in Blood and Cecal Contents of Fasting GF and CONV Rats Sacrificed 72 Hr After Sham or Bilateral Nephrectomy

Microbial status and procedure	No. of rats	Blood urea nitrogen,	No. of rats	Cecal urea nitrogen,
		mg %		*mg %*
GF sham	12	47.6±4.1	12	123.2±14.2
CONV sham	8	45.4±2.4	7	101.8±16.5
GF nephrectomized	19	359.4±14.5* $P < 0.1\%$	19	427.0±52.2* $P < 0.1\%$
CONV nephrectomized	20	515.0±32.8*	9	108.4±26.9

Mean ± SE of mean.

* Statistically significant difference ($P < 0.1\%$) between sham vs. nephrectomized rats

total calcium was used. Fluorescence was measured directly on an Aminco fluorometer with an activation wavelength of 475 mμ and fluorescent wavelength of 535 mμ; no chelating agent was used.

Indoxyl sulfate was measured by a method devised by one of us (H.R.): plasma proteins were precipitated by heating 1 ml of plasma for 5 min at 100°C. After centrifugation, the supernatant was evaporated and 5 ml of ethyl alcohol were added to the dry material and shaken. This step extracted any indoleacetic acid and/or indole. After centrifugation, 5 ml of water were added and the supernatant was shaken with Dowex-50 in the hydrogen form. Basic indole compounds were removed in this way. Indoxyl sulfate was then assayed fluorometrically with an activation wavelength of 285 mμ and fluorescent wavelength of 375 mμ.

Histopathology.—Rats used in the previous experiments (1) and a random sample of those sacrificed for chemical determinations were studied. At death, all animals were autopsied and gross observations were made. The skin incisions were removed and placed in fixative. The body was opened longitudinally and all organs from the floor of the mouth to the anus were removed en bloc and placed in 10% buffered formalin. At the conclusion of the experiment, selected tissues were prepared for histologic study. The following organs were regularly examined: heart, aorta, small and large intestine, and cecum; the skin incision, stomach, parathyroid glands, adrenal glands, spleen, and mesenteric lymph nodes were also studied in some animals. Lymph nodes and spleens were examined for lymphocyte depletion and immunologic activity as described before (8). The heart was cut longitudinally and embedded to

present all four chambers and the origin of the great vessels for sectioning. Aortas were sampled longitudinally and in multiple cross-sections. The gastrointestinal tract was opened in its entirety and examined under a dissecting microscope. Visible lesions were excised and 6 to 7 cm strips from each segment were rolled on applicator sticks and sectioned after paraffin embedding to yield "Swiss Roll" type preparations. Multiple sections were cut from all tissues and stained with hematoxylin and eosin. Giemsa, periodic acid Schiff, and von Kossá's stains were employed where appropriate.

Statistical analyses: Statistical significance of the differences between mean values and that between relative frequencies of occurrence were assessed, respectively, by the "t" test and by χ^2 using Yates' correction (9). A probability level of 1% or less was chosen for statistical significance.

TABLE II

Sodium and Potassium Concentrations in Plasma and Skeletal Muscle of Fasting GF and CONV Rats Sacrificed 72 Hr After Sham or Bilateral Nephrectomy

Variable	Sham nephrectomy		Nephrectomy	
	Germfree	CONV	Germfree	CONV
Plasma K, *meq/liter*	3.8±0.6 (12)	4.4±0.5 (9)	7.1±1.2* (18)	8.4±0.2* (9)
Muscle K, *meq/Kg* dry weight	330±17 (8)	299±18 (7)	367±17 (12)	333±0.7 (11)
Plasma Na, *meq/liter*	153.8±6.2 (5)	155 (1)	160.2±8.1 (5)	149.1±3.8 (8)
Muscle Na, *meq/kg* dry weight	82±5 (8)	79±11 (7)	89±4 (12)	75±7 (11)

Mean ± SE of mean. Number of animals used in parentheses.

* Statistically significant difference ($P < 0.1$ %) between sham vs. nephrectomized rats.

RESULTS

Chemical Findings

Blood urea nitrogen (BUN) rose significantly higher in the CONV than in the GF rats after nephrectomy. BUN values at 72 hr after nephrectomy are shown in Table I. A less pronounced rise in BUN (to about 300 mg %) was shown by both GF and CONV rats at 48 hr. The urea nitrogen concentration in the cecal contents rose significantly in the GF-nephrectomized rats, but not in the CONV-nephrectomized rats at 72 hr (Table I). The total amount of urea in the ceca of GF rats was consistently 50 to 60 times that of the CONV rats, and relates to the considerably greater volume of cecal contents of the former.

Table II shows the values obtained for *plasma and skeletal muscle sodium and potassium* at 72 hr. No difference between GF and CONV values for plasma or muscle was observed. Potassium in plasma, but not in muscle, was significantly elevated in both GF- and CONV-nephrectomized rats. No significant change in plasma and skeletal muscle sodium was demonstrated for the nephrectomized GF and CONV rats.

Table III shows the plasma concentrations of total calcium and total inorganic phosphorus at 72 hr. *Total calcium* concentration was significantly higher in CONV controls than in GF controls; however, no difference between the controls and the nephrectomized rats was observed. *Total inorganic phosphorus* concentration was significantly greater in the GF-nephrectomized rats than in the CONV-nephrectomized rats and both were significantly greater than that observed in the controls.

Hematocrit values were significantly reduced in both the GF ($P < 0.1\%$) and CONV ($P < 1\%$) rats 72 hr after nephrectomy when compared with their respective controls. The 8 GF and 8 CONV controls had virtually identical

TABLE III

Total Calcium and Inorganic Phosphorus Concentrations in Plasma of Fasting GF and CONV Rats Sacrificed 72 Hr After Sham or Bilateral Nephrectomy

Microbial status and procedure	No. of rats	Calcium	No. of rats	Phosphorus
		mM/liter		*mM/liter*
GF sham	8	2.5±0.11	11	2.2±0.3
		$P < 0.1\%$		
CONV sham	8	3.5±0.3	8	2.1±0.2
GF nephrectomized	12	2.7±0.2	16	6.7±0.7*
				$P < 0.1\%$
Conv nephrectomized	11	3.3±0.2	11	5.1±0.4*

Mean ± SE of mean.

