Numerous studies have shown variations in pesticide content among selected tissues and species (Casarett et al., 1968; Durham, 1967; Matthesse et al., 1968; Rumsey et al., 1967; Wesley et al., 1966), but little information is available concerning the distribution of pesticides within lipid-containing material. Hayes (1965) stated that the neutral fat content of tissues other than adipose tissue has some influence on the accumulation of organochlorine pesticide residues, but the distribution of DDT in viscera before and after starvation suggests factors other than fat content per se are involved. Hugunin and Bradley (1969) studied the distribution of dieldrin and Kelthane in components of buttermilk and reported residue association with phospholipids in milk fat. A better understanding of the mode of pesticide association within the lipid-containing material of the organism may lead to theories of how pesticides are stored and then mobilized and possibly may also lead to a better understanding of their action with the organism.

Egg yolk lipoproteins differ considerably in amount of lipid material and in the relative proportion of neutral to phospholipids. The low density lipoprotein, lipovitellin, contains 80-90% lipid, of which approximately 26% is phospholipid; whereas the high density lipoprotein, lipovitellenin, contains 18-23% fat which is made up of about 50% phospholipid (Cook, 1968). Several studies have shown that feeding pesticide-contaminated feed to hens results in deposition of the pesticide in the eggs (Cummings et al., 1966; Herrick et al., 1969; Stadelman et al., 1965; Wesley et al., 1969).

The effect of the amount and type of lipid present on the amount of selected pesticides associated with selected egg fractions was investigated using eggs obtained from hens fed contaminated feed. Pesticide residues in the eggs were determined in albumen, in the whole yolk, in the lipovitellin and lipovitellenin fractions, as well as in the protein fractions, phosphitin, and livitelin.

The chlorinated hydrocarbon pesticides have been grouped as DDT and its derivatives and related compounds including Kelthane and methoxychlor, cyclodiene compounds including aldrin, dieldrin, endrin, and heptachlor, and miscellaneous compounds such as BHC and lindane. Lindane, dieldrin, and p,p'-DDT were used in the current study to represent these groups. The slight differences in their water solubilities could also influence association with various lipid components.

METHODS AND MATERIALS

Three 1-week groups of eggs, designated as Groups I, II, and III, respectively, were collected from ten 10-month-old White Leghorn hens starting 1 week after the hens were fed ad libitum a standard laying ration which was contaminated with 25.0 ± 0.1 ppm analytical grade each of lindane (γ isomer of benzene hexachloride), dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethanaphthalene), and p,p'-DDT (1,1,1-trichloro-2-bis[p-chlorophenyl]ethane). Eggs were collected daily and held at 4°C until the entire group was collected; all eggs collected were used in each group.

The albumen from each group of eggs was separated and pooled. Adhering albumen was removed from the yolk, the vitelline membrane was punctured, and yolk contents from each group of eggs were pooled with 0.02% butylated hydroxytoluene added to minimize oxidation.

The yolk was further subdivided into the following four fractions: lipovitellin, lipovitellenin, phosphitin, and livetinin. Approximately 340 g of yolk from each group was fractionated according to the scheme presented in Figure 1. Lipovitellin, lipovitellenin, and livetinin fractions were dialyzed free of Cl and/or SO4 before total yield was obtained.

Chemical and Pesticide Residue Analyses. Percentage solids were determined by drying samples to a constant weight at 60°C under vacuum (Steer et al., 1968). Protein was determined using the microKjeldahl method (AOAC, 1960). Percentage phosphorus of the egg yolk fractions and total lipids of each fraction was obtained using procedures outlined by Morrison (1964).

Lipid Analyses. Total lipids of the albumen and whole yolk, as well as the lipovitellin, lipovitellenin, and livetinin fractions, were obtained by chloroform–methanol extraction (Bligh and Dyer, 1959). The total lipids of the phosphitin samples were estimated from aliquots of hexane solution following the hexane–methanol extraction for pesticide determinations.

The proportion of neutral lipids to phospholipids was obtained in two ways. The amount of phospholipids was estimated by multiplying the percentage phosphorus of the total lipids by 25, as outlined in several previous studies (Augustyniak et al., 1964; Martin et al., 1963; Noble and Moore, 1967). The second method utilized differential solubility of the lipid components in chloroform and methanol after the total lipid had been absorbed on activated silicic acid.

Pesticide Analyses. Sample sizes for pesticide residue analyses were as follows: 50 g for albumen, lipovitellin, lipovitellenin, phosphitin, and livetinin.
lipovitellenin, and livetin; 25 g for whole yolk; and 7–10 g for phosvitin. Following extraction with hexane-methanol (1:1), pesticide solutions were analyzed using a Beckman GC-4 chromatograph equipped with a discharge electron capture detector as described by Zabik and Dugan (1971). Concentrations were expressed on the wet weight and on the weights of the fat components, as well as on the total pesticide in each fraction.

RESULTS AND DISCUSSION

Egg production averaged 64.1%, which was well within the number of eggs anticipated from the previous laying history of these hens, and substantiates the conclusion of Cummings et al. (1966) that low level pesticide contamination does not affect egg production.

