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2,4-Dichlorophenoxyacetic Acid (2,4-D) and Paranitrophenol (PNP) Interactions with Gills of *Anodonta californiensis* and *Mytilus californianus:* Uptake and Effects on Membrane Fluxes

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The effects of pesticides and their breakdown products on membrane fluxes is a subject of interest in assessing the potential ecological impact of these substances. As part of a continuing program we have examined the effects of 2,4-dichlorophenoxyacetic acid (2,4-D) and paranitrophenol (PNP) on divalent cation and primary amine losses from and glycine uptake by gills of the bivalve molluscs *Anodonta californiensis* (fresh water) and *Mytilus californianus* (marine). Both 2,4-D and PNP reduce glycine influx into gills of *M. californianus*. This observation is consistent with the view that glycine is bound to the gill surface as a Mg^{2+} complex prior to active transport.

For gills of *A. californiensis*, Ca^{2+} and Mg^{2+} losses are increased by 10^{-3} *M* 2,4-D relative to that into distilled water. Primary amine losses are increased for both *A. californiensis* and *M. californianus* gills at low 2,4-D concentrations.

For *A. californiensis* and *M. californianus* gills the uptake of both 2,4-D and PNP are reduced by increasing concentrations of Ca^{2+} and Mg^{2+}. In the case of *A. californiensis*, Ca^{2+} has a larger effect than Mg^{2+}, which is consistent with the demonstrated stabilizing effect of Ca^{2+} on biological membranes. The uptake of PNP is larger than that of 2,4-D and glycine for gills of *A. californiensis*. The uptake of 2,4-D is reduced by the presence of an excess concentration of glycine but this is probably a physical effect. The uptake of 2,4-D, PNP, and glycine are all passive processes for *A. californiensis* gills. Glycine does not reduce 2,4-D uptake into *M. californianus* gills. For *M. californianus* the transport mechanism for glycine is not the same as that for 2,4-D; the former being active transport and the latter passive transport. However, 2,4-D does interfer with the active transport of glycine.

INTRODUCTION

The outer membrane of the gills of bivalve molluscs are the sites of active and passive transport of a variety of chemicals between the organism and the surrounding aquatic environment. Chemicals involved in such transport processes are often critical to the biological functions of the organism. An alteration in the balance of these processes by pollutant-induced inhibition of influxes and/or increases in effluxes can disrupt the biological processes dependent on these fluxes. The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) has been shown to be toxic to animals (1). 2.4-D disrupts a number of biological processes (2, 3) and degenerates muscle tissue (4, 5). The interaction of this chemical with membranes and its effect on membrane fluxes would be of interest in light of these observations.

The specific aims of this work are to determine what effects 2,4-dichlorophenoxyacetic acid (2,4-D), a herbicide, and paranitrophenol (PNP), a breakdown product of some agriculturally applied chemicals, have on membrane fluxes of gills of the bivalve molluscs *Anodonta californiensis* (freshwater) and *Mytilus californianus* (marine). Additionally, information on the uptake of these chemicals and factors affecting their uptake will be determined.

This work will identify pollutants which strongly effect membrane fluxes. In previous work on the effects of heavy metals on membrane fluxes of gills of *M. californianus* (6), it has been shown that Hg^{2+}

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causes excessive losses of primary amines. These primary amine losses are, in fact, an indication of the presence of Hg^{2+}. The mechanism of this efflux process is currently under investigation and it is the ultimate aim of this continuing study to understand at a molecular level the mechanisms by which these pollutants affect membrane fluxes.

MATERIALS AND METHODS

Mussels and solutions. Specimens of *Anodonta californiensis* were collected in irrigation canals approximately 5 miles north of Knights Landing, California and *Mytilus californianus* were collected from the open coast at the Bodega Marine Laboratory, University of California, Bodega Bay, California. Specimens were used within a week of collection and were maintained in aquaria having water and temperature conditions of the natural environment.