* Statistically significant difference ($P < 0.1\%$) between sham vs. nephrectomized rats.

mean values (45.0 ± 1.4% and 45.6 ± 2.9%, respectively), while the 12 GF- and 11 CONV-nephrectomized rats had values, respectively, of 28.8 ± 1.4% and 33.2 ± 1.7%. *Total plasma protein*, however, was not different between control and nephrectomized rats, nor between GF and CONV. The mean value for 3 control GF rats was 5.1 ± 0.4 g% and that for 5 nephrectomized GF was 5.2 ± 0.2 g%; the mean value for 2 control CONV rats was 6.4 g% and that for 8 nephrectomized CONV was 5.8 ± 0.4 g%.

Blood glucose levels were unchanged by nephrectomy or microbial status. Seven GF-nephrectomized rats at 72 hr had a blood glucose of 97 ± 13.7 mg %, while 9 CONV-nephrectomized rats, at this time, had a blood glucose of 104 ± 9.6 mg %. Similar values were found at 48 hr postnephrectomy.

Table IV shows the values for *plasma indoxyl sulfate* at 72 hr. This compound was present in significantly greater concentration in the CONV than in the GF rats after nephrectomy. The presence of an apparent small amount of indoxyl sulfate in the GF plasma probably indicates as yet unidentified indoxyl sulfate-like compounds.

Histopathologic Findings

Gastrointestinal Tissues.—Lesions were found only in the *cecum*, only in rats *dying from uremia*, and only in the *CONV* group (group E, Table V). No lesions were seen in a group of 31 *germfree* rats that died for reasons other than uremia; this group included: 19 rats sacrificed 48 or 72 hr after sham or bilateral nephrectomy, 2 unoperated rats sacrificed after 7 days of fasting, and 10 rats that died from starvation after sham nephrectomy. Lesions were not found in a group of 33 CONV rats that also died for reasons other than uremia; this group included: 21 rats sacrificed 48 or 72 hr after sham nephrectomy, 2 unoperated rats sacrificed after 7 days of fasting, and 10 rats that died from starvation after sham nephrectomy. A minimal inflammatory cell infiltration of the cecal lamina propria was found in 1 of 4 CONV rats sacrificed 48 hr and in 1 of 9 rats sacrificed at 72 hr after nephrectomy.

TABLE IV

Plasma Concentration of Indoxyl Sulfate in Fasting GF and CONV Rats Sacrificed 72 Hr After Sham or Bilateral Nephrectomy

Microbial status and procedure	No. of rats	Indoxyl sulfate, μg/100 ml plasma
GF sham	3	120±20
CONV sham	4	270±180
GF nephrectomized	9	90±10
		$P < 0.1\%$
CONV nephrectomized	5	540±80

Mean ± SE of mean.

TABLE V

Influence of Microbial Status on the Occurrence of Lesions of the Cecal Mucosa in Fasting Rats Dying of Bilateral Nephrectomy

Group	Microbial status at Nephrectomy*	No. of rats	Incidence,
			%
A	Germfree	20	0
B	*Staphylococcus albus*	10	0
C	*S. albus* and *Proteus mirabilis*	8	0
D	*S. albus*, *P. mirabilis*, *Streptococcus faecalis*, and *Escherichia coli*	8	0
E	Conventionalized (flora includes that of group D)	23	57‡

* For method of contamination of groups B to E, (see reference 1).

‡ Statistically significant ($P < 1\%$) difference as compared with germfree.

The histological characteristics of the cecum of CONV (Fig. 1), limited-flora, and GF (Fig. 2) control rats showed no change. The ceca of 13 nephrectomized CONV rats with lesions (Table V) all showed marked submucosal inflammation and edema; 11 ceca showed, in addition, foci of mucosal necrosis (Fig. 3), and in 6 of these, necrosis was associated with frank

mucosal ulceration (Figs. 4, 5, and 6). The muscular layer was not involved in any of the animals, however. Giemsa stain revealed masses of bacteria in the ulcerations.

As shown in Table VI, the uremic CONV rats *with* cecal lesions had a statistically significantly shorter survival time than those *without* cecal lesions. The incidence of cecal lesions also correlated with the duration of survival after nephrectomy: of the 13 CONV rats that endured

TABLE VI

Correlation of Cecal Lesions with Survival Time of Fasting Conventionalized Rats Dying After Bilateral Nephrectomy

Cecal Pathology	No. of Rats	Survival Time	
		Mean ± SE mean	Observed range
		hr	*hr*
No mucosal lesions	10	86.2±3.6	60–100
		$P < 1\%$	
Mucosal lesions	13	67.9±4.6	41–86

TABLE VII

Occurrence of Cardiovascular Lesions in Fasting Germfree, Limited-Flora, and Conventionalized Rats Dying of Uremia

Group*	Microbial status at nephrectomy	No. of rats	Incidence			
			Myocardial necrosis	Myocardial calcification	Coronary	Aortic
					Necrosis and/or calcification	
			%	%	%	%
A	Germfree	20	100	90	60	50‡
B	Monocontaminated	10	100	100	50	60
C	Dicontaminated	8	100	100	84	75
D	Tetracontaminated	8	100	100	88	100
E	Conventionalized	23	74	48§	17§	22

* For contaminants present, see Table V and reference 1 for methods of contamination.

‡ Only 16 rats studied.

§ Statistically significant difference ($P < 1\%$) as compared with germfree.

anuria *less than 80 hr*, 86% showed cecal lesions, whereas only 30% of the 7 rats that survived *from 81 hr to the observed maximum of 100 hr* showed cecal lesions. Moreover, the rats with the shorter periods of survival had all the cecal ulcerations and all but one of the foci of mucosal necrosis. By contrast, the ceca of the 10 rats which lived longest showed either no lesions or only marked edema and inflammation. However, even those nephrectomized CONV rats which had no cecal lesions at death uniformly had shorter survival times than the uremic GF rats (1).

