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INHIBITION OF AMINO ACID ACCUMULATION IN SLICES OF RAT KIDNEY CORTEX BY DIAMIDE

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SUMMARY

1. The oxidizing agent diazenedicarboxylic acid bis-(*N*,*N*-dimethylamide), common name diamide, has been used to lower the concentration of GSH in rat kidney cortex slices.

2. The ability to accumulate L-amino acids against a concentration gradient was decreased in diamide-treated slices.

3. Studies with six different amino acids have shown that this inhibition of amino acid accumulation is not specific for one amino acid or one group of related amino acids.

4. The addition of exogenous GSH to slices that have been treated with diamide leads to a significant recovery of the ability to concentrate amino acids.

5. Dithiothreitol and mercaptoethanol also reverse the effect of diamide, but are less effective than GSH.

INTRODUCTION

GSH, the tripeptide γ-glutamylcysteinylglycine, is found in large concentrations ubiquitously in nature. This fact has led to a great deal of speculation as to its possible metabolic roles beyond its well-established co-enzymatic activity. GSH has been implicated in such diverse phenomena as regulation of hexose monophosphate shunt activity [1, 2] protection against radiation damage [3], participation in mitotic events [4], regulation of protein [5–7] and RNA [8] synthesis and cataract formation [9]. In view of the fact that GSH represents over 95 % of the acid-soluble, non-protein sulfhydryl content of most tissues [10], these divergent effects of GSH may be related to the oxidation–reduction state of the cell.

Kosower et al. [11] have synthesized various reagents in an attempt to find one that is specific for the oxidation of GSH [11]. Diazenedicarboxylic acid bis-(*N*,*N*-dimethylamide), commonly called diamide, has been shown to be highly specific for GSH, although there is some suggestion that oxidation of NADPH also

Abbreviation: DTNB, 5,5-dithio-bis-(2-nitrobenzoic acid).

occurs [12]. Addition of diamide to human red blood cells led to stoichiometric oxidation of intracellular GSH, and the conversion of GSH to GSSG was very rapid even at low temperature. This effect of diamide on red cell GSH was completely reversible [11]. Diamide has also been used to lower GSH levels in the lens of the rat [13], in Ehrlich ascites tumor cells [14] and in adipose tissue cells [15].

Many studies on the effects of sulfhydryl reagents such as *p*-chloromercuribenzoate, and its sulfonate, and *N*-ethylmaleimide on sugar and amino acid transport have been reported. However, these reagents have been demonstrated to act primarily at the membrane level [16], whereas diamide easily penetrates the cell [11, 13] and has been demonstrated to react with intracellular GSH. A role for GSH in the transport and absorption of amino acids in kidney and small intestine was suggested by Binkley [17, 18] several years ago. More recently Orlowski and Meister [19] presented a model for amino acid transport based on GSH and the enzymes involved in its metabolism. We undertook the present investigation to explore the effect of diamide on amino acid accumulation in slices of rat kidney cortex. Preliminary reports of this work have been presented [20, 21].

EXPERIMENTAL PROCEDURE

Materials

Sprague–Dawley rats weighing from 200–300 g were used in all experiments. Reagents were obtained as follows: mercaptoethanol from Eastman–Kodak Co.; diamide, 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) and dithiothreitol from Nutritional Biochemicals Corp.; GSH, L-[^{14}C]lysine (spec. act. = 315 Ci/mole), and L-[^{14}C]glycine (spec. act. = 106 Ci/mole) from Schwarz-Mann; L-[^{14}C]leucine (spec. act. = 216 Ci/mole) and L-[^{14}C]arginine (spec. act. = 216 Ci/mole) from Cal Atomic; α-amino[^{14}C]isobutyric acid (spec. act. = 56 Ci/mole) from ICN Corp.; L-[^{14}C]methionine (spec. act. = 41.3 Ci/mole) from New England Nuclear; sodium [^{131}I]iodohippurate (spec. act. = 0.17 mCi/mg) from E. R. Squibb and Sons; inulin from Matheson, Coleman and Bell. All other chemicals were reagent grade and were purchased from Fisher Scientific Co.

Tissue preparation

Animals were fed Purina rat chow and water ad libitum until sacrificed by cervical fracture. The kidneys were quickly removed and placed in a beaker containing 0.90 % NaCl on ice. The capsule was removed and the kidney was bisected. A Stadie–Riggs tissue slice apparatus was then used to make three cortical slices from each hemi-kidney, the first of which was discarded. Each rat yielded eight slices of renal cortex and a typical experiment involved three rats.

