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EFFECT OF *S*-(1,2-*trans*-DICHLOROVINYL)-L-CYSTEINE ON THE TRANSPORT OF AMINO ACIDS AND GLUCOSE BY EVERTED SEGMENTS OF RAT SMALL INTESTINE*

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SUMMARY

1. Using everted sacs of rat small intestine, *S*-(1,2-*trans*-dichlorovinyl)-L-cysteine (DCVC) was found to suppress the active transport of glucose, glycine and a number of L-amino acids including phenylalanine, leucine, valine, alanine and histidine.

2. The inhibition appeared to be proportional to the concentration of DCVC, and was not readily reversed by simple washing technics.

3. There was no marked alteration in the permeability of the intestinal sacs in the presence of DCVC, as measured by passage of D-phenylalanine in either direction.

4. DCVC inhibition of L-phenylalanine uptake by intestinal tissue appears to be a competitive process.

5. DCVC itself undergoes active transport at low concentrations, with greatest transport occurring in the mid-jejunum region of rat small intestine, and least in the duodenum.

6. DCVC transport through everted intestinal sacs was suppressed by dinitrophenol, anaerobiosis, absence of Na^+, and by low temperatures.

INTRODUCTION

DCVC was synthesized by McKINNEY *et al.*[1] nearly 10 years ago in an attempt to define the chemical nature of the toxic agent in trichloroethylene-extracted soy meal which produced a fatal hemorrhagic syndrome in young calves, cattle and horses[2]. SCHULTZE *et al.*[3] reported that doses of DCVC as low as 0.22 mg/kg of body weight, administered daily to young calves for 10 days, produced a severe bone-marrow hypoplasia and a blood dyscrasia which reached its greatest severity about

Abbreviations: DCVC, *S*-(1,2-*trans*-dichlorovinyl)-L-cysteine; DPO, 2,5-diphenyloxazole; POPOP, 1,4-bis-(5-phenyloxazolyl-2)benzene.

* A preliminary report of this work was made to the American Federation for Clinical Research[44].

1 month after the start of treatment; higher doses produced thrombocytopenia, leukopenia, lymphopenia and fatal termination. However, these effects on the hematopoietic system were not seen in a wide variety of laboratory animals receiving either the toxic soy meal preparation[2] or synthetic DCVC (ref. 4).

The administration of DCVC to rats resulted in a dose-related inhibition of growth and renal damage which could be prevented by the simultaneous administration of massive doses of D- or L-phenylalanine; whereas L-phenylalanine administered to calves failed to reduce the toxic effects of DCVC upon the hematopoietic system[5]. DCVC also inhibited the growth of *Escherichia coli* and produced changes in morphology, which were prevented by high concentrations of L-phenylalanine or L-tyrosine, but not by their D-isomers[6]. Increasing concentrations of DCVC diminished the cellular uptake of [^{14}C]phenylalanine and glycine by *E. coli* and subsequent incorporation into protein and nucleic acid[7], presumably by interference with synthesis mechanisms[8]. The relationships with phenylalanine were of particular interest to us because of our earlier observations with drugs as inhibitors of active transport[9], suggesting that some of the effects of DCVC might be due to interference with membrane transport systems.

METHODS AND MATERIALS

Active transport studies

The everted intestinal sac procedure of WILSON AND WISEMAN[10] and the open-sac technic of CRANE AND WILSON[11] were used in this study. Albino male rats (Holtzman) weighing 180–240 g were killed by decapitation and the small intestine was removed immediately. After washing with KREBS–HENSELEIT[12] bicarbonate buffer (pH 7.4) containing 0.3% glucose, the portion including jejunum and ileum was gently everted on a glass rod. Sacs approx. 5 cm in length were prepared by tying off one end of each section with cotton thread, and 1 ml of buffer containing known concentrations of isotope-labelled compound and unlabelled inhibitor was transferred to the sac. The open end was then closed off with a ligature. Alternate sections of intestine were run as controls in the absence of inhibitor. The everted sacs were then placed in an erlenmeyer flask containing 5 ml of the same identical solution, and incubated in a Dubnoff metabolic shaking incubator at 37° under an atmosphere of 95% O_2–5% CO_2 for 1 h. At the end of the incubation period, the sacs were removed, the volumes of solutions inside and outside the sac were measured, and samples were taken for radioactivity measurements. The concentration gradient developed across the intestinal wall could then be expressed as the ratio of serosal:mucosal concentration calculated directly from the counting data (disint./min per ml of solution).

