some have three to five lobes. In none of the cells are the lobes sharply segmented as in mammalian granulocytes. The cytoplasm of the heterophils is filled with approximately 125 red, rod-shaped granules about 0.5 μ in diameter and 1.0 to 1.5 μ in length. Electron microscopy revealed mitochrondria and a single type of osmiophilic elongated granule in the cytoplasm. A moderate number of cells approximately 14 μ in diameter are present in the smears. A scant rim of dark blue cytoplasm arranged in a polar fashion on opposite sides of the nuclei is present in these cells. Although they resemble morphologically the lymphocytes of higher vertebrates, the dense chromatin pattern and the consistent polarity suggest to us the probability that these cells are derived from the erythrocytic or thrombocytic lines.

The presence of apparently only one type of granulocyte with only one type of granule is quite interesting from a phylogenetic standpoint. Higher vertebrates generally have three types of granulocytes and the predominate type of cell usually has at least two types of granules.

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DDE-INDUCED EGGSHELL THINNING IN WHITE PEKIN DUCKS *Anas platyrynchos:* STRUCTURAL, PHYSIOLOGICAL, AND BIOCHEMICAL STUDIES

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While the occurrence of eggshell thinning due to DDT and its metabolites has been widely noted in many species of birds, the physiological consequences of this thinning have received little attention. Egg breakage as a result of thinning has been noted but no systematic studies of the relationship of shell thickness to breaking strength has been made for eggs from birds exposed to DDT and its metabolites. Changes in shell permeability during eggshell thinning have not been studied. Finally studies on the biochemical basis for DDT-induced thinning have indicated that direct effects on shell gland enzymes seem to be involved (Risebrough, et al., *The Biological Impact of Pesticides in the Environment*, J.W. Gilbert, editor, Oregon State Univ., Corvalis, 1970, pp 40-53).

White Pekin layers weighing 7-9 lb (C. & R. Duck Farm, Westhampton, Long Island) were kept in two flocks of about 10 birds each and fed *ad lib* duck breeder mash (kindly donated by Agway, Inc.). The experimental flock had 40 ppm p,p -DDE (Aldrich Chemical Co.) added to the mash before pelletization. Eggs were collected daily and washed briefly in warm water to remove surface dirt. Length and breadth were then measured and shell thickness was later measured at the waist of the egg (average of 10 readings) using an Ames Model 25E thickness guage. Permeability to water vapor was measured by maintaining unfertilized eggs in a constant temperature desiccator containing anhydrous $CaSO_4$. Addition of an open dish of P_2O_5 did not increase the rate of water loss. The eggs were weighed daily for four consecutive days. Breaking strength of eggs was determined using an Instron Universal Testing machine and scanning electron micrographs of the shell surface were prepared with a Cambridge Stereoscan Model 2A microscope. Biochemical studies were carried out on freshly scraped mucosa from functioning shell glands (contained an egg undergoing

calcification when duck was sacrificed) and utilized standard homogenization and enzyme assay procedures. Residue analysis by gas-liquid chromatography was obtained for frozen mucosa, egg yolk, and whole blood samples shipped to Dr. Risebrough's laboratory.

Regarding DDE-induced eggshell thinning, it was found that the readily available White Pekin duck was somewhat more sensitive than its wild forbear, the Mallard *Anas platyrhynchos*. A dietary intake of 40 ppm DDE caused a 20 percent decrease in eggshell thickness (Table 1) and eggshell thinning was essentially maximal after four days on the DDE-diet. The breaking strength of the eggshell was also significantly lower in the DDE-treated birds (Table 1). Regression analysis of shell thickness and breaking strength data indicates different relationships for the control and DDE-treated groups. The water vapor permeability of the shell was evaluated by placing eggs in a 40°C desiccator and weighing at intervals. Surprisingly the weight loss was less for eggs from DDE-treated birds (Table 1) despite significantly thinner eggshells. Thus O_2 and CO_2 exchange for embryonic development would also have been reduced; calculations indicate that the effective pore area was decreased by 38 percent in the DDE-treated group. Scanning electron micrographs proved to be difficult to quantify due to variations from area to area, but confirmed the decreased number of pores and also revealed a rougher surface with more globular inclusions in eggshells from the DDE–treated ducks.

