and remained at this level until 72 hours. Putrescine- and spermidine-stimulated SAMD activities were depressed two hours after treatment and remained depressed until 16 hours, when they returned to basal levels.

The liver and kidney enzyme patterns seem to correlate with differences in the accumulation patterns of the methylmercury in these organs. The initial inhibition of ODC and SAMD activities would seem to be a direct reflection of the cellular damage that occurs after methylmercury administration. The temporal increases in ODC and SAMD activities are consistent with the reported temporal increase in RNA. In both liver and kidney ODC and SAMD are maximal before there is any change reported in RNA concentration. Also the increase in the number of small ribosomal subunits (Expt. Mol. Pathol. 18: 263-280, 1973) after mercury poisoning supports the hypothesis that the polyamines are intimately involved in the synthesis of ribosomes.

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1974 #22

FAILURE OF DDT TO INHIBIT CARBONIC ANHYDRASE IN VITRO IN SHELL GLAND OF THE DUCK, *Anas platyrhynchos*

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There continues to be speculation (Peakall, D.B., Chem.-Biol. Interactions, in press, 1974) that DDT causes thinning of eggshells by inhibition of carbonic anhydrase. Classical carbonic anhydrase inhibitors, such as sulfanilamide and acetazolamide, do cause egg-shell thinning in all species in which they have been tested and the mechanism is quite clear. These

specific drugs slow carbonate formation by inhibiting carbonic anhydrase in the secretory shell gland and thus repress the laying down of $CaCO_3$. The problem with DDT and its analogs is more complex since varying results have been obtained on the basic question of whether DDT inhibits the enzyme *in vitro* (reviewed by A. S. Cooke, Environmental Pollution, 4, 85, 1973). Many other mechanisms of course have also been put forward for the action of DDT in those species in which thinning is observed (not in all birds as with the sulfonamide inhibitors) but none is certain. In previous publications blood carbonic anhydrase was used to test the *in vitro* effect of DDT-DDE; we report here the effect of DDT, DDE, and methazolamide (or acetazolamide) on the enzyme from the shell gland itself.

Laying White Pekin ducks (*Anas platyrhynchos*) about one-year old were sacrificed by decapitation and the shell glands removed and placed in ice-cold 0.25 M sucrose. The mucosa was dissected and homogenized in approximately 60 volumes of the sucrose solution. Aliquots (1 ml) were frozen and lyophilized and the freeze-dried material was stored at -20° C. Each aliquot was reconstituted with 10 ml water, resulting in a 600-fold dilution of the original mucosal tissue. Carbonic anhydrase activity and inhibition were measured as previously described (J. Pharm. Expt. Therap. 130, 26, 1960) except that in some experiments 0.3 ml of 25 mM barbital buff (pH 7.9) was used in place of carbonate. Protein was determined by the method of Lowry *et al.* (J. Biol. Chem. 193, 265, 1951).

Table 1 shows that 0.1 ml of the diluted tissue had about one unit of activity in this assay where activity is defined as (uncatalyzed time-catalyzed time)/catalyzed time. Thus the activity in the original mucosa was approximately 6000 units/gram. A similar assay for mammalian or bird red cells yielded a three to four-fold higher concentration. These activity

TABLE 1

ABSENCE OF EFFECT OF DDE ON DUCK SHELL GLAND CARBONIC ANHYDRASE

	Reaction Time (sec)	Activity (units/mg prot)
Uncatalyzed time	35	
Experiment #1		
Control (bird #1)	17	20
+ 5% DMF[1]	16	22
+ 5% DMF + 150 ppm DDE	17.5	19
+ 5% DMF + 150 ppm DDT	17	20
Experiment #2		
Control (bird #2)	25	7.4
+ 0.5% DMF + 7.5 ppm DDE[2]	24	8.1
Experiment #3		
Control (bird #3)	20	14
Sonicated control[3]	20	15
Sonicated + 6 ppm DDE	18.5	16
Sonicated + 6 ppm DDE + 5% DMF	19	15
Experiment #4		
DDE-homogenate[4]	25	12
+ 1 ppm methazolamide	35	0

[1]DMF or DMF + DDE added directly to reaction vessel to give these final concentrations.

[2]DDE in DMF was added to shell gland homogenate 20 minutes prior to assay (concentration in homogenate 5% DMF, 75 ppm DDE); concentrations shown are those present in reaction vessel.

[3]Homogenate re-constituted with water (control) or with a sonicated aqueous suspension of 6 ppm DDE (no DMF present). These suspensions were sonicated for an additional 20 min and then assayed. Concentrations shown are those in homogenate. The concentrations in reaction vessel were one-tenth of those shown here.

