and β globulins, at least 3 proteins of slow mobility, which migrate electrophoretically with the γ-globulins. Formation of γ-globulin and β_2-macroglobulin, the major known immune globulins in other species(9), could not be shown. The relationship, if any exists, of the 3 unidentified proteins of gamma mobility to immunity has not been determined. Orlans *et al.*(10) have demonstrated, in chicken serum, 2 proteins of gamma mobility which may contain antibody, but the precipitation patterns produced in immunoelectrophoresis by their antisera differ from those described here, and cannot, therefore, be readily compared. The inability of neonatal chickens to form antibody(11) and the results of transfer experiments(12,13) indicate that these animals and embryos lack immune mechanisms present in the adult. The finding that embryos and neonatal chicks do not synthesize the major identified immune globulins is consistent with such experimental results, and is additional evidence that all of the γ-globulin found in neonatal sera is obtained from the mother *via* the yolk. The presence of proteins of slow mobility, other than γ-globulin, is of importance in evaluation of observations in free electrophoresis concerning the γ-globulin content of sera from germfree chickens(13). These preliminary findings also suggest that the prealbumin present in sera of laying hens(1) and of embryos is obtained by the embryos, from the yolk.

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Effect of Phenylalanine on Toxicity of S-(Dichlorovinyl)-L-Cysteine in the Rat and the Calf.* (27835)

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It is well documented that S-(1,2-dichlorovinyl)-L-cysteine (DCVC)(1) is very toxic for the bovine specie and for *E. coli* B. In the former it induces fatal aplastic anemia(2) while in the latter it causes inhibited and abnormal, filamentous growth(3). In contrast, the rat is much more resistant to the toxic effects of this compound.† When DCVC (about 10-20 mg/kg) is injected daily into young rats over prolonged periods, it can produce inhibition and arrest of growth, kidney

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† Unpublished observations.

TABLE I. Effect of DL-Phenylalanine on Toxicity of DCVC in the Rat.

Group	Treatment 1st 10 days I.P.; thereafter, none	Wt increment, g 1st 10 days (injected)	2nd 10 days (not inj)	Died
1	DCVC only, 26 mg/kg/day	8.7	3.9	0/14
2	DCVC + DL-Phe* simultaneous	19.7	35.1	1/14
3	DCVC, 1 hr later DL-Phe*	8.9	11.5	1/14
4	DCVC, 2 " " DL-Phe*	−.8	14.3	2/14

* Mole ratio DL-Phe/DCVC = 80.

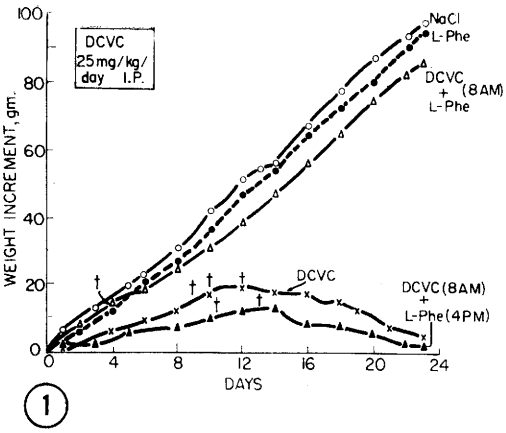
damage and death, although no blood dyscrasia is observed. Inasmuch as the effects of DCVC in *E. coli* B can be prevented by L-phenylalanine(3), but not by the D-isomer, it was of interest to determine whether this amino acid could also alter the toxicity of DCVC in the rat and the calf.

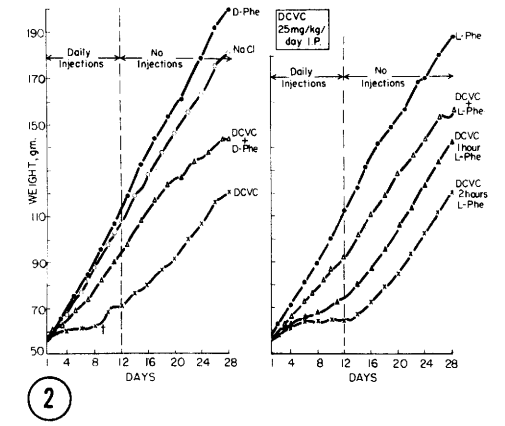
Materials and methods. Work with rats. Weanling male rats from a hooded strain of our colony, weighing about 50 g, were allotted randomly to the various experimental groups each comprising at least 10 rats. They were housed in lots of 2-3 in wire mesh cages and fed an adequate stock diet of natural products. The volume of the various solutions which were injected intraperitoneally, daily or for the stated periods, was based on daily body weight. DCVC (M.P. 160°C with decomposition) was synthesized in this laboratory(2) and dissolved either in 0.9% aqueous, sterile NaCl solution or in the appropriate sterile solution of phenylalanine (Phe) isomers.‡ Depending on the solubility of these the total volume injected intraperitoneally into the rats ranged from 0.05 to 0.10 ml (the latter for the DL-mixture) per g body weight. The same relative volume of DCVC or NaCl solution was injected into the control groups. The mole ratio Phe/DCVC was 80:1.