The artificial sea water (ASW), and Ca^{2+} and Mg^{2+} = deficient sea waters used in experiments involving *M. californianus* are described elsewhere (7). Additionally, ASW solutions of varying pH were used in which the pH was adjusted with hydrochloric acid. The medium used for experiments on gills of *A. californiensis* was distilled water, or distilled water containing Ca^{2+} or Mg^{2+}. The divalent cations were added as the chloride hydrates and the concentrations of the divalent cations were determined by atomic absorption spectroscopy. To these solutions the organic compounds glycine, 2,4-D, and PNP with or without their radiotracers were added, and the uptake or loss experiments described below were carried out.

Glycine, 2,4-D, and PNP uptake experiments. When the uptake of a particular organic compound was to be studied , a solution 10^{-4} *M* in that compound containing a small concentration of the carbon-14 labeled radiotracer was prepared. Typically the concentration of the radiotracer was about 10^{-8} *M*. In some experiments another organic compound was added to this solution in order to determine the effect of the compound on the uptake of the radiolabeled material.

Prior to the experiments, excised gills were treated in the following manner. Gills of the freshwater species *A. californiensis* and the marine species *M. californianus* were incubated in unchlorinated natural freshwater and ASW, respectively, for periods of up to 30 min. The gills were then allowed to drain, dried lightly on a paper towel, and placed in the solution used in the experiment. The beakers containing the experimental media and gills were placed on a shaker table. At predetermined times gill samples were removed from the solution, washed for several seconds in the experimental solution devoid of the organic compounds, and blotted on filter paper. The gills were placed on tared scintillation vials, dried to constant weight at 60–70°C, and weighed to the nearest 0.1 mg. One milliliter of 0.1 *M* nitric acid was added to the dried gills; the mixture was allowed to stand for 2 hr; 10 ml of scintillation fluid was added; the mixture was incubated for at least 12 hr; and the radioactivity of the vials was determined with a scintillation counter. The data were computed as micromoles of organic compound uptake per gram dry weight of sample using the activity of a known volume of the experimental solution, and thus a known amount of organic material, before the gills were added. All data points are an average of two experiments with each point run in duplicate.

Experiments involving divalent cation and primary amine losses. The experiments were carried out as described in the previous section except radiolabeled compounds were not used and aliquots were removed from the experimental solution at predetermined intervals. These aliquots were analyzed for primary amines, Ca^{2+} and Mg^{2+}. Primary amines were determined by the fluorescamine procedure of Udenfriend *et al.* (8), as modified by North (9), using a Turner II fluorometer. The con-

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RESUL

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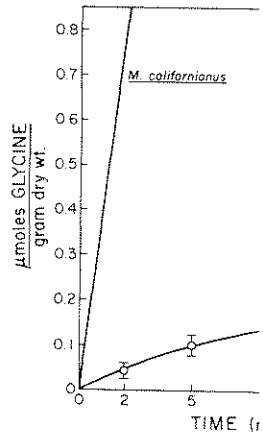

FIG. 1. *Uptake of 10^{-4} M g*
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centrations of primary amines were calculated using glycine standards and were expressed as "glycine equivalents" (10–12). The concentrations of the divalent cations were measured with an Instrumentation Laboratory Model 751 atomic absorption spectrometer.

RESULTS

Incorporation of 2,4-D and PNP, and Glycine as Affected by 2,4-D and PNP

As a reference point for the results on the incorporation of 2,4-D and PNP to be reported in this paper, Fig. 1 shows the uptake of 10^{-4} *M* glycine into gills from *M. californianus*, a marine bivalve mollusc (7), and *A. californiensis* (13), a freshwater bivalve mollusc. The uptake experiments reported in this paper were carried out at the same concentrations as the glycine experiments reported in Fig. 1.

A. californiensis. Figure 2 displays the uptake of 2,4-D as affected by the divalent cations Ca^{2+} and Mg^{2+}, and the uptake of PNP. Figure 2 shows that the incorporation of 2,4-D at 10^{-4} *M* is reduced relative to that in distilled water by 10^{-3} *M* Ca^{2+} and Mg^{2+}. Calcium^{2+} is more effective than