In the CRANE AND WILSON[11] technic, a 4-cm length of the washed, everted intestine was tied off at one end; the open end was mounted on the apparatus. 0.8 ml of buffer containing ^{14}C-labelled amino acid (10 mM = 0.2 μC) was placed inside the sac, which was immersed immediately into 8 ml of solution having the same composition. The entire apparatus was lowered into a 37° water bath. 20-μl samples of the solution inside the sac were drawn with a microsyringe at designated time intervals for radioactivity measurements. In all active transport studies, the concentrations of labelled compounds and inhibitor were the same in the inside and outside solutions at the start of the experiment.

Tissue uptake studies

The technic described by AGAR, HIRD AND SIDHU[13] was used for uptake studies. Intestinal segments approx. 1 cm long were cut longitudinally to expose the mucosa. Each segment was bathed in 2 ml buffer containing the desired concentration of ^{14}C-labelled amino acid, and incubated at 37° under an atmosphere of 95% O_2–5% CO_2 for 20 min. After incubation, the segments were quickly removed and rinsed free of adherent solution. Total radioactivity accumulated by each segment was determined by liquid-scintillation counting following O_2-flask combustion.

Analytical methods

Radioactivity measurements of ^{14}C- and ^{35}S-labelled compounds in solution were obtained by liquid-scintillation counting in a Packard Tri-Carb instrument. Samples of serosal and mucosal solutions were taken in duplicate. Each counting vial contained an 0.2-ml aliquot of sample solution, 1 ml of water, 1 ml of methanol, and 15 ml of a dioxane scintillation solution (23.3 g DPO, 0.29 g POPOP, and 320 g naphthalene dissolved in 2.91 l of 1,4-dioxane). Two or more counting periods of 5–10 min were used for each sample, and counting efficiency was determined following addition of an internal standard.

Total radioactivity in intestinal segments was determined by liquid-scintillation counting following O_2-flask combustion. Segments were dried overnight and combusted in O_2-filled flasks, using a modification of the procedure described by KELLY *et al.*[14] and by KALBERER AND RUTSCHMANN[15]. $^{14}CO_2$ was trapped in phenylethylamine in methanol, and a sample was added to a toluene–DPO–POPOP solution for the scintillation counts.

D-*Phenylalanine* was assayed by the fluorimetric procedure described by WONG, O'FLYNN AND INOUYE[16], based upon a modification of the MCCAMEN AND ROBINS procedure[17].

Paper chromatography of [^{35}S]DCVC was performed on Whatman No. 1 paper strips using the descending technic, with *n*-butanol–acetic acid–water (12:3:5, by vol.) as developing solvent[18]. Radioactivity was detected by eluting 1-cm segments of the paper strips with methanol, followed by liquid-scintillation counting. Unlabelled DCVC and cysteine were detected on the paper strips by spraying with 0.1% ninhydrin in *n*-butanol and heating briefly at 110°.

Materials

L-Phenylalanine, L-valine (Aldrich Chemical Co., Milwaukee, Wisc.); L-leucine, glycine, L-histidine (Eastman Kodak Co., Rochester, N.Y.); L-alanine (Nutritional Biochemicals Corp., Cleveland, Ohio); D-phenylalanine (Sigma Chemical Co., St. Louis, Mo.); uniformly ^{14}C-labelled L-amino acids and D-glucose (New England Nuclear Corp., Boston, Mass.); [^{35}S]DCVC (courtesy of Dr. M. O. SCHULTZE, University of Minnesota); unlabelled DCVC was synthesized in the Parke, Davis laboratories by Mrs. M. LIPNIK and Dr. H. M. CROOKS.

RESULTS

Inhibition of intestinal transport of amino acids and glucose by DCVC

Preliminary observations with the closed-sac technic of WILSON AND WISEMAN[10]

TABLE I

EFFECT OF DCVC ON THE ACTIVE TRANSPORT OF AMINO ACIDS AND GLUCOSE

Identical concentrations of substrate in bicarbonate buffer were placed on the serosal (1 ml) and mucosal (5 ml) sides of everted sacs of rat small intestine. When present, DCVC concentration was 4.6 mM. Following 1 h incubation at 37° assays for ^{14}C activity were carried out by liquid-scintillation counting. Ratios are given for the individual sacs in each series.

