Possible Effect of Lead on Egg-Shell Thickness in Kestrels 1874-1974

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The environmental pollution with lead, especially from automobile exhaust, has without doubt increased the intake of lead for man as well as for other organisms (GRANDJEAN 1975). The highest levels of lead are found in urban areas and near highways where such organisms as earthworms (GISH & CHRISTENSEN 1973) and small mammals (RAYMOND & FORBES 1975) retain a high burden of lead. Birds have recently been found of value to compare the exposure in urban and rural areas. Thus a tendency towards a higher lead retention in urban compared to rural starlings (*Sturnus vulgaris*) has been reported by MARTIN & NICKERSON (1973), and in different species of rather sedentary pigeons a high accumulation of lead in metropolitan areas occurs (TANSY & ROTH 1970, SIEGFRIED *et al.* 1972, and OHI *et al.* 1974).

The effects of the accumulation of lead in birds have not yet been established. Birds of prey are especially sensitive to environmental chemicals, and the kestrel (*Falco tinnunculus*) was one of the first raptor species to respond to the pollution with pesticides by a decrease in egg shell thickness (RATCLIFFE 1970). Several environmental factors influence the thickness of egg-shells (COOKE 1973), but lead has until now not been found of relevance (HAEGELE *et al.* 1974, LINCER & MCDUFFIE 1974).

Because of the present decline of the raptor populations it is only reasonable to collect unhatched eggs or other material which does not disturb the reproduction of the species. This study concerns the excretion of lead in the egg-shells of the kestrel in the time period of 1874-1974. Nowadays the kestrel often nests in urban areas where it is exposed to high levels of lead. The data of HAEGELE *et al.* (1974) show that more lead is excreted in the egg-shells of *Anas platyrhynchos* when the lead dosage is increased. Thus, an increase in the lead excretion should be expected in the contemporary eggs-shells of the kestrel compared to older eggs.

Materials and methods

From the period of 1874-1953 twelve clutches of in all 69 eggs were obtained from the Zoological Museum, University of Copenhagen. All of these clutches except one were from the pre-pesticide aera. During the years 1972-1974 twenty-five unhatched eggs were collected from eleven nests when the birds had stopped brooding. Seven of these nests were placed in rural areas, four of them in urban areas.

Egg-shell thickness was measured with a micrometer screw on the dried shells from the fresh eggs without the inner membranes and on the museum specimens on the shells including the dried membranes. All shells were measured at the equator, the new shells at three places, the old shells only at one point. The coefficient of variance within the clutches was 4.2% for the old eggs and 5.8% for the fresh eggs. The different clutches are only represented by the mean thickness.

Lead analyses were made on shells rinsed in redistilled water and the inner membranes removed by means of a rhodium-coated scalpel. After drying at 110° in an oven the shell fragments were cleaned for 30 seconds with Freon TF in a Millipore ultrasonic bath. This cleaning method has earlier been found very effective for the cleaning of hair (GRANDJEAN, unpublished). About 10 mg shell was weighed on a Beckmann Microbalance LM-500 and then transferred to a polystyrene cup rinsed with dilute hydrochloric acid. The shells were then dissolved in 2 ml suprapure nitric acid (Merck). Twenty microliters of this solution was injected into the graphite tube of the Perkin-Elmer HGA-72 apparatus for flameless atomic absorption spectrophotometry according to the method described by the makers. A spectrophotometer model 300S with deuterium background corrector was used. Standards containing 10 and 50 ng lead and blinds were analyzed as well. Double determinations differed with a coefficient of variance of 3.9%.

Chlorinated hydrocarbons were analyzed in the following way: The content of the recent eggs without the embryos was homogenized with seasand and anhydrous sodium sulphate. In a glass column the lipid soluble phase was extracted with acetone, n-hexane, petroleum ether and ether. The combined and washed solvents were evaporated in a water bath at 80°, and the isolated fat was dissolved in n-heptane and divided into two portions. One portion was treated with fuming sulphuric acid in order to destruct the fat, and the second portion was cleaned up by passing through an Al_2O_3 - column according to the method described by HOLDEN & MARSDEN (1969). The purified extracts were analyzed by electron-capture gas chromatography with the column containing 8% QF-1 and 4% SF-96 on Chromosorb W, 100/120 Mesh mixed in the ratio 65:35. Both DDE, dieldrin and PCB peaks could be found, and standards of Clophen A60 proved favourable for the estimation of PCB concentrations. The results are presented with reference to the amount of extractable fat as the most valid basis for expressing organochlorine residues in eggs of different origins.

