The Renal Erythropoietic Factor (REF). IV. Distribution in Mammalian Kidneys.* (32499)

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Our laboratory has demonstrated that a hypotonic extract of the light mitochondrial fraction of kidneys from hypoxic rats contains a factor (renal erythropoietic factor, REF), that generates erythropoietin (ESF) when incubated with normal serum(1,2). We have also shown that the REF exerts no vasopressor action either before or after incubation with normal serum(3). Kinetic studies of the REF-serum interaction strongly suggest that the REF is an enzyme acting upon a protein substrate in serum to form the ESF(4).

The present research was undertaken to determine the location of the REF in the kidneys of 6 mammalian species including the human.

Materials and methods. In all experiments, adult male rats (250-300 g) of the Long-Evans strain were employed as serum donors. Since dialysis of serum against ethylenediamine tetra-acetate (EDTA) eliminates an ESF-inactivating agent from the system, all sera used were previously dialyzed against Na_2-EDTA(3).

Rat kidneys. Fifty female rats (220-250 g) of the Long-Evans strain were exposed to 0.42 atmosphere of air for 16 hours. Immediately thereafter, the rats were exsanguinated and their kidneys removed, decapsulated, and kept on ice, but not frozen. Thirty of the 100 kidneys obtained were processed for extraction of the REF(5,6). The remaining 70 kidneys were first separated into cortical, medullary and cortico-medullary portions, the latter precluding contamination of the cortical and medullary regions with each other. Each of these 3 zones was then subjected to the procedure for REF extraction. REF preparations were also made from similar regions of kidneys from 20 unexposed control rats.

An additional 30 rats were rendered hypoxic and their kidneys prepared for REF extraction. However, in these experiments, the cortical tissue was further separated into glomerular and tubular regions (the latter consisting essentially of renal tubules with some connective tissue and a few disrupted glomeruli), according to the procedure of Nagano and Schafer(7). These tissues were then processed for the REF.

Kidneys of other animal species. In all cases, kidneys from normal adult animals were used. These included 4 rabbits, 2 dogs, 2 sheep and 2 pigs. Their kidneys were separated into cortex, medulla and corticomedullary regions. Procedures for extracting the REF were then applied to these portions. The porcine and ovine kidneys were secured from a slaughterhouse and processed within 2 hours after death of the animals. Kidneys were also obtained from 2 adult humans. One was a 24 year-old woman who died of barbiturate overdosage and the other was a 65 year-old man whose death was ascribed to a cerebral thrombosis. These kidneys were extracted for the REF within 12 hours after death of these subjects.

Incubation techniques. The incubation procedure consisted of adding 6 ml of the REF-containing fluid (derived from 3 g of kidney tissue) to 6 ml of EDTA-dialyzed serum, and incubating for 60 minutes. All incubations were conducted in a water bath shaken at 37°C. Reaction vessels were left open to the air and the reactions were stopped at the end of the incubation period by plunging the flasks into ice water.

Assay methods. The REF-serum incubation mixtures were assayed for erythropoietic activity in hypoxia-induced polycythemic mice (8). Five mice were used to test each sample. Each mouse received a single 2 ml i.p. in-

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jection of the incubation mixture on day 3 post-hypoxia. On day 5, they were injected i.v. with 0.5 uC ^{59}Fe in 0.2 ml saline. Percent RBC-radioiron incorporation values were estimated on day 7. These values were then converted into ESF International units by reference to the dose-response curve for assay mice injected with the International Reference Preparation for ESF(9). The method as now developed(9) permits detection of quantities of ESF as low as 0.02 units. Each experiment was conducted on 3 separate occasions using similar amounts of material and the mouse assays were performed twice. All data have been combined in Fig. 1 and Table I.

Results. Fig. 1 indicates the total quantity of ESF generated in a mixture of 6 ml of EDTA-dialyzed serum and 6 ml of REF-containing fluid extracted from 3 g of various regions of hypoxic rat kidneys. In general, the REF activity was approximately equally distributed throughout the kidney. Although the activity in the cortical tubules appears lower than the glomerular fraction (Fig. 1), the difference was not significant ($P>0.05$). Approximately equal distribution of REF activity was also noted in the renal cortical and medullary tissues of normal unexposed rats (Table I). However, the REF activity of these normal rat renal regions was lower than that derived from similar zones of hypoxic rat kidneys. Table I also shows that extracts of the cortical and medullary regions from normal rabbit, dog, sheep, pig and human kidneys all possessed detectable REF activity.