^{14}C-labelled substrate (10 mM)	*Serosal:mucosal concentration ratios*	
	No inhibitor added	*DCVC (4.6 mM) added*
L-Phenylalanine	2.3—2.3—2.2	1.1—1.1—1.0
	1.6—1.7—2.0	1.1—1.1—1.0
L-Leucine	2.1—2.1—2.4	1.1—1.1—1.2
Glycine	1.5—1.7—2.2	0.9—0.9—0.8
L-Valine	2.0—2.7—3.0	1.1—1.1—1.1
L-Alanine	2.0—2.2—2.2	1.1—1.1—1.1
L-Histidine	1.5—1.9—2.0	0.8—0.9—0.8
D-Glucose (5.6 mM)	4.8—7.6—12.5	2.1—2.5—2.9

indicated that a DCVC concentration of 4.6 mM produced almost complete suppression of L-phenylalanine transport. The effect of DCVC on the transport of a number of other amino acids and glucose was investigated in the same manner. The results are shown in Table I, together with control data obtained in the absence of inhibitor. DCVC effectively suppressed the transport of L-phenylalanine, L-leucine, L-valine, glycine, L-alanine and L-histidine; whereas the transport of D-glucose was only partially inhibited.

The effect of DCVC concentration on the transport of L-phenylalanine was studied in greater detail using the open-sac technic of CRANE AND WILSON[11], which

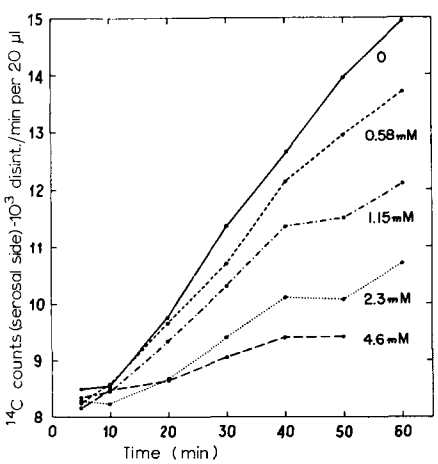

Fig. 1. Effect of DCVC on active transport of L-phenylalanine. 10 mM concentrations of L-[^{14}C]-phenylalanine in bicarbonate buffer were placed inside and outside the everted intestinal sacs. The concentrations of DCVC ranged from zero to 4.6 mM. Assays were run on 20-µl samples of the solution inside the sacs by liquid-scintillation counting, with samples being taken at 10-min intervals during the course of the experiment.

permits repeated sampling from the serosal and mucosal solutions at different time intervals. All segments of intestine were taken from the same animal. The concentration of L-phenylalanine at the start of the experiment was 10 mM on both sides of the intestinal sac. Identical concentrations of DCVC were used in the serosal and mucosal solutions in each experiment; but different concentrations were used with each segment of intestine. The results are shown in Fig. 1.

The net transport of L-phenylalanine appeared to be significantly decreased at the lowest concentrations of DCVC employed (0.58 mM). Greater inhibitory effect was evident at higher concentrations of DCVC, with nearly complete inhibition of transport occurring at a concentration of 4.6 mM. The inhibition occurred within a very short time after the addition of DCVC, with no extensive pre-exposure period being required to produce this effect.

Reversibility of inhibition

With high concentrations of DCVC the inhibitory effect on L-phenylalanine transport appeared to be irreversible. Using the CRANE–WILSON technic[11], three sections of rat intestine were set up in solutions containing (A) L-phenylalanine; (B) L-phenylalanine *plus* DCVC (2.3 mM); and (C) same conditions as (B). Samples of serosal solution were withdrawn at 5-min intervals for 20 min. The preparations were then rinsed thoroughly for 10 min with fresh buffer containing no DCVC or phenylalanine. At the end of this wash-out period, solutions of the same composition as those used previously were introduced into segments (A) and (C); but the solution added to (B) contained phenylalanine with no DCVC present. The rate of phenylalanine transport was then followed for an additional 40–50 min. The results are shown in Fig. 2.