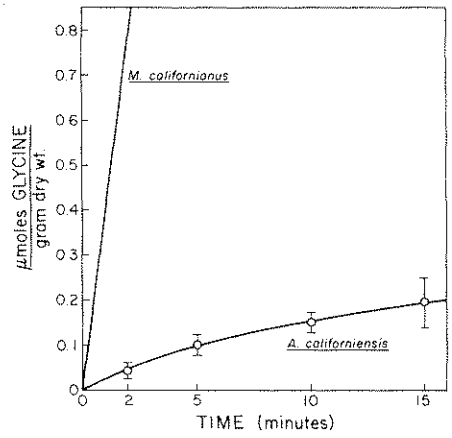

FIG. 1. *Uptake of 10^{-4} M glycine into gills of M. californianus and A. californiensis. Volume 200 cc. Line for M. californianus influx comes from previously published data (6). A. californiensis data are average of two experiments with each point run in duplicate.*

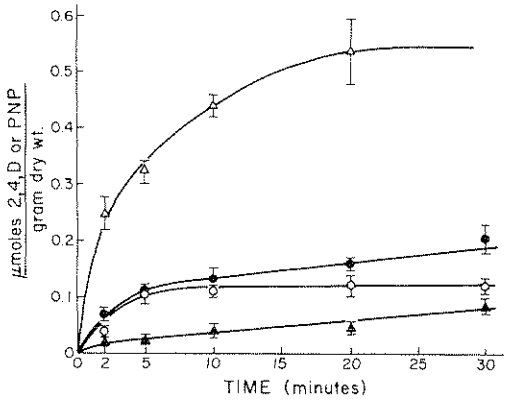

FIG. 2. *Uptake of 10^{-4} M PNP (△), and 10^{-4} M 2,4-D (●) into gills of A. californiensis as affected by added 10^{-3} M Mg^{2+} (○) and Ca^{2+} (▲). Volume 200 cc.*

Mg^{2+} in reducing the incorporation of 2,4-D. In distilled water the incorporation of PNP under comparable conditions is over twice that of 2,4-D (Fig. 2) or glycine (see Fig. 1). Although the data is not shown, the addition of Ca^{2+} or Mg^{2+} reduces the uptake of PNP compared to that in distilled water.

The data contained in Fig. 3 show the effect of glycine on the uptake of 10^{-4} *M* 2,4-D. At 10^{-4} *M* glycine the uptake of 2,4-D is the same as that in distilled water, but increasing the concentration of glycine from 10^{-4} to 10^{-3} *M* reduces the 2,4-D uptake. At 10^{-3} *M* glycine the 2-min 2,4-D

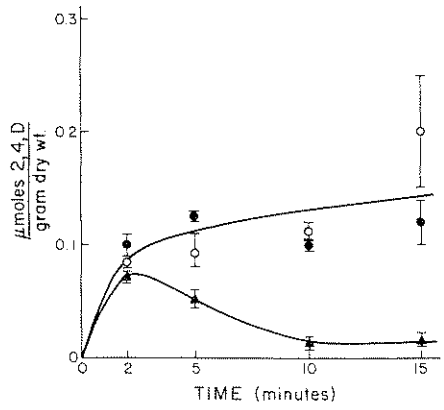

FIG. 3. *Uptake of 10^{-4} M 2,4-D (●) into gills of A. californiensis as affected by 10^{-4} M (○) and 10^{-3} M (▲) glycine. Volume 200 cc.*

influx point is within experimental error the same as those in distilled water and 10^{-4} *M* glycine, and only at longer times is the 2,4-D uptake reduced. In distilled water the uptake of 2,4-D (Figs. 2 and 3) by *A. californiensis* gills is the same as that of glycine (Fig. 1).

M. californianus. The incorporation of 2,4-D into gills of *M. californianus* as affected by Ca^{2+}, Mg^{2+}, and glycine is shown in Fig. 4. Referring to Figs. 1 and 2, the 2,4-D uptake into *M. californianus* gills is less than that of glycine into *M. californianus* and slightly greater than that of 2,4-D into *A. californiensis* gills. The removal of Ca^{2+} or Mg^{2+} from ASW and replacement of the divalent cation with Na^{+} increases slightly the uptake relative to ASW. If glycine is added to ASW it has no effect on the uptake of 2,4-D.

Although not shown, the uptake of PNP into *M. californianus* gills is the same within experimental error as that of 2,4-D, and Ca^{2+} and Mg^{2+} have the same effect on PNP uptake as on the uptake of 2,4-D.

