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The Effect of Uremic Serum on Normal Human and Guinea Pig Lymphocytes (38722)

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(Introduced by G. O. Broun, Sr.)

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Acute and chronic renal failure are generally associated with a marked peripheral blood lymphocytopenia (1, 2). We have previously shown (3) in the rat, rabbit, and guinea pig that lymphocytopenia may develop as early as 1 day after the experimental induction of uremia. Such an early onset of lymphocytic loss would tend to rule out decreased production as the cause of peripheral blood lymphocytopenia in uremia. In addition, we showed that adrenalectomy did not diminish the severity of the lymphocytopenia in the rat after induction of uremia. This finding would appear to eliminate elevation of corticosteroid levels as a causative factor for the lymphocytopenia. The mechanism of uremic lymphocytopenia remains unknown, although the most prominently mentioned suggestion has been that this abnormality is linked with an increased destruction or shortened half-life of lymphocytes (4). The present study was undertaken to ascertain the toxic effect of uremic serum and guanidinosuccinic acid (GSA), a toxic metabolite of uremia, on normal lymphocytes.

Materials and Methods. Uremic guinea pig serum. Adult male guinea pigs of Swiss Strain weighing at least 500 g were anesthetized with ip Nembutal, 29 mg/kg body wt. Under surgically clean conditions, a midline suprapubic incision through the peritoneum exposed the urinary bladder and the ureters which were ligated with silk sutures just above the point of entrance into the bladder. Three days later blood was obtained by cardiac puncture, the serum separated, and stored in a frozen state.

Uremic human serum. Blood was drawn from uremic patients on the wards of the St. Louis University Medical Center. The serum was separated and frozen.

Preparation of labeled guinea pig and human lymphocytes. Ten milliliters of blood was drawn from a normal guinea pig via cardiac puncture into a syringe containing 100 units of heparin (Panheprin, Abbott Laboratories, North Chicago, IL). Lymphocytes were separated on a layer of Ficoll–Hypaque by centrifugation at 400*g* for 40 min (5). The cells were washed twice in veronal-buffered saline (VBS) containing 10% fetal bovine serum (FBS). After the second washing, the cells were resuspended in 1 ml VBS–FBS with 0.1 ml ^{51}Cr in isotonic saline. (The Radio Chemical Centre, Amersham 1 mCi/ml, 6 μg Cr/ml.) The mixture was incubated at 37° for 45 min with frequent agitation. The cells were then washed three times with 5 ml VBS–FBS and resuspended in VBS–FBS. A cell count was made and the suspension diluted to a final concentration of 2.0×10^6 cells per ml. The same procedure was carried out on human venous blood.

Incubation of lymphocytes. One hundred microliters of guinea pig cell suspension, containing the radioactive isotope, was added to tubes containing 100 μl of the following: uremic guinea pig serum, autologous serum, VBS–FBS (negative control), and distilled water. The human cells were treated in the same way except for incubation with human uremic serum and an added tube containing 100 μl of GSA (Sigma Chemical Company, St. Louis, MO) 1.3×10^{-3} *M* in VBS.

The tube containing only distilled water and cells was rapidly frozen and thawed several times to obtain 100% ^{51}Cr release. All tubes were then incubated at room temperature for at least 2 hr. After incubation, 2 ml of cold VBS–FBS was added to each tube and spun at 1500 rpm for 10 min. One and a half milliliters of the supernatant was carefully removed from each tube to avoid disturbing the pellet, and was counted in a gamma scintillation counter (Nuclear Chi-

TABLE I. INCUBATION OF ^{51}Cr-LABELED NORMAL GUINEA PIG LYMPHOCYTES WITH AUTOLOGOUS OR UREMIC SERUM.

	2-Hr incubation			6-Hr incubation			24-Hr incubation		
	Uremic serum	Autologous serum	Negative control	Uremic serum	Autologous serum	Negative control	Uremic serum	Autologous serum	Negative control
Mean	10.59[a]	11.51	12.47	15.00	18.34	15.47	35.14	51.54	75.07
Standard deviation	2.33	2.77	3.81	7.21	13.54	6.69	9.00	19.64	19.13

[a] Expressed as percentage of Cr release using freeze–thaw value as 100%.

TABLE II. INCUBATION OF ^{51}Cr-LABELED NORMAL HUMAN LYMPHOCYTES WITH AUTOLOGOUS OR UREMIC SERUM FOR 2 HOURS.

