TABLE II. LD_{50} and Potency of M.A.O. Inhibitors in Protecting against *E. coli* Endotoxin.

	P.I.H.	Saline control	Nialamide	Saline control	S.K.F. 556	Saline control
LD_{50} (mg/kg)	15.85	9.12	17.38	10.72	17.38	10.72
Potency ratio	1.74 (1.74>1.00 at P ≤.01)		1.62 (1.62>1.00 at P ≤.05)		1.62 (1.62>1.00 at P ≤.01)	

P.I.H. and Nialamide both contain the hydrazine moiety and inhibit diamine oxidase (D.A.O.) as well as M.A.O., whereas S.K.F. 556 does not contain the hydrazine moiety and inhibits only M.A.O.(7). These data showed that when both M.A.O. and D.A.O. were inhibited, the effect was not one of simple potency reduction of the endotoxin effect, but also one of greater variation in tolerance to endotoxin, best shown in the wide dose range for Nialamide in which the mortality was between 0 and 100% (Table I).

Although a different mechanism of protection may be operative for animals given drugs which inhibit both M.A.O. and D.A.O., inhibition of M.A.O. alone is complex, affecting serotonin, tryptamine, dopamine, and catechol amine metabolism. Although inhibition of endogenous serotonin destruction alone may diminish the lethality of endotoxin, any protection afforded by serotonin may be mediated through adrenal corticoids, for serotonin administration has been shown to increase ACTH output(8) and adrenal corticoids are known to protect against endotoxin (9).

Summary. M.A.O. inhibitors P.I.H., Nialamide and S.K.F. 556 were shown to have a small but significant effect in reducing mortality to intraperitoneal injection of *E. coli* endotoxin. Inhibition of M.A.O. alone appeared to have an effect distinct from inhibition of both M.A.O. and D.A.O. though the 3 drugs were not of significantly different potency.

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Some Aspects of the Relationship Between Plasma and Urine Erythropoietin.* (27223)

T. C. PRENTICE AND E. A. MIRAND
Roswell Park Memorial Institute, Buffalo, N. Y.

Anemia and hypoxia will induce elevated levels of erythropoietin (EPF) in plasma and/or urine(1,2). However, the relationship of EPF in plasma to that in urine is unclear. Systematic assays of EPF in plasma and urine taken during the same time interval from the same animals have not been reported. The present study deals with this aspect of the EPF problem, and reports data as to time of appearance and amounts of EPF present in plasma and urine after varying stimuli.

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Methods and materials. The experimental

animals were New Zealand White rabbits. Three types of stimulation were used: (1) Rabbits were bled serially from the heart to predetermined hematocrits which varied from 25 to 10%. (2) 2.5% phenylhydrazine (1 cc daily, subcutaneously) was given until the desired hematocrit was reached. Again a range of 25 to 10% was selected. (3) Rabbits were kept at 10 vol % O_2 concentration for varying periods of time by methods previously described(3,4,5).

Plasma and urinary collections were matched in the following way: Where anemia was induced by bleeding or phenylhydrazine, daily hematocrits were measured until the desired level was reached. At this time the 24-hour urine collection was started. At the end of the 24-hour period the rabbit was bled and the plasma reserved for comparison with the urine. This procedure was repeated at each hematocrit level. At no time did any hematocrit change by more than ± 3% during the 24-hour period of urine collection. In the hypoxic studies the first urine collection was started at time 0 and continued through 24 hours. This 0-24-hour urine was compared with plasma collected at the 24-hour interval. Likewise the 24-48-hour urine collection was compared with the 48-hour plasma sample. This system of collection was continued at 24-hour intervals throughout the 120-hour hypoxic period. Since concentration was considered more important than volume, plasma and urine collected from each individual rabbit was not measured but added to pools derived from the other animals in the particular experimental group.† EPF assay was then performed using aliquots of this pooled material from several animals.

Plasma and urine were assayed using the polycythemic mouse(6,7). Some assay groups received whole plasma and urine which had undergone no antecedent preparation. Others received plasma deproteinized by the method of Borsook(8) and concentrated to ⅓ its original volume. For comparison, a triply concentrated extract of urine was made using the method of Hodgson *et al.* (9).