The effect of 2,4-D on the uptake of glycine into gills of *M. californianus* is shown in Fig. 5. The incorporation of glycine into *M. californianus* gills is known to be an active transport process. At 10^{-4} *M* glycine the addition of 10^{-4} or 10^{-3} *M* 2,4-D reduces the uptake of glycine to less than a fifth of the value in ASW at 15 min.

The uptake of glycine is affected by the

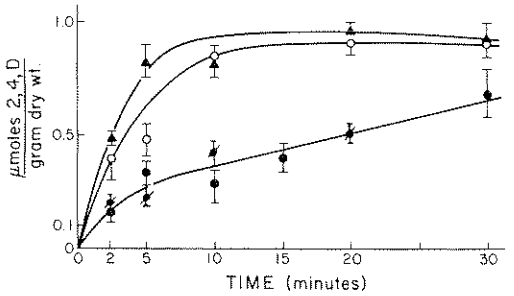

FIG. 4. *Uptake of 10^{-4} M 2,4-D (●) into gills of M. californianus as affected by 10^{-4} and 10^{-3} M glycine (ø), −Mg ASW (○), and −CA ASW (▲). Points for glycine at 10^{-4} and 10^{-3} M are averaged. Volume 200 cc.*

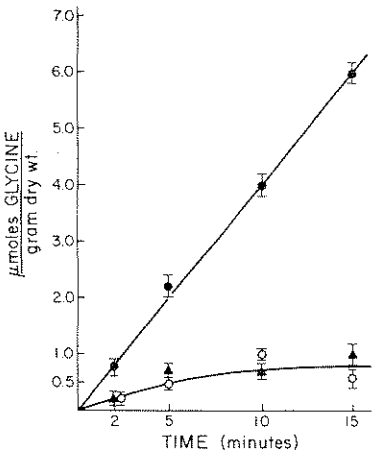

FIG. 5. *Uptake of 10^{-4} M glycine (●) into gills of M. californianus as affected by 10^{-4} (○) and 10^{-3} M (▲) 2,4-D. Volume 200 cc.*

presence of PNP (Fig. 6). However, the effect of PNP on glycine uptake is not as great as that of 2,4-D (Fig. 5). At a concentration of 10^{-4} *M* PNP the uptake of 10^{-4} *M* glycine is about 50% that of glycine alone. It requires 10^{-3} *M* PNP to reduce the uptake of 10^{-4} *M* glycine to levels observed for 10^{-4} *M* 2,4-D on 10^{-4} *M* glycine.

The variation in acidity of a solution containing 2,4-D or PNP added to 10^{-4} *M* glycine could affect the uptake of a particu-

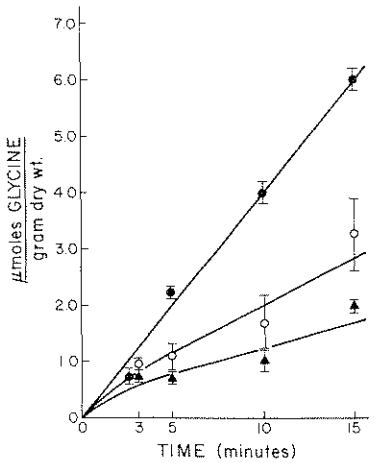

FIG. 6. *Uptake of 10^{-4} M glycine (●) into gills of M. californianus as affected by 10^{-4} (○) and 10^{-3} M (▲) PNP. Volume 200 cc.*

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Effects of 2,4-D on Ca²⁺ Primary Amines Loss

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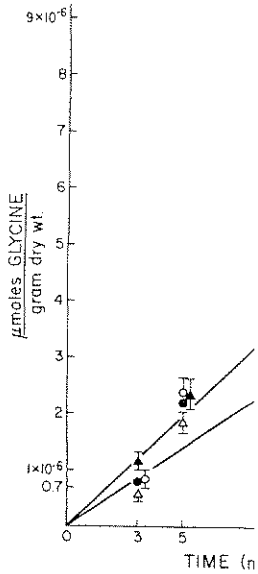