[4]Shell gland enzyme from duck fed 40 ppm DDE for one month (see text).

units, since they are based on the micro-method (1 ml) and different buffer, must be divided by 20 (this factor takes into account the volume ratio of 7 and the higher sensitivity in barbital over carbonate) to align them with the carbonic anhydrase concentration of various tissues as reported in Tables 15 and 16 in Physiol. Rev. 47, 595, 1967. In this original system of unitage, the shell gland then has about 300 units/gram mucosa, a value which is in the normal range for secretory tissue. Table 1 also shows the activity of the glands in terms of units in the assay procedure per mg protein. The approximate relation is that 20 activity units/mg protein is equivalent to 300 "standard units"/gram mucosa.

In preliminary experiments performed during the summer of 1973 (carbonate buffer system), shell gland carbonic anhydrase activity was essentially abolished by 0.3 µg acetazolamide in the 1 ml reaction vessel (10^{-6}M). An in vitro dose response curve for acetazolamide against shell gland enzyme was similar to that for human red cell enzyme. On hundred ppm DDT or DDE was added as 0.1 ml of 1000 ppm organochlorine in dimethylformamide (DMF) giving a final DMF concentration of 10 percent. There was no effect on enzyme activity. DMF alone in this concentration did not affect either the uncatalyzed or catalyzed rate.

Table 1 shows the results of additional experiments done in 1974 using the more sensitive barbital system. Organochlorine concentrations of 150 ppm in the reaction system had no effect on shell gland carbonic anhydrase activity (experiment #1); nor did prior addition of 75 ppm DDE to the re-constituted shell gland homogenate (experiment #2). Similar results were obtained when the freeze-dried material was re-constituted with a sonicated suspension of DDE in distilled water (experiment #3). The results of these experiments clearly show that neither DDT nor DDE inhibits duck shell gland

carbonic anhydrase *in vitro*.

In order to determine if shell gland carbonic anhydrase was affected by DDE feeding, we assayed tissue from a duck that had been fed 40 ppm DDE for one month. This bird layed eggs with shells that were approximately 20 percent thinner than controls. As shown in Table 1 (experiment #4), shell gland enzyme activity in this bird was certainly within the range of control values (experiments 1 - 3).

Our results with DDE/DDT in the shell gland *in vitro* are compatible with previous data from the literature using blood carbonic anhydrase with the single exception of the abstract by Serine and Schraer (Fed. Proc. 31, 2869, 1972). Other results showing inhibition *in vitro* have always been with DDT in suspension and have been explained by Pocker *et al.* as due to a coprecipitation phenomenon rather than "true inhibition" (Biochem. 10, 1390, 1971). The results of feeding DDE in the duck (bird #4) agree with those of Pritchard *et al.* (Bull. MDIBL 12, 77, 1972) in showing relatively little decrease in carbonic anhydrase activity in the shell gland. They differ from those of Peakall in the ring dove (Chem.-Biol. Interactions, in press, 1974) since he showed about 50 percent decrease in gland carbonic anhydrase activity when the birds were fed 40 ppm DDE; curiously this effect could be reversed by adding DMF to the assay system. Since we found no inhibition under these conditions (bird #4), we could not study this DMF effect. However there is abundant evidence to show that 50 percent inhibition of carbonic anhydrase produces no physiological effect in any *in vivo* system (Physiol. Rev. 47, 595, 1967) and Peakall's data (op. cit.) with acetazolamide bear this out for the shell gland, since notable thinning (15 percent decrease in Ratcliffe Index) was not observed until gland enzyme was 60 percent of normal by an assay system which greatly underestimates inhibition.

We conclude that there is no evidence to suggest that the thinning of egg-shells by DDT or DDE in ducks is due to the inhibition of carbonic anhydrase.

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1974 #23

EFFECTS OF DDE ON PLASMA OSMOREGULATION AND NASAL GLAND Na-K-ATPASE IN THE BLACK GUILLEMOT *Cephus grylle* AND THE COMMON PUFFIN *Fratercula arctica*

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To assess the possibility that organochlorine pesticide disruption of osmoregulation is responsible for recent large kills of young sea birds (e.g., shearwaters and puffins), we previously studied the effect of DDE feeding (10 - 250 ppm) on plasma osmoregulation and nasal gland function in mallard and white Pekin ducks (both *Anas platyrhynchos*) and found that neither of these closely related strains was sensitive enough to demonstrate conclusive DDE effects at environmental levels (Miller, et al., Bull. MDIBL, 13: 77, 1973; Fed. Proc. 33: 220, 1974). Since the genus *Anas* are not strictly marine we speculated that, due to species differences, increased sensitivity might be found in true pelagic species, members of the genus *Alcidae*. The present study was undertaken to determine the effect of DDE feeding on osmoregulation and nasal salt gland Na-K-ATPase in two alcids, the black guillemot, *Cephus grylle*, and the common puffin, *Fratercula arctica*.

Sixteen black guillemots approximately 25 days old were collected from nests on Old Man Island off Cutler, Maine and transported to the Laboratory