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BACTERIOLOGIC STUDIES OF THE X-RADIATED DOG*

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Aureomycin, an antibiotic with a broad bacterial spectrum, has been used in the therapy of the radiation syndrome in dogs and has been found to delay the onset of morbidity and mortality. Clinical and pathologic observations of this study have been reported in the preceding paper.[1] The bacteriologic observations made in this study are in agreement with the reports of Chrom,[2] Lawrence and Tennant,[3] Bennett, Rekers, and Howland,[4] and Miller, Hammond, and Tompkins,[5] which indicate that the intestinal tract is an important source of infection in the x-radiated animal. Vincent[6] studied the flora of the small intestine of normal and radiated rats and found that the coliform and pathogenic staphylococci increased markedly following radiation. He suggested that there is a possible relationship between radiation damage to the small intestine and alteration of the flora of the intestinal tract.

This report concerns the bacteriologic studies made on a group of 24 dogs that received 450 r. of whole body x-radiation. Twelve of these animals were treated with aureomycin. The remainder served as controls. Since blood cultures obtained from x-radiated dogs show a preponderance of gram-negative bacilli,[4] it is important to study in detail the flora of the intestinal tract, which is the major potential reservoir of such organisms. In this study analysis of the feces showed an increase in the number of coliform bacteria and staphylococci in all animals post-radiation. The coliform bacteria, however, increased to an even greater extent in irradiated dogs treated with aureomycin. Blood culture studies following irradiation indicated that the number of positive cultures in the control dogs increased, while the number of positive cultures in the treated dogs decreased.

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METHODS

In this study 24 adult, healthy, mongrel dogs received 450 r. of whole body x-radiation. Immediately following irradiation one-half of the animals received aureomycin* at the rate of 100 mg. per kg. per 24 hours. This medication was continued every 6 hours for 28 days. Details of animal care and radiation factors were given in the preceding paper.[1]

During the course of the experiment venous blood was obtained from the external jugular vein after preparation of the skin by shaving and applying tincture of iodine. For aerobic culture, thioglycollate broth was inoculated with 2 cc. of blood, and after 24 hours of incubation at 37° C., a loopful of the broth was transferred to a blood agar plate. For anaerobic cultures, after 48 hours of incubation, 1 cc. of broth was pipetted from the bottom of the thioglycollate tube and transferred to anaerobic agar. These plates were then incubated in an anaerobic jar for 48 hours.

Cultures of heart's blood, liver, spleen, and lung for aerobic and anaerobic culture were obtained at necropsy. All necropsies were carried out within 4 hours after death, and in most instances, within the first hour.

Complete taxonomic studies with exact species and strain differentiation were not done on the bacteria isolated, so that a positive identification was made only as to genera.

A quantitative analysis of fecal flora was used to study coliform bacteria, staphylococci, and streptococci. A uniform suspension of fecal material was made by shaking a 0.5 gm. sample of fresh feces in 100 cc. of sterile saline solution in a mechanical shaker for 20 minutes. Pour plates, using various dilutions of this saline suspension, were made with mannitol salt agar for staphylococci identification and with EMB† media for the coliform organisms. For streptococci isolation the saline suspension was distributed on the surface of Mitis salivarius† media with a sterile glass spreader. The colonies on the plate were counted after 24 hours of incubation at 37° C. The feces were also plated on Shigella-Salmonella† media for the isolation of pathogens. No attempt was made in this study to differentiate between species. The daily fecal bacterial counts for each group of dogs were averaged and the log of this daily average is plotted in Text-figures 1 to 4.

The sensitivity to aureomycin of the various organisms isolated from

* Supplied by Lederle Laboratories Division, American Cyanamid Corporation, New York, N.Y.

† Difco Laboratories, Detroit, Mich.

blood cultures, necropsy material, and feces was determined by a tube dilution method, using serial dilutions of the antibiotic with a standard amount of a 24 hour broth culture of the organism. Readings were made at the end of 24 hours. The end point is defined in terms of the aureomycin content of the tube containing the least amount of aureomycin in which no growth has occurred. These readings, therefore, presumably represent the bactericidal level.

The above bacteriologic studies were made for a 2-week period prior to x-radiation and for a 4 weeks post-radiation period.