FIG. 7. *Uptake of 10^{-4} M g californianus as affected by pH (▲), and 5.0 (△). Volume 200 c*

lar component of the solution. For solutions 10^{-4} *M* in 2,4-D or PNP the pH = 7.6 to 7.8, and for 10^{-3} *M* in 2,4-D or PNP the pH = 6.6 to 6.8, compared to pH = 7.8 for ASW or ASW containing 10^{-4} *M* glycine. When gills are added to a solution which is 10^{-3} *M* in 2,4-D or PNP and 10^{-4} *M* in glycine the pH increases to 7.0 within 15 min. To test the effect of acidity on the uptake of glycine, measurements of the incorporation of 10^{-4} *M* glycine at pH's of 7.8 (ASW alone), 6.5, 6.0, and 5.0 were made (Fig. 7). The pH was held constant over the course of the experiment by the addition of acid. No discernable reduction of glycine uptake is observed until pH 5 is reached indicating that acidity changes are not a factor in the observed effect of 2,4-D and PNP on glycine influx.

Effects of 2,4-D on Ca^{2+}, Mg^{2+}, and Primary Amines Losses

Figure 8 shows the effect of 2,4-D at 10^{-4} and 10^{-3} *M* on the loss of Ca^{2+} from the gills of *A. californiensis*. The loss of Mg^{2+} as affected by 2,4-D shows the same trend. The losses of both Ca^{2+} and Mg^{2+} are enhanced relative to that in distilled water alone by the presence of 2,4-D at 10^{-3} *M*. The loss of primary amines is increased relative to that in distilled water at 10^{-4} *M*, but is reduced at 10^{-3} *M*. For gills of *M. californianus*, the loss of primary amines is increased upon the addition of 10^{-4} and 10^{-3} *M* 2,4-D to ASW. At 10^{-4} *M* 2,4-D the loss of primary amines is approximately twice that observed in ASW. When the concentration of 2,4-D is increased to 10^{-3} *M* the loss of primary amines increases to three times the value of ASW alone.

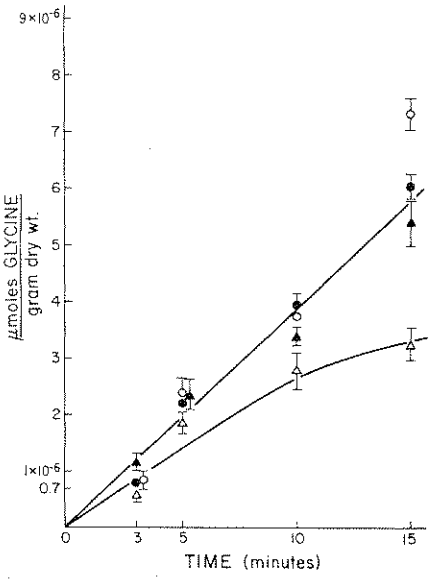

FIG. 7. *Uptake of 10^{-4} M glycine into gills of M. californianus as affected by pH; 7.5 (●), 6.5 (○), 6.0 (▲), and 5.0 (△). Volume 200 cc.*

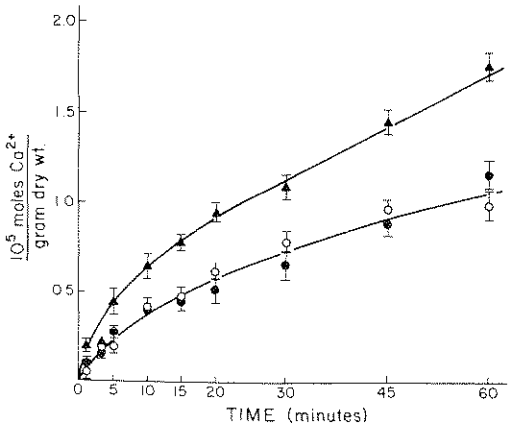

FIG. 8. *The loss of Ca^{2+} from gills of A. californiensis into distilled water (●), 10^{-4} (○), and 10^{-3} M (▲) 2,4-D. Volume 100 cc and gill weights approximately 0.1 g.*

DISCUSSION

Incorporation Experiments

In the case of the gills of *A. californiensis*, the uptake of 2,4-D (Fig. 2) and glycine (Fig. 1) (13) are nearly the same, while that of PNP (Fig. 2) is a factor of three larger than both 2,4-D and glycine. All of these experiments were executed at concentrations of 10^{-4} *M* in the carrier compound with the radiotracer at negligible concentrations compared to the carrier. It seems likely from the data in Fig. 1 that glycine uptake into *A. californiensis* gills is not an active transport process as it is for gills of *M. californianus*. Thus, it can be concluded that the uptake of 2,4-D and PNP into gills

of *A. californiensis* is also a passive process. Calculations of the "concentrations" of these substances in the gills versus the water substantiates this view.

