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THE EFFECT OF PHLORIZIN ON AMINO ACID TRANSPORT IN RAT-KIDNEY-CORTEX SLICES

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SUMMARY

Phlorizin has been shown to enhance the accumulation of amino acids by the rat-kidney-cortex slice. Evidence has been presented that this drug has no effect on the affinity of the carrier site(s) for amino acids but inhibits the efflux of amino acid from the intracellular fluid, possibly by increasing the intracellular binding of amino acids. Phlorizin was found to diminish the incorporation of amino acids into protein and to cause an increased oxidation of amino acids.

INTRODUCTION

Phlorizin has been of considerable interest as a drug capable of providing insight into cellular transport mechanisms. The initial stimuli for this interest were the discovery by VON MERING that phlorizin causes glycosuria in the dog[1] and the observation that this substance inhibits renal tubular reabsorption of glucose[2,3]. Subsequent studies have revealed that phlorizin is a potent inhibitor of the penetration of hexoses and other sugars in gut preparations[4], kidney slices[5], ascites-tumor cells[6] and red cells[7]. The effects of phlorizin on cellular transport and metabolic functions have been reviewed by LOTSPEICH[8] and CRANE[9].

Previous studies from this laboratory have shown that rat-kidney-cortex slices accumulate a variety of α-amino acids against concentration gradients *in vitro*[10]. This concentrative transfer is dependent on aerobic metabolism, is temperature dependent, and is markedly altered by the presence of a variety of substances including other amino acids[11], 2,4-dinitrophenol[12] and maleic acid[13].

The observation has also been reported that in contrast to its inhibition of glucose uptake by rat-kidney-cortex slices, phlorizin causes an increase in the intracellular accumulation of amino acids[14]. Further investigation into the nature of this phenomenon has been carried out and the results are reported in the present paper. Evidence is offered that phlorizin increases amino acid accumulation not by increasing the transport of amino acid into the cell but by reducing the efflux of amino acids from the cell. In addition it has been found that this substance enhances the oxidation of amino acids but inhibits their incorporation into protein by the kidney slice.

EXPERIMENTAL PROCEDURE

Materials

[2-^{14}C]Glycine (sp. act. 1.19 mC/mmole), uniformly labeled L-[^{14}C]lysine (sp. act. 1.62 mC/mmole), uniformly labeled L-[^{14}C]phenylalanine (sp. act. 1.30 mC/mmole)

and uniformly labeled L-[^{14}C]histidine (sp. act. 1.06 mC/mmole) were obtained from the Volk Radiochemical Company. α-[1-^{14}C]Aminoisobutyric acid (sp. act. 3.39 mC per mmole) was purchased from Isotope Specialties Co. Each of these labeled amino acids was chromatographically pure in single-dimension ascending paper system using butanol – acetic acid – water (4:1:2, v/v). Unlabeled α-aminoisobutyric acid, phlorizin, and phloretin were purchased from the Mann Research Lab. The phlorizin used was twice recrystallized from hot water although the observed effects of the purchased material and the recrystallized compound were identical. Phloretin was once recrystallized from hot ethanol. Phloroglucinol was obtained from Eastman Organic Chemicals.

Methods

Male Sprague-Dawley rats weighing 140–180 g were used in all experiments, and were fed a Purina(R) chow diet and water *ad libitum* until sacrificed by stunning and decapitation. The techniques for preparation of kidney-cortex slices, incubation in Krebs–Ringer bicarbonate buffer (pH 7.4) at 37° and assessment of intracellular and extracellular radioactivity have been described in detail previously[10]. Total tissue water determined by the difference between tissue weight after blotting and weight after drying at 105° for 24 h was found to be 80.2 ± 1 % of the wet tissue weight. Extracellular space as estimated with [^{14}C]inulin was 25.7 ± 1.1 % of the wet-tissue weight. No change in these parameters was found in the presence of phlorizin as indicated previously[15]. Chromatography of tissue extracts revealed greater than 90 % of the radioactivity to be found with the appropriate R_F for the specific amino acid studied.