Within the clutches large variations in the concentrations of environmental chemicals were found. Though some of the clutches were represented by only one or two eggs, it was decided only to present the means of the single clutches. All results were treated with non-parametric statistical methods (SIEGEL 1956).

Results

The lead concentration in egg-shells from recent eggs is compared to the concentration in old eggs in Table 1. The confidence limits to the medians overlap very much, and there is no difference between the lead concentration in the two samples of eggs.

TABLE 1

Lead concentration in egg-shells from different periods of time.

Period	Number of clutches	Lead concentration (μg/g)		
		Median	95% confidence limits to median	Range
1874-1953	12	1.73	1.27 - 3.38	0.89 - 4.33
1972-1974	11	2.13	1.35 - 3.09	1.02 - 4.77

TABLE 2

Lead concentration and thickness of egg-shells from urban and rural areas 1972-1974.

Origin	Number of clutches	Lead concentration (μg/g)		Thickness (mm)	
		Median	Range	Median	Range
Urban	4	2.80	2.38 - 4.77	0.153	0.133 - 0.178
Rural	7	1.52	1.02 - 2.19	0.200	0.180 - 0.220

TABLE 3

Organochlorine concentrations in eggs from 1972-1974.

Residue	Number of clutches	Concentration (μg/g fat)	
		Median	Range
DDE	11	16	0.3 - 42
PCB	11	23	5.3 - 271
Dieldrin	10	0.95	0.20 - 5.9

From Table 2 it is seen that the lead concentration is greater and the egg-shell thickness smaller in the urban than in the rural areas. The two groups do not overlap, and these differencies are significant, as determined by a two-tailed Mann-Whitney U-Test ($P = 0.006$).

The contents of chlorinated hydrocarbons in the recent eggs appear in Table 3. Most of these concentrations are quite low.

It is seen from Fig.1 that there is a correlation between lead concentration in the old egg-shells and the shell thickness, but this is disturbed by one clutch with a mean shell thickness of 0.208 mm and a mean lead concentration of 0.89 µg/g. The Kendall rank correlation coefficient of -0.26 is not significant, but when excluding the divergent clutch, the correlation coefficient is increased to -0.59 which is significant in a two-tailed test (P = 0.011). The same correlation appears between the lead concentration and the shell thickness of recent eggs which also contain chlorinated hydrocarbons (Fig.2). The Kendall rank correlation coefficient is in this instance -0.56 which is also significant (P = 0.016). The same rank correlation coefficient between shell thickness and DDE concentration in the egg content is -0.37 (P = 0.11), between shell thickness and PCB -0.52 (P = 0.026), and between shell thickness and dieldrin -0.41 (P = 0.10). The organochlorine concentrations are strongly interrelated, but PCB is the only residue which correlates significantly to the lead concentration in the shells. The Kendall partial rank correlation coefficient between lead concentration and shell thickness is reduced by PCB to -0.39 (P = 0.10), and PCB is the only residue which reduces the partial coefficient between lead concentration and shell thickness to a non-significant level.

Discussion

When comparing Fig. 1 and Fig. 2 it is seen that a certain increase in the lead concentration corresponds to almost the same reduction in the shell thickness. The thickness of the inner membranes is about 0.05 mm (KRAUL, unpublished), and if this amount is subtracted from the thickness of the old shells it is seen that the curves are quite similar. This means that the influence of the chlorinated pesticides on the shell thickness of the new eggs is masked. Investigations of recent Danish sparrow-hawk (*Accipiter nisus*) eggs indicate that also here a correlation exists between thin shells and relatively high concentrations of lead (GRANDJEAN & DYCK, unpublished).