Work with calves. Female Holstein calves, weighing about 45 kg were purchased in the open market, housed in pens with clean shavings and fed cow's whole milk and good quality alfalfa hay. They had access to mineralized salt. Before the experiments began the calves were observed and conditioned for about 2 weeks until their blood counts were stable. Blood was drawn daily from a jugular vein. Two technics were used to study the effect of Phe on DCVC toxicity. In the first procedure 2 calves were injected intravenously on consecutive days with a sterile (Selas filter) isotonic solution which provided 202 mg of L-Phe per kg body weight. On the second day, after administration of L-Phe, each calf was also injected, intravenously, with 3.3 mg of DCVC per kg body weight in 3 divided portions, 4 hr apart in sterile 0.9% NaCl. The mole ratio of L-Phe/DCVC was 80 to 1. In the second procedure 2 calves were injected intravenously for 10 consecutive days with a sterile aqueous solution of a mixture (made fresh, daily) of 0.20 mg DCVC and 12.2 mg L-Phe per kg body weight per day, the mole ratio L-Phe/DCVC also being 80 to 1. Daily blood counts and clinical examinations were used to evaluate the effects of the treatments. Control calves were treated with DCVC or L-Phe respectively, the latter being well tolerated. The 9 control calves which received a dose of 3.3 mg DCVC in 3 divided portions per kg body weight in one day, and the 6 calves which were injected with 0.20 mg DCVC per kg body weight daily, for 10 days, were also used in conjunction with other experiments.

Results and discussion. With rats. The data in Fig. 1 show that DCVC, when administered daily, can severely depress and arrest the growth of young rats. This effect was largely overcome by simultaneous administration of L-Phe in a mole ratio of 80:1, but not when L-Phe was given 8 hours after DCVC. The D-isomer of Phe was also effective in this respect, as shown in Fig. 2, which also demonstrates that the preventive effect of L-Phe becomes smaller the longer the interval between its administration and that of DCVC. Moreover, the rat can resume rapid growth soon after treatment with DCVC has been discontinued, provided the dosage

‡ Purchased from Mann Research Laboratories, New York.





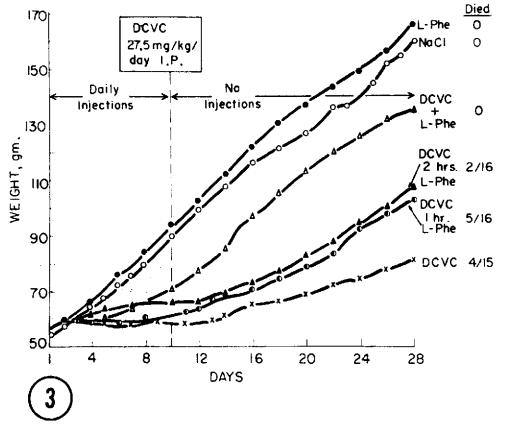


FIG. 1. Effects of L-Phe on toxicity of DCVC in rats. Daily I.P. injections of DCVC and/or L-Phe. When both compounds were inj into the same animal they were administered either simultaneously at 8 A.M. or 8 hr apart, as shown. Control animals were treated with the same volumes of 0.9% NaCl solution or of L-Phe solution. † represents death of an animal. 10 rats/group. Mole ratio of L-Phe/DCVC administered was 80:1.

was not too high or the treatment too prolonged. In this respect much individual variation was encountered. When rats were treated with a higher dosage of DCVC for 10 days, they were affected so that growth was resumed only after a delay and slowly, some individuals, in fact, losing weight and dying after treatment had ceased. As shown in Fig. 3, under these conditions L-Phe also diminished the effects of DCVC if it was administered without delay. Such treatment with Phe not only reduced the growth inhibition but also greatly decreased the residual effects of DCVC which manifest themselves in persistent inhibition of growth (Table I).