Discussion. Several reports have appeared implicating both the juxtaglomerular apparatus (JGA) and tubules as sites of ESF

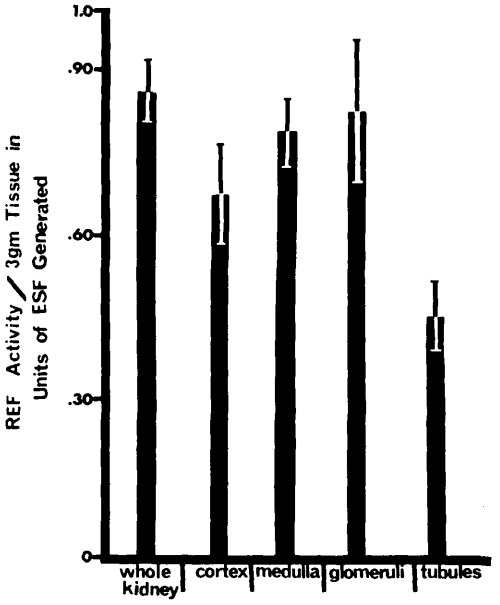

FIG. 1. Mean units of ESF generated by incubating REF extracted from 3 g of various fractions of hypoxic rat kidneys with 6 ml of EDTA-dialyzed rat serum for 60 min. Whole kidneys, cortex and medullary portions were used. In addition, separated cortical glomeruli and tubules were employed. Vertical lines at tops of bars represent ± 1 SEM.

formation in the kidney(10-16). Based on findings that increased granularity of the JG cells accompanied increased erythropoietic activity following hemorrhage(12) or phenylhydrazine treatment(17), the JGA was proposed as the site of ESF production. Supporting evidence was provided by the finding that plethora induced by hypertransfusion, caused a decrease in JG granularity in rats (12). The renal tubules have also been suggested as a site of ESF formation since erythropoiesis was depressed following $HgCl_2$ in-

TABLE I. Distribution of REF in Renal Tissues of 6 Mammalian Species.

Tissue from which REF was extracted	ESF-generating capacity* of the REF (mean units ESF ± SEM) †						
	Hypoxic rat	Normal rat	Normal rabbit	Normal dog	Normal sheep	Normal pig	Human
Whole kidney	.90 ± .04	.32 ± .03	.30 ± .01	.42 ± .02	.54 ± .03	.35 ± .02	.40 ± .04
Cortex	.84 ± .05	.22 ± .02	.25 ± .01	.40 ± .03	.60 ± .08	.36 ± .03	.30 ± .02
Medulla	.86 ± .06	.29 ± .01	.36 ± .01	.38 ± .03	.46 ± .02	.42 ± .05	.32 ± .03
Cortico-medullary junction	.60 ± .04	.30 ± .02	.32 ± .05	.40 ± .03	—	—	.28 ± .03

* In all cases 6 ml EDTA-dialyzed normal rat serum was added to 6 ml of REF-containing fluid.

† Total units of ESF produced in 12 ml of incubation mixture.

jections in rats treated with phenylhydrazine (10), and ESF production was inhibited in cobalt-treated rats receiving mercury-containing drugs(11). Considerable tubular necrosis was observable in these mercury-poisoned rats.

Although these investigators were concerned with determining the site of ESF formation, in the light of our studies(1-6) it is likely they were actually measuring the effects of the various erythropoietic stimuli and depressants on REF production by the kidney. The present studies indicate convincingly that the REF is distributed throughout the kidney, a conclusion based on our finding that approximately equal activity was present in the renal cortex and medulla in the 6 species of mammals examined.

The relative hypoxia that exists normally in the renal tubules(18) may serve as a mild continuous stimulus for the daily production of the REF in the normal animal. It would seem that more severe hypoxia induces augmented production of the REF as a result of greater stimulation of both the cortical and medullary sites of formation. Conversely, in situations resulting in renal hyperoxygenation, it would be anticipated that REF production in both the cortical and medullary regions of the kidney should be depressed. This point is presently under investigation.

Summary. The REF has been demonstrated in the kidneys of rats, rabbits, dogs, sheep, pigs and humans. In these 6 species, approximately equal quantities of the REF were found in the renal cortical and medullary tissues. Exposure of rats to hypoxia induced an increase in both cortical and medullary REF activity.

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Interferon Production in Neonatally Thymectomized Mice.* (32500)

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The host response to certain viruses may be altered by neonatal thymectomy. Evidence has been provided that neonatally thymectomized mice are more susceptible to coxsackievirus B5 infection(1), and in mice inoculated with the LDH agent, increased levels of serum lactate dehydrogenase as well

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