The effect of organochlorine compounds on the shell thickness has been proved beyond doubt (RATCLIFFE 1970 and COOKE 1973). Certainly, part of the correlation between lead concentration and shell thickness must be explained by an association between lead and PCB's and perhaps other chlorinated hydrocarbons. But lead is an important confounding factor.

Lead has a high affinity for sulfhydryl groups and several other groups, but only a few enzymes are described to be inhibited by the lead concentrations normally occurring (VALLEE & ULMER 1972). The role of lead as a possible inhibitor of egg-shell production therefore needs elucidation.

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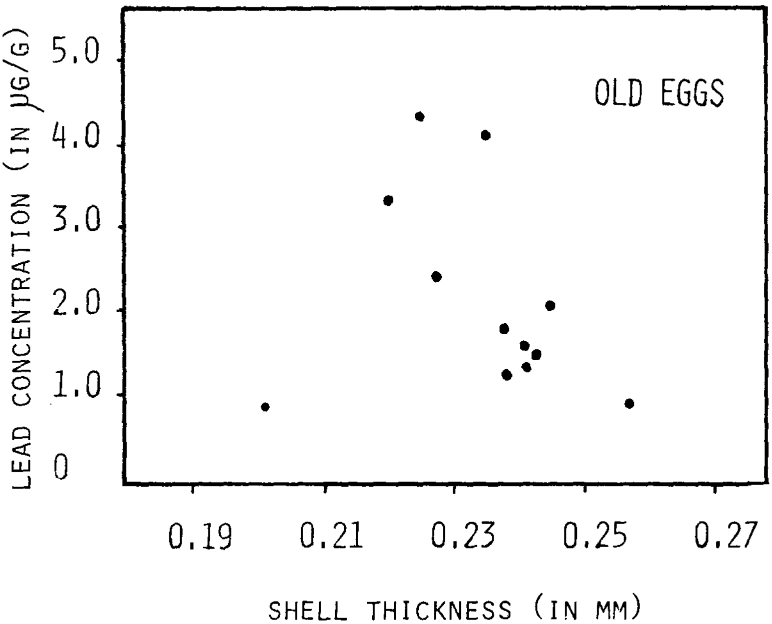

FIGURE 1. Lead concentration and egg-shell thickness (including inner membranes) during 1874-1953.

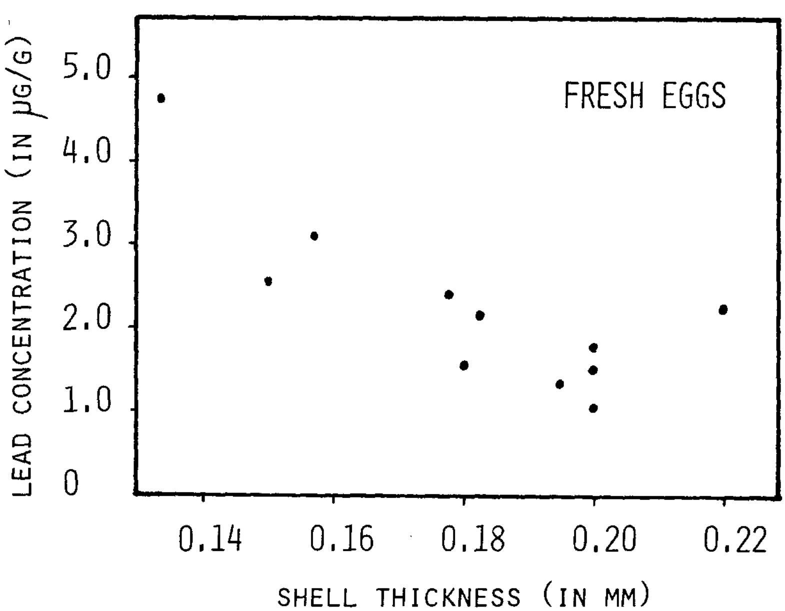

FIGURE 2. Lead concentration and egg-shell thickness (without inner membranes) during 1972-1974.

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