TABLE I
Percentage of Positive Blood Cultures

	Pre-radiation, aerobes and anaerobes	Post-radiation, aerobes and anaerobes
Treated group		
Number cultured	71	176
Positive cultures	8.5%	6.3%
Control group		
Number cultured	71	149
Positive cultures	1.4%	14.8%

RESULTS

The percentage of positive blood cultures in the control group increased following irradiation and exceeded the number of positive cultures in the treated group following irradiation. This is shown in Table I.

Of the positive cultures in both groups post-radiation, 22 per cent occurred during the first week, 6 per cent in the second week, 36 per cent in the third week, and 36 per cent in the fourth week. Of all the positive anaerobic cultures post-radiation, 12 showed gram-positive bacilli which were of the Clostridia group, 3 were gram-negative bacilli, and 11 were gram-positive cocci.

The incidence of positive blood cultures was no higher in the dogs that died. Blood cultures were obtained within the 24-hour period before death in 8 of the 12 dogs that died. Only 2 were positive. One from a control dog showed *Escherichia coli,* which was isolated also from the blood and tissues of the dog when it died 6 hours later. The other culture was taken from one of the treated dogs and was positive for Pseudomonas which was cultured also from the heart's blood at necropsy. Seven of the control and 5 of the treated dogs died post-radiation. Cultures obtained at necropsy from 6 of the control dogs

were positive for *E. coli*. *Aerobacter aerogenes* was recovered from the other control dog that died. A Pseudomonas organism was recovered from 2 of the treated dogs and Proteus from another treated dog that died. Necropsy cultures from 2 of the treated dogs were negative. Table II shows the types of aerobic bacteria found in blood and necropsy cultures following radiation.

TABLE II

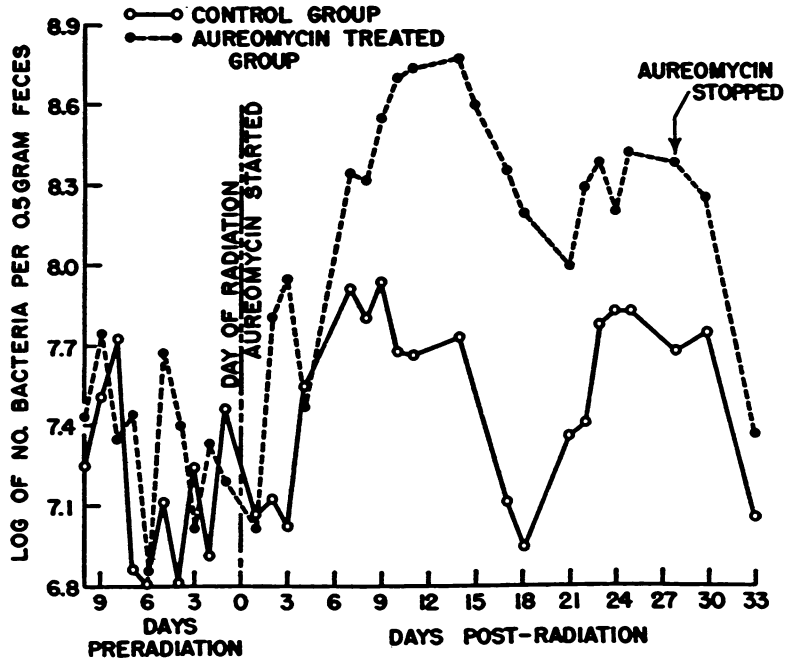
Aerobic Bacteria Found in Blood and Necropsy Material Following Radiation

Dog no.	Week post-radiation	Source	Organism	Sensitivity
1 Control	2	Blood	Non-hemolytic streptococcus	0.195
2 Control	2	Blood	*A. aerogenes*	
3 Control	2	Necropsy	*E. coli*	3.12
4 Control	2	Necropsy	*E. coli*	1.56
5 Control	3	Blood	*Staph. albus*	25.00
	3	Blood	Non-hemolytic streptococcus	0.195
6 Control	3	Necropsy	*A. aerogenes*	3.12
7 Control	3	Necropsy	*E. coli*	3.12
8 Control	3	Necropsy	*E. coli*	1.56
9 Control	3	Necropsy	*E. coli*	3.12
10 Control	3	Blood	Non-hemolytic streptococcus	0.195
	4	Blood	*E. coli*	
	4	Necropsy	*E. coli*	1.56
11 Control	4	Blood	Non-hemolytic streptococcus	
12 Control	4	Blood	*Strep. viridans*	12.5
13 Treated	2	Blood	Diphtheroids	
	3	Blood	Pseudomonas	12.5
	3	Necropsy	Pseudomonas	25.0
14 Treated	3	Blood	*Strep. viridans*	0.195
15 Treated	3	Blood	*Staph. albus*	0.195
16 Treated	4	Necropsy	Proteus	50.0
17 Treated	4	Necropsy	Pseudomonas	25.0