The method of assaying incorporation of labeled amino acid into kidney-slice protein used in this laboratory and reported in detail[12] is based on the trichloroacetic acid precipitation of protein and the radioactivity measurement of the trichloroacetic precipitate in hyamine by the method of STEINBERG *et al.*[16]. Studies to determine the oxidation of amino acids to $^{14}CO_2$ were carried out in modified Warburg flasks with trapping of $^{14}CO_2$ in hyamine by the method described by ROSENBERG, WEINBERG AND SEGAL[17]. All radioactive samples were counted in a liquid scintillation spectrometer with efficiency of counting as noted in the methods references above[10, 12, 17].

Techniques similar to those described for kidney-cortex slices[10] were employed for the determination of intracellular and extracellular amino acid concentration in rat diaphragm and intestinal segments. The isolated "cut" diaphragm was used and calculations were based on the values for extracellular water and total tissue water reported by KIPNIS AND CORI[18]. Everted intestinal segments weighing 15–35 mg were prepared from a portion of the jejunum excised 15–27 cm from the pylorus. Segments from several animals were pooled in chilled buffer at 4° and three segments were randomly selected for incubation together in a single flask.

RESULTS

Effect of phlorizin on the concentrative transfer of amino acids by kidney slices

As shown in Table I, α-aminoisobutyric acid, glycine, L-phenylalanine, L-lysine and L-histidine are accumulated against a concentration gradient by cells of the renal cortex. During the 90-min incubation phlorizin caused a significant increase

TABLE I

EFFECT OF PHLORIZIN ON UPTAKE OF AMINO ACIDS BY KIDNEY-CORTEX SLICES

Three slices weighing a total of 80–120 mg were incubated aerobically ($O_2 - CO_2$, 95:5) for 90 min in 2 ml Krebs–Ringer bicarbonate buffer (pH 7.4) at 37°. Total radioactivity per flask ranged from 0.2 to 0.5 μC. Phlorizin concentration 3 mM. Triplicate determinations were averaged.

Amino acid	*Medium concentration (mM)*	*Medium (ECF)**		*Intracellular fluid (ICF)*		*Distribution ratio***		*P*
		Control	*Phlorizin*	*Control*	*Phlorizin*	*Control*	*Phlorizin*	
α-Aminoisobutyric acid	0.13	304 000	283 000	1 177 000	2 141 000	3.86 ± 0.19	7.57 ± 0.25	<0.001
Glycine	0.18	165 000	159 000	944 000	1 507 000	5.73 ± 0.35	9.49 ± 0.11	<0.001
L-Lysine	0.045	224 000	201 000	783 000	917 000	3.50 ± 0.17	4.55 ± 0.27	<0.05
L-Phenylalanine	0.075	56 500	59 200	105 000	203 000	1.86 ± 0.12	3.42 ± 0.27	<0.01
L-Histidine	0.095	162 000	165 000	371 000	564 000	2.28 ± 0.39	3.41 ± 0.04	<0.05

* Medium values are those at the termination of incubation.

** Distribution ratio $= \frac{\text{counts/min/ml ICF}}{\text{counts/min/ml ECF}}$ · Mean ± SD.

TABLE II

EFFECTS OF PHLORIZIN, PHLORETIN AND PHLOROGLUCINOL ON α-AMINOISOBUTYRIC ACID ACCUMULATION

Incubation conditions as in Table I. α-Aminoisobutyric acid concentration was 0.065 mM. Compounds were tested at a concentration of 3 mM. Incubation time was 90 min. Triplicate determinations were averaged. Phloretin was added in 20 μl of ethanol. This quantity of ethanol was found to have little or no affect on the distribution ratio.

Substance	*Distribution ratio*
None	3.53
Phlorizin	6.99
Phloretin	1.41
Phloroglucinol	3.51

TABLE III

INFLUENCE OF PREINCUBATION OF KIDNEY-CORTEX SLICES WITH PHLORIZIN ON THE UPTAKE OF α-AMINOISOBUTYRIC ACID

Tissues were preincubated 45 min at 37° in Krebs–Ringer bicarbonate buffer (pH 7.4) with or without phlorizin (3 mM). Tissues were then transferred to flasks containing α-[1-^{14}C]aminoisobutyric acid (0.065 mM) in the presence or absence of phlorizin and the incubation continued for 60 min. Triplicate determinations were averaged.

