Presence of Erythropoietin in Plasma of Non-Anemic Rats with Renal Adenocarcinomas.* (31181)

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The erythropoietic response to anemia, anoxia, cobalt(1) and androgen(2) is mediated by a humoral erythropoietic stimulating factor, erythropoietin (EPF). Jacobson *et al* (3), later confirmed by others(4-6), suggested that the kidney was a site for the production or activation of EPF. Although there are many experimental and clinical observations implicating the kidney in EPF production or activation, there is a paucity of data demonstrating the ability of animals, particularly during a non-anemic state and with renal tumors, to produce and to activate erythropoietin. Therefore, this communication provides evidence that non-anemic rats bearing renal adenocarcinomas induced by a single low dose of dimethylnitrosamine (DMN)(7,8) display a significant level of plasma erythropoietin.

Materials and methods. One hundred and fifty-six male Wistar rats received one dose (18 mg/kg) of dimethylnitrosamine intramuscularly, intraperitoneally, retroperitoneally or intrarenally at 5 months of age. Aliquot groups were serially sacrificed later at 14, 15, 16 and 17 months of age. This corresponds to 9, 10, 11 or 12 months following the single dose of carcinogen (Table I). Plasmas were collected from individual animals which were not anemic; anemic animals were excluded from these studies. Plasmas were then appropriately pooled for EPF assay in the hypertransfused polycythemic Ha/ICR Swiss mice(2). Any assay animal with a hematocrit below 60% at the end of the assay was excluded.

Kidney and heart weights to the nearest 0.1 g were obtained on all animals. A meticulous gross autopsy was performed. Multiple sections were obtained from any suspicious organs and routinely from both kidneys.

All animals received an I.P. dose of 3.0 μc of ^{203}Hg-chlormerodrin. Twenty-four hours after injection, urine samples were collected and assayed for radioactivity in a well-type scintillation counter. Following sacrifice, aliquots of kidney tissue were similarly assayed. The net 3-minute count obtained from each specimen was expressed as counts/min/ml of urine or counts/min/g of tissue.

Results and conclusions. An overall renal adenocarcinoma incidence of 18.0% was obtained with DMN (Table I). There was no particular enhancement of tumor induction dependent on the route of administration, whether intramuscular, intraperitoneal, retroperitoneal or intrarenal. The intrarenal location of all tumors was essentially cortical except in those instances where the expanding tumor extended directly into the medulla. Early tumors were generally of microscopic size but large masses deforming the kidney were found in the final periods. Pulmonary, liver and other metastases were usually observed in those animals with expanding tumors which caused gross deformity of the kidney.

Variable increases in the heart/kidney weight ratio and kidney ^{203}Hg concentration were particularly observed at all ages in rats which developed tumors and also at 17 months in non-tumor bearing animals which were injected with DMN 12 months previously (Table II). Also, a decreased urinary excretion of ^{203}Hg was observed in animals from both of these groups (Table II).

Only non-anemic rats were used for our EPF studies (Table III). For that reason, only 75% of the tumor bearing rats were utilized. Pooled plasmas from animals with microscopically and grossly proven renal adenocarcinomas showed a significant level of EPF as judged by RBC Fe^{59} uptake of

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1.33% (SD ± 0.19, SE 0.01). The RBC Fe^{59} uptake of pooled plasmas from uninjected controls was 0.31% (SD ± 0.06, SE 0.03). Pooled plasmas from DMN injected but non-tumor bearing animals at 14, 15 or 16 months did not have significant increases of EPF levels. However, pooled plasmas of non-tumor bearing animals at 17 months previously injected with DMN had significant EPF activity, 1.24% (SD ±0.17, SE 0.08).

Discussion. The renal tumors induced by DMN are due to the by-product of an alkylation reaction which is widespread and are not contact-dependent(9). Intrarenal injection of the carcinogen failed to increase the tumor incidence rate. DMN-induced renal tumor bearing rats at 14 to 17 months of age and non-tumor bearing rats at 17 months of age

TABLE I. Renal Adenocarcinoma Incidence in Rats Injected with Dimethylnitrosamine.