The amount of organic compound (2,4-D, PNP, or glycine) that accumulates in *A. californiensis* gills compared to the level present in the surrounding water is of interest in assessing the permeability of the gill to the compound and to what extent the "concentration" of compound in the gill is a reflection of concentration in the surrounding water. In these experiments the compound concentration in the surroundings was 10^{-4} *M*. If 2×10^{-7} mol/g dry wt is taken as the accumulation of 2,4-D after 30 min of contact with the surrounding water (Fig. 2) and a gill of 0.1 g dry wt has a wet wt of 1 g, the "concentration" of 2,4-D in the gill is 2×10^{-5} *M*. Thus, after 30 min, the level of 2,4-D in the gill is approximately 20% of the concentration of the surrounding water. This is true of the glycine as well. For PNP the level found in the gill after 30 min is about 60% of that found in the water. In neither case do the "concentrations" of 2,4-D or PNP exceed that of the surrounding media.

In natural waters the "concentration" reached in the gills will certainly be less due to the lower levels of the compounds in the water and the fact that Ca^{2+} and Mg^{2+} are present. The data presented in Fig. 2 show that the presence of these divalent cations in a 2,4-D-containing solution reduces the rate of uptake of this compound by a substantial amount, Ca^{2+} being more effective than Mg^{2+}. The same result is found for the effect of divalent cations on the uptake of PNP. In an earlier study (13) it has been shown that increasing concentrations of Ca^{2+} and Mg^{2+} reduce the uptake of glycine into gills of *Corbicula manilensis* and *A. californiensis*, both freshwater bivalve molluscs. In the same study, primary amine losses from the gills of these species are reduced by Mg^{2+} and Ca^{2+}, and Ca^{2+} is more effective than Mg^{2+}. It has been suggested in a number of studies that divalent cations induce changes in membrane packing properties and reduce membrane permeability (14–16). It has been shown that Ca^{2+} binds more strongly than Mg^{2+} to phospholipid vesicles (17). The stronger binding of Ca^{2+} compared to Mg^{2+} could account for the observed variation in properties between these divalent cations.

For the gills of *M. californianus* the uptake of PNP and 2,4-D are approximately the same, but considerably less than the influx of glycine which is known to occur by an active transport process. Figure 4 shows that the removal of Ca^{2+} or Mg^{2+} from ASW increases the uptake of 2,4-D and PNP (data not shown). Thus the effect of the divalent cations on the uptake of 2,4-D and PNP is the same for freshwater species, *A. californiensis*, and the marine species, *M. californianus*. However, for the influx of glycine into the gills of *M. californianus*, an active transport process, the effects of the divalent cations are entirely different (7). In this case the removal of Mg^{2+} from the ASW reduces the influx of glycine, but the loss of Ca^{2+} has no effect on the process. As reflected in the effects of Ca^{2+} and Mg^{2+} on the uptake process by gills, PNP, 2,4-D, and glycine for *A. californiensis* and PNP and 2,4-D for *M. californianus* have the same properties, but glycine for *M. californianus* is entirely different. Again, this suggests entirely different processes based on the effects of Ca^{2+} and Mg^{2+} on uptake.

