Erythropoietin Alterations in Renoprival and Renal Allografted Patients* (33434)

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Erythropoietin (ESF) is a hormone active in the regulation of erythropoiesis (1–10). Jacobson *et al.* (5) postulated the kidney to be the only source of ESF. In 1957, however, Mirand (6, 7, 11) showed that bilaterally nephrectomized, hypophysectomized and/or splenectomized rats stimulated by hypoxic hypoxia could produce erythropoietin but at a lower level. These observations were confirmed by Piliero *et al.* (13), Gallagher *et al.* (14), and Baciu *et al.* (15) and others in bilaterally nephrectomized animals. Just as the rat and rabbit have an extrarenal source for ESF, so does the tamarin, a small primate (10). Nathan *et al.* (16) reported the persistence of erythropoiesis in anephric man without the detection of erythropoietin in the plasma. However, Mirand *et al.* (11, 12) reported in a single human renal transplant candidate the presence of high titers of plasma erythropoietin in such a patient while he was in a renoprival state. Naets and Wittek (17) confirmed this finding in a recent communication. Naets and Wittek reported assaying the plasmas of eleven anephric patients and only one determination out of 65 showed a striking elevation in ESF plasma titer. This report describes further evidence of erythropoietin elevations in humans during renoprival and allograft states.

Materials and Methods. Plasmas from each patient were collected and frozen. All specimens were assayed in adult Ha/ICR Swiss mice rendered polycythemic (8, 18). Five mice were used to determine the ESF activity for each plasma sample. Each test mouse received subcutaneous injections of 0.5 ml of plasma daily for 3 successive days. On the fourth day they were injected intravenously with 1 μCi of ^{59}Fe in 0.5 ml of saline. Twenty-four hr later they were bled from the dorsal aorta and the radioactivity of the blood sample was measured in a well-type scintillation counter. The percentage incorporation of radioactive iron into the circulating red cells was then calculated. Assays from mice with hematocrit levels of less than 60% at the end of the experiment were discarded. A concise clinical statement on the status of each of the 4 patients is presented.

Patient I (V.W.), a 12-year-old male, with end-stage glomerulonephritis underwent bilateral nephrectomy and chronic hemodialysis. Hypertension and heart failure improved following bilateral nephrectomy with salt restriction. A series of 2 cadaver allografts were performed. The first was rejected and the second failed to function due to extensive tubular necrosis. At present he is anephric, in otherwise good health on intermittent dialysis, and awaiting another renal graft. The finding of high plasma ESF titers in this patient was initially reported at a meeting of South African Medical Society on February 15, 1968.

Patient II (P.), a 45-year-old male, with end-stage glomerulonephritis had bilateral nephrectomy. Hypertension improved, but allografting was deferred due to a pulmonary embolus with partial persistent atelectasis of one lung. Following anticoagulation therapy, he was stabilized and is being managed by intermittent hemodialysis.

Patient III (F.), a 56-year-old male was seen and studied only before successful renal

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TABLE I. ESF Plasma Levels[a] in Bilaterally Nephrectomized Humans with and without Renal Homografts.

Patient		Before nephrectomy	After nephrectomy	After first renal homograft	After second renal homograft	Anephric state
(I) V. W. 9/28/67[b]	Av ESF unit	—	.18 (.13–.36)[c]	.20 (.12–.37)	.10 (.07–.14)	.14 (.03–.38)
	Av HCT	—	22 (17–30)[c]	26 (15–31)	25 (22–28)	23 (20–26)
			66 days[d]	56 days	17 days	48 days
(II) P. 1/30/68	Av ESF unit	.06 (.03–.12)	0.08 (.03–.20)			
	Av HCT	28 (20–38)	36 (21–50)			
		46 days	62 days			
(III) F. 3/1/67	Av ESF unit	—	.03 (.02–.04)	.03 (.03–.04)		
	Av HCT	—	20 (19–22)	23 (21–24)		
			316 days	39 days		
(IV) S. 12/18/67	Av ESF unit	.06 (.05–.07)	.17 (.04–.19)	.11 (.10–.13)		
	Av HCT	18 (16–20)	20 (18–25)	27 (17–31)		
		5 days	67 days	50 days		

[a] ESF levels are expressed in units of ESF, 1 unit of ESF in our laboratory induces a 24-hr ^{59}Fe uptake of 26% in polycythemic mice.

[b] Date of nephrectomy.

[c] Range.