Amino acid uptake

Uptake experiments were run as follows: the slices were placed into eight 25-ml flasks which contained either 3.0 ml of Krebs–Ringer bicarbonate buffer or 3.0 ml of $3 \cdot 10^{-2}$ M diamide in Krebs–Ringer bicarbonate buffer. The buffer was gassed for 1 h with O_2–CO_2 (95 : 5, v/v) before use, and the amount of $NaHCO_3$ originally added was adjusted to give a final pH of 7.4. The flasks were sealed, placed in an ice bucket, and shaken for 15 min (Incubation 1). The slices were removed from

the flasks, dipped in Krebs–Ringer bicarbonate buffer and placed in new 25 ml flasks that contained either 3.0 ml Krebs–Ringer bicarbonate buffer or 3.0 ml of $2 \cdot 10^{-2}$ M GSH in Krebs–Ringer bicarbonate buffer. The flasks were sealed and shaken at room temperature for 15 min (Incubation 2). The slices were then removed from the flasks, dipped in Krebs–Ringer bicarbonate buffer and placed in new 25-ml flasks that contained 2.0 ml Krebs–Ringer bicarbonate buffer plus an amino acid at the following concentrations: lysine, 65 μM and 0.083 μCi/ml; leucine, 55 μM and 0.079 μCi/ml; arginine, 65 μM and 0.032 μCi/ml; glycine, 83 μM and 0.042 μCi/ml; methionine, 100 μM and 0.055 μCi/ml; α-aminoisobutyric acid, 65 μM and 0.085 μCi/ml. The flasks were gassed with O_2–CO_2 (95 : 5, v/v) for 15 s, sealed and placed in a Dubnoff metabolic shaker bath at 37 °C for various times (uptake period). At the appropriate time, the flask was removed from the water bath, the tissue slices were removed from the flasks, dipped in Krebs–Ringer bicarbonate buffer, and gently blotted. The slices were weighed and placed in conical glass centrifuge tubes containing 2.0 ml of distilled water. The centrifuge tubes were covered, placed in a boiling water bath for 10 min, cooled, and centrifuged at 3000 rev./min for 10 min. A 0.2-ml aliquot of the supernatant fraction, and of the media used for incubation, were placed in counting vials with 2.8 ml of absolute ethanol, and 7.0 ml of a solution of toluene, PPO (0.457 %) and POPOP (0.010 %). The vials were sealed, shaken, and counted in a Tricarb liquid scintillation spectrometer (with a counting efficiency of 83 %). In those experiments involving hippuran accumulation, sodium[^{131}I]iodohippurate was used. Aliquots of the aqueous tissue supernatant and media were counted in a Packard scintillation γ counter.

Distribution ratios

The distribution ratios were calculated according to Rosenberg et al. [22]. Total tissue water was determined by the difference between tissue weight after blotting and the weight after freeze-drying for 24 h. Extracellular space measurements were made using inulin [23]. Chromatography of tissue extracts in butanol–acetic acid–water (4 : 1 : 2, v/v/v) using a descending paper system revealed that over 90 % of the radioactivity appeared at the appropriate R_F for the amino acid studied.

Concentration of GSH

The concentration of GSH was estimated by the following procedure: tissue slices were weighed and placed in a Kontes glass homogenizer with sufficient 6 % trichloroacetic acid to give a 5 % homogenate. The homogenate was spun in an International clinical centrifuge (3000 rev./min) for 10 min. An aliquot of the supernatant was diluted to 2.0 ml with 6 % trichloroacetic acid, and 8.0 ml of 0.3 M Na_2HPO_4, and 1.0 ml of 0.04 % DTNB in 1 % sodium citrate were added. The increase in absorbance which developed was then measured on a Beckman DB spectrophotometer at 412 nm. GSH content was determined by comparison with a standard curve. The concentration of GSH did not appear to be affected by the presence of amino acids in the incubation medium and the data presented are from a series of experiments with Krebs–Ringer bicarbonate buffer in the incubation mixture.