The stomach, small intestine, and colon showed no mucosal, submucosal, muscular, or vascular abnormality in any of the experimental animals. The small and, to a lesser extent, the large bowel of the selectively contaminated uremic and control rats showed an increase in

cellularity of the lamina propria as compared with the GF; this was most marked in group D which had an established flora of 4 microorganisms. However, the degree of change was slight as compared with that of the CONV rats. The ceca of the mono-, di-, and tetracontaminated rats were grossly and histologically similar to those of the GF (Fig. 2) and distinctly different from those of the CONV rats (Fig. 1) which had a thicker muscle and mucosa which was composed of longer and narrower glands.

Cardiovascular Tissues.—No abnormalities were found in rats which did not have bilateral nephrectomy. Cardiovascular lesions were found in nephrectomized rats whether they died from uremia or were sacrificed 48 or 72 hr after nephrectomy. Pericarditis was not seen in any of the rats. The GF and limited-flora animals dying from uremia generally had a higher incidence of cardiovascular damage than their CONV counterparts (Table VII). The data of

TABLE VIII

Occurrence of Cardiovascular Lesions in Fasting GF and CONV Rats Sacrificed 48 or 72 Hr After Nephrectomy or Dying of Uremia

Microbial status at nephrectomy	Cause of death	No.of rats	Incidence			
			Myocardial Necrosis	Myocardial Calcification	Coronary	Aortic
					Necrosis and/or Calcification	
			%	%	%	%
Germfree	Sacrificed 48 hr	7	57	29	29	0
	Sacrificed 72 hr	7	100	100	100	86
	Uremia	20	100	90	60	50*
Conventionalized	Sacrificed 48 hr	4	25	25	0	0
	Sacrificed 72 hr	9	89	78	22‡	0‡
	Uremia	23	74	48‡	17‡	22

* Only 16 rats studied.

‡ Statistically significant difference ($P < 1\%$) as compared with corresponding germfree.

Table VIII also suggest that GF rats develop cardiovascular damage earlier after nephrectomy than do CONV rats.

Myocardium: Myocardial necrosis occurred in all GF and selectively contaminated rats dying from bilateral nephrectomy and in three-fourths of the corresponding CONV rats (Table VII). Similarly, when sacrificed 48 or 72 hr after nephrectomy, the GF tended to show a higher incidence of necrosis than the CONV rats (Table VIII). Lesions ranged in severity from eosinophilic and PAS-positive focal swelling and vacuolization of the sarcoplasm (Fig. 7) to complete disintegration of the fibers attended by mild mononuclear cell reaction (Fig. 8). Lesions occurred more frequently in the wall of the left ventricle than in the right, and were often located subepicardially or near vessels (Fig. 9). The atria were spared. As regards severity of necrosis, the GF and limited-flora rats generally fared worse than the CONV rats (Tables IX and X).

Myocardial calcification occurred in or near necrotic foci and also was less marked in the CONV rats 48 or 72 hr after nephrectomy (Tables VIII and X) or at death from uremia (Tables VII and IX). As is evident from Tables VII to Table X, the anuric GF and limited-flora animals at death had a higher incidence and greater severity of myocardial calcification

TABLE IX

Severity of Myocardial Necrosis and Calcification in Fasting Germfree, Limited-Flora, and Conventionalized Rats Dying of Uremia

Groups*	Microbial status at nephrectomy	No. of rats	No. of rats						Mean score‡
			4+	3+	2+	1+	0.5+	0	
			Myocardial necrosis						
A	Germfree	20	5	2	6	5	2	0	2.2+
B	Monocontaminated	10	0	0	1	9	0	0	1.1+
C	Dicontaminated	8	0	1	3	4	0	0	1.6+
D	Tetracontaminated	8	0	3	2	3	0	0	2.0+
E	Conventionalized	23	0	1	7	7	2	6	1.1+
			Myocardial calcification						
A	Germfree	20	3	5	3	5	2	2	2.0+
B	Monocontaminated	10	0	0	7	3	0	0	1.7+
C	Dicontaminated	8	1	2	2	3	0	0	2.1+
D	Tetracontaminated	8	3	2	2	1	0	0	2.9+
E	Conventionalized	23	0	1	6	4	0	12	0.8+

* For contaminants present, see Table V and reference 1 for methods of contamination.
‡ Score based on arbitrary scale of 0 to 4+; trace scored 0.5+.

TABLE X

Severity of Myocardial Necrosis and Calcification of Fasting GF and CONV Rats Sacrificed 48 or 72 Hr After Bilateral Nephrectomy or Dying of Uremia

Microbial status at nephrectomy	Cause of death	No. of rats	Mean score*	
			Myocardial necrosis	Myocardial calcification
Germfree	Sacrificed 48 hr	7	0.4+	0.2+
	Sacrificed 72 hr	7	1.5+	2.9+
	Uremia	20	2.2+	2.0+
Conventionalized	Sacrificed 48 hr	4	0.3+	0.3+
	Sacrificed 72 hr	9	0.6+	1.0+
	Uremia	23	1.1+	0.8+

* Score based on arbitrary scale of 0 to 4+; trace scored 0.5+.

than their CONV counterparts; the difference in incidence between the GF and CONV rats is statistically significant. The calcification ranged from a fine stippling that covered apparently intact fibers to heavy deposits of calcium associated with loss of nuclei, muscle breakdown, and muscle necrosis (Fig. 9).

Blood vessels: Lesions were found only in the aorta, coronary artery branches, and in two cecal ulcers (Figs. 5 and 6). No vascular abnormalities were encountered elsewhere.

Calcification of the coronary arteries and aorta was prominent in all but the CONV rats and was most prevalent in the di- and tetracontaminated rats (groups C and D, Table VII), and in the GF as compared with the CONV rats sacrificed 48 or 72 hr postnephrectomy (Table VIII). Coronary artery calcification usually involved the inner portion of the media. In the aorta, lesions appeared to begin with hydropic degeneration of smooth muscle (Fig. 10) with or without calcium deposition. The latter usually involved the middle third of the media and spared the elastic lamina (Fig. 11), but in some cases calcium also permeated the elastic tissue (Fig. 12). No inflammatory cell infiltration was seen around the lesions in the aorta or coronary arteries. Although coronary artery and aortic calcification usually occurred together, especially in the dying anuric GF and limited-flora rats and in GF rats sacrificed 48 or 72 hr after nephrectomy, they occasionally occurred separately in rats from all nephrectomized groups.