No renewal of phenylalanine transport was observed, since the slope of (B) was nearly the same as (C). DCVC concentrations of 1.15 mM were also tried, with similar

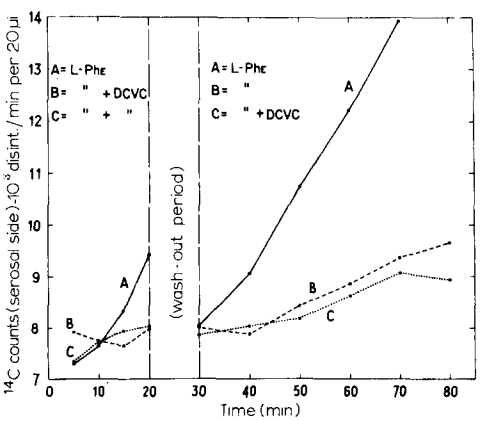

Fig. 2. Attempted reversal of DCVC inhibition of L-phenylalanine transport. 10 mM concentrations of L-[^{14}C]phenylalanine in bicarbonate buffer were placed inside and outside the sacs. Initially, DCVC in a concentration of 2.3 mM was also added to the solutions inside and outside of sacs B and C. Samples of 20 µl were taken from inside the sacs (serosal side) for radioactivity counts at 5-min intervals over a 20-min period. All sacs were then washed with fresh buffer for 10 min, and L-phenylalanine was again added to the serosal and mucosal solutions; but DCVC was added only to sac C in the same concentration used previously. Sampling was continued at 10-min intervals for the duration of the experiment.

results. Using the same technic with other inhibitors of active transport such as chloramphenicol[9], a rapid recovery of phenylalanine transport was observed following the buffer wash, with the transport rate being equal to or greater than that of the control. Since the transport of phenylalanine from the mucosal to the serosal side of the gut involves passage across a number of structural elements in the intestinal wall, these observations may have little bearing upon the reversibility of DCVC inhibition in other test systems, such as the cell suspensions which are often used in active transport studies.

Effect of DCVC upon diffusion of D-*phenylalanine through the intestinal wall*

Using the everted-sac technic, the passage of D-phenylalanine in both directions was studied to determine whether DCVC had any influence on the permeability of the intestinal wall. These experiments were carried out with unlabelled D-phenylalanine, which appears to be absorbed primarily by diffusion processes and undergoes no significant degree of active transport[19]. A solution of the D-isomer of phenylalanine (10 mM in glucose–bicarbonate buffer) was added either to the mucosal side (5 ml) or to the serosal side (1 ml) of the everted intestinal sac; and the same buffer with no

TABLE II

EFFECT OF DCVC ON THE PASSAGE OF D-PHENYLALANINE THROUGH EVERTED SACS OF RAT SMALL INTESTINE

D-Phenylalanine (10 mM) was added to the bicarbonate-buffered solutions either on the mucosal side (5 ml) or serosal side (1 ml) of the everted rat intestinal sacs. DCVC, when present, was added to the solution on both sides of the sac in equal concentration (4.6 mM). D-Phenylalanine concentrations were determined fluorimetrically following 1 h incubation at 37° under 95% O_2–5% CO_2.

D-*Phenylalanine at start*		*DCVC (4.6 mM)*	*Final distribution of* D-*phenylalanine*			*% transported*
Location	*Amount (μg)*		*Serosal soln. (μg)*	*Mucosal soln. (μg)*	*Tissue* (μg)*	
Mucosal side	8085	Absent	389	6350	1346	4.8
	8085	Absent	428	6780	877	5.3
	8045	Present	262	7370	413	3.3
	8045	Present	293	6990	762	3.6
Serosal side	1617	Absent	720	465	432	28.8
	1617	Absent	750	469	398	29.0
	1609	Present	842	488	279	30.3
	1609	Present	856	451	302	28.0

* Calculated by difference following assay of serosal and mucosal solutions.

D-phenylalanine present was added to the opposite side. When present, DCVC was added to the solutions inside and outside the sac in equal concentrations (4.6 mM). Following incubation for 1 h in the O_2–CO_2 atmosphere, the concentration of D-phenylalanine was determined in serosal–mucosal solutions, the volumes of the serosal and mucosal solutions were measured, and from this the amounts of D-phenylalanine passing through the intestinal wall were calculated. The results are summarized in Table II.

