tiveness by the larger doses of phenylalanine is consistent with the *in vitro* studies of Woolley(2). Large doses of L-phenylalanine are known to directly inhibit antibody response, so this may be another factor in the diminished host response to infection(12).

The relevance of these findings to infections with other organisms or to human infections remains to be established. In pervious studies, phenylalanine has been given to patients only after 3 to 8 days of effective chloramphenicol therapy(1). The results presented, however, suggest that further investigation of the combined use of L-phenylalanine and chloramphenicol in clinical situations would be both worthwhile and appropriate.

Summary. Parenteral administration of phenylalanine in a dose equivalent to that of chloramphenicol enhanced the antibacterial effect of chloramphenicol in mice infected with *Klebsiella pneumoniae*. Larger doses of phenylalanine, however, reduced the drug's antibacterial efficacy. The interaction of phenylalanine and chloramphenicol is discussed in relation to bacterial and mammalian cell protein synthesis.

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Dose-Response Relationships for Erythropoietin Given in Serum or in Saline.* (30605)

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The stimulation of radioiron incorporation into the red cells of the polycythemic mouse has become accepted as the most sensitive method currently available for assay of erythropoietin. The polycythemic state has been induced in the assay mice either by transfusion of isologous red blood cells or by exposure of mice to an hypoxic environment. We have noted, from time to time, that the injection of serum or plasma of anemic or hypoxic animals into such polycythemic mice often gave assay values which were remarkably high when compared to a dose-response curve obtained when erythropoietin was injected in saline. With this in mind, we decided to compare the dose-response relationships, using serum as well as saline as diluent for erythropoietin.

Methods. Mice with transfusion-induced polycythemia were used as the erythropoietin assay animals in a procedure essentially the same as that of DeGowin *et al*(1). Groups of 10 female Swiss mice, weighing 20-25 g, were given 1 ml of washed red blood cells (hematocrit approximately 80%) intraperitoneally on 2 successive days. Various doses of erythropoietin were given in single or fractionated amounts so as to establish 4 different dose-response curves. The erythropoie-

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TABLE I. Dose-Response Relationships of Sheep Plasma Erythropoietin Given in Normal Rat Serum and in Fractionated Doses in Saline.

Erythropoietin total dose	Serum	Saline		
	1 injection	1 injection	2 injections	4 injections
.1875 cobalt unit	10.7 ± .9*(10)†	5.7 ± .3 (8)	10.3 ± .7 (8)	9.9 ± .7 (9)
.375 " "	20.0 ± 1.0 (10)	9.5 ± .9 (8)	13.4 ± 1.1 (8)	19.0 ± 1.1 (10)
.75 " "	28.6 ± 1.5 (10)	13.6 ± 1.4 (8)	20.6 ± 1.4 (9)	31.6 ± 1.9 (9)
1.5 " units	35.2 ± 1.6 (10)	18.2 ± 1.9 (8)	31.3 ± 2.4 (8)	35.2 ± 1.4 (9)
3.0 " "	38.0 ± 1.3 (10)	28.0 ± 2.7 (6)	31.7 ± 2.3 (10)	44.6 ± 1.6 (8)

Fe^{59} incorporation into red cells of transfusion-induced polycythemic mice assuming a blood volume of 7% body wt.

* S.E. of mean. † No. of assay animals.

tin dissolved in saline was given in a single injection on the 5th day, divided into 2 injections separated by 6 hours on the 5th day, or divided into 4 injections and given on the 5th and 6th days following the last transfusion. Another dose-response curve was obtained, using the same doses of erythropoietin dissolved in normal rat serum and given in a single injection on the 5th day following the last transfusion. Each assay animal received a total volume of one milliliter of erythropoietin solution, and in all cases this was given subcutaneously. The erythropoietin used was an extract from phenylhydrazine anemic sheep plasma.†

On the 7th day following the last transfusion, 0.5 μC of Fe^{59} as iron citrate was given intraperitoneally. The mice were bled by cardiac puncture 3 days later, and the radioiron in 0.5 ml of blood was determined. Microhematocrits were also done on this blood. Values from any animal losing weight or whose hematocrit was less than 55% of the time of bleeding were discarded. The results are expressed as per cent of the injected amount of Fe^{59} in the calculated total blood volume. The blood volume of the hypertransfused mice was assumed to be 7% of total body weight.

