DDE in the puffin and the absence of an in vivo effect in the duck (Miller, et al., Bull. MDIBL, 13: 77, 1973) correlates well with the higher sensitivity of the puffin ATPase to DDE added in vitro (Miller and Kinter, unpublished observations). A similar comparison cannot be made for the guillemot enzyme due to the short DDE dosing period.

In conclusion DDE feeding does not affect plasma osmoregulation or nasal gland function in fasting, immature puffins and guillemots. These findings for two pelagic species, one of which (the puffin) is declining in population (Flegg, Bird Study, 19: 7, 1972) provide strong evidence against the osmoregulatory failure hypothesis. Our results in the puffin however do suggest that organochlorine toxicity, at a site other than the nasal gland, cannot be ruled out as a possible contributing factor in seabird kills.

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1974 #24

POSSIBLE ENZYMATIC BASIS OF DDE-INDUCED EGGSHELL THINNING IN THE WHITE PEKIN DUCK, *Anas platyrhynchos*

DAVID S. MILLER, ALLYN SEYMOUR, JR., DAVID SHOEMAKER, MARTHA H. WINSOR, DAVID B. PEAKALL, AND WILLIAM B. KINTER. MOUNT DESERT ISLAND BIOLOGICAL LABORATORY; AND CORNELL UNIVERSITY, ITHACA, NEW YORK

Although the occurrence of DDE-induced eggshell thinning is well documented in many avian species, the biochemical mechanism of action remains unclear. Since there is considerable evidence (see review by Cooke) suggesting that thinning results from inhibitory action close to the site of shell formation (Cooke, Environ. Pollut. 4: 85, 1973), we have investigated the effect of DDE on several ion-activated ATPases found in avian shell gland mucosa.

Female white Pekin ducks, *Anas platyrhynchos*, approximately one year of age were divided into control and experimental flocks and maintained as previously described (Peakall, et al., J. Gen. Pharmacol. 4: 305, 1973). The experimental flock had 40 ppm DDE added to the feed before pelletization.

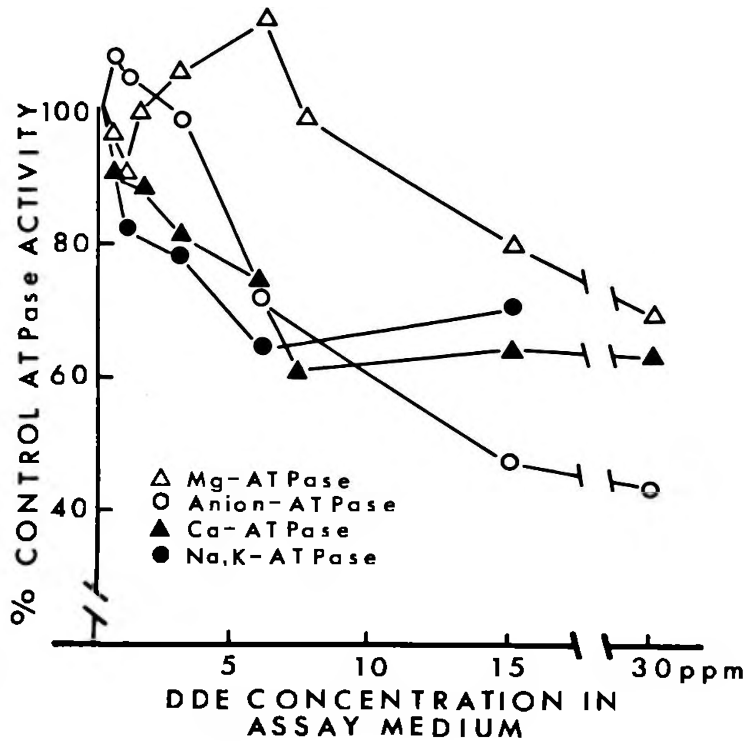

Figure 1: Inhibition of duck shell gland ion-activated ATPases by DDE added to the in vitro assay media. Each point represents the mean of 3 - 12 assays on pooled tissue from 2 - 4 ducks.

In the white Pekin this concentration of DDE produces maximum (20 percent) eggshell thinning within four days. After one to three months on DDE ducks with active shell glands -- containing an egg undergoing calcification -- were sacrificed by decapitation, the gland removed, and the mucosa scraped, homogenized, and freeze-dried. Tissue samples were also set aside for DDE residue analysis (data not yet available) and determination of calcium binding protein (Wasserman and Taylor, Science, 152: 791, 1966). ATPase assays were performed on reconstituted freeze-dried homogenates using standard procedures and optimized conditions. Protein was determined by the procedure of Lowry, et al. (J. Biol. Chem., 193: 265, 1951) using bovine serum albumin as standard.