Observations concerning the effect of one organic compound (2,4-D, PNP, or glycine) on the uptake of another can give information about the sites at which uptake occurs. An interesting case is the effect of 2,4-D or PNP on the influx of glycine into the gills of *M. californianus*. Glycine influx, an active transport process, is reduced by the presence of 2,4-D (Fig. 5) and to a lesser extent by PNP (Fig. 6). At 10^{-4} *M* glycine, an equimolar concentration of 2,4-2-D reduces glycine influx by a factor of five, while PNP at 10^{-4} *M* reduces the influx by a factor of two. If glycine initially binds to the gill surface in a Mg^{2+} complex (10), 2,4-

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Losses of Ca^{2+}, Mg^{2+}, a Amines

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D or PNP could effectively compete with glycine for Mg^{2+} and reduce the amount of glycine available for transport across the membrane. The functional group that can complex Mg^{2+} is a carboxylate for 2,4-D and phenolate for PNP. The former group is a better in complexing Mg^{2+} than the latter, and thus would produce a greater reduction in the glycine bound in a Mg^{2+}-glycine complex (18, 19). Interference with this surface complexation process has been proposed to explain the reduction of glycine influx into gills of *M. californianus* by Hg^{2+}, Cu^{2+}, and Fe^{3+} (13).

The uptake of 10^{-4} *M* 2,4-D by gills of *M. californianus*, a passive process, is unaffected by the addition of glycine at 10^{-4} and 10^{-3} *M* (Fig. 4). Although 2,4-D can block the influx of glycine, 2,4-D uptake does not occur by the glycine active transport process. Since glycine does not effect 2,4-D uptake, this process is distinct from that of glycine and does not involve divalent cation complexation.

The uptake of 10^{-4} *M* 2,4-D by gills of *A. californiensis* is unaffected by an equimolar concentration of glycine, but is reduced by 10^{-3} *M* glycine (Fig. 3). The uptake of 2,4-D at the 2-min value is the same in distilled water, 10^{-4} and 10^{-3} *M* glycine, but at 5 min and longer the uptake of 2,4-D at 10^{-3} *M* glycine is increasingly reduced below that of the rising uptakes of the other solutions and the 2-min value for all solutions. Several explanations are possible. The 2,4-D could bind rapidly to surface sites but be displaced from these sites by a slower binding but more thermodynamically favored glycine association. Another possibility is that glycine at 10^{-3} *M* slowly alters the physical properties of the surface and reduces the surface area available for 2,4-D association.

Losses of Ca^{2+}, Mg^{2+}, and Primary Amines

For gills of *A. californiensis* the losses of Ca^{2+} (Fig. 8) and Mg^{2+} are increased over that found in distilled water by the addition of 2,4-D at concentrations of 10^{-4} *M* or greater. Primary amines loss is increased over that found in distilled water by 10^{-4} *M* 2,4-D, but is reduced below that found in distilled water by 10^{-3} *M* 2,4-D. There probably is a connection between this observation and that of the effect of glycine on the uptake of 2,4-D by *A. californiensis* gills described in the previous paragraph. A possible explanation involves physical changes in the gill induced by the addition of glycine or 2,4-D. For the marine species, *M. californianus*, the addition of 2,4-D at the levels used for *A. californiensis* increases the loss of primary amines with increasing concentration of 2,4-D.

Potential Ecological Impact

In summary, in terms of their potential ecological impacts, both 2,4-D and PNP reduce the influx of glycine into the gills *M. californianus*. The inhibition of this active transport process decreases the accumulation of a biologically important compound used by the organism in its amino acid pool (20).

For *A. californiensis* gills the losses of Ca^{2+} and Mg^{2+}, cations known to stabilize membranes, and primary amines from the amino acid pool are enhanced at low concentrations of 2,4-D. For *M. californianus* gills 2,4-D enhances primary amine loss. The data show that 2,4-D acts to disrupt the membranes of these gills and cause excessive losses of amino acids.

Thus, the net effect of the presence of 2,4-D at the levels studied is a reduction in the amino acid pool. Changes in the size of amino acid pools as a result of stress imposed upon bivalve molluscs have been the subject of several studies (20, 21). The work of Sanson *et al.* (21) suggests that stress resulting from pollutants causes changes in both the composition and amount of the free amino acid pool. This work suggests the same result and indicates on a molecular level why the amino acid pool is altered.