Results are presented in Table I. Comparison of the results obtained with a particular dose of erythropoietin given in a single subcutaneous injection in serum shows a much greater stimulation of Fe^{59} incorporation into red cells of polycythemic mice than the same dose of erythropoietin given as a single injection in saline. This was true throughout the entire dose range studied, the difference for each dose being highly significant with a p value of less than 0.001. However, if a particular dose of erythropoietin in saline is divided into 2 or 4 fractions and injected over 1 to 2 days, the response observed is increased over that seen when the same dose is given in a single injection in saline. In fact, the response seen when erythropoietin is injected in 4 fractions in saline is very similar to that seen when the erythropoietin is given in a single injection in serum.

Discussion. One possible explanation for the greater response seen when erythropoietin is given subcutaneously in serum is that the serum acts as an adjuvant and slows down the absorption of erythropoietin from the injection site. Thus, a particular dose of erythropoietin, when injected in serum, acts for a longer time in the assay mouse than when injected in saline, and during this time increases occur in the number of erythropoietin sensitive cells in the marrow. Some recent experiments certainly suggest that increased numbers of erythropoietin sensitive cells occur in the polycythemic mouse following the initial exposure to erythropoietin(2). Such an explanation is consistent with the increased erythropoietic responses seen when erythropoietin is given subcutaneously in fractionated doses in saline. A slower absorption of erythropoietin from the injection site, due to the presence of serum proteins or the fractionation of the injected dose of erythropoietin in saline, presumably more closely approximates the ideal of a continuous exposure of erythropoietin sensitive cells to erythropoietin. Previous work in which sheep plasma erythropoie-

† A1-0336, No. K103194A kindly supplied by Hematology Study Section, NIH.

tin was injected intravenously resulted in erythropoietic responses for given doses of erythropoietin much reduced from any of the responses to subcutaneous erythropoietin observed here(2).

In contrast to our findings, Weintraub *et al* (3) have observed "no change or only a slight insignificant rise in the potency" when an extract of sheep plasma erythropoietin was injected in combination with dog plasma as compared to saline; however, their studies were different from ours in that the route of administration was intraperitoneal rather than subcutaneous.

Another possible explanation for the observed results may reside in the suggestion of Goldwasser *et al*(4) that erythropoietin may exist in plasma as a complex with some binding protein, and that the unbound form is unstable in contrast to the relatively stable bound form. Recently Kuratowska *et al*(5) have found a renal factor in the perfusate of hypoxic rabbit kidneys which is erythropoietically inactive when injected subcutaneously into fasted rats, unless it is preincubated with plasma alpha globulins. They suggest that the alpha globulins may protect the active structure of the renal factor against destruction in subcutaneous tissue, or that they may serve as a carrier enabling the active substance to penetrate into the blood. These experiments, which suggest a role for carrier protein, may be of importance in explaining our experimental observations; however, the relative amounts of bound and unbound erythropoietin in our sheep erythropoietin preparations are unknown.

Mirand *et al*(6) recently observed significant increases in erythropoietic activity when whole human plasma was injected subcutaneously into polycythemic mice, but similar injections of plasma extracted by a slight modification of the Borsook procedure gave very few significant increases in erythropoietic activity. Possibly the addition of these plasma extracts to erythropoietically inactive plasma proteins or alpha globulins might have given results more similar to the unextracted whole plasma.

Regardless of the explanation of the observed results, we feel that the present observations are important, since the erythropoietin activity of various body fluids is often compared to dose-response curves obtained with saline as the vehicle. It is perhaps more reasonable in estimating the erythropoietic activity of biological fluids to make such comparisons on the basis of dose-response curves obtained with erythropoietin in a diluent as close as possible to the assayed biological fluid. Finally, we would suggest that the effect observed here may be, in part, responsible for the apparent loss during the extraction of erythropoietin from plasma when extracted erythropoietin is compared for its biological activity to the original plasma source.

Summary. Dose-response curves for sheep erythropoietin given subcutaneously to polycythemic mice are presented comparing saline to serum as a vehicle. The response observed in radioiron incorporation into red cells is greater for a particular dose of erythropoietin when that dose is given in serum. However, if erythropoietin dissolved in saline is given in fractionated doses over a period of time, the response of a given dose of erythropoietin will increase to that observed with the same dose given in serum. It is suggested that the plasma protein may slow down the absorption of erythropoietin from the subcutaneous site, resulting in a more continuous exposure of erythropoietin sensitive cells to erythropoietin.

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