In most cases the volume of the mucosal solutions decreased from the initial

5 ml to about 4.5 ml; serosal solution volumes showed no significant change (0.9–1.1 ml). The passage of D-phenylalanine from the mucosal to the serosal side ranged from 262 to 428 μg, representing about 5% of the starting quantity of phenylalanine in the absence of DCVC, and a little less in the presence of DCVC (3.3–3.6%). Starting with D-phenylalanine on the serosal side, only one-fifth as much amino acid was present, although the concentration was the same as in the first series. In this case, 451–488 μg were recovered on the mucosal side, representing 28–30% of the amount originally added, with no significant differences observed in the presence or absence of DCVC. The DCVC unaccounted for by assay of the serosal–mucosal solutions was probably retained by the tissue, although direct assays were not carried out to confirm this point. This amounted to 300–400 μg in the serosal series, and about double this quantity in the mucosal series.

Kinetics of DCVC inhibition of L-*phenylalanine transport*

The tissue uptake technic described under METHODS was used for these studies. Duplicate tissue specimens were used in each run. The initial concentrations of L-phenylalanine in the incubation medium ranged from 1 mM to 8 mM. The uptake

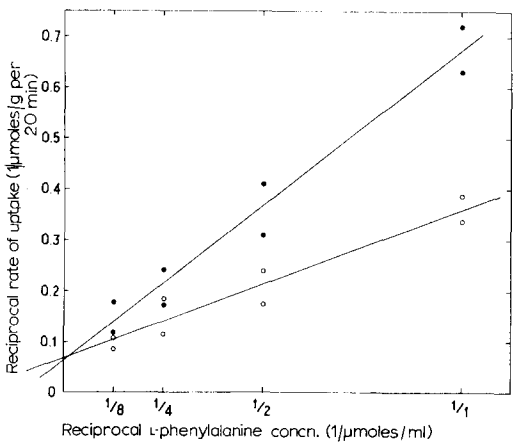

Fig. 3. LINEWEAVER–BURK plot of L-phenylalanine uptake in the presence and absence of DCVC. The initial concentration of L-[^{14}C]phenylalanine in the incubation medium ranged from 1 to 8 mM. In a parallel run, the DCVC concentration was kept constant at 2.3 mM. Segments of rat small intestine approx. 1 cm in length were cut open and dropped into these solutions, using duplicate samples for each time period. These were incubated for 20 min at 37° under an atmosphere of 95% O_2–5% CO_2, following which they were rinsed briefly and assayed for ^{14}C activity by O_2-flask combustion and liquid-scintillation counting. The best straight lines for each set of data were calculated by the method of least squares. Lower line represents the control series; upper line represents the series with inhibitor present.

studies were run in the presence or absence of DCVC (2.3 mM). The incubation time was fixed at 20 min, which adequately reflected the initial rate of uptake. A LINEWEAVER–BURK[20] plot of the experimental data is shown in Fig. 3. The best straight lines were calculated by the method of least squares. The direct proportionality noted between the reciprocals of uptake velocity and substrate concentration suggests that the transport of L-phenylalanine conforms with the pattern of saturation kinetics[21].

The addition of DCVC produced a greater slope of the line, but the *y*-axis intercept was essentially unchanged, which is characteristic of competitive inhibition processes.

Active transport of DCVC in vitro

DANIEL AND SCHULTZE[22] reported that the *S*-dichlorovinyl group must be attached to a C_3-chain containing free α-amino and carboxyl groups in L-configuration for maximum effect on the growth of *E. coli* and suppression of hematopoietic function in calves. All structural modifications of the carbon skeleton, substitution on the amino group, or steric inversion, produced a marked decrease in the effects of DCVC. These structural requirements for activity closely resemble those of the amino acids for maximum transport across the intestinal mucosa[23]. Experiments were therefore set up to determine whether DCVC itself could undergo active transport with everted sacs of rat small intestine. We also learned that Dr. R. P. SPENCER* made some preliminary observations in this area some years ago; but his results have not appeared in the literature.

Using the everted-sac procedure, DCVC transport was studied with a ^{35}S-labelled preparation, using three different concentrations of DCVC. The results are shown in Table III. With a concentration of 0.58 mM inside and outside of the sac at

TABLE III

ACTIVE TRANSPORT OF [^{35}S]DCVC AS A FUNCTION OF INITIAL CONCENTRATION

Buffered solutions containing equal concentrations of [^{35}S]DCVC were placed inside and outside of everted sacs of rat small intestine. These were incubated for 1 h at 37° under an 95% O_2–5% CO_2 atmosphere, and assays for ^{35}S were then run by liquid-scintillation counting. Values are given for the individual sacs in this series.