Comparison of the data for individual animals showed that neither the total plasma calcium or inorganic phosphorus levels at 72 hr postnephrectomy, nor the time to death from anuria correlated consistently with the presence or absence of cardiovascular damage, its location or its severity.

Other tissues.—No pneumonitis was seen. There were no consistent changes in the cytologic appearance of the parathyroid glands. The adrenals appeared normal. Lymphatic tissue showed lymphocyte depletion, the extent of which increased with survival time. All selectively contaminated and CONV-uremic rats showed more plasma cells than the GF, but immunoblasts were rare in all nodes. The lymph nodes of all limited-flora animals (groups B to D) contained more immunoblasts and plasma cells than did those of the GF, but fewer than those of the CONV rats.

The skin incisions were healing poorly in nephrectomized as compared to control rats. No granulation tissue was observed in the wounds of the uremic rats. Epidermal regeneration and subcutaneous fibroblastic proliferation were slightly more advanced in the GF rats. The wounds of the CONV rats showed more inflammation.

Infection was never in evidence at autopsy and was only rarely detected histologically. No infection was seen in CONV rats sacrificed 48 or 72 hr after nephrectomy or in those dying from starvation after sham nephrectomy. Only in CONV rats dying from uremia were foci of superficial infection seen in the skin wounds (3 rats) and bacteria detected in the retroperitoneal nephrectomy sites (2 rats); the duration of survival after nephrectomy did not correlate with either of these findings.

DISCUSSION

Our present survey of some of the chemical and histological changes of fasting germfree, limited-flora, and conventionalized rats rendered acutely uremic by bilateral nephrectomy has revealed important differences which are attributable to the known difference in microbial status of these animals. These differences provide a tangible indication of the ways by which an animal's indigenous microflora may affect the lethal course of uremia. Thus, our present findings lend support to the observations of the preceding paper (1), in which it was clearly shown that the survival time of fasting rats after nephrectomy was altered not only by the presence of a microbial flora but also by its composition.

A high incidence of cecal erosions was found in the CONV rats dying from

uremia while no such lesion was found in any of the CONV rats sacrificed 48 or 72 hr after nephrectomy, nor in CONV rats that died from starvation, nor in any of the GF or selectively contaminated rats. Cecal lesions have been observed to occur in ordinary open room rats after bilateral nephrectomy and after silk encapsulation of one kidney and removal of the other (10). The exclusive occurrence of these lesions in our CONV rats dying from uremia suggests that living bacteria play a significant role in their production, that certain unspecified bacteria alone or in combination with those used for selective contamination are responsible, and that a prerequisite for their development is the biochemical environment and/or physiological status of the animal during the terminal stages of acute uremia. The importance of these lesions is suggested because their incidence and severity correlated with a shorter postnephrectomy survival time.

A primary vascular basis for the cecal lesion seems unlikely, since vascular lesions were found only in the vicinity of two of the most severe cecal lesions. Furthermore, the location and appearance of the necrotic arteries indicate that they were the result rather than the cause of the ulcerations. It is noteworthy that the GF rat has been reported to have more mucus in its cecum than the bacteria-laden rat (11). Thus, the GF cecum might be protected by the greater amount of mucus present which is apparently degraded by bacterial enzymes (11) but not by digestive proteolytic enzymes (12). More cecal damage does, however, occur in the GF than in the CONV rat after temporary or permanent occlusion of the superior mesenteric artery (13).

The fermentative activity of the intestinal flora has long been associated with the occurrence of lesions of the alimentary tract in uremia (14, 15). Bacterial urease activity resulting in the hydrolysis of the urea in the intestinal contents and the liberation of ammonia has been considered important. It may be pertinent that 72 hr after nephrectomy the CONV rats had much lower concentrations of urea nitrogen in their cecal contents than in their blood, while the GF rats 72 hr after nephrectomy had similar concentrations of urea nitrogen in their cecal contents and in their blood. Because there is no bacterial urease in the GF rat, breakdown of urea does not occur (16), and the urea entering the alimentary tract of the uremic GF rat may accumulate. Bacterial ureolysis does occur in CONV rats, however, and considerable quantities of ammonia may have been produced in the alimentary tract of the CONV rats dying from uremia. As Warren and Newton (17) have shown, portal blood ammonia concentrations of intact GF guinea pigs average only one-fourth those of their CONV counterparts. Thus, the local irritative properties of ammonia (14, 15), together with a direct microbial involvement of the cecal mucosa, as observed in this study, may have contributed to the production of the lesions. The exact species of bacteria which may be responsible for the changes which lead to mucosal ulceration remain to be determined. From our observations on the limited-flora

animals, however, certain combinations have been excluded. Further work is necessary to elucidate the mechanism underlying the cecal erosions we have described, and to determine their significance to the uremic animal. While we have shown the cecal lesions of the acutely uremic rat to be associated with the presence of a complex intestinal microflora, the full role of the microflora in accelerating the lethal course of uremia must include other, still more important factors since the tetracontaminated rats had a significantly shorter survival time than the GF rats after nephrectomy (1), but had no cecal lesions. Similarly, the CONV rats that died from uremia without manifesting cecal lesions also had a significantly shorter survival time than the uremic GF rats.

In contrast to the cecal lesions, the cardiovascular lesions were found in all groups of uremic rats, whether they had been sacrificed or had died post-nephrectomy and regardless of microbial status, but in none of the control rats with kidneys. These lesions were generally more severe, appeared earlier and were more frequent in the anuric GF and limited-flora animals than in the corresponding CONV rats. In individual animals, however, survival time and biochemical findings showed no consistent relationship to the lesions, even though the average total inorganic phosphorus was higher in the anuric GF rats and the average length of survival of the groups (1) tended to correlate directly with the general incidence and severity of these lesions. Therefore, it would seem that in acute uremia the presence of a complex microbial flora plays an adverse or enhancing role in the development of cecal lesions, but plays a beneficial or attenuating role with respect to the development of the cardiovascular lesions, especially calcification of the media of the coronary arteries and aorta. This ambivalence of the microbial flora was also observed in the preceding study (1), in which it was found that the microbial flora shortened life after anuria, but prolonged life after starvation.