Examination of Feces

Text-figure 1 shows that the number of coliform organisms in the feces increased markedly in both the control and treated groups following irradiation, reaching maximum levels during the second week. The coliform bacteria increased to a much greater extent in the aureomycin treated dogs, and remained at a high level until therapy was discontinued, after which the count dropped abruptly to the pre-radiation value. The decrease in the counts from the 15th to the 25th days may be accounted for in part by the anorexia which occurred in both groups during this period. Text-figures 2 and 3 show that similar increases occurred in the staphylococcus and streptococcus counts in the treated animals and in the staphylococcus count in the controls. In

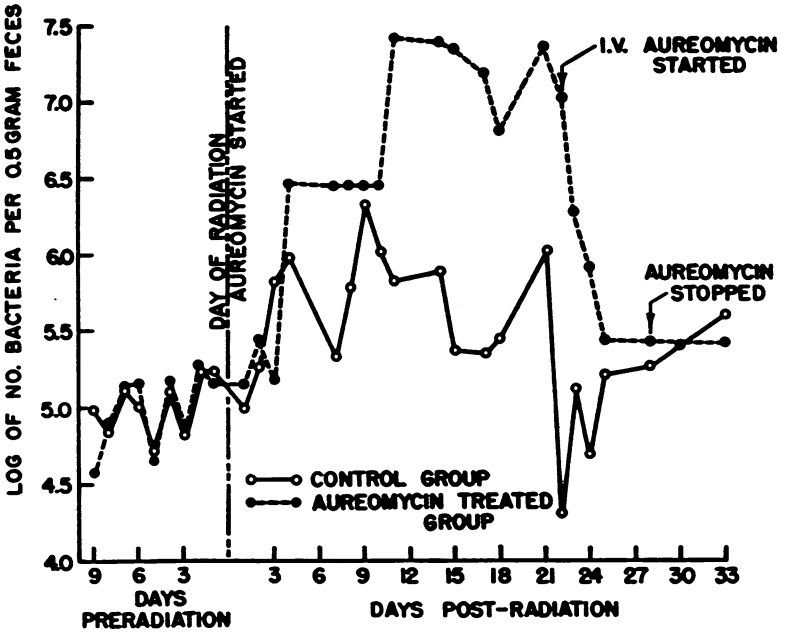
certain of the treated dogs a sudden drop in staphylococci coincided with the start of intravenous aureomycin therapy. The incidence of Proteus in the treated group also increased post-radiation. No organisms ordinarily considered to be pathogenic were isolated. The increases in fecal bacterial counts occurred during the period when the leukocyte counts of the blood were lowest.



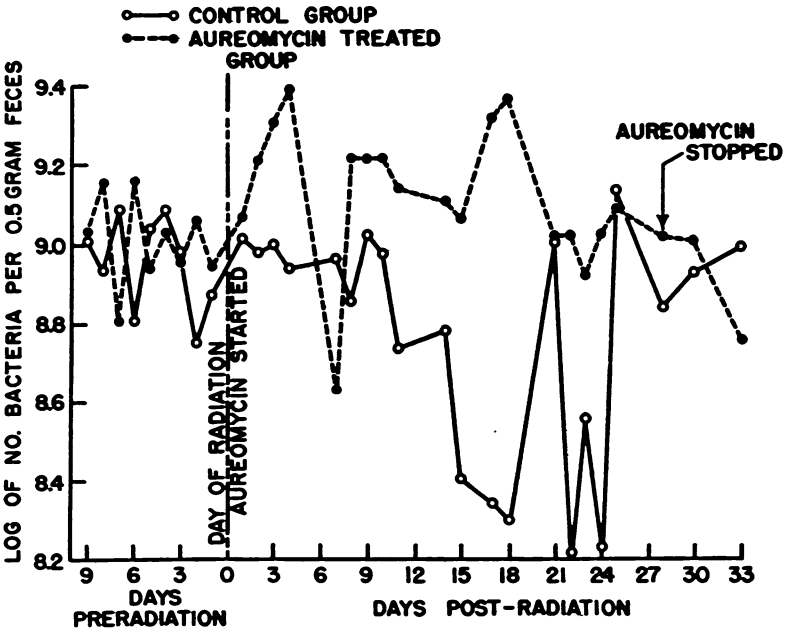
Text-figure 1. Fecal coliform bacterial count.