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REFERENCES

1. Berwick, P., 2,4-Dichlorophenoxyacetic acid poisoning in man, *J. Amer. Med. Assoc.* **214,** 1114 (1970).
2. Overbeek, J. V., Survey of mechanisms of herbicide action, *In* "Physiology and Biochemistry of Herbicides" (L. J. Hudus, Ed.), p. 387, Academic Press, New York, 1964.
3. Lotlikar, P. D., L. F. Remmert, and V. H. Freed, Effects of 2,4-D and other herbicides on oxidative phosphorylation in mitochondria of cabbage, *Weed Sci.* **16,** 161 (1968).
4. Dux, E., I. Toth, L. Dux, F. Joo, and G. Kysely, The possible cellular mechanism of 2,4-dichlorophenoxyacetate-induced myopathy, *Fed. Eur. Biochem. Soc. Lett.* **82,** 219 (1977).
5. Hofmann, W. W., W. Walston, and G. Rowe, A study of individual neuromuscular junction in myotonia, *Electroencephalogr. Clin. Neurophysiol.* **21,** 521 (1966).
6. Lee, W., J. H. Swinehart, and J. H. Crowe, The effects of copper (II), mercury(II) and iron(III) on primary amine and divalent cation losses from and glycine incorporation into gills of the bivalve mollusc, *Mytilus californianus*, *Mol. Physiol.*, in press.
7. Swinehart, J. H., J. H. Crowe, A. P. Giannini, and D. A. Rosenbaum, Effects of divalent cations on amino acid and divalent cation fluxes in gills of the bivalve mollusc, *Mytilus californianus*, *J. Exp. Zool.* **212,** 396 (1980).
8. Udenfriend, S., S. Stein, P. Bohlen, W. Dairman, W. Leimgruber, and M. Weigele, Fluorescamine: A reagent for assay of amino acids, peptides, and primary amines in the picomole range, *Science* **178,** 871 (1972).
9. North, B. B. (1975). Primary amines in California coastal waters: Utilization by phytoplankton, *Limnol. Oceanogr.* **20,** 20 (1975).
10. Crowe, J. H., K. A. Dickman, J. L. Otto, R. D. Colon, and K. K. Farley, Uptake of amino acids by the mussel *Modiolus demissus*, *J. Exp. Zool.* **202,** 323 (1977).
11. Stephens, G. C., Uptake of naturally occurring primary amines by marine annelids, *Biol. Bull.* **149,** 397 (1975).
12. Wright, S. H., and G. C. Stephens, Characteristics of influx and net flux of amino acids in *Mytilus californianus*, *Biol. Bull.* **152,** 295 (1977).
13. Swinehart, J. H., A. P. Gianinni, D. A. Rosenbaum, M. A. Cheney, and J. H. Crowe, Effects of divalent cations of amino acid and divalent cation losses from and glycine influx into gills of the freshwater bivalve molluscs *Anodonta californiensis* and *Corbicula Manilensis*, submitted for publication.
14. Watts, J. A., and S. K. Pierce, Characterization of the divalent cation activated Adenosine Triphosphatase from the membranes of the cardiac cell of *Modiolus demissus demissus*, *J. Exp. Zool.* **204,** 43 (1978).
15. Watts, J. A., and S. K. Pierce, A correlation between the activity of divalent cation activated adenosine triphosphatase in the cell membrane and low salinity tolerance of the ribbed mussel, *Modiolus demissus demissus*, *J. Exp. Zool.* **204,** 49 (1978).
16. Papahadjopoulos, D., Role of ionic environments on self-assembly properties of phospholipid membranes, *In* "Light Transducing Membranes" (D. W. Deamer, Ed.), p. 77, Academic Press, New York.
17. Nir, S., C. Newton, and D. Papahadjopoulos, Binding of cations to phosphatidylserine vesicles, *Bioelectrochem. Bioenerget.* **5,** 116 (1978).
18. Sillen, L. G., and A. E. Martel, "Stability Constants of Metal–Ion Complexes," Special Publ. No. 17, The Chemical Society, London, 1964.
19. Sillen, L. G., and A. E. Martell, "Stability Constants of Metal–Ion Complexes," Supplement No. 1. Special Publ. No. 25, The Chemical Society, London, 1971.
20. Peirce, S. K., Jr., A source of solute for volume regulation in marine mussels, *Comp. Biochem. Physiol. A* **38,** 619 (1971).
21. Sanson, G., A. Biondi, and L. Noviello, Free amino acids in fluids and tissues of *Mytilus galloprovincialis* in relation to the environment. Then behaviors as an index of normality of metabolism, *Comp. Biochem. Physiol. A* **61,** 133 (1978).