Results. The rabbits with anemia induced by bleeding showed considerably lower levels of EPF in both plasma and urine than the phenylhydrazine anemic animals, both groups being compared at the same hematocrit levels. The bled anemic animals had no elevation of EPF in either plasma or urine until a hematocrit level of 15% was reached. At this point increased titers of EPF were present in both plasma and urine, the urine being only 1/6 as active as the plasma, however. At a hematocrit level of 10% the plasma retained increased EPF activity, though less than at the 15% hematocrit level, and level of EPF in the urine had dropped back to control levels. The phenylhydrazine anemic animals had increased titers of EPF in both plasma and urine at all hematocrit levels. In all instances urine was less active than plasma with an average of ⅓ to ½ the stimulating potency (Tables I and II).

The hypoxic rabbits evidenced a remarkably parallel response in both the levels and the times of appearance and disappearance of EPF from plasma and urine. These results are summarized in Table III.

Discussion. Whether the EPF of plasma and urine is one substance is not known at the present time. Urinary EPF may represent partial excretion of plasma content. Or, if the kidney is truly an important source of this material, it could conceivably appear in urine directly, independent of any plasma source. The increasing use being made of urinary extracts of high potency for physiologic, chemical and clinical studies(10) makes it of further interest and importance to establish the nature of urinary EPF and its relationship to plasma EPF.

Previous studies in our laboratory(11) have shown differences in heat and pH stability as well as response to proteolytic enzymes between the active material in urine and plasma. However, these might be explainable on the basis of the discrepancy in protein concentration of plasma and urine. Extension of these studies with normalization of protein content is now under way.

The present data do not suggest a separate

† Urine collected simultaneously from 3 rabbits in the same metabolic cage, although variable, averaged approximately 200cc over a 24-hour period.

TABLE I. Erythropoietin Titer, Determined by 24 Hour Fe^{59} Uptake, of Polycythemic Mice That Received Plasma and Urine from Rabbits Bled to Hematocrit of 25 Down to 10%.

Plasma and/or urine from rabbits bled to hematocrit	No. of rabbits	Plasma, whole, 0.5 cc/day × 2		Urine, raw, 0.5 cc/day × 2	
		24 hr Fe^{59} uptake, %	Hematocrit, %	24 hr Fe^{59} uptake, %	Hematocrit, %
25	5	.2 ± .2 * (5)†	72.2	.3 ± .4 (5)	70.8
20	5	.3 ± .2 (5)	67.8	.3 ± .2 (5)	71.4
15	5	7.5 ± 1.8 (5)	71.6	1.2 ± .9 (4)	70.0
10	5	3.2 ± 1.0 (5)	71.8	.2 ± .0 (4)	75.3
Normal control (38)	4	.2 ± .06 (5)	68.8	.2 ± .05 (5)	68.5

* Stand. dev. † No. of polycythemic mice used in assay for erythropoietin.

TABLE II. Erythropoietin Titer, Determined by 24 Hour Fe^{59} Uptake, of Polycythemic Mice That Received Plasma and Urine from Rabbits Injected with Phenylhydrazine to Hematocrit of 25 Down to 10%.

Plasma and/or urine from rabbits inj. with phenylhydrazine to hematocrit	No. of rabbits	Plasma, whole, 0.5 cc/day × 2		Urine, raw, 0.5 cc/day × 2	
		24 hr Fe^{59} uptake, %	Hematocrit, %	24 hr Fe^{59} uptake, %	Hematocrit, %
25	4	3.5 ± 1.1 * (5)†	72.4	.7 ± .3 (4)	72.3
20	4	6.6 ± 2.7 (4)	69.3	2.0 ± .6 (5)	70.6
15	4	4.6 ± 3.3 (5)	73.2	3.1 ± 2.8 (4)	64.3
10	4	7.1 ± 3.4 (5)	67.0	2.0 ± .3 (5)	69.8
Normal control (37)	4	.2 ± .05 (5)	68.6	.2 ± .06 (5)	68.3

* Stand. dev. † No. of polycythemic mice used in assay for erythropoietin.

TABLE III. Erythropoietin Titer, Determined by 24 Hour Fe^{59} Uptake, of Polycythemic Mice That Received Plasma and Urine from Rabbits Exposed to 10% O_2 for 0 to 120 Hours.