	Uremic serum	Autologous serum	Negative control
Mean	6.88[a]	7.60	6.85
Standard deviation	2.43	3.96	2.43

[a] Expressed as percentage of Cr release using freeze–thaw value as 100%.

TABLE III. INCUBATION OF ^{51}Cr-LABELED NORMAL HUMAN LPMPHOCYTES WITH GUANIDINOSUCCINIC ACID (GSA) OR AUTOLOGOUS SERUM.

	GSA (1.3×10^{-3} *M*)	Autologous serum	Negative control
Mean	7.28[a]	9.54	7.91
Standard deviation	2.96	7.73	3.06

[a] Expressed as percentage of Cr release using freeze–thaw value as 100%.

cago). The percentage of ^{52}Cr release was calculated, with the results obtained from the freeze–thawed tube considered as 100% release. ^{51}Cr release was regarded as an index of cytotoxicity.

Electron microscopy. Lymphocytes were suspended for 2 hr in 3% phosphate-buffered glutaraldehyde. After a buffer rinse, the lymphocyte pellet, obtained by centrifugation, was osmicated for 1 hr in Millonig's osmium tetroxide. Dehydration was carried out in a series of graded ethanol solutions, prior to embedment in Epon–Araldite. Thin sections were cut with a Huxley-LKB microtome, placed on collodionized grids, stained with uranyl acetate and lead citrate, and viewed in a Philips 300 electron microscope. For orientation, sections were also cut 1½ μm thick, and stained with 1% methylene blue in sodium borate.

Results. Incubation of normal guinea pig lymphocytes. Table I shows the results of 10 experiments. After 2 hr of incubation there was no significant change in ^{51}Cr release between the negative control tubes, the tubes containing normal guinea pig lymphocytes in autologous serum, and the tube containing normal guinea pig lymphocytes in uremic guinea pig serum. In seven experiments the incubation time was carried out from 6 to 24 hr. While, in general, the release of ^{51}Cr appeared to rise with the length of incubation, no increase was seen in the uremic serum tubes; on the contrary, the level of ^{51}Cr was decreased in these tubes.

Incubation of normal human lymphocytes. In Table II the results of 20 experiments are seen. In all instances the incubation of normal human lymphocytes with uremic serum resulted in no greater release of ^{51}Cr than incubation with autologous serum. In another part of this study, shown in Table III, incubation of normal human lymphocytes with GSA resulted in no significant release of ^{51}Cr.

Electron microscopy. Figure 1 shows a normal human lymphocyte after incubation with human uremic serum. The cell presents normal morphologic characteristics; the subcellular organelles, including mitochondria, rough endoplasmic reticulum, and free ribosomes, appear to be intact, and offer no electronmicroscopic evidence of cytological derangement.

Discussion. A consistent finding in uremia is a falling in numbers of circulating lymphocytes. Previously reported studies demonstrated that neither decreased production nor