Preincubation	*Incubation*	*Distribution ratio*
Buffer alone	Buffer alone	4.54
Phlorizin	Buffer alone	5.76
Phlorizin	Phlorizin	5.91

in the intracellular amino acid accumulation. This effect was dependent on the phlorizin concentration above $5 \cdot 10^{-4}$ M with an increasing effect observed up to $3 \cdot 10^{-3}$ M, the highest concentration employed in these studies. Little or no effect was seen below $5 \cdot 10^{-4}$ M.

The time course of the phlorizin effect on α-aminoisobutyric acid and glycine accumulation is shown in Fig. 1. Of particular interest is the fact that phlorizin causes little increase in intracellular amino acid accumulation until the lapse of 20 min of incubation time and that the principal effect is an increase in the distribution ratios at equilibrium. These results are identical to those obtained in the presence of glucose, 100 mg % (5 mM) in the medium.

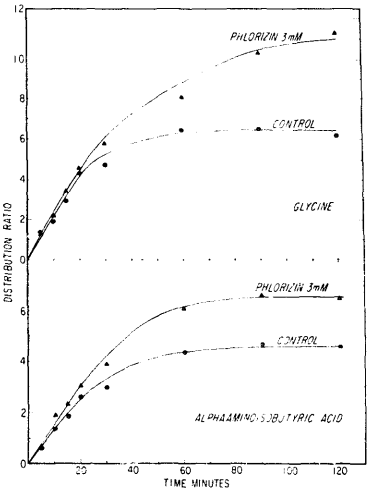


Fig. 1. Effect of phlorizin on the intracellular accumulation of glycine and α-aminoisobutyric acid by rat-kidney-cortex slices. The distribution ratio is the counts/min/ml intracellular fluid divided by counts/min/ml extracellular fluid.

Comparative effects of phlorizin, phloretin, and phloroglucinol on α-aminoisobutyric acid accumulation

Phloretin is the aglycone of phlorizin and studies were performed to ascertain the importance of the glycoside moiety for the effect on amino acid accumulation. In addition phloroglucinol, a principal component in phlorizin structure, was also included in the experiment. The results are shown in Table II. Contrary to the phlorizin enhancement of amino acid concentration in intracellular water, phloretin inhibited intracellular accumulation. Phloroglucinol had no effect. It is apparent, therefore, that the complete phlorizin molecule is necessary for the amino acid effect. It is

surprising that removal of the glucose moiety to form phloretin converts the molecule to a very potent inhibitor of amino acid transport. The nature of this inhibitory effect remains to be investigated.

Effect of preincubation with phlorizin on α-aminoisobutyric acid accumulation

Table III describes the results of an experiment in which tissue was preincubated with and without phlorizin for 45 min. At that time the tissues were removed, dipped rapidly in buffer, gently blotted and transferred to flasks which contained the labeled amino acid, phlorizin being omitted from one group of tissues which had previously been exposed to the drug. After incubation for an additional 60 min, tissues exposed to phlorizin during the preincubation period alone accumulated α-aminoisobutyric acid to the same extent as tissues incubated with phlorizin for the entire 105 min of the experiment. The possibility thus appears that phlorizin remained attached to the cell or caused a metabolic alteration during the preincubation which continued during the subsequent incubation period.

The effect of phlorizin in the presence of anaerobiosis

Previous studies have demonstrated that the concentrative transfer of amino acids in the kidney-cortex slice is dependent on aerobic metabolism and that under anaerobic conditions at equilibrium the ratio of intracellular to extracellular amino acids is approx. 1 (see ref. 10). This value would be expected on the basis of a diffusion process alone as an explanation for amino acid entry into intracellular fluid. Table IV shows the results of anaerobic incubation with and without phlorizin in the medium. In the control, the distribution ratio is slightly above 1 while in the presence of phlorizin the ratio is approx. 2. Since diffusion as a transfer mechanism cannot account for the existing concentration gradient between extra- and intracellular fluid, the existence of some other process for producing the gradient must be entertained.