RESULTS

Effect of diamide on GSH levels

The effect of diamide treatment on the level of reduced glutathione in rat kidney cortex slices is shown in Fig. 1. It can be seen that the level of GSH steadily decreases in slices incubated in Krebs–Ringer bicarbonate buffer. However, slices that are incubated in diamide show an even more marked decrease in the level of GSH. Subsequent incubation in Krebs–Ringer bicarbonate buffer allows the tissue to regenerate a significant amount of GSH, but the level never reaches that of untreated slices. In addition, slices not removed from diamide show no regeneration of GSH, and the concentration of reduced glutathione remained at less than 0.05 mg/g tissue (unpublished data). It should be noted that high concentrations ($3 \cdot 10^{-2}$ M) of diamide are used in this experiment. These high concentrations permit the use of short incubation times (15 min) and reduced temperatures (4 °C) and still cause a complete oxidation of the GSH in the tissue. Subsequently, it will be demonstrated that even higher diamide concentrations are necessary to completely inhibit amino acid accumulation when using these techniques. The data referred to in Fig. 1 were obtained using DTNB which reacts with nonprotein sulfhydryl groups other than GSH. However, Revesz and Modig [10] have demonstrated that over 98 % of the acid-soluble sulfhydryl content of cells is GSH. This fact coupled with the demonstration that diamide lowers the DTNB-reactive material of tissue by approx. 95 % is a strong indication of the suitability of diamide as a GSH-oxidizing agent.

Amino acid accumulation

Total tissue water was calculated to be 80.0±0.2 %, while extracellular space was found to be 28.0±1.2 %, and did not vary significantly with the different conditions of incubation. From these values, the intracellular space of 52 % was determined.

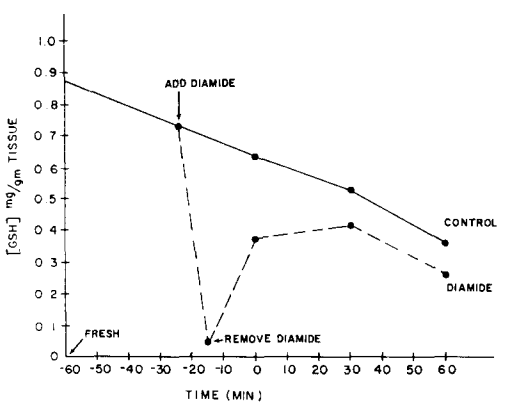

Fig. 1. Glutathione concentration in control and diamide-treated slices of rat kidney cortex. Slices, prepared as described under Materials and Methods, were incubated in Krebs–Ringer bicarbonate buffer in the absence or presence of diamide ($3 \cdot 10^{-2}$ M) for 15 min at 4 °C. Slices were incubated in buffer for 15 min at 25 °C, and then transferred to fresh buffer and incubated at 37 °C for an additional 60 min. The slices were removed at various times and GSH was determined as described under Materials and Methods. Time 0 refers to the start of the incubation at 37 °C.

Using this information, a distribution ratio could be calculated for uptake experiments as follows:

$$\text{distribution ratio} = \frac{\text{cpm/ml intracellular fluid}}{\text{cpm/ml extracellular fluid}}$$

Fig. 2A shows the distribution ratios calculated for L-[^{14}C]methionine in slices of rat kidney cortex in the presence and absence of diamide. It can be seen that slices which were incubated in $3 \cdot 10^{-2}$ M diamide show significantly lower distri-

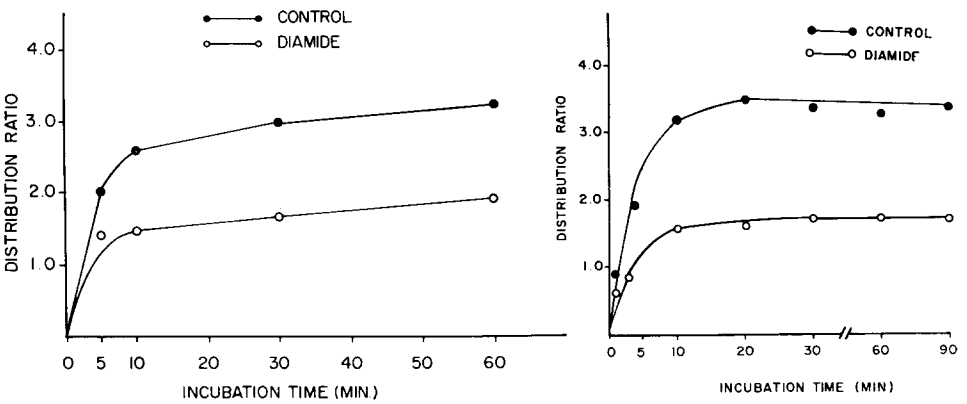

Fig. 2. Accumulation of L-methionine (A) and L-lysine (B) in control and diamide-treated slices of rat kidney cortex. Tissues were preincubated in the presence and absence of diamide for 15 min at 4 °C. They were then incubated in fresh Krebs–Ringer bicarbonate for 15 min at 25 °C and then placed in flasks containing 100 μM L-methionine or 65 μM L-lysine and incubated at 37 °C for various time intervals.

