Summary. A new antiviral agent with activity both *in vivo* and *in vitro* has been obtained from lambda infected *E. coli.*

A study of the antiviral activity of the lysate in chick embryo monolayers shows that this material is effective in completely suppressing both vaccinia and herpes simplex when added before and as long as 6 hours after infection, and the activity is retained by chick embryo monolayers that have been preincubated with the lysate and were washed thoroughly prior to infection.

The lysate does not block absorption of the virus, but rather inhibits intracellular replication. The activity of the lysate is not due to the infective lambda particle *per se* but to a soluble protein fraction.

In vivo, this material has significant therapeutic activity in the treatment of rabbit corneal ulcers.

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Chloramphenicol and L-Phenylalanine Therapy in Experimental *Klebsiella pneumoniae* Infection in Mice.* (30604)

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Ingestion of L-phenylalanine has been shown to reverse one of the early toxic effects of chloramphenicol on human bone marrow, namely, the cytoplasmic vacuolization of erythrygones(1). The relationship of these vacuoles to the aplastic anemia associated with chloramphenicol treatment is not clear. Before investigating the possibility that prophylactic administration of phenylalanine might prevent the later more serious toxic effects of chloramphenicol, it was necessary to evaluate the effect of administration of the amino acid upon the antibacterial efficacy of chloramphenicol.

The need for the study was also suggested by the possibility that the cytotoxic effect of chloramphenicol in clinical situations and the antibiotic activity of the drug might involve a common mechanism. In addition, Woolley(2) reported that phenylalanine inhibits the antibacterial action of chloramphenicol *in vitro.*

Materials and methods. The following materials in varying combinations were injected into Swiss White male mice (25 $\pm$ 2 g initial weight) in each test. A strain of *Klebsiella pneumoniae* isolated from the blood culture of a patient was used throughout. The organism was maintained and diluted in brain-

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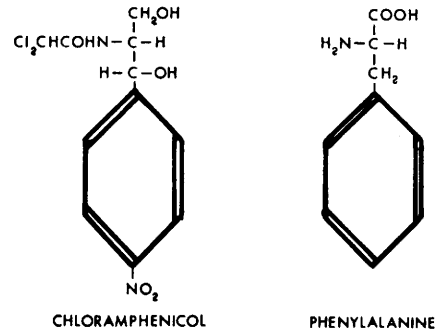

FIG. 1

heart infusion broth. Mice were infected intraperitoneally with 0.5 ml of an overnight culture diluted 10^{-2} in all but 2 experiments which used 10^{-4} dilutions. An overnight culture resulted in approximately 10^{10} organisms/ml with an LD_{50} of $10^{-3.6}$. The minimum inhibiting concentration of chloramphenicol for the infecting strain was 6.2 μg/ml.

L-phenylalanine was injected subcutaneously in a volume of 0.4 ml suspended in 0.89% sodium chloride acidified to pH 4 with 1 N HCl. Acidified saline, 0.4 ml, was used as a control solution.

Chloramphenicol succinate was given subcutaneously in a volume of 0.2 ml at a site distant to that employed for the phenylalanine.

Mice under light ether anesthesia were given the intraperitoneal injection of organisms followed in rapid sequence by the subcutaneous chloramphenicol and phenylalanine or control solution in all but one test. In the latter, the phenylalanine or control solution was injected 4 hours before the other injections. Cages were inspected twice daily and numbers of dead mice recorded. The animals were observed for 10 days, but the final mortality was reached in all experiments by 72 hours, most deaths occurring in the first 36 hours. In 2 experiments, the dose of chloramphenicol was varied between 10 and 70 mg/kg; in all others, the dose was 25 to 40 mg/kg.

Results. All the infected animals that did not receive chloramphenicol died whether or not they received phenylalanine in doses from 50 to 400 mg/kg. All non-infected animals given phenylalanine or saline with or without chloramphenicol survived.

Table I presents data from these experiments. The results include those of the experiments with pre-loading of phenylalanine as there was no difference in host response between this group and all the others where the animals were given phenylalanine simultaneously with the infecting organisms and chloramphenicol. The results are grouped in the Table both by dose of chloramphenicol and by ratio of phenylalanine to chloramphenicol dosage. The ratio is given both as

TABLE I. Mouse Mortality from *Klebsiella pneumoniae* Infection Treated with Chloramphenicol and L-Phenylalanine.