The myocardial lesions we observed resemble those described in uremic patients (18), renoprival dogs (19), and rats (10, 20). The destructive arterial lesions often seen in the renoprival state were not observed in the present study. The reasons for their absence are not apparent, but may be related to the type of diet given before surgery, and the withdrawal of food and water the night before surgery and during the development of uremia.

The calcifications in the media of the aorta and coronary arteries have been described in renoprival animals (10, 20) and, as discussed above, were generally less prominent in our fasting uremic CONV rats for reasons that we do not know. In seeking a possible explanation for this difference in terms of microbial status, we considered the following information: (*a*) Wooley, on the basis of his investigations (21, 22), has postulated that "the basic mechanism of action of serotonin seems to be to arrange for the entry of calcium into cells" (reference 23, p. 81); (*b*) injection of calcium and serotonin into rats has shown that ". . . a histochemically demonstrable amount of blood calcium can be attracted

into the arterial wall", an observation considered by the author to be " . . . compatible with Wooley's theory" (24); (*c*) the largest concentration and store of serotonin in the body is found in the mucosa of the gastrointestinal tract (reference 23, p. 63); (*d*) serotonin levels in the intestinal wall are generally higher in the absence of an intestinal flora than in its presence (25); and (*e*) in vitro studies have shown that urea in concentrations encountered in patients with the uremic syndrome depresses the activity of monoamine oxidase, one of the enzymes known to destroy serotonin in the body (26). If we assume, from the foregoing, that more serotonin was available and active in the uremic GF rat than in the CONV, and we accept Wooley's theory, then we have an explanation of the generally greater prominence of the calcific lesions in the uremic GF rats and, perhaps, of that in the uremic limited-flora rats, too. In any event, our findings demonstrate that myocardial and vascular lesions occur in uremia in the absence of living bacteria.

Wound healing was equally poor in GF- and CONV-uremic rats, and may have represented a greater hazard to the latter as five of these showed secondary infection at surgical sites. The retardation of healing in uremic as compared to sham-nephrectomized rats cannot be attributed to food and water deprivation as both groups were so deprived. The lymph nodes and spleens of all uremic rats showed severe depletion of lymphocytes which eliminates living bacteria as major etiologic factors in this phenomenon.

The plasma potassium levels 72 hr postnephrectomy were similarly elevated in the GF and CONV rats. If this reflects the pattern to the time of death, then it seems that the longer survival of the anuric GF rats is not explicable on the basis of a lower blood level of potassium which could mean a delay in the attainment of levels toxic to the heart (27). The level of potassium in skeletal muscle was also similar in the anuric GF and CONV rats. To the extent that the plasma potassium level reflects general catabolism, these observations are compatible with the data of the preceding paper (1), in which no difference in the rate of body weight loss was found between the fasting GF and CONV rats. Our observations on the changes in the plasma glucose level and the plasma and skeletal muscle sodium levels revealed no differences which we would interpret as influencing survival time postnephrectomy. The hematocrit of both the GF and CONV rats was reduced 72 hr after nephrectomy relative to the sham-nephrectomized controls; since total plasma protein was not significantly altered at this time, it may be that the reduction in hematocrit reflects a reduction in the number of red blood cells. Thus, it appears that an anemia occurred with uremia irrespective of microbial status.

The failure of the GF animal to experience as sharp a rise in BUN as the CONV 72 hr after nephrectomy poses an interesting question: Was this the result of a failure of urea synthesis by the uremic GF animals, a super efficient gastrointestinal urea excretory mechanism, or the result of no bacterial ureo-

lysis? While we do not have firm conclusions on this point, it is noteworthy that (*a*) the total cecal urea of the GF rat is much larger than that of the CONV, and (*b*) the total urea of the germfree cecum rose about 2-fold after nephrectomy, while the total urea of the conventional cecum rose only minimally after nephrectomy. The various schools of thought on the question of the potential toxicity of urea in uremia are thoroughly discussed in the review of Schreiner and Maher (15) and will not be further discussed here.

Because the animals were nephrectomized and deprived of food and water, they were, in effect, a "closed system." Thus, the foregoing differences in chemical changes should reflect the modification of endogenous processes by the microflora.

The blood in uremia has been shown to contain an incompletely characterized group of organic substances which have been correlated with uremic toxicity and which, presumably, are largely organic anions or acids (15, 28, 29). Recently, some of these organic substances in the hemodialysis fluids from uremic patients have been carefully analyzed, and are considered to be either metabolic products of the tissues or of the intestinal bacteria (30). The opinion was expressed that they may play a role in the uremic syndrome (30). In this connection, it may be pertinent that the plasma concentration of indoxyl sulfate was elevated in our CONV rats 72 hr after nephrectomy. As is well known, indoxyl is produced from indole by the liver which may also conjugate indoxyl to yield indoxyl sulfate which is excreted in the urine as the potassium salt (indican) (31). Indole has long been considered to be a product of the action of the intestinal bacteria on tryptophane. These indolic substances have been found only in trace amounts or not at all in the urine of germfree animals (32, 33). Our finding of an increased plasma level of indoxyl sulfate in the anuric CONV rats carries the implication that other substances associated with bacterial action in the intestine may, like indole, enter the blood stream and be retained by the anuric animal. The theories and conflicts regarding the possible deleterious role of "toxic" substances in uremia have been extensively reviewed (15). In view of our demonstration of indoxyl sulfate in plasma, it seems that further detailed analyses of blood from uremic germfree and bacteria-laden animals should uniquely enable differentiation of those substances that are metabolic products of bacterial origin from those that are of tissue origin, and, ultimately, lead to the assessment of their relative importance to the uremic animal.

SUMMARY

Uremic colitis of varying severity occurred in the majority of conventionalized rats dying after removal of both kidneys, but was not found in uremic conventionalized and germfree rats sacrificed preterminally, or in germfree and limited-flora rats dying from uremia, or in any of the controls. The lesions were

restricted to the cecum and their incidence and severity paralleled a shorter duration of survival. Cardiovascular damage including focal myocardial necrosis and calcification and patchy aortic and coronary calcification were observed in uremic rats regardless of their microbial status. These lesions had a higher incidence, developed more rapidly, and were more severe in the germfree and limited-flora rats than in the conventionalized animals. The presence or severity of the lesions, however, did not correlate with survival time of rats dying from uremia or with total plasma calcium and inorganic phosphorus levels of individual animals. Generalized necrotizing arteritis was not observed. Wound healing was poor in all uremic rats regardless of microbial status. Focal infection was noted in a few conventionalized rats dying from uremia, did not correlate with survival time, and was absent in all other groups.