In order to determine whether this shift in bowel flora was primarily an effect of irradiation or of aureomycin, to a group of 4 normal dogs aureomycin was administered in identical dosage for 4 weeks. As shown in Text-figure 4, the counts of coliform bacteria and staphylococci showed increases during the 3rd week of aureomycin administration similar to, but less marked than, those demonstrated by the irradiated treated animals from the 10th to the 15th days post-radiation. There was essentially no change in the count of streptococci.

The results of the sensitivity tests of the bacteria isolated from blood and necropsy cultures are shown in Table II. All organisms isolated at necropsy from the control dogs were sensitive to aureomycin whereas those isolated at necropsy from the treated animals were resistant. The sensitivities of the organisms isolated from the feces before, and at weekly intervals after, irradiation are shown in Tables III and IV. The sensitivity of the staphylococci remained essentially

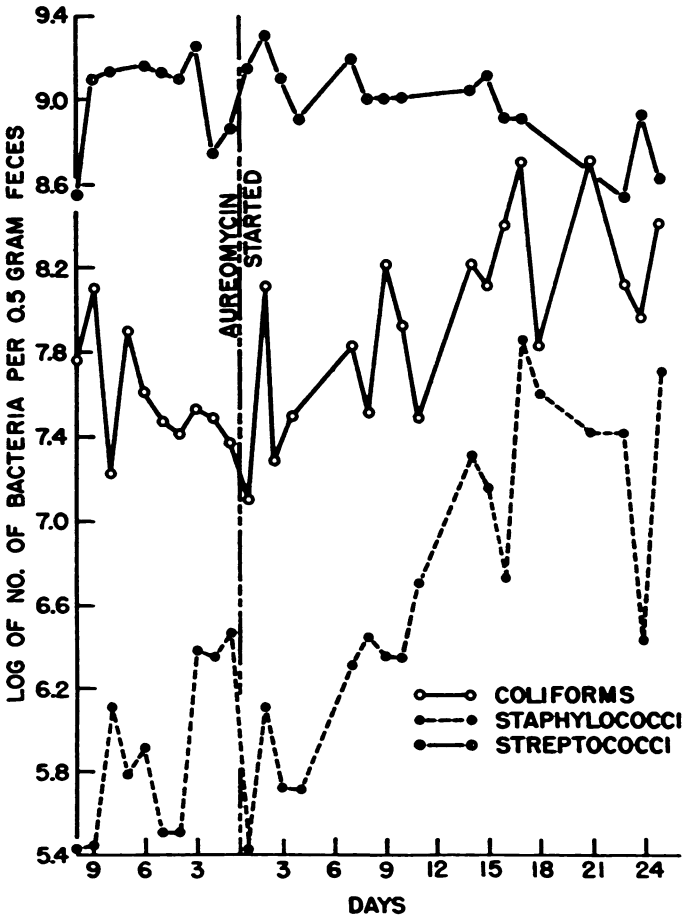


Text-figure 2. Fecal staphylococcus count.



Text-figure 3. Fecal streptococcus count.

unchanged in both groups, whereas the sensitivity of the streptococci decreased in both groups following irradiation. All Proteus strains were resistant to aureomycin before and after irradiation. The sensi-



Text-figure 4. Fecal bacterial counts in non-radiated dogs.

tivity to aureomycin of the coliform organisms from the treated dogs decreased markedly after irradiation but remained unchanged in the untreated group.