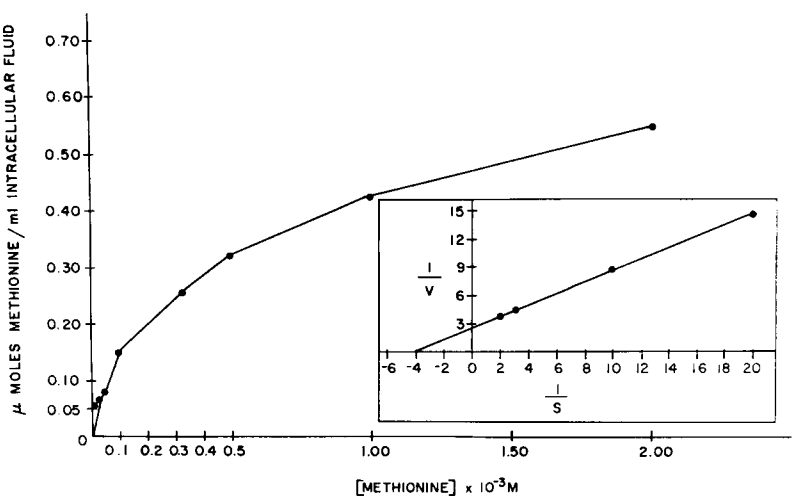

Fig. 3. Effect of L-methionine concentration on the velocity of L-methionine uptake. Rat renal cortex slices were incubated with varying amounts of L-[^{14}C]methionine for 60 min. The ordinate in this graph refers to the μmoles of L-methionine accumulated/ml intracellular fluid, calculated by using the specific activity of the ^{14}C-labelled amino acid, and corrected for diffusion. The inset shows a double reciprocal plot of the data.

bution ratios. Fig. 2B shows similar data for L-lysine; in this case, samples were measured for radioactivity after a very short exposure to the labeled amino acid. From these experiments it can be seen that diamide affects the initial rate of uptake of amino acids as well as the steady-state level of accumulation. These two figures show a similar effect of diamide on two amino acids which are not believed to share the same transport mechanism [24].

Fig. 3 shows the effect of methionine concentration on the velocity of accumulation of this amino acid by kidney slices. The results shown have been corrected for diffusion, and are consistent with a saturable, carrier-mediated process [22]. From the data of the Lineweaver–Burk plot presented in the inset of Fig. 3, a K_m of $2.7 \cdot 10^{-4}$ M can be calculated.

In order to obtain further information about the specificity and reversibility of the diamide inhibition of amino acid uptake, the following experiment was undertaken. Duplicate samples were incubated at 4 °C for 15 min in the presence or absence of diamide and then incubated at 25 °C for 15 min in the presence or absence of GSH. All samples were then incubated for 60 min at 37 °C with a ^{14}C-labeled amino acid. Table I shows the results of these experiments; the values are listed as percent of control distribution ratios in order to facilitate comparisons between amino acids which have a wide range of distribution ratios. It is evident that incubation in the presence of reduced glutathione leads to quite diverse effects, most notably a 25 % increase in the ability of tissues to accumulate [^{14}C]glycine. The effect of diamide treatment, however, is shown to be remarkably consistent for these six amino acids. Since a distribution ratio of 1.0 can be expected from simple diffusion, the decrease of approx. 40 % of the distribution ratio presented in Table I actually reflects an inhibition of active transport of over 60 % in some cases. Finally, it can be seen that the

TABLE I

EFFECT OF DIAMIDE ON THE DISTRIBUTION RATIOS FOR SEVERAL AMINO ACIDS

Tissues were incubated in Krebs–Ringer bicarbonate buffer with or without diamide ($3 \cdot 10^{-2}$ M) for 15 min at 4 °C (Incubation 1). Tissues were then placed in Krebs–Ringer bicarbonate buffer with or without GSH ($2 \cdot 10^{-2}$ M) for 15 min at 25 °C (Incubation 2). The tissues were then incubated for 60 min at 37 °C in the presence of the labelled and unlabelled amino acid. Samples which were incubated in buffer only served as controls and were assigned a value of 100 %. The distribution ratios calculated for the treated samples were then compared with these control values. The number of experiments is in parentheses.