Compared with DCVC, its sulfoxide,† prepared from L-DCVC by treatment with performic acid, was much less toxic, 20 mg per kg per day injected I.P. daily for 28 days producing only slight growth inhibition and no apparent kidney damage.

The effects of Phe on DCVC toxicity in rats have only a partial counterpart in those observed in *E. coli* B. While both isomers are effective in the rat, the D-isomer is ineffective in *E. coli* B(3). Until the mechanisms of the effects of DCVC or of the resistance thereto by various species are better understood, no valid explanation can be given of the manner in which Phe exerts its protective effects. Recent work in these laboratories has disclosed that in rats(4), *E. coli* B† and in the calf(5) as well, DCVC undergoes rapid metabolic changes with the formation of several different products(4,5,†). Some of these reactions may be susceptible to interference by one or both isomers of Phe, a suggestion which is underlined by the fact that, for greatest effectiveness, Phe must be administered to the rat simultaneously with DCVC. Using C^{14}-labeled Phe and S^{35}-labeled DCVC(4) we have failed to find evidence for interaction of

FIG. 2. Effects of Phe-isomers on toxicity of DCVC in rats. Daily injections of all compounds were made for 12 consecutive days only. See legend to Fig. 1.

FIG. 3. Effects of L-Phe on toxicity of DCVC. Daily injections of all compounds were made for 10 consecutive days only. Time schedule of injections and proportion of rats dying, as shown with each line. No deaths occurred before 12th day. See legend to Fig. 1.

TABLE II. Effect of L-Phenylalanine on Toxicity of DCVC in the Calf.

Calf No.	Treatment	mg/kg/day	Days	Effects
2265	L-Phe	202	2	None
2266	"	202	2	"
2320	L-Phe	202	2	Aplastic anemia, died 28th day
	DCVC	3.3	2nd	
2367	L-Phe	202	2	*Idem*
	DCVC	3.3	2nd	
2546	L-Phe	12.2	10	Aplastic anemia, died 26th day
	DCVC	.20		
2547	L-Phe	12.2	10	Severe hematol. crisis 24th to 30th day. Gradual recovery.
	DCVC	.20		
Nine	DCVC	3.3	1	Aplastic anemia, died 23rd to 33rd day
Six	"	.20	10	2: Aplastic anemia, died 28th, 29th day 4: Severe hematol. crisis. Survived.

DCVC and L-Phe *in vitro*.§

With the calf. Contrary to the results with the rat and with *E. coli* B no evidence was obtained that L-Phe reduced the toxicity of DCVC in the calf. The results are summarized in Table II. All 9 calves that received a dose of 3.3 mg DCVC per kg body weight for one day only, developed the typical syndrome of aplastic anemia previously(2) described and died after 23 to 33 days. Their response with respect to time course of the clinical manifestations, onset, nature, progress and severity of the blood dyscrasia and time of death was in no way different from that observed in calves No. 2320 and 2367 which were treated with DCVC and L-Phe. Similarly no differentiation could be made in the response of the calves treated for 10 days with 0.20 mg DCVC per kg with or without simultaneous administration of L-Phe. Of the 6 control calves 2 died after showing the typical ante- and post-mortem evidence of aplastic anemia; 4 others experienced a severe hematologic crisis with thrombocyte counts as low as 10,000 per mm^3 and temporary complete absence of neutrophils between the 24th and 27th day after which time they made first a slow, then a more rapid hematologic recovery, approaching normal values about the 60th day. Calf 2547 which received L-Phe together with DCVC showed exactly the same response, with a severe hematologic crisis, followed by recovery, whereas calf 2546 died on the 26th day. With the amounts of Phe administered, no evidence of toxic effects were observed in either the calf or the rat. This is in accord with observations on rats reported by Goldstein(6).

We also injected, I.V., L-cysteine, in a mole ratio of 300 to 1 into 2 calves treated with 3.3 mg DCVC per kg of body weight, as outlined above. This treatment did not prevent the development of fatal aplastic anemia or alter the nature and course of the blood dyscrasia.

Summary. Both enantiomers of phenylalanine when injected intraperitoneally into rats can greatly reduce the toxicity of S-(1,2-dichlorovinyl)-L-cysteine. To be most effective the DCVC and Phe must be administered simultaneously, a delay of one hour in injection of phenylalanine reducing its effectiveness markedly. Administration of L-phenylalanine to calves failed to reduce the toxic effects of S-(dichlorovinyl)-L-cysteine and development of fatal aplastic anemia.

§ Dr. Nester Dickie and Mr. P. M. Anderson gave valuable aid in this phase of the work.

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