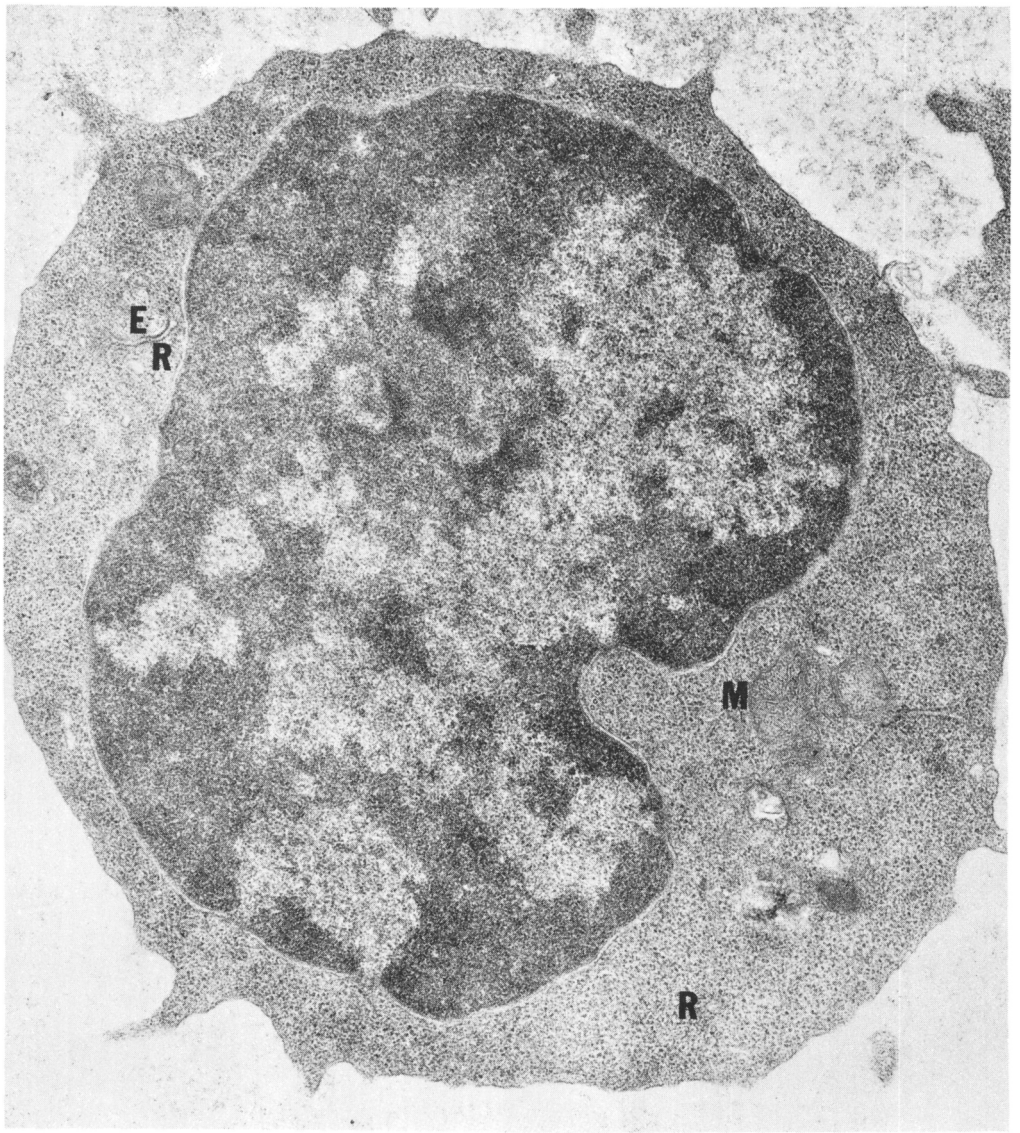

FIG. 1. Peripheral blood; normal human lymphocyte after incubation in uremic serum. The cell presents an essentially normal profile with intact mitochondria (M), scattered endoplasmic reticulum (ER), and free ribosomes (R). Glutaraldehyde-osmium tetroxide fixation; orig. magnification ×6800.

elevated levels of corticosteroids appeared to be causes of lymphocytopenia of uremia (3). The rapid development of lymphocytopenia in uremia would favor increased destruction of these cells. Daniels *et al.* (4) have shown that the *in vitro* survival of lymphocytes from uremic patients was significantly decreased over normal subjects using supravital staining as an index of cytotoxicity; however, incubation of normal lymphocytes in uremic plasma showed no decreases in survival time (6).

Our studies, utilizing ^{51}Cr-labeled lymphocytes, tend to corroborate this latter finding. Incubation of normal human and guinea pig lymphocytes in uremic serum resulted in no marked release of ^{51}Cr, beyond that observed in the control, indicating no evidence for the

presence of cytotoxicity. Addition of GSA, a surplus metabolite in uremia, resulted in similar findings.

It would, therefore, appear that the lymphocytopenia of uremia is not due to any cytotoxic factor present in serum based on these limited *in vitro* conditions including the time of incubation. A defect intrinsic to the lymphocyte may be postulated as one explanation for this abnormality, however, electronmicroscopic examination of normal lymphocytes incubated in uremic serum and lymphocytes of uremic humans revealed no ultrastructural changes. Electron microscopic appearance, however, has no essential relationship to functional integrity. In addition, a cellular derangement may exist which cannot be demonstrated with presently available technics.

Another explanation for the lymphocytopenia might be provided by a sequestration or redistribution of lymphocytes to certain body compartments. The situation in uremia might be analogous to that seen in the hydrocortisone-treated animal in which sequestration of peripheral T lymphocytes in bone marrow has been reported (7). Studies in our laboratory are in progress, to determine the validity of this hypothesis.

Summary. Incubation of normal ^{51}Cr-labeled human and guinea pig lymphocytes with uremic serum or guanidinosuccinic acid did not cause an increase in ^{51}Cr release. Electron microscopic examination of these lymphocytes revealed no abnormalities. It is suggested that the lymphocytopenia of uremia is not due to increased destruction of cells but may be a result of redistribution to other body compartments.

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