DCVC concentration (mM)	*Serosal:mucosal ratio*
0.58	4.5—4.2
1.16	2.9—3.2
4.65	1.5—1.2

the start, a high concentration gradient developed across the intestinal wall, indicating active transport (serosal:mucosal ratio of 4.2, 4.5). This ratio decreased with increasing concentrations of DCVC, suggesting that a saturable carrier mechanism might be involved. A similar type of behavior has been observed with naturally occurring amino acids which undergo active transport[24,25]. Paper chromatography of the mucosal and serosal solutions following 1 h incubation revealed only one spot corresponding with unchanged DCVC, indicating that this compound was stable under the conditions of the experiment.

DCVC transport in different sections of rat small intestine

The pattern of DCVC transport along the entire length of rat small intestine

* Personal communication from Dr. M. O. SCHULTZE, University of Minnesota.

TABLE IV

TRANSPORT OF DCVC IN DIFFERENT SECTIONS OF RAT SMALL INTESTINE USING THE EVERTED-SAC TECHNIC

Equal concentrations of [^{35}S]DCVC (0.46 mM) were placed in buffered solutions inside and outside of everted sacs of rat small intestine. Assays for ^{35}S were run after 1 h incubation at 37° under an 95% O_2–5% CO_2 atmosphere.

Rat No.	*Section of intestine*	*Radioactivity (disint./min·ml)*		*Serosal:mucosal ratio*
		Serosal	*Mucosal*	
1	Duodenum	44 440	20 330	2.2
2		44 840	20 630	2.2
1	Proximal	67 180	12 860	5.2
2	jejunum	65 850	13 120	5.0
1	Mid-	75 450	9 100	8.3
2	jejunum	74 420	9 390	7.9
1	Ileum	85 710	10 040	8.5
2		68 210	12 220	5.6

was also studied, using the everted-sac technic. Sections of the rat small intestine were removed from two animals, representing the duodenum, proximal jejunum, mid-jejunum and ileum. Everted sacs were prepared from these sections, and the experiments were run with [^{35}S]DCVC (0.46 mM) present inside and outside of the sacs. The radioactivity assays and the serosal:mucosal ratios which appeared in 1 h are shown in Table IV.

The data indicate that the concentration gradients varied considerably in different sections of the small intestine. Maximum serosal:mucosal ratios of DCVC were found in the mid-sections of the jejunum, with somewhat lower concentration gradients in the proximal jejunum and ileum. The duodenum exhibited the lowest

TABLE V

TRANSPORT OF [^{35}S]DCVC ACROSS EVERTED SACS OF RAT SMALL INTESTINE UNDER VARIOUS CONDITIONS

Equal concentrations of [^{35}S]DCVC (0.46 mM) were placed inside and outside of the sacs. Variations in composition of the buffer or in physical conditions are indicated in the table. Assays for ^{35}S were made by liquid-scintillation counting after 1 h incubation. Values given in the table are for individual sacs in the series.

Treatment	*Serosal:mucosal concentration ratio*	
	Normal controls	*plus treatment*
2,4-Dinitrophenol (1 mM)	7.1—6.3—4.1	1.6—1.6—1.6
Anaerobiosis*	5.8—5.7—5.4	1.8—1.6—1.8
Na^+-free medium**	4.8—6.0—6.7	1.1—1.0—1.2
Room temperature (21°)	(mean 5.8; range 4.1 to 7.1)	3.2—3.9—3.6—3.3

* 95% N_2–5% CO_2.
** The sodium salts in the buffer[12] were completely replaced by potassium salts.

concentration gradient. Similar observations have been reported in the literature for a number of other amino acids[24-26].

Inhibition of DCVC transport

A number of experimental factors which are known to affect the active transport of amino acids were also examined. These included a metabolic inhibitor (2,4-dinitrophenol), presence or absence of O_2, effect of temperature, and dependence upon Na^+ (refs. 27,28). The influence of these factors on the transport of DCVC by everted segments of rat intestine are shown in Table V.

