response to stressors would be markedly influenced by this factor. This point is of special significance in studies of enzyme induction by stressful stimuli.

Any influence of housing conditions on the plasma corticosterone elevation produced by histamine or saline administration can be prevented by conditioning to intravenous saline administration.

In general, a small variation was noted in resting levels of plasma corticosterone in the male rat over a 12-month period, while the variation in adrenal ascorbic acid levels was much greater. This observation leads us to question the reliability of adrenal ascorbic acid depletion as an indicator of adrenocortical response to stressors. In other experiments(1) we have noted further discrepancies in the relationship of adrenal ascorbic acid depletion and plasma corticosterone elevation in response to stressors. This relationship has been questioned by other authors(7,8).

It therefore appears that great care must be taken in all studies of adrenocortical response to stressors to ensure: (a) that basal or resting levels of plasma corticosterone are relatively constant in the population of experimental animals employed, and (b) that animals employed as "controls" be refractive to handling and drug administration.

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Potentiation of Purified Erythropoietin with Serum Proteins. II: Serial Dose Response Relationships.* (31560)

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The relationship of plasma proteins to erythropoietic activity has been the subject of much interest(1,2). We have reported the enhancement of erythropoietically active urinary fraction II + III(3) by normal human, rabbit and mouse sera and non-neutralizing antisera and suggested that the enhancing protein either a) provides a protective carrier, b) neutralizes an inhibitor of erythropoietin or c) activates an erythropoietin precursor (4). Recently Garcia and Schooley have shown enhancement of erythropoietin by normal serum when the material was injected subcutaneously into the test animals. However, this did not exceed the activity of a comparable amount of saline suspended erythropoietin given in divided doses subcutaneously(5). They suggested that the apparent enhancement of activity by the normal serum may actually be merely a reflection of slower and more prolonged absorption from the subcutaneous tissues when serum is added. The following experiments were carried out to evaluate this suggestion.

Materials and methods. The erythropoietically active human urinary concentrate (fraction II + III) was prepared by DEAE cellulose column chromatography(3). One milligram of this material is approximately equal to 0.2 unit of Erythropoietin Standard B (Medical Research Council, National Insti-

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tute for Medical Research, London). Erythropoietin was assayed in the hypertransfused polycythemic CD-1 female mouse, using the 48-hour incorporation of Fe^{59} into the red blood cells of the test animal as an indicator of erythropoiesis(6). The routine procedure in our laboratory is to give one-half of the total dose of erythropoietin (mixed in saline) intraperitoneally on day 6 and again on day 7 following transfusion. For the purposes of this study, one milligram of fraction II + III was mixed in either saline or normal human serum and was given intraperitoneally or subcutaneously either as a single dose on day 6 or as fractional doses (either 2 or 4 doses) on days 6 and 7. When 2 doses were given, one was given on day 6 and one on day 7; when 4 doses were given, they were given as 2 doses 5 hours apart on day 6, and another 2 doses 5 hours apart on day 7.

Results. The results of 2 separate experiments, which were virtually identical, were combined and are shown in Table I and Fig. 1 and 2. These data show that erythropoietin is more active in serum than in saline in all dosage schedules; these figures are highly significant statistically (for 1 and 2 doses subcutaneously, $p = .01$; for all others, $p = .005$). Also, activity was enhanced by giving fractional doses in all except the intraperitoneal route using saline diluted erythropoietin; however, the increase in activity when the saline diluted material was given fractionally subcutaneously was not of the magnitude that has been reported by others(5), and was not statistically significant. Fractional doses of saline and normal human serum without erythropoietin were also assayed and had no erythropoietic activity.

TABLE I. % Fe^{59} Incorporation in Red Cells of Polycythemic Mice.

Fraction II + III in saline		Fraction II + III in serum	
a) Intraperitoneal			
1 dose	3.1	1 dose	5.4
2 doses	2.6	2 doses	10.6
4 "	1.7	4 "	11.9
b) Subcutaneous			
1 dose	3.5	1 dose	10.1
2 doses	4.7	2 doses	13.4
4 "	6.4	4 "	21.7

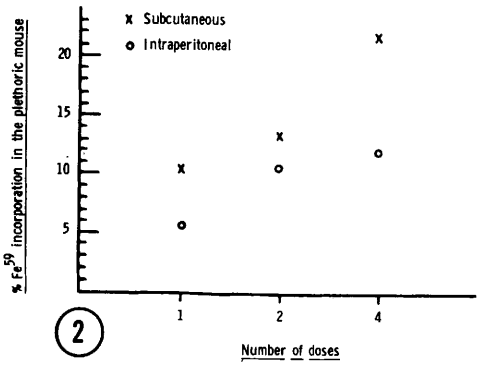

FIG. 1. Dose response relationship to fractionalization of the test material: Erythropoietin in saline.

FIG. 2. Dose response relationship to fractionalization of the test material: Erythropoietin in serum.

Discussion. The apparent association of erythropoietic activity with plasma globulins has long been appreciated(7,8,9). However, only recently has some clarification of this relationship come about. Kuratowska *et al* (1) have reported a factor, present in perfusates from isolated hypoxic rabbit kidney, which is erythropoietically inactive, but which acquires activity when incubated with the globulin fraction of serum. Contrera *et al*(2) have described 2 erythropoietically active renal fractions obtained by DEAE chromatography, one of which (the particulate fraction) exhibits activity only after incubation with normal rat serum. Our recent studies on potentiation of erythropoietin have shown enhancement of activity by a highly purified α-1 acidic-glycoprotein (orosomucoid) as well as by normal mouse, rabbit and human sera (4). Garcia and Schooley found that a single dose of erythropoietin in serum was more

active than a single dose of erythropoietin in saline; however, when the latter was given in divided doses, the activity approached that of the single dose of the serum diluted material. However, erythropoietin in serum was not given in divided doses as a control. In the present study, fractionation of the erythropoietin in serum showed even greater enhancement of activity, with the activity of the serum diluted material being greater than the saline diluted material in any given dosage schedule. While these studies in no way eliminate an adjuvant effect of serum merely causing delayed absorption, they do seem to indicate a definite potentiating effect of serum which is consistent with the work cited above.

Summary. Erythropoietin (urinary fraction II + III) was diluted with either saline or normal serum and injected into assay animals as a single dose or as 2 or 4 fractional doses either intraperitoneally or subcutaneously. Erythropoietic activity increased as the dose was fractionated; however, the erythropoietin plus serum showed greater activity than erythropoietin plus saline in all dosage schedules. This suggests that normal serum actually enhances the activity of erythropoietin rather than merely acting as an adjuvant causing delayed absorption of the material.

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Absence of Transferable Endogenous Substances Altering Vascular Reactivity in Endotoxin Shock.* (31561)

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Late phase of endotoxin shock in the rabbit is consistently accompanied by hyperreactivity of pulmonary vasoconstrictor mechanisms to acetylcholine (Ach) (1,2,3). A latent period of 10 to 70 minutes is found between injection of endotoxin and onset of hyperreactivity to Ach(1). It is conceivable that substances produced by tissue, either from endotoxin or in response to endotoxin, are responsible for the alteration in pulmonary vascular response to Ach, rather than endotoxin *per se*. The latent period would then correspond with the slow formation of such products. In such a case, one might expect that blood incubated with endotoxin *in vitro* or *in vivo* would contain substances capable of inducing hyperreactivity to Ach in a recipient rabbit after a significantly shorter latent period than that observed after injection of

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