Incubation 1	Incubation 2	% of control distribution ratio					
		Arg	Lys	Leu	Gly	Met	α-Amino-isobutyric acid
Krebs–Ringer bicarbonate buffer	GSH	113 (4)	95 (15)	97 (8)	125 (4)	112 (8)	101 (14)
Diamide	Krebs–Ringer bicarbonate buffer	67 (10)	59 (9)	55 (8)	63 (4)	59 (9)	54 (15)
Diamide	GSH	87 (6)	87 (11)	83 (8)	94 (4)	83 (8)	73 (14)

effect of incubation with diamide is reversible. Diamide-treated slices which are subsequently incubated in the presence of GSH demonstrate a significant recovery in their ability to accumulate amino acids. These results indicate that diamide has a reversible effect on tissue slices.

In order to determine if the reversal of the diamide inhibition of uptake was specific for GSH or if other sulfhydryl compounds are equally effective, dithiothreitol and mercaptoethanol were added to diamide-treated slices. The results in Table II indicate that dithiothreitol and mercaptoethanol can reverse the inhibition of amino acid uptake caused by diamide, but are less efficient than GSH. The reversibility of the effects of diamide would strongly indicate that diamide is not lowering amino

TABLE II

THE EFFECT OF THIOL COMPOUNDS ON THE DISTRIBUTION RATIO OF METHIONINE IN DIAMIDE-TREATED SLICES

Slices were incubated in Krebs–Ringer bicarbonate buffer in the absence or presence of $3 \cdot 10^{-2}$ M diamide for 15 min at 4 °C (Incubation 1). Tissues were then placed in Krebs–Ringer bicarbonate buffer with or without thiol compounds ($2 \cdot 10^{-2}$ M) for 15 min at 25 °C (Incubation 2). The tissues were then incubated for 60 min at 37 °C in the presence of labelled and unlabelled L-methionine. Distribution ratios were calculated as under methods. Values represent mean distribution ratio ± S.E. from four separate experiments.

Incubation 1	Incubation 2	Distribution ratio
Krebs–Ringer bicarbonate buffer	Krebs–Ringer bicarbonate buffer	2.87±0.20
Diamide	Krebs–Ringer bicarbonate buffer	1.54±0.16
Diamide	GSH	2.60±0.19
Diamide	Dithiothreitol	2.24±0.14
Diamide	Mercaptoethanol	2.12±0.19

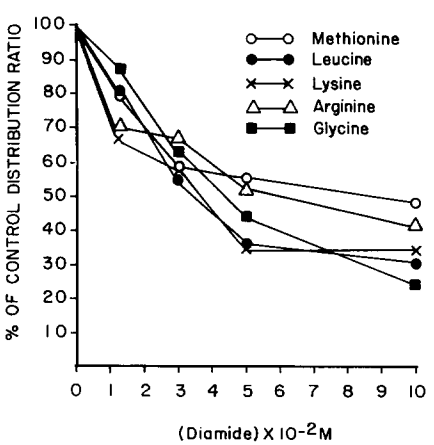

Fig. 4. The effect of diamide concentration on the active accumulation of amino acids. Rat renal cortex slices were placed in various concentrations of diamide for 15 min at 4 °C. The slices were placed in fresh buffer for 15 min at 25 °C, and subsequently incubated with a labelled amino acid for 60 min at 37 °C. Slices which are not treated with diamide were run simultaneously as controls. Results are presented as percent of control distribution ratios.

acid uptake by virtue of tissue toxicity or membrane damage. A further indication that diamide does not lead to tissue damage was found in our experiments with *p*-aminohippuric acid uptake. Tissues were incubated in the absence and presence of 30 mM diamide as described under Materials and Methods. Control slices gave a distribution ratio of 7.51 ± 0.31 as compared to diamide treated slices which gave a ratio of 7.13 ± 0.38. The uptake of *p*-aminohippuric acid was used as an indication of mitochondrial function [25] and demonstrated that diamide treated slices were capable of concentrative uptake.