[d] Period of days in which patient was studied.

allografting and after bilateral nephrectomy. His clinical course is good and, although on steroids and azathioprine, there are no signs of decline in renal function or renal rejection.

Patient IV (S.), an 18-year-old female was studied before bilateral nephrectomy for severe glomerulonephritis and hypertension. During the renoprival state she was well controlled with intermittent hemodialysis and has recently received a successful renal allograft.

Results. The ESF plasma levels in bilaterally nephrectomized humans with and without renal allografts are shown in Table I. This table expresses the plasma ESF level in units of ESF.

During the 66 renoprival days, patient I (*V.W.*) had considerable evidence of ESF activity. Assay of plasma ESF had been performed nine times in this patient. All nine determinations were positive for ESF activity. The first determination was made at renoprival day 19 and the ^{59}Fe uptake was 4 times above control levels. At this time, he was anemic and ESF activity was unrelated to dialysis or transfusions. During the ischemic rejection crises after the first renal allograft, further increases in ESF activity was apparent. This level of ESF fell following removal of the first allograft and insertion of the second allograft. The level of ESF was, however, still above control states. Following removal of the second allograft, a lower but significant ESF level of activity has re-

mained. Thus, this patient has had significant ESF release during the anephric state, during renal allograft rejections, and again during the second renoprival period.

Patient II (P) had some elevation in ESF activity prior to bilateral nephrectomy (Table I). This level increased somewhat rather than fell during the 62 days renoprival state observation period and was unrelated to the degree of azotemia, hemodialysis, or transfusion.

Patient III (F) in Table I represents a lack of ESF activity during the 316-day renoprival state. The other 3 patients all had significant ESF elevations from 62 to 67 days postbilateral nephrectomy. The allograft functioned well and there was no detectable renal ischemia. His clinical state correlates well with the lack of increased ESF activity in the samples assayed from this period.

Patient IV (S) also showed a rise in ESF activity in her samples after bilateral nephrectomy. Recently, she received a renal allograft and a significant amount of ESF activity was seen in her plasma.

Discussion. These investigations reaffirm our original observation that an extrarenal source for ESF exists in humans (11, 12). It is unlikely that any blood transfusion or preexisting renal insufficiency state could account for these ESF variations. The half-life of ESF in the plasma at best is only a few hours (19), and all of these 4 patients were studied for periods of 62–316 days during the renoprival state.

Extrarenal ESF was also demonstrated recently in our laboratory in baboons (20). Anephric baboons maintained by peritoneal dialysis had substantial release of ESF in response to repeated bleeding episodes (20). The release of ESF in response to hemorrhage indicates that it is similar to renal ESF released in the intact animal under similar conditions. However, there is some apparent species difference for anephric dogs similarly maintained show no detectable ESF in response to hemorrhage (21).

An implication for future investigation is to locate the extrarenal site or sites which produce the ESF. Perhaps the liver may be the extrarenal site or one of the sites for ESF production although there is no definite data at this time. Despite our lack of knowledge of the site or sites of ESF production, one can say that an extrarenal ESF production does occur in man (11, 12, 17), baboons (20), tamarins (10), rats and rabbits (6, 7, 11, 13–15), but not in the dog (21).

To what extent extrarenal ESF contributed to ESF elevations postallotransplantation is indeterminate. Angiotensin II could conceivably play some role since the mechanisms underlying ESF and angiotensin production appear basically similar and both hormones serve to maintain hemodynamic balance in the organism (4). Burgoignie *et al.* (22) feel that there is a clear dissociation between the activities of angiotensin II and ESF even though the same stimuli can apparently induce the production of renin and ESF. However, because 3 of the 4 patients had significant ESF activity in their plasma preparation during the renoprival state, angiotensin would thus appear not to be a critical factor in ESF release. Moreover, Gould *et al.* (23) in their study on the presence of a renin-like activity in ESF preparations concluded that renin, renin substrate, and angiotensin II in physiologic amounts had no measurable erythropoietic activity.

Westerman *et al.* (24) postulated that when erythrocytosis and increased ESF secretions occur in patients with renal transplants, this may be related to a chronic rejection reaction. We have observed in a single patient (Patient I, V.W., Table I) ESF elevations in response to a rejected graft and also in another graft that was simply ischemic. In our studies on simian (25) and baboon (26) renal auto- and allotransplants, we have observed ESF in response to acute ischemic and cellular destruction. Moreover, the variations in ESF activity we observed in these animals indicate, too, the ability of the denervated transplanted kidney to respond to normal ESF stimuli. In concert with other clinical tests, ESF assay could be a prognostic sign as to allograft viability and successful function.