Phenylalanine/Chloramphenicol		Dose chloramphenicol,	No.		% Mortality	Significance of difference from comparable controls	
mg/mg	mmol/mmol	mg/kg	animals	No. dead		χ^2	p
0	0	10–20	20	16	80		
0	0	25–40	159 | 179	71 | 87	45 | 49		
0	0	50–70	15	1	7		
		Total	194	88	45		
1.25–2.5	.6–1.3	10–20	15	9	60		
1.25–2.5	.6–1.3	25–40	155 | 170	51 | 60	33 | 35	4.6 | 6.3	<.02 | <.02
1.25–2.5	.6–1.3	50–70	15	2	13		
		Total	185	62	33	5.6	<.02
4.0–10.0	2.0–5.1	10–20	20	16	80		
4.0–10.0	2.0–5.1	25–40	176	98	56	4.1	<.05
		Total	196	114	58	3.4	>.05
16.0–40.0	8.2–20.4	10–20	20	16	80		
16.0–40.0	8.2–20.4	25–40	30	26	87		
		Total	50	42	84	20.0	<.01

mg/mg and mmol/mmol. The significance of the differences was calculated by the 4-fold table, and the values of both χ^2 and p are given.

It may be seen that addition of phenylalanine in doses approximately equimolar to the doses of chloramphenicol (0.6-1.3 mmol/mmol) reduced mortality significantly from that of the animals which received the same amount of chloramphenicol without supplemental phenylalanine.

Doses of phenylalanine which on a molar basis were 2 to 5 times those of chloramphenicol had no significant effect on overall mortality. When only those animals which received 25 to 40 mg/kg of chloramphenicol are considered, however, these doses of phenylalanine had an adverse effect which was statistically significant ($p<.05$).

Larger amounts of phenylalanine (molar ratio to chloramphenicol of 8-20/1) markedly increased mortality.

Thus, equimolar doses of phenylalanine augmented the beneficial effects of chloramphenicol while 8 to 20 times as much phenylalanine inhibited the antibiotic effect. Intermediate doses of phenylalanine (2-5 times those of chloramphenicol) probably inhibited antibiotic effect.

Discussion. The interaction of phenylalanine and chloramphenicol in relation to mammalian protein synthesis and bacterial growth is one of great interest but of uncertain significance in therapeutic situations. Woolley (2) proposed that the antibiotic could be regarded as a metabolic analogue of phenylalanine.

Yamada and Matsuo(3) used L-phenylalanine for synthesis of chloramphenicol. The similarity of chemical structure is apparent in Fig. 1.

More recently, Jardetsky(4,5) has shown that the steric configuration of chloramphenicol in solution is very similar to uridine 5′ phosphate. He suggested that the site of action of chloramphenicol is probably on the ribosome and that it acts by interfering with uridine coding. The genetic code for phenylalanine as carried by messenger RNA to the ribosome is a triplet of uridylic acid (UUU) or two uridylic residues with one cytodilic (UCU)(6). Thus, any interference with uridine should in theory preferentially inhibit the incorporation of phenylalanine into protein.

Gale(8) has demonstrated that chloramphenicol interferes with synthesis of both protein and nucleic acid *in vitro.* His experiments showed that this interference leads to the accumulation of glutamic acid and adenine-labelled substances which he thought represented a piling up of biosynthetic precursors whose utilization had been blocked by chloramphenicol. These substances have not been identified but possibly represent an amino-acyl nucleotide complex, a precursor of nucleoprotein synthesis. When chloramphenicol is removed from an inhibited bacterial culture, the material which accumulates in the presence of chloramphenicol will then be incorporated in more complex molecules. It is interesting to speculate that the red cell precursor vacuoles described in patients treated with chloramphenicol might contain substances analogous to those demonstrated by Gale in bacteria.

Phenylalanine has also been reported to prevent the decrease in liver glucose-6-phosphatase produced in ducks by chloramphenicol(7).

Whatever the final mechanism, the present study suggests that parenteral L-phenylalanine given in a dose equivalent to that of the chloramphenicol will not inhibit the drug's antibacterial activity in the experimentally infected mouse treated with a single injection of the antibiotic. Indeed, there is a potentiation of the host resistance to Klebsiella infection. This is not a direct effect of the amino acid since phenylalanine by itself had no demonstrable antibiotic activity. A preferential uptake of phenylalanine by the host cells might block the site of activity of chloramphenicol on the host cell and yet allow a more effective antibacterial concentration of chloramphenicol outside the host cells. Another possible explanation is that this dose of phenylalanine might prevent the inhibitory effect which chloramphenicol has on some protein dependent body defense mechanism such as antibody formation(9,10,11).

The inhibition of chloramphenicol's effec-

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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