TABLE 1

EFFECT OF DDE FEEDING (40 ppm) ON DUCK SHELL GLAND ION-ACTIVATED ATPases AND CALCIUM BINDING PROTEIN (CaBP)*

Enzyme	Control	DDE-Fed	P
Ca-ATPase	16.7±1.6 (6)	10.9±1.2 (5)	<.02
Mg-ATPase	23.5±3.1 (6)	21.2±3.0 (5)	>.05
Na-K-ATPase	5.1±0.5 (6)	5.1±0.7 (5)	>.05
$HCO_3^=$-ATPase	4.6±0.5 (5)	4.8±0.6 (4)	>.05
$SO_3^=$-ATPase	25.4±2.3 (5)	29.6±3.6 (4)	>.05
CaBP	5.01±1.14 (5)	4.07±0.74 (4)	>.05

*Values given as Mean ± SE(n), where n is the number of ducks. Data expressed as μmoles P_i released/mg protein/hr for ATPases and nmoles Ca^{++} bound/mg protein for CaBP.

As shown in Figure 1 addition of DDE in DMF to the enzyme assay medium (final DMF concentration in medium, one percent; concentrations up to five percent did not affect ATPase activity) resulted in substantial inhibition of all duck shell gland ATPases. However the *in vitro* sensitivity of the various enzymes to low concentrations of DDE varied markedly, with only the Ca- and Na-K-activated ATPases being affected by DDE concentrations below 3 ppm. Not shown in Figure 1 are additional data demonstrating inhibition of gland Ca-ATPase by DDE concentrations as low as 0.2 ppm. These *in vitro* data are significant because of the low DDE residue values (1.5 ppm in gland mucosa)

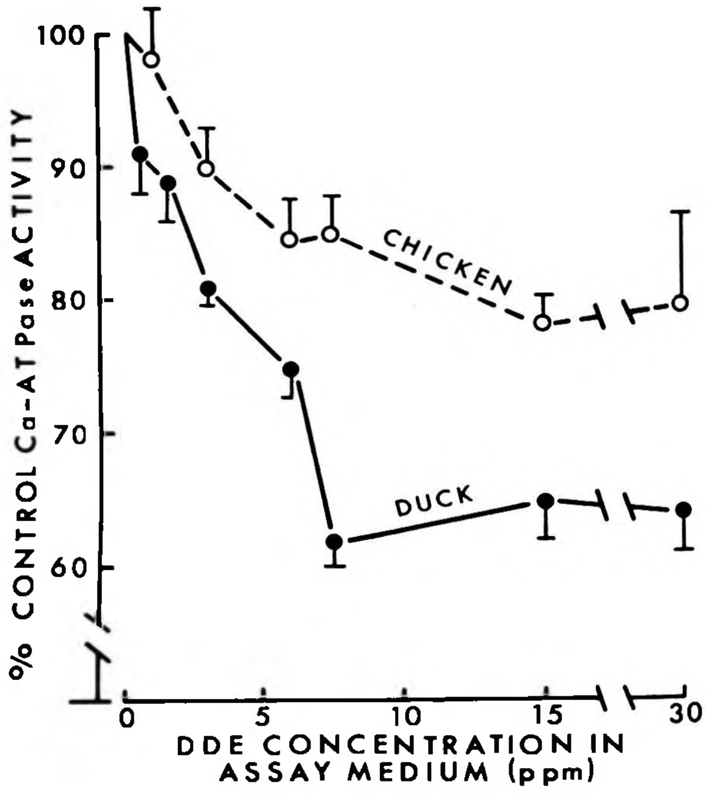

Figure 2: Inhibition of chicken and duck shell gland Ca-ATPase by DDE added to the *in vitro* assay media. Each point represents the mean of 4 - 12 assays on pooled tissue from 2 chickens or 4 ducks. Variability is given by SE bars.

reported for ducks laying eggs with 20 percent thinned shells (Peakall, et al., J. Gen. Pharmacol. 4: 305, 1973). In addition we have surveyed shell gland ATPase and calcium binding protein activities in DDE-fed ducks laying thin-shelled eggs and found that only the Ca-ATPase activity was significantly reduced in vivo (Table 1). The results of preliminary experiments with chicken, *Gallus domesticus*, shell gland mucosa show the Ca-ATPase to be less sensitive to DDE inhibition in vitro than the duck enzyme (Figure 2). The chicken is generally regarded as a species that does not lay thin-shelled eggs in response to DDE feeding.

In conclusion we have found a Ca-activated ATPase in duck shell gland mucosa which is inhibited by DDE in vivo as well as in vitro. Since calcium transport across avian shell gland is known to be an active process (Ehrenspeck et al., Amer. J. Physiol. 220: 967, 1970; Pearson and Goldner, Amer. J. Physiol. 225: 1508, 1973) this Ca-ATPase may function as a "Ca^{++}-pump" similar to the pumps already described in other tissues -- muscle sarcoplasmic reticulum. If so, partial inhibition of the enzyme by DDE would result in reduced calcium transport across the shell gland to the calcifying egg.

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EFFECTS OF MAGNESIUM ON POTASSIUM EXCRETION IN *Squalus acanthias*

J.D. MYERS, V.E. REA, E.W. MURDAUGH, AND H.V. MURDAUGH, JR. UNIVERSITY OF PITTSBURGH, SCHOOL OF MEDICINE, PITTSBURG, PENNSYLVANIA

Earlier studies of cation excretion in *Squalus acanthias* in our laboratory focused attention on attempts separately to induce natriuresis or urea