Discussion

The delayed onset of morbidity and mortality in the aureomycin treated dog that has received a large dose of whole body x-radiation supports the concept that infection plays an important rôle in the radiation syndrome. The view that the bacterial flora of the bowel is an important source of infection to the x-radiated animal is substantiated by the isolation from necropsy material of bacteria which are

TABLE III

In Vitro Aureomycin Sensitivities in μg. per cc.

	Dog no.	Fecal coliforms				Fecal Proteus			
		Pre-treatment	7 days after starting aureomycin	14 days after starting aureomycin	21 days after starting aureomycin	Pre-treatment	7 days after starting aureomycin	14 days after starting aureomycin	21 days after starting aureomycin
Controls	1516	1.56	3.12	3.12	1.56			100	
	1548	3.12	1.56			50			
	1552	25.0	12.5	3.12	3.12		50	100	
	1553	3.12	1.56	3.12		50			
	1559	1.56	3.12	3.12	3.12	100	3.12		
Average		7.0	4.0	3.0	3.0	66	27	100	
Treated	1554	1.56	100	12.5	50	100	100+	100	100
	1557	3.2	100	12.5	25	100+	100	50	100+
	1558	3.12	100	12.5		100+	100+	50	
	1565	1.56	50	6.25	50	100	100	100	50
	1567	3.12	50	12.5	12.5	100+	100	100	25
Average		2.5	80	11	35	100+	100+	80	70

the normal inhabitants of the intestinal tract. These types of bacteria were recovered also 6 to 12 hours pre-mortem in blood cultures from 2 of the dogs. Clostridia, which are also normally found in the bowel flora, were recovered in a large number of the anaerobic pre-mortem blood cultures. It is believed by some workers that a positive blood culture can be obtained uniformly from animals in the agonal state. However, blood cultures obtained a few hours prior to death and necropsy cultures from many of the treated dogs have been negative. All organisms isolated from necropsy material of the control dogs were sensitive to aureomycin. This may indicate that sepsis might have been prevented had these dogs received effective antibiotic therapy. All organisms isolated from the treated dogs at necropsy were resistant to high concentrations of aureomycin.

Basic factors contributing to the spread of enteric infection to other parts of the body of the radiated animal are damaged capillaries and ulcerations of the intestinal mucosa, damage of the reticulo-endothelial barriers, destruction of mesenteric lymph nodes, and leukopenia. In guinea-pigs which have been infected with the cholera vibrio, Burrows, Deupree, and Moore[7] reported a marked fall in coproantibody titer in the feces coincident with the invasion of other body tissues by the vibrio following irradiation. They stated that if natural immunity to intestinal bacteria is related to coproantibody, then the inhibition of this immune mechanism in the irradiated animal may account for a bacteremia of intestinal origin. Studies to determine the rôle of coproantibody and its correlation with fecal bacterial counts in the dog are now being made in this laboratory.

The increased number of coliform bacteria and staphylococci in the bowel of these radiated dogs may be of significance in the radiation syndrome. Alterations in environmental conditions in the intestinal tract may be responsible in part for the increase of the bacteria in the bowel. It has been shown that the pH of gastric juice is increased following irradiation.[8] There may also be qualitative and/or quantitative changes in the intestinal and pancreatic secretions.

The greater increase in fecal bacterial counts in the treated dogs than in the control dogs is of considerable interest. Dearing and Heilman[9] reported that coliform and gram-positive organisms are practically eliminated from the feces of humans who have received a therapeutic dose of aureomycin for from 1 to 3 days. In this laboratory comparatively long-term administration of aureomycin to normal dogs is followed by an increase in the number of coliforms and staphylococci in the feces. This increase is detected after 7 days of

TABLE IV

In Vitro Aureomycin Sensitivities in μg. per cc.