Summary and Conclusions. Erythropoietin (ESF) is a hormone active in the regulation of RBC formation. It was commonly thought

to originate only in the kidney and is believed to be activated or produced in the kidney in response to hypoxia. Three of 4 human renal allograft patients described here demonstrate that significant ESF activity occurs in the renoprival state. This observation in humans is consistent with previous experimental findings that an extrarenal source or sources of ESF do exist, and that humoral regulation of erythropoiesis persists in anephric man and animals. The validity of ESF detection as a prognostic indicator of graft viability and function is discussed.

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1. Erslev, A., Blood **8**, 349 (1953).
2. Gordon, A. S., Physiol. Rev. **1**, 39 (1959).
3. Stohlman, F., New Engl. J. Med. **267**, 342 (1962).
4. Gordon, A. S., Cooper, G. W., and Zanjani, E. D., Seminars Hematol. **4**, 337 (1967).
5. Jacobson, L. O., Goldwasser, E., Fried, W., and Plzak, L., Nature **179**, 633 (1957).
6. Mirand, E. A. and Prentice, T. C., Proc. Soc. Exptl. Biol. Med. **96**, 49 (1957).
7. Mirand, E. A., Prentice, T. C., and Slaunwhite, W. R., Ann. N. Y. Acad. Sci. **77**, 677 (1959).
8. Mirand, E. A., Gordon, A, S., and Wenig, J., Nature **206**, 270 (1965).
9. Mirand, E. A., Weintraub, A. H., Gordon, A. S., Prentice, T. C., and Grace, J. T., Proc. Soc. Exptl. Biol. Med. **118**, 823 (1965).
10. Mirand, E. A., Invest. Urol. **2**, 579 (1965).
11. Mirand, E. A., S. African Med. J. **42**, 462 (1968).
12. Mirand, E. A., Murphy, G. P., Steeves, R. A., Weber, H. W., and Retief, F. P., Acta Haematol. **39**, 359 (1968).
13. Piliero, S. J., Medici, P. T., and Gordon, A. S., Proc. 7th Congr. Intern. Soc. Hematol. **2**, 1 (1958).
14. Gallagher, M. I., McCarthy, J. J., and Lange, R. D., J. Lab. Clin. Med. **57**, 281 (1961).
15. Baciu, I., Oprisiu, C., Dorofteiu, M., Rosenfeld, E., Rusiu, M., and Cherebetiu, C., J. Physiol. (Paris) **55**, 551 (1963).
16. Nathan, D. G., Schupak, E., Stohlman, F., Jr., and Merrill, J. P., J. Clin. Invest. **43**, 2158 (1964).
17. Naets, J. P., and Wittek, M., Lancet **1**, 941 (1968).
18. Lange, R. D., Simmons, M. L., and Dibelius, N. R., Proc. Soc. Exptl. Biol. Med. **122**, 761 (1966).
19. Weintraub, A. H., Gordon, A. S., Becker, E. L., Camiscoli, J. F., and Contrera, J. F., Am. J. Physiol. **207**, 523 (1964).
20. Mirand, E. A., Steeves, R. A., Groenewald, J. H., van Zyl, J. J. W., and Murphy, G. P., Proc. Soc. Exptl. Biol. Med., in press (1968).
21. Mirand, E. A., Murphy, G. P., Bennett, T. E., and Grace, J. T., Life Science **7**, 689 (1968).
22. Bourgoignie, J. J., Gallagher, N. I., Perry, H. M., Jr., Kurz, L., Warnecke, M. A., and Donati, R. M., J. Lab. Clin. Med. **71**, 523 (1968).
23. Gould, A. B., Keighley, G., and Lowy, P. H., Lab. Invest. **18**, 2 (1968).
24. Westerman, M., Jenkins, J. L., Dekker, A., Kreutner, A., Jr., and Fisher, B., Lancet **2**, 755 (1967).
25. Mirand, E. A. Johnston, G. S., Huser, H. J., and Murphy, G. P., Invest. Urol. **2**, 574 (1965).
26. Mirand, E. A., Steeves, R. A., van Zyl, J. J. W., and Murphy, G. P., Proc. Soc. Exptl. Biol. Med. **128**, 785 (1968).

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