The effect of increasing diamide concentration on the distribution ratios of various amino acids was also investigated. The results presented in Fig. 4 show that a value of $3 \cdot 10^{-2}$ M which was used in all the other experiments produced approx. a 40 % reduction of distribution ratios for all five amino acids tested. The values are again presented as percent of control distribution ratios (diamide concn = 0), to facilitate comparisons.

DISCUSSION

The use of diamide as a thiol-oxidizing agent has increased over the past several years, because of its specificity for reduced glutathione, and the ease with which its effects can be reversed in biological systems, in marked contrast to other sulfhydryl reagents. The ability of various sulfhydryl reagents to inhibit amino acid transport is well documented [16]. This is believed to be the first report demonstrating inhibition of amino acid transport by diamide in a mammalian system.

The transport of amino acids is believed to be mediated by membrane proteins. Much work in the past has demonstrated that sulfhydryl reagents can interfere with amino acid uptake, and hence that there is a sulfhydryl group(s) associated with the activity of transport proteins. Evidence has been presented in this investigation that the uptake of amino acids by slices of rat renal cortex is inhibited after incubation of tissue in diamide. Since diamide does not react appreciably with protein thiol groups [26] this observation indicates that the integrity of these membrane sulfhydryl groups, and of the transport process itself, is dependent on reduced glutathione, a suggestion made many years ago [27].

The inhibitory effect of diamide on amino acid uptake can be explained in several ways: (1) Diamide may react with membrane sulfhydryl groups which are an essential component of the amino acid transport process. (2) Intracellular GSH may be a necessary component of the amino acid transport system and diamide might inhibit transport by reacting specifically with GSH. (3) Diamide may disrupt the normal metabolic processes of kidney slices which are required to produce the energy involved in active transport. (4) Diamide may react specifically with intracellular GSH, and the lack of GSH secondarily produces an oxidation of membrane sulfhydryl groups.

The first alternative, that diamide reacts with membrane sulfhydryl groups, cannot be completely ruled out. The work of Kosower and his associates [11, 26], however, indicates that diamide is highly specific for the oxidation of GSH. Furthermore, results in Table II demonstrate that the addition of exogenous thiol compounds to diamide-treated slices does not lead to complete recovery of amino acid uptake. If diamide were reacting merely with membrane sulfhydryl groups then one would

expect complete reversibility of its effects, as has recently been shown for *p*-chloromercuribenzoic acid sulfonate [28].

Meister [30] has proposed that glutathione serves as a donor of γ-glutamyl groups in a cyclic series of enzymatic reactions which mediate amino acid transport. The effect of diamide on amino acid transport in kidney slices cannot be explained in terms of this cycle for two reasons. Firstly, γ-glutamyl transpeptidase isolated from the kidney is capable of using GSSG as a γ-glutamyl donor [29], and hence conversion of GSH to GSSG should not inhibit amino acid uptake in this tissue. Secondly, the inhibition of amino acid transport seen after diamide treatment was also found for the non-metabolizable amino acid α-aminoisobutyric acid which is not an acceptor of the γ-glutamyl moiety [30]. The inhibition of α-aminoisobutyric acid uptake by diamide therefore cannot be explained in terms of a decrease of γ-glutamyl cycle activity.

The possibility that diamide disrupts the metabolic activity of tissues must be considered. Studies by Epstein and Kinoshita [13] in the lens and by Kosower et al. [11] in erythrocytes have shown no such inhibition of metabolic activity to occur. The observation that diamide treated slices have the ability to regenerate significant quantities of GSH and to accumulate hippuran against a concentration gradient would also suggest that energy yielding reactions have not been interfered with.

Finally the possibility that diamide reacts specifically with intracellular GSH, and that the inhibition of amino acid uptake is a secondary result, should be considered. Epstein and Kinoshita [13], in explaining the inhibition of Rb^+ transport in the lens following diamide treatment, have proposed the following scheme: (1) Diamide reacts with intracellular GSH, converting it to GSSG. (2) Membrane sulfhydryl groups are oxidized in normal metabolic processes to form disulfides. (3) GSH is usually present in large enough quantities in tissues to reduce these disulfides on membrane proteins, but in diamide-treated slices, there is less GSH available and the membrane proteins remain in the disulfide form. This explanation of diamide inhibition of transport appears to be the most attractive.