TABLE IV

ACCUMULATION OF α-AMINOISOBUTYRIC ACID UNDER ANAEROBIC CONDITIONS

Incubation conditions as in Table I. Buffer was gassed with $N_2 - CO_2$ (95:5) and this gas mixture was used as the atmosphere to produce anaerobiosis, α-aminoisobutyric acid concentration 0.065 mM. In the third part of the experiment slices were incubated with $O_2 - CO_2$ (95:5) and then transferred to anaerobic flasks containing the same amount of α-[1-^{14}C]aminoisobutyric acid initially present in the aerobic incubation.

Incubation condition	*Incubation time (min)*	*Distribution ratio*	
		Control	*Phlorizin*
1. Anaerobic	90	1.36	1.97
2. Aerobic	90	5.09	8.48
3. Aerobic plus anaerobic	90 15	2.64	5.54

Other data worthy of note are to be found in Table IV. Transfer of slices after a 90-min aerobic incubation to a 15-min anaerobic period results in a very rapid efflux of amino acid from the cell water as evidenced by the marked decrease in the distribution ratio. At the end of the 15-min anaerobic period 52 % of the amino acid remained

in the intracellular fluid in the control whereas 66% remained in the presence of phlorizin. This suggested that phlorizin may be inhibitory to the loss of amino acid from the cell. Such a possibility will be discussed further below.

Michaelis–Menten analysis of the phlorizin effect on amino acid accumulation

Evidence exists[19] that biological transport systems consist of two general components, one that is saturable and one that follows Fick's law, the latter process being either physical diffusion or mediated passage by an overabundant reaction site. The first of these processes may be analyzed in the kidney slice by the Michaelis–Menten formulation[10,11]. The expression for this is according to AKEDO AND CHRISTENSEN[20]

$$Y = \frac{V_{max} A_f}{K_m + A_f}$$

where Y is the velocity of uptake by the saturable component and is expressed in the present experiments as mmoles per liter of intracellular water per 30 min, V_{max} the maximum velocity, K_m the apparent affinity of the binding site(s) and A_f, the the medium amino acid concentration. In the present studies Y was estimated as described previously[10,11] by subtracting a value equal to A_f to separate the diffusion factor from the saturable transfer process.

Although this type of analysis is more frequently used to study the effects of inhibitors on V_{max} or K_m it is applied here to a stimulatory process to ascertain if phlorizin alters the apparent affinity of the binding site(s) for transport. LINEWEAVER–BURK plots[21] of data with α-aminoisobutyric acid as substrate are shown in Fig. 2.

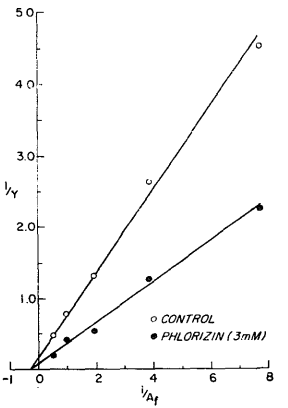


Fig. 2. LINEWEAVER–BURK plot of the effect of alteration of external α-aminoisobutyric acid concentration (A_f) on the saturable transfer, Y, with and without phlorizin. Duration of incubation was 30 min and triplicates were averaged. The reciprocals of the observed Y values (mmoles/l/30 min) are plotted against the reciprocal of the external concentration (A_f) expressed in mmoles/l.

The slope of the phlorizin α-aminoisobutyric acid curve is less, compatible with an enhanced accumulation of the amino acid. The intersection of the curves at an identical value on the abscissa indicates that no change of the K_m value of 3.3 mM was caused by phlorizin.

Analysis of the phlorizin effect on intracellular concentration when diffusion is the major mode of cellular amino acid penetration

AKEDO AND CHRISTENSEN[20] have published a method for the analysis of apparent diffusion constant in a system similar to that under present study. This involves plotting the distribution ratio A_c/A_f, where A_c and A_f are intracellular and extracellular fluid (or medium) concentration, respectively, against $1/A_f$. Extrapolation of the curve obtained at high A_f values to the ordinate indicates the distribution ratio obtained at infinitely large A_f concentrations. Where the time of incubation is sufficiently long and complete diffusion equilibration has occurred A_c/A_f reaches a value of 1, there being no concentration gradient possible due to diffusion alone[11].