A mean serosal:mucosal ratio of 5.8 obtained with DCVC under normal conditions was reduced to 1.6 in the presence of 1 mM 2,4-dinitrophenol, and to 1.7 in an O_2-free atmosphere. Active transport appeared to be completely suppressed in media containing K^+ in place of Na^+. In addition, the transport of DCVC was significantly reduced by lowering the temperature to 21°. These observations support the conclusion that a saturable, energy-dependent transfer mechanism may be involved in the transport of DCVC by everted sacs of rat small intestine.

DISCUSSION

The inhibition of amino acid transport by DCVC is not surprising in view of the similarities in chemical structure. MATTHEWS AND LASTER[29] demonstrated that glycine, alanine, valine, leucine, phenylalanine and methionine shared a common transport mechanism in hamster small intestine, and that the mutual inhibition was competitive. HINDMARSH, KILBY AND WISEMAN[30] reported inhibition of D-glucose transport by L-histidine. Other hexoses have been found to suppress the intestinal transport of amino acids in a non-competitive manner[31]. More recently, ALVARADO[32] postulated a polyfunctional common carrier having separate binding sites for each group of compounds and for Na^+, in which allosteric interactions between the binding sites could occur. With DCVC, complete suppression of active transport of amino acids occurred with DCVC concentrations high enough to inhibit its own transport. Conceivably, DCVC may share a common transport mechanism with the amino acids. A greater affinity of DCVC for the carrier sites could saturate the transport mechanism at higher concentrations, thereby blocking the transport of other amino acids as well as its own. The data obtained with D-phenylalanine indicates that DCVC had no marked effect upon permeability.

The present observations on the inhibition of active transport processes by DCVC in rat intestine may perhaps be extended to include other cellular systems. OXENDER AND CHRISTENSEN[33] indicated that closely allied transport mechanisms for amino acids may be found in the intestinal mucosa, erythrocytes, Ehrlich's ascites tumor cells and in the isolated diaphragm. However, following oral administration of DCVC higher concentrations of this compound would be expected to occur in the intestinal tract than in the blood and tissues of the body, with a correspondingly greater effect on the intestinal mucosa. This may result in a decreased intestinal absorption of amino acids and glucose, possibly leading to nutritional deficiencies. Absorption by passive diffusion mechanisms in the rat intestine appears to be unaffected by DCVC, suggesting that dietary supplements may be used to increase the absorption of essential nutrients. This may be a factor in the observed reversal of

renal toxicity to DCVC in the rat with massive doses of phenylalanine or tyrosine[5].

The precise effect of DCVC in protein synthesis has not been clearly defined. Earlier observations by DICKIE AND SCHULTZE[7] and by PARKER[8] indicate that it may have a direct effect on protein synthesis at the molecular level. KLUBES AND SCHULTZE[18] found that *E. coli* incorporated the ^{35}S-labelled portion of DCVC into cellular protein to a greater extent than the C-3 atom of the cysteine chain, indicating possible cleavage of the C–S bond. The dichlorovinyl residue could then act as an alkylating agent, or combine with proteins to form a variety of derivatives. However, as pointed out by others[34,35], amino acid transport mechanisms are not necessarily dependent upon protein synthesis *per se*.

The observations reported here may provide some basis for understanding the mode of action of DCVC in biological systems. Perhaps the most striking effect of this agent is on the bone marrow of young calves, cattle and horses[2,3], and its relative lack of effect on this organ system in laboratory animals such as the rat, mouse, guinea-pig, rabbit, cat and dog[8]. In all species tested at high dose levels there was evidence of renal damage[5,8]. In the rat, the renal effects were prevented by simultaneous administration of massive doses of either D- or L-phenylalanine in a molar ratio of 80:1 or more; but toxicity to the bone marrow cells of calves was not influenced by phenylalanine[5]. In bacterial systems, DANIEL AND SCHULTZE[6] found that DCVC produced an inhibition of bacterial growth and changes in morphology which could be prevented by high concentrations of DL-phenylalanine or L-tyrosine, while L-tryptophane was only partly effective. The morphological changes resulted in the appearance of a filamentous form of *E. coli*, similar to the forms produced by exposure to ionizing radiation[36] and a variety of chemical agents including various mustards[37,38] and some antibiotics[39,40]. The effects of azaserine on *E. coli* can be prevented by phenylalanine[41], tyrosine[38] or tryptophane[42], and those of 5-diazouracil can be prevented by tyrosine[43]. A closer examination of these phenomena from the standpoint of possible interference with transport processes is clearly indicated.

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