Comparison of biochemical findings between uremic germfree and conventionalized rats show a higher blood urea nitrogen and elevated plasma indoxyl sulfate in the presence of a microbial flora and a greater amount of plasma inorganic phosphorus in its absence. Uremia resulted in a decrease in hematocrit and increase in plasma and muscle potassium that were similar for germfree and conventionalized rats. Plasma and muscle sodium, total plasma calcium, glucose, and total protein were essentially unchanged by microbial status or uremia.

Because the foregoing differences in the metabolic and histopathologic changes of uremia are linked to the known difference in microbial status of the fasting bilaterally-nephrectomized rats that were studied, they are a tangible indication of ways by which the indigenous microbial flora and its composition may affect the course of acute uremia.

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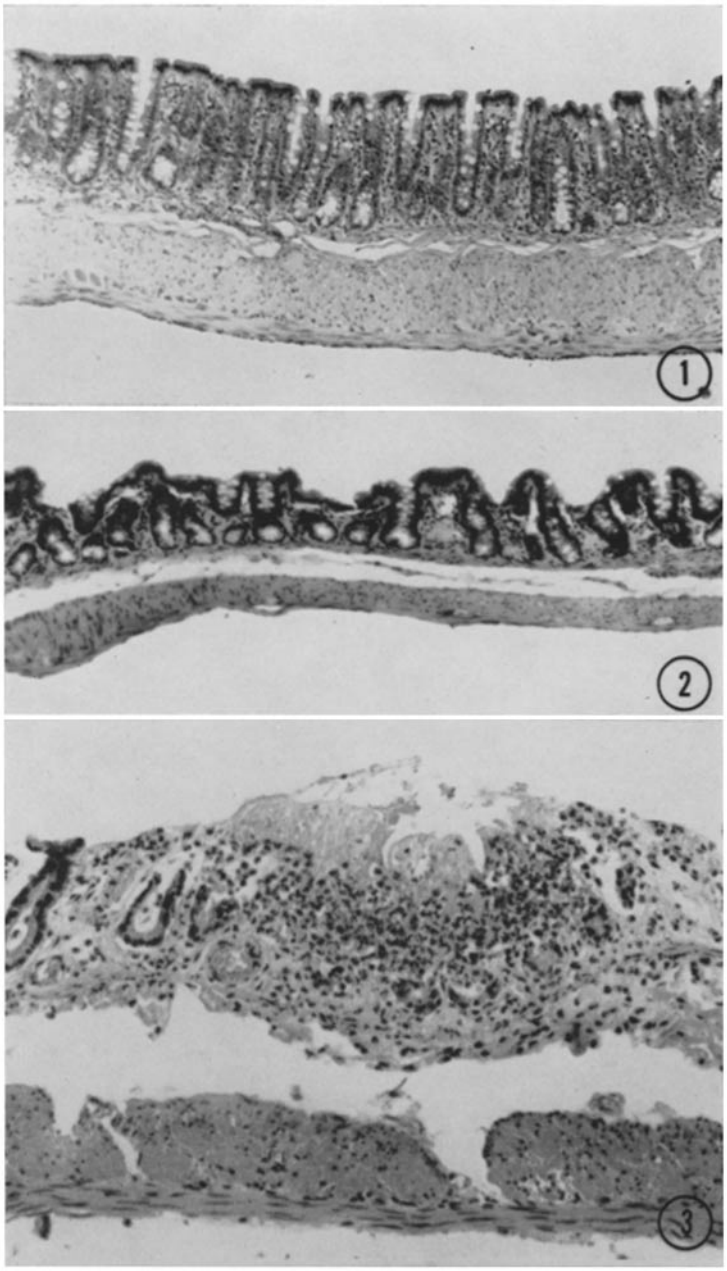
EXPLANATION OF PLATES

PLATE 40

FIG. 1. Cecum of normal conventionalized rat. Note thickness of mucosa and muscle. Hematoxylin and eosin. × 80.

FIG. 2. Cecum of normal germfree rat. Note thinness of mucosa and muscle. Hematoxylin and eosin. × 80.

FIG. 3. Cecum of conventionalized rat dying 41 hr after bilateral nephrectomy. Note superficial necrosis and ulceration of mucosa with acute inflammation. Hematoxylin and eosin. × 100.