A plot of A_c/A_f *vs.* $1/A_f$ for a 30-min incubation with α-aminoisobutyric acid is shown in Fig. 3. Extrapolation of the control curve to the ordinate gives a distribution ratio of 1.2 whereas this value in the presence of phlorizin is 1.6. The control value of 1.2 though close to 1 is above the theoretical value of 1 and suggests some degree of α-aminoisobutyric acid accumulation at infinite concentration. The ability of phlorizin to increase the extrapolated A_c/A_f to 1.6 cannot be explained by either physical diffusion or the small amount of saturable transport of α-aminoisobutyric acid at infinitely large A_f.

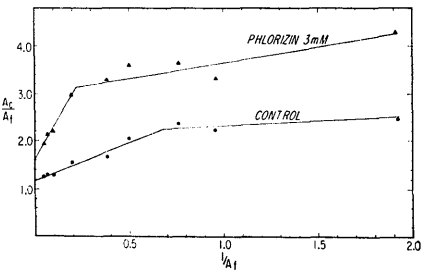


Fig. 3. Plot of the α-aminoisobutyric acid distribution ratio, A_c/A_f, where A_c is intracellular fluid concentration and A_f is the extracellular fluid (or medium) concentration, *versus* the reciprocal of the extracellular fluid concentration. Duration of incubation was 30 min, and triplicate determinations were averaged.

Model analysis of the phlorizin effect on transport

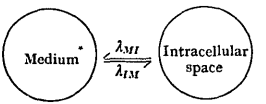
The multi-compartment model approach of BERMAN *et al.*[22] has been applied previously to the analysis of amino acid transport by rat-kidney-cortex slices[12]. With this procedure both influx and efflux rates of amino acid passage between the medium and intracellular fluid may be calculated. Application of this technique to data collected during experiments for α-aminoisobutyric acid and glycine uptake *vs.*

time shown in Fig. 1 resulted in values for the influx and efflux rate constants (λ_{IM} and λ_{MI}) shown in Table V. There is essentially no alteration in the entry rate but a decrease in the exit rate of the amino acids. This is reflected in the data in Fig. 1 which reveals little difference in the initial distribution ratios in the presence of phlorizin but an increase in the eventual equilibrium attained.

TABLE V

EFFECT OF PHLORIZIN ON KINETICS OF α-AMINOISOBUTYRIC ACID AND GLYCINE TRANSPORT IN RAT-KIDNEY-CORTEX SLICES

Incubation conditions as in Table I.



Amino acid	*Experimental condition*	*Fractional turnover rate** (min^{-1})*		*Difference from control (%)*	
		λ_{IM}	λ_{MI}	λ_{IM}	λ_{MI}
α-Aminoisobutyric acid	Control	0.00492 ± 0.00018	0.0290 ± 0.0020		
	Phlorizin (3 mM)	0.00521 ± 0.00020	0.0246 ± 0.0019	+6***	−15
Glycine	Control	0.00733 ± 0.00062	0.0425 ± 0.0058		
	Phlorizin (3 mM)	0.00707 ± 0.00044	0.0260 ± 0.0032	−3.5	−39

* Site of initial radioactivity.

** Expressed as turnover rate ± standard deviation: λ_{IM}, rate constant for movement into intracellular space from medium; λ_{MI}, rate constant for movement into medium from intracellular space

*** Not significant.

Study of efflux of amino acid from the kidney slice

In order to experimentally verify the above analysis indicating a specific effect of phlorizin on amino acid efflux an experiment was performed in which slices were incubated to equilibrium in buffer and then transferred to medium free of amino acid with and without phlorizin. The appearance of labeled amino acid was measured in the medium. Results are shown in Fig. 4 which is a semilogarithmic plot of the per cent of the equilibrium amount of amino acid remaining in the tissue after transfer to amino acid free solution *versus* time. It may be seen that in the presence of phlorizin the amino acid efflux occurs at a rate slower than that of the controls.