	Dog no.	Fecal staphylococci				Fecal streptococci			
		Pre-treatment	7 days after starting aureomycin	14 days after starting aureomycin	21 days after starting aureomycin	Pre-treatment	7 days after starting aureomycin	14 days after starting aureomycin	21 days after starting aureomycin
Controls	1516	12.5		12.5	12.5	0.195	0.195	25	12.5
	1548	25	12.5			0.195	12.5		
	1552	25	12.5	25	12.5	25		12.5	12.5
	1553	0.195		12.5		25	12.5		
	1559	0.195	12.5	25	12.5	0.195	1.56	0.195	12.5
Average		13	12.5	19	12.5	10	5	12.5	12.5
Treated	1554	50			12.5		12.5	25	25
	1557	12.5			12.5	3.12	12.5	12.5	12.5
	1558	25	12.5			12.5		25	
	1565	0.195	12.5	12.5	12.5	0.195	3.12	25	12.5
	1567	0.195			12.5	25	0.195	12.5	12.5
Average		18	12.5	12.5	12.5	8	7	20	15

oral aureomycin administration. These results are supported by the work of Marshall, Palmer, and Kirsner,[10] who found that, after administration of aureomycin, an initial decrease in fecal bacterial count occurs, followed by a rise of varying degree with *E. coli* becoming the predominant organism.

The secondary increase in the fecal coliform count may be partially explained with reference to the sensitivity tests, which showed that the coliform organisms from irradiated-treated and normal-treated dogs became resistant to aureomycin after 7 days of administration. It is also possible that aureomycin-dependent organisms may develop. A growth-promoting effect of aureomycin on these bacteria must also be considered, although initial experiments in this laboratory do not lend evidence to support this concept.[11] The increase in bacterial flora in irradiated dogs may follow the decrease in coproantibody titer reported by Burrows *et al.*,[7] since the withdrawal of this defense mechanism could allow bacteria in the bowel to multiply unchecked. It may be possible that a bacteriophage, normally present in the intestinal contents, is either inhibited or destroyed by x-radiation and/or by aureomycin.

It was found that following intravenous administration of aureomycin there was a sudden decrease in the fecal staphylococcus count. This may indicate that a bactericidal level of aureomycin was attained in the bowel following excretion of high concentrations of the antibiotic in the bile.[12]

It is of interest to note that the changes in fecal flora were somewhat different in a separate group of dogs that received oral aureomycin, streptomycin, and penicillin, in clinical dosage range, simultaneously.[11] The coliform bacteria in the treated animals again showed a marked increase following radiation. However, the gram-positive organisms, presumably as a result of penicillin therapy, remained at the pre-radiation level.

The deaths observed in both groups of animals were in most instances related to hemorrhagic tendencies present in various parts of the body. The mechanism by which this hemorrhage is produced is undoubtedly related to derangements in the vascular system and in the coagulation mechanisms. Oral administration of aureomycin decreases the extent of the ulceration and hemorrhage in the bowel wall. However, the hemorrhagic tendencies elsewhere are not altered. Certain of the toxic products of enteric organisms have been shown to cause alteration in vascular integrity.[13] If the increase in coliform organisms in the bowel causes an overproduction of lytic toxins which in turn

may cause hemorrhage, one might anticipate an increased tendency to local bleeding within the bowel in the treated animals. This did not occur. Even with the greater increase of fecal organisms as the result of aureomycin therapy, the absence of a hemorrhagic tendency within the bowel in the treated dogs would point toward the hemorrhage as a direct effect of the irradiation on the vascular walls and coagulation mechanism rather than a secondary effect of toxins elaborated in the lumen of the bowel from these organisms and circulated elsewhere.

This hemorrhagic diathesis contributes directly to the mortality from x-radiation. However, it can be reasoned from the evidence presented here that bacterial infection also must contribute to the morbidity and mortality of the radiation syndrome and in certain of the animals may have been the major cause of death, particularly in early stages.

SUMMARY

Twenty-four adult dogs received 450 r. of total body x-radiation. Of these dogs, 12 received aureomycin at the rate of 100 mg. per kg. per 24 hours, for 28 days following radiation. Twelve animals exposed simultaneously served as controls. Bacteriologic studies of blood, necropsy material, and feces of radiated and normal dogs were made.

The incidence of positive blood cultures post-radiation was greater in the control group than in the treated group.

The fecal coliform bacteria, staphylococci, and streptococci increased to a greater extent post-radiation in the treated than in the control group.

Aureomycin resistant organisms were obtained from necropsy cultures from the treated dogs. The bacteria isolated from the control dogs were sensitive to aureomycin. The sensitivity to aureomycin of the fecal coliform bacteria and streptococci decreased post-radiation in the treated dogs. Bacterial strains resistant to high concentrations of aureomycin developed.

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