Plasma and/or urine from hypoxic rabbits at hr	No. of rabbits	Plasma, modified Borsook extract, 0.5 cc/day × 4 (= 6 cc whole)		Urine, Hodgson alcohol extract, 0.5 cc/day × 4 (= 6 cc raw)	
		24 hr Fe^{59} uptake, %	Hematocrit, %	24 hr Fe^{59} uptake, %	Hematocrit, %
0	6	.2 ± .08* (8) †	67.6	.2 ± .1 (11)	66.9
24	6	4.6 ± 1.6 (10)	63.7	5.4 ± 1.5 (14)	60.7
48	6	5.2 ± 2.8 (9)	67.3	3.9 ± 1.2 (13)	63.5
72	6	.7 ± .4 (10)	67.7	.3 ± .2 (10)	66.2
96	6	2.3 ± .6 (8)	64.8	1.1 ± .4 (7)	71.4
120	6	.2 ± .1 (6)	68.7	.2 ± .1 (6)	64.7

Data represents avg of several assays, all of which showed the same pattern of response.
* Stand. dev. † No. of polycythemic mice used in assay for erythropoietin.

identity of plasma and urinary EPF. Rather the fact that they appear and disappear simultaneously and at similar levels under hypoxic conditions points to their similarity rather than their difference. However, these parallels do not establish that the two are the same chemically, or that the EPF activity in urine is simply an excretion product of EPF activity in plasma. Nevertheless these experiments demonstrate that EPF does not appear independently in the urine under the experimental conditions used. EPF was never demonstrable in the urine unless comparable or, usually, higher levels were present in the plasma.

Others have noted previously the tendency for EPF levels to be higher in phenylhydrazine induced anemia than in the anemia of bleeding. Jacobsen *et al.*(12) attributed this to liver damage and methemoglobin formation. Previous data from our laboratory (3,4) likewise suggested that the liver might play

a part in the inactivation of EPF. The present studies illustrate again that both plasma and urine EPF levels commonly reach higher levels in phenylhydrazine anemia than at comparable degrees of anemia due to bleeding. Why levels of urinary EPF, as compared with corresponding plasma levels, are lowest in bled anemic, intermediate in phenylhydrazine anemic, and highest in hypoxic animals is not immediately apparent. Further chemical characterization of both plasma and urinary EPF is needed to establish whether or not their structure is the same.

Summary and conclusions. Simultaneous assays of plasma and urinary EPF were done in animals with varying degrees of anemia induced by bleeding or phenylhydrazine. Likewise comparisons of plasma and urine, collected after similar intervals of hypoxia from the same rabbits, were carried out. Diverse results were seen in the rabbits with induced anemia. a) Bled anemic rabbits showed distinct EPF elevations in plasma at hematocrit levels of 15 and 10% Only minimal corresponding elevation appeared in the urine, occurring only at the 15% hematocrit level. b) Phenylhydrazine anemic rabbits showed marked elevation of plasma EPF at all hematocrit levels from 25 to 10%. Urinary EPF was also present, although in lesser amounts, at these same hematocrit levels. At no time, in either the bled or phenylhydrazine anemic groups, were EPF levels as high in urine as in plasma.

The time of appearance and potency of EPF in plasma and urine of hypoxic rabbits were strikingly similar. Highest levels were present in both plasma and urine at 24 and 48 hours. Return to control levels was reached at 120 hours in both plasma and urine. At no time were EPF levels higher in urine than in plasma. In our opinion the above results suggest more the similarity than the separate identity of plasma and urinary EPF. They are compatible with the concept that urinary EPF represents partial excretion of plasma content.

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Loss of Neurotropic Pathogenicity and Hemagglutinating Property of Columbia SK Virus by *in vitro* Cultivation in Sarcoma 180 Ascites Cells.* (27224)

EIICHI FURUSAWA AND WINDSOR CUTTING
Dept. of Medical Microbiology, Stanford University, Calif.

We have recently reported that Columbia SK (Col SK) virus adapted to Ehrlich ascites tumor cells *in vivo*(1,2) could also be adapted to Sarcoma 180 (S180) ascites cells both *in vivo* and *in vitro*(3). After 30 passages of the virus in S180 ascites cells *in vi-*

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