Other data related to efflux may be found in Table IV and has been mentioned in the section on anaerobiosis. The 52 and 66 % of the equilibrium value observed 15 min after transfer to anaerobic conditions in the absence and presence of phlorizin agrees very favorably to the corresponding 15-min values of 52 and 63 % found in Fig. 4.

Incorporation of amino acid into protein and oxidation to CO_2 in the presence of phlorizin

Table VI presents data on protein incorporation and oxidation of several amino acids. Phlorizin increased the amount of radioactivity appearing in $^{14}CO_2$ but inhibited the incorporation of amino acid into protein. Since the rate of protein synthesis is

only 1/70 that of the rate of influx of amino acid in this tissue and appears to be independent of the build up of the intracellular amino acid pool[12] the decrease in protein incorporation is in no way responsible for the increased accumulation seen with phlorizin. The increase in $^{14}CO_2$ in the presence of this drug may be a reflection of the increase in size of the intracellular amino acid pool.

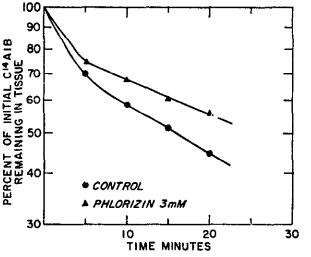


Fig. 4. The effect of phlorizin on the efflux of α-aminoisobutyric acid (AIB) from rat-kidney-cortex slices. Six flasks of kidney slices were incubated with α-[1-^{14}C]aminoisobutyric acid (0.13 mM) for 90 min under conditions described in Table I. At the end of this incubation period, during which complete equilibrium takes place, tissues in two flasks were removed for analysis of the distribution ratio, A_e/A_f. Tissues from two other flasks were transferred to 2 ml of α-aminoisobutyric acid-free buffer containing phlorizin 3 mM while tissues of the remaining two flasks were transferred to buffer without phlorizin. At the intervals shown, small samples of the media were removed from the latter flasks and assayed for radioactivity. From the distribution ratios for the group of tissues obtained after the initial 90-min incubation and the assay for α-aminoisobutyric acid of medium from which the other tissues had been transferred, the amount of α-aminoisobutyric acid in transferred tissues could be estimated. By assay of the media at intervals during the second period of incubation the cumulative release of α-aminoisobutyric acid from the slices was calculated. The ordinate represents the α-aminoisobutyric acid present in the slices at the start of the second incubation period minus the amount released into the medium divided by the amount present at the start × 100, plotted on a logarithmic scale.

TABLE VI

EFFECT OF PHLORIZIN ON AMINO ACID INCORPORATION INTO PROTEIN AND OXIDATION TO CO_2

Incubation conditions as in Table I. Phlorizin concentration was 3 mM. Radioactivity added per flask was L-lysine 0.37 μC, glycine 0.27 μC and L-phenylalanine 0.15 μC. Counting efficiency for protein was 44% and for CO_2 57%, 90 min incubation. Triplicate determinations were averaged.

Amino acid	*Concentration (mM)*	*Radioactivity*			
		Protein (counts/min/mg)		*CO_2 (counts/min/100 mg slice)*	
		Control	*Phlorizin*	*Control*	*Phlorizin*
L-Lysine	0.045	1465	982	35 350	62 466
Glycine	0.17	453	324		
L-Phenylalanine	0.075	684	392	42 450	69 500

Tissue specificity of the phlorizin effect on amino acid transport

Experiments to determine amino acid accumulation in the presence and absence of phlorizin were carried out using the isolated rat diaphragm and everted jejunal

segments. No stimulation of amino acid accumulation was seen in these tissues. Similar negative results with phlorizin in the diaphragm have been reported previously[23]. On the other hand, GUROFF has observed phlorizin enhancement of amino acid accumulation by brain slices[24].