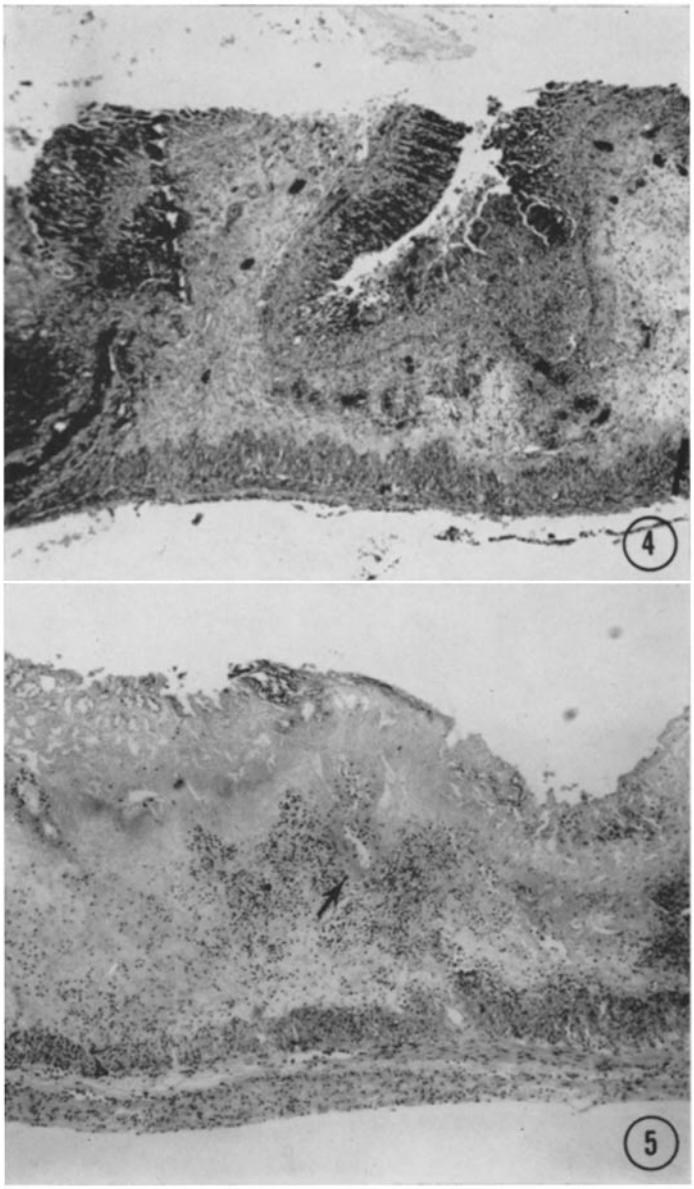


(Carter et al.: Uremia in germfree rats. II)

PLATE 41

FIG. 4. Cecum of conventionalized rat dying 72 hr after bilateral nephrectomy. Note complete mucosal and early submucosal necrosis. Hematoxylin and eosin. × 100.

FIG. 5. Cecum of conventionalized rat dying 67 hr after bilateral nephrectomy. Note complete mucosal and submucosal necrosis with necrotic superficial artery (arrow). Hematoxylin and eosin. × 100.



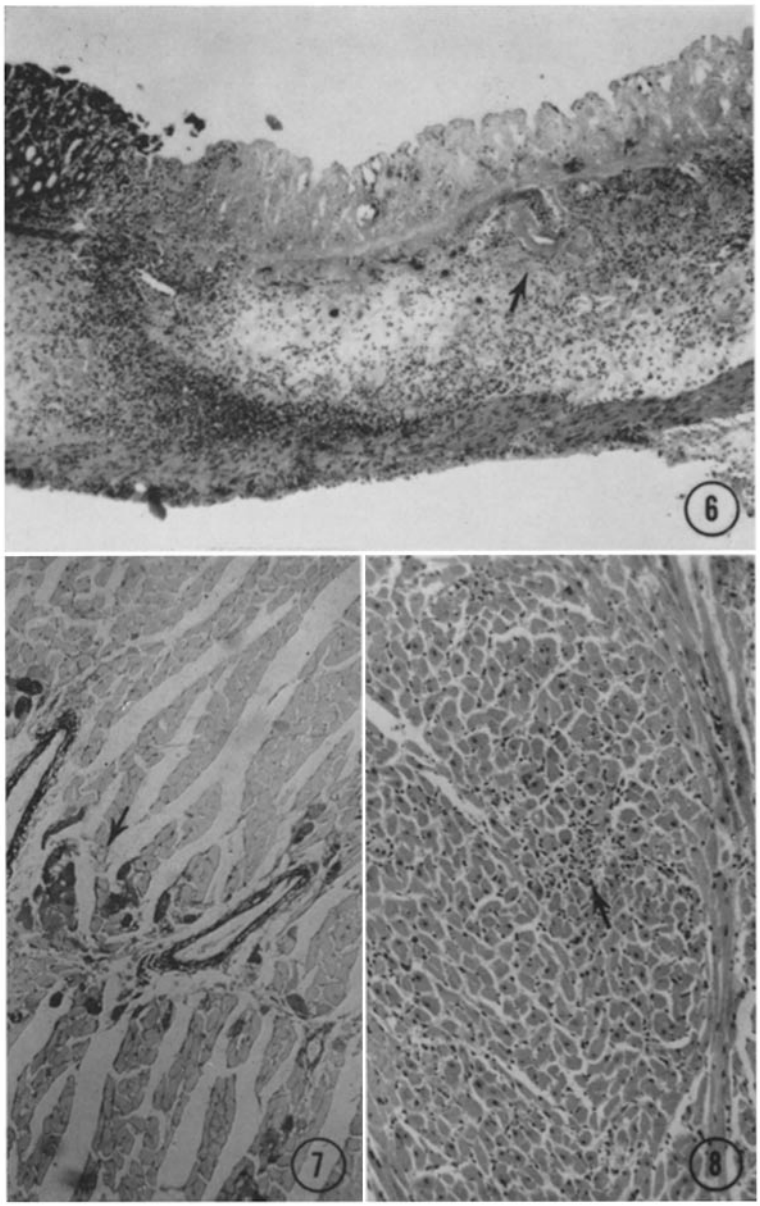
(Carter et al.: Uremia in germfree rats. II)

PLATE 42

FIG. 6. Cecum of conventionalized rat dying 69 hr after bilateral nephrectomy. Note demarcation of mucosal and submucosal necrosis by acute inflammation and necrotic superficial artery (arrow). Hematoxylin and eosin. × 100.

FIG. 7. Heart of germfree rat dying 128 hr after bilateral nephrectomy. Note swollen and PAS-positive myocardial fibers (arrow). Periodic acid Schiff. × 100.

FIG. 8. Heart of germfree rat dying 108 hr after bilateral nephrectomy. Note disintegrating necrotic myocardial fibers attended by inflammation (arrow). Hematoxylin and eosin. × 100.