TABLE 1

EFFECTS OF DIETARY DDE (40 ppm for 2-3 wk) IN LAYING DUCKS

	Control		DDE Fed		Significance Level of Difference
	mean ± SD	(n)	mean ± SD	(n)	
EGGSHELL					
Thickness mm	0.50 ± 0.23	(18)	0.40 ± 0.04	(22)	<0.1%
Breaking Strength lb	10.4 ± 1.5	(20	7.7 ± 1.2	(17	<0.1%
Water Permeability mg/hr x cm^2 at 40 C	0.22 ± 0.05	(18)	0.18 ± 0.05	(22)	5%
EGG YOLK					
DDE ppm wet weight	0.21 ± 0.03	(4)	39.3 ± 10.6	(8)	<0.1%
SHELL GLAND MUCOSA					
DDE ppm wet weight	0.03 ± 0.01	(4)	1.44 ± 0.68	(8)	<0.1%
Ca Binding Protein %ca/mg protein	17.5 ± 3.2	(4)	18.3 ± 4.2	(8)	>5%
Carbonic Anhydrase units/g tissue	329 ± 9	(4)	293 ± 32	(8)	2%

ATPases - preliminary, see text

When the ducks were sacrificed after several weeks of treatment, the DDE level in shell gland mucosa was about 1/20 and in egg yolk about equal the dietary level of 40 ppm (Table 1). The corresponding blood level in treated ducks was only about 0.4 ppm DDE compared to about 0.004 ppm for untreated controls and not surprisingly the carbonic anhydrase activity of whole blood was unaffected (kindly measured by R.M. Woodworth and T.H. Maren). In the shell gland mucosa, three likely components of the calcium carbonate forming mechanism were also examined, i.e., calcium binding protein, carbonic anhydrase, and in a preliminary way several ATPases. No difference was found in the activity of calcium binding protein extracted from mucosa of ducks treated with DDE as compared to controls (Table 1) and no effects was observed with the addition of 4, 20, or 40 ppm DDE under *in vitro* conditions.

On the other hand carbonic anhydrase did decrease with increased DDE in the mucosa of individual birds (correlation coefficient 0.78, mean values in Table 1). Although moderate inhibition of mucosal carbonic anhydrase after exposure of birds to DDE has been noted before (Peakall, *Science* 168,592-594, 1970), a dose-response has not previously been reported. In addition to ATPase dependent on Mg++ alone, both a Na+-K+ and a Ca++ activated ATPase were demonstrable in the shell gland mucosa of laying ducks. The latter two ATPases are generally thought to be associated with transport of the activating ion(s) and Ca++ ATPase activity (approximately 0.2 micromoles Pi/mg protein x hr at 40 C) has not been reported heretofore in shell gland mucosa. Although activities of all three ATPases appeared unchanged in fresh mucosa from the DDE-treated birds, preliminary work with frozen control mucosa suggests that as little as 2 ppm DDE added under *in vitro* conditions inhibits both ion transporting ATPases by about 15 percent.

In conclusion the laying White Pekin, commercially available year-round, is a suitable experimental bird for further investigation of DDE-induced eggshell thinning in general and the roles of shell gland ATPases and carbonic anhydrase in particular.

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EGG SHELL POROSITY AND GAS CONDUCTANCE DIFFERENCES IN A PRECOCIAL CHICKEN AND AN ALTRICIAL BIRD (DOUBLE-CRESTED CORMORANT *Phalocrocorax auritus)*

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The normal development of an avian embryo depends upon a continuous gas exchange between the internal and external environment. This process is by simple diffusion of gases through fixed pores in the egg shell which in turn establish the precise diffusive gas conductance for O_2, CO_2, and water vapor. These pores must be designed in such a manner that diffusion will supply the increasing oxygen demands of the embryo right up to the pipping stage before hatching. These pores will therefore also determine the CO_2 level of the embryo at all stages as well as the water loss during incubation.