DISCUSSION

Several types of experimental approaches have been utilized in an attempt to elucidate the mechanism whereby phlorizin enhances the intracellular accumulation of amino acid in cortical slices of rat kidney. The multi-compartmental model analysis has revealed that phlorizin inhibits the efflux of amino acid from the cell, and this conclusion received confirmatory evidence in direct studies of the efflux of amino acid from the cell. Consistant with this interpretation is the Michaelis–Menten analysis which indicated no alteration in the apparent K_m or affinity of the reactive site(s) for the amino acid. A change might be expected if phlorizin enhanced the saturable component for entrance into the cell.

In our experience with the kidney slice we have observed that compounds such as maleic acid[13] or dinitrophenol[12], reduced temperature[12] or competitive amino acids[11] have effects both on influx and efflux rates. Phlorizin is the first substance encountered which has a specific effect on efflux alone. Phlorizin is known to inhibit secretion of organic acids by kidney[25], an effect which may be analogous to the observed effect *in vitro* on efflux. Phlorizin itself is secreted by the tubular cell[25].

The basic mechanism of the phlorizin effect on efflux cannot be stated with certainty. Results obtained in the analysis of diffusion kinetics showing accumulation against a gradient as the substrate concentration approaches infinity suggest that underlying the phenomenon may be an increase in binding of amino acids in the cell. The enhancement of amino acid accumulation caused by phlorizin under anaerobic conditions lends some support to this idea.

Phlorizin is known to inhibit oxidative phosphorylation by homogenates of kidney cortex[26] at the concentrations used in the present studies. That such a metabolic alteration could explain the effect of phlorizin on amino acid accumulation appears unlikely in view of the fact that 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation, has the opposite effect, namely, to decrease accumulation by accelerating efflux from the cell[12]. In other instances, substances which inhibit oxidative metabolism reduce accumulation of substrate[19].

Phlorizin appears to stimulate kidney tubular transport of phosphate *in vivo*[27] as well as the accumulation of sulfate by kidney-cortex slices[28]. It is not clear at present whether the effect *in vivo* on phosphate is secondary to the prevention of glucose penetration by phlorizin. The mechanism of the effect on sulfate has not been elucidated. It is of interest to note that amino acids inhibit sulfate transport by the renal tubule[29]. The similar effect of phlorizin on the accumulation of both sulfate and amino acids may bespeak of common factors involved in transmembranous movement of these substrates.

The difference in the phlorizin and phloretin effect presented here is striking. The presence of the sugar moiety in the former completely alters the effect of the aglycone from a suppression to an enhancement of amino acid acumulation. Phlorizin and phloretin behave differently in other systems also. Sugar transport by kidney

is only slightly affected by phloretin but markedly inhibited by phlorizin[30]. The converse appears to be true for red cells[31].

Although there is suggestive evidence that in order to be effective phlorizin and derivatives must penetrate the cell[32], much evidence has been accumulated to support the thesis that phlorizin acts to inhibit sugar transport at the cell membrane. In the kidney slice, KRANE AND CRANE[5] showed that D-galactose uptake was prevented by phlorizin even under conditions where active transport was inhibited, and penetration occurred by diffusion alone. From what is known about inhibition of sugar transport by phlorizin[6] and from data presented here concerning the increase in amino acid accumulation, no connection between the two phenomena is apparent. In our studies the phlorizin effect on amino acid accumulation has been found to be independent of the presence of glucose in the medium.

The inhibition of kidney tubular reabsorption of glucose by phlorizin is easily discernible in experiments *in vivo*[3]. A stimulatory effect on amino acid clearance by the kidney *in vivo* on theoretical grounds should be difficult to detect since amino acids are so extensively reabsorbed under ordinary conditions. Indeed, no effect of phlorizin on amino acid clearance has been observed[33].

Two metabolic fates of amino acids, incorporation into protein and oxidation are affected by phlorizin. The recent kinetic analysis of these two fates with regard to their relationship to the accumulation of amino acid has revealed that oxidation is dependent on build up of the intracellular amino acid pool while incorporation into protein is not[12]. Therefore, since phlorizin increases the intracellular amino acid concentration, an increase in oxidation of amino acid might be expected. The inhibition of incorporation of amino acid into protein was not expected and the explanation for this phenomenon is not known. The divergence of the phlorizin effect on amino acid incorporation and oxidation is consistent, however, with the model previously proposed[12].

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