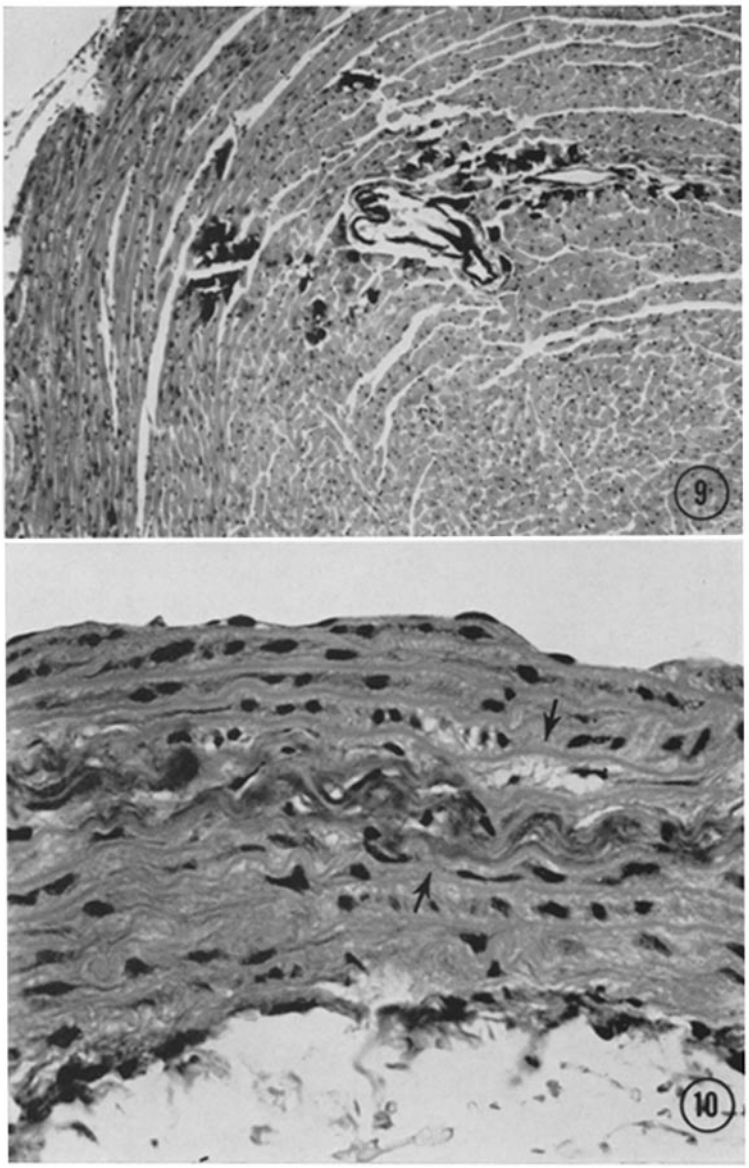


(Carter et al.: Uremia in germfree rats. II)

PLATE 43

FIG. 9. Heart of germfree rat sacrificed 72 hr after bilateral nephrectomy. Note subepicardial location of lesions, necrosis, and calcification of myocardium, and calcified coronary artery branch. Hematoxylin and eosin. × 120.

FIG. 10. Aorta of germfree rat sacrificed 72 hr after bilateral nephrectomy. Note focal hydropic degeneration of smooth muscle (upper arrow), intact elastic lamina, and early diffuse calcification (lower arrow). Hematoxylin and eosin. × 630.

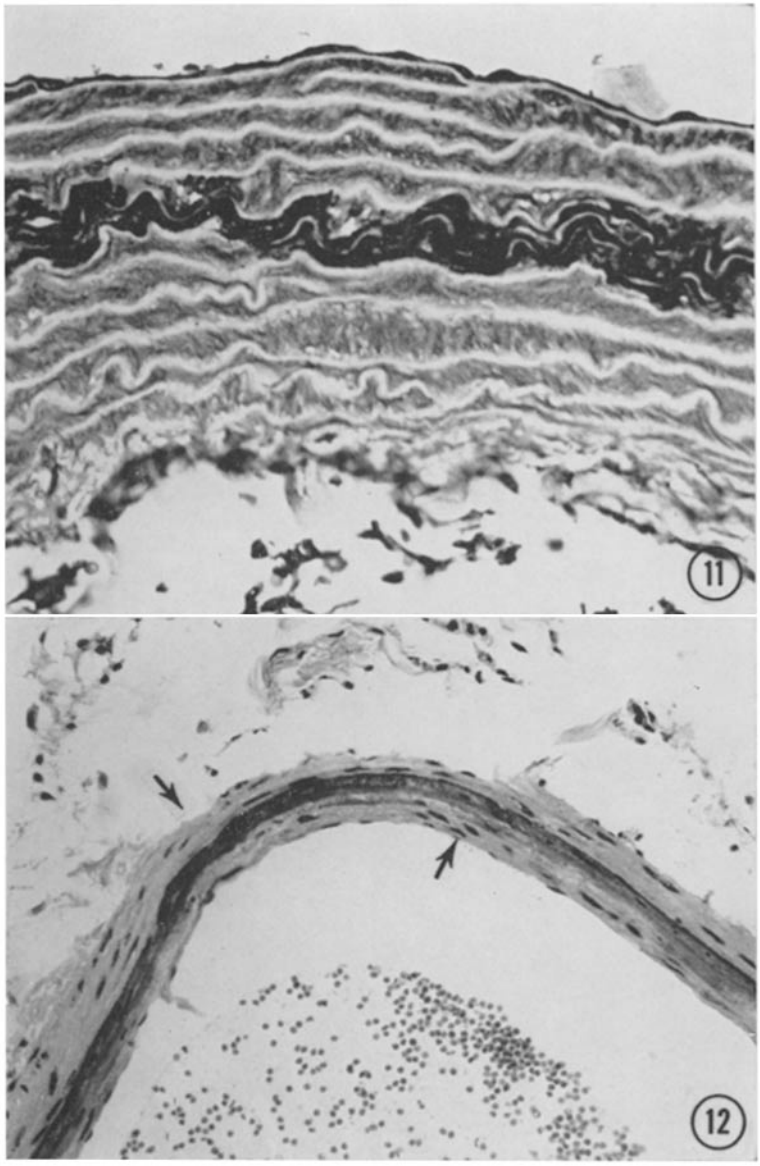


(Carter et al.: Uremia in germfree rats. II)

PLATE 44

FIG. 11. Aorta of germfree rat sacrificed 72 hr after bilateral nephrectomy. Note calcification of necrotic smooth muscle between intact elastic lamina (light colored wavy lines). von Kossá. × 630.

FIG. 12. Aorta of germfree rat dying 108 hr after bilateral nephrectomy. Note hydropic degeneration and early diffuse calcification of smooth muscle (arrow on right) and adjacent heavy calcification of media (arrow on left). Hematoxylin and eosin. × 180.



(Carter et al.: Uremia in germfree rats. II)