It is interesting to note that the results of the present investigation with amino acid uptake in slices of rat kidney cortex can be interpreted on the same basis as those obtained in the studies of Rb^+ transport in the lens. Recent work in our laboratory has indicated that diamide also inhibits the active accumulation of α-methyl-D-glucoside in slices of rat and rabbit kidney cortex (Pillion, D., Hewitt, J. and Leibach, F., manuscript in preparation). The fact that diamide can inhibit the transport of cations, several amino acids and α-methyl-D-glucoside is a strong argument in favor of the concept that GSH serves as an intracellular reducing agent for membrane proteins, and suggests that diamide may be a useful agent for transport studies in many different tissues.

In this investigation, slices of rat renal cortex were used. The tissue slice inherently has the disadvantage of containing cut surfaces. Hence the results presented here may not reflect the in vivo situation. Studies are currently in progress in which the effects of diamide on the uptake of sugars and amino acids in the perfused kidney, as well as in kidney slices, are being investigated.

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REFERENCES

1 Jacob, H. S. and Jandl, J. H. (1966) J. Biol. Chem. 241, 4243–4250
2 Eggleston, L. V. and Krebs, H. A. (1974) Biochem. J. 138, 425–435
3 Revesz, L., Bergstrand, H. and Modig, H. (1963) Nature 198, 1275–1277
4 Mazia, D. (1961) in The Cell (Brachet, J. and Mirsky, A. E., eds), Vol. 3, p. 77, Academic Press, London
5 Nolan, R. D. and Hoagland, M. B. (1971) Biochim. Biophys. Acta 247, 609–620
6 Harris, J. W. (1969) Exp. Cell Res. 56, 299–303
7 Kosower, N. S., Vanderhoff, G. A. and Kosower, E. M. (1971) Biochim. Biophys. Acta 272, 623–637
8 Zehavi-Willner, T., Wax, R. and Kosower, E. M. (1970) FEBS Lett. 9, 100–102
9 Kinoshita, J. H. (1964) Arch. Opthalmol. 72, 554–572
10 Revesz, L., and Modig, H. (1965) Nature 207, 430–431
11 Kosower, N. S., Kosower, E. M. and Wertheim, B. (1969) Biochem. Biophys. Res. Commun. 37, 593–596
12 Brown, J. S. (1971) Biochem. J. 124, 665–667
13 Epstein, D. L. and Kinoshita, J. H. (1970) Invest. Opthalmol. 9, 629–638
14 Biaglow, J. E. and Nygaard, O. F. (1973) Biochem. Biophys. Res. Commun. 54, 874–881
15 Czech, M. P., Lawrence, Jr, J. C. and Lynn, W. S. (1974) J. Biol. Chem. 249, 1001–1006
16 Rothstein, A. (1970) in Current Topics in Membranes and Transport, Vol. 1, pp. 135–176, Academic Press, New York
17 Binkley, F. (1951) Nature 167, 888–889
18 Binkley, F. (1954) In Glutathione (Colowick, S., Lazarow, A., Racker, E., Schwartz, D. R., Stadtman, E. and Waelsch, H., eds), p. 160, Academic Press, New York
19 Orlowski, M. and Meister, A. (1970) Proc. Natl. Acad. Aci. U.S. 57, 1248–1255
20 Hewitt, T. and Leibach, F. (1973) Fed. Proc. 32, 4082
21 Pillion, D. and Leibach, F. (1973) Am. Chem. Soc. SE Regional Meet. Charleston, S. C., 175
22 Rosenberg, L. E., Blair, A. and Segal, S. (1961) Biochim. Biophys. Acta 54, 479–488
23 Walser, M., Davidson, D. G. and Orloff, J. (1955) J. Clin. Invest. 34, 1520–1523
24 Christensen, H. N. (1969) Adv. Enzymol. 32, 1–20
25 Reimer, K. A. and Jennings, R. B. (1971) Lab. Invest. 25, 176–184
26 Kosower, E. M. and Kosower, N. S. (1969) Nature 224, 117–120
27 Barron, E. S. G. and Singer, T. P. (1943) Science 97, 356–358
28 Schaeffer, J. F., Preston, R. L. and Curran, P. F. (1973) J. Gen. Physiol. 62, 131–146
29 Hanes, C. S., Hird, F. J. R. and Isherwood, F. A. (1952) Biochem. J. 51, 25–35
30 Meister, A. (1973) Science 180, 33–39