Group			No. of animals	Tumor incidence (%) Month 14	15	16	17	Total avg (%)
A) Injected	I	Intramuscular	47	0	25.0	14.3	25.0	19.2
	II	Intraperitoneal	47	20.0	8.3	8.3	11.8	10.6
	III	Retroperitoneal	45	60.0	5.5	21.4	20.0	24.4
	IV	Intrarenal	17	0	25.0	0	43.0	23.5
		Avg total		20.0	18.0	15.7	21.8	19.4
B) Non-injected	V	Control	29	0	0	0	0	0

30 out of 156 injected animals contained renal adenocarcinoma; only 75% of these were non-anemic and were used for EPF studies.

TABLE II. Representative Values of Heart/Kidney Weight Ratio and Kidney and Urine ^{203}Hg Uptakes of Rats Receiving Dimethylnitrosamine (DMN).

Group	Ratio heart/kidney wt (g)	Avg ^{203}Hg uptake Kidney (counts/min/g)	Urine 24 hr volume (counts/min/ml)
A) DMN-induced renal tumor rats at 14, 15, 16, 17 mo	.43	643,754	14,980
B) Non-tumor rats receiving DMN at:			
14 and 15 mo	.35	160,761	52,657
16 mo	.34	135,519	29,011
17 mo	.41	516,946	18,127
C) Control rats, not injected	.26	108,683	23,265

TABLE III. Presence of Erythropoietin in Plasmas of Non-Anemic Rats with Dimethylnitrosamine (DMN) Induced Renal Adenocarcinomas.

Pooled plasmas from	Polycythemic mouse assay No. of assay mice	Avg body wt (g)	Avg ht	Avg blood 24 hr Fe^{59} uptake
A) DMN-induced renal tumor rats at 14, 15, 16, 17 mo	5	33	71.3	1.33 (1.26–1.43) SD ±.19, SE .01
B) Non-tumor rats receiving DMN at:				
14, 15 mo	3	33	65.0	.39 (.26–.64) SD ±.22, SE .13
16 mo	5	34	67.3	.31 (.22–.39) SD ±.09, SE .05
17 mo	7	33	69.6	1.24 (1.17–1.31) SD ±.17, SE .08
C) Control rats at 14, 15, 16, 17 mo	4	34	60.0	.31 (.24–.37) SD ±.06, SE .03

injected with DMN previously, which showed significant levels of plasma EPF, also demonstrated increases in the heart/kidney weight ratio and in radioactive ^{203}Hg concentration in the kidney (Table II). This correlation may not be directly related to renal tumor formation but may possibly reflect a state of hypertension following DMN treatment. Cardiac hypertrophy(10) and decreased urinary isotope excretion(11) may indicate a renal hypertensive state. The presence of a significant degree of renal ischemia and hypertension might also explain the presence of increases in plasma EPF in a non-anemic state (Table III). With the DMN-induced renal tumor model, one might be able to study the critical intrarenal factors that may bring about EPF production or activation by the kidney in the non-anemic and anemic states. Because samples from all tumor animals were pooled and included both microscopic and gross renal cortical tumors, critical separation of a possible EPF stimulus by the expanding tumor lesions with resultant intrarenal hypoxia *per se* cannot be determined from these studies.

Nephrogenic erythrocytosis in humans, possibly caused by increased production of EPF, has been reported in association with several types of renal lesions(12,13), and EPF has been demonstrated in the plasma(12,14), in the cyst wall and fluid(15), and in tumor tissue(16,17) in some of these cases. Although these experimental and clinical findings further support the theory that the kidney is a site of EPF production or activation (4,5), it is important to know what the specific locus and type of effect(s) is necessary to induce the kidney to form and release erythropoietin. To some degree, this can be done in human subjects; however, precise methods will have to be carried out in animals with various renal abnormalities of the type which are reported herein. It is also appreciated that anephric humans(18) can respond to hypoxia with EPF release. These and other studies lend support to previous suggestions (4,5) that more than one source of EPF or different varieties may exsit. Renal tumor EPF may represent a variant.

Summary. Non-anemic and hypertensive rats bearing renal adenocarcinomas induced by a single low dose of dimethylnitrosamine had a significant level of plasma erythropoietin (EPF) at 14-17 months of age as compared to non-injected controls. Carcinogen-injected, non-tumor bearing rats without hypertension had undetectable levels of EPF at 14, 15 and 16 months but had increased levels at 17 months of age. The detection of EPF in these animals might be accounted for by the presence of a significant degree of renal ischemia and hypertension.

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