The percentage of yolk solids varied from 83.7 to 90.5%. The percentage distributions among the egg yolk fractions were fairly consistent and are in the range anticipated from the values reported by Cook (1968), except that the low density fraction in the granules is not separated from the lipovitellenin in the current study. The protein, lipid, and phosphorus values were used to verify the fraction identification.

LIPID COMPOSITION. Only traces of lipid material were found in the albumen (0.193, 0.236, and 0.101% based on solids for Groups I, II, and III, respectively). Phosphorus determinations of the albumen lipid material established low phospholipid contents of 15.9, 12.5, and 5.6% for Groups I, II, and III, respectively. However, Smith (1959) reported that diffusing yolk lipids are primarily triglyceride in nature. Thus, some of the very small quantity of lipid present in the albumen could have resulted by diffusion from the yolk during refrigerated storage.

Unfractionated yolk had total lipid contents of 32.38, 33.49, and 32.13% for Groups I, II, and III, respectively. Total lipid determinations, neutral lipids and phospholipids, and phospholipid estimated from the percentage lipid phosphorus for the egg yolk fractions are summarized in Table I. Phosvitin was found to be lipid-free as previously reported (Joubert and Cook, 1958).

The total lipid composition of lipovitellenin varied from 81 to 89% but is well within the range expected from previous studies (Martin et al., 1963; Sugano and Watanabe, 1961; Evans et al., 1969). Agreeing closely with the data of Martin et al. (1963), phospholipid accounted for approximately 25% of the lipovitellenin's total lipid for Groups I and II and approximately 20% for Group III.

Lipovitellin consisted of approximately 26%, of which about 40% was phospholipid. Previous reports had shown

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**Figure 1.** Separation scheme for obtaining egg yolk fractions

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**Table I.** Percentage Total Lipid, Neutral Lipid, and Phospholipids in Lipovitellin, Lipovitellenin, and Livetin

<table>
<thead>
<tr>
<th>Group</th>
<th>Fraction</th>
<th>Total lipid, %</th>
<th>Neutral lipid, %</th>
<th>Phospholipid, %</th>
<th>% P method, %</th>
<th>Average phospholipid, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Lipovitellin</td>
<td>25.74</td>
<td>63.02</td>
<td>37.98</td>
<td>38.20</td>
<td>38.09</td>
</tr>
<tr>
<td>I</td>
<td>Lipovitellenin</td>
<td>88.81</td>
<td>74.14</td>
<td>25.86</td>
<td>23.25</td>
<td>24.56</td>
</tr>
<tr>
<td>I</td>
<td>Livetin</td>
<td>42.23</td>
<td>80.16</td>
<td>19.84</td>
<td>13.78</td>
<td>16.81</td>
</tr>
<tr>
<td>II</td>
<td>Lipovitellin</td>
<td>83.60</td>
<td>71.58</td>
<td>28.42</td>
<td>25.97</td>
<td>27.20</td>
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<tr>
<td>II</td>
<td>Livetin</td>
<td>38.84</td>
<td>75.10</td>
<td>24.90</td>
<td>12.03</td>
<td>18.47</td>
</tr>
<tr>
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<td>25.58</td>
<td>56.26</td>
<td>43.74</td>
<td>39.25</td>
<td>41.67</td>
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<tr>
<td>III</td>
<td>Lipovitellin</td>
<td>80.56</td>
<td>81.99</td>
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<tr>
<td>III</td>
<td>Livetin</td>
<td>41.32</td>
<td>79.03</td>
<td>20.97</td>
<td>8.85</td>
<td>14.91</td>
</tr>
</tbody>
</table>

* Based on solids.  b Average of duplicate determinations.
Purified lipovitellin to have as high as 60% phospholipid (Martin et al., 1963); however, the small amount of low density fraction which is associated with lipovitellin in the granules, and is called LDFG, was not separated by the fractionation procedures used in this study. Since the LDFG has a lipid composition similar to other low density lipoproteins (Cook, 1968), its low phospholipid content would result in lowering of the proportion of phospholipid in the lipovitellin fraction. Nevertheless, the proportion of phospholipid in these lipovitellin preparations was still 1.5 to 2 times that in lipovitellenin.

The relatively high proportion of lipid in the livetin fraction resulted from free lipid released from the lipovitellenin during the extensive centrifugations. The high proportion of neutral lipid supports the postulation that it originated in lipovitellenin. Evans et al. (1969) reported that the easily extractable lipid of lipovitellenin was higher in neutral lipid than that of the total lipid. Probably some of this lipid material also resulted from contamination with lipovitellenin per se, since precise separation of the two materials during the fractionation was extremely difficult.

**Pesticide Analyses.** Gas chromatographic analyses revealed that a portion of the p,p'-DDT fed to the hens was dechlorinated to form DDE (1,1-dichloro-2,2-bis[p-chlorophenylethane]). This conversion of DDT to DDE by fowl has been reported by Cummings et al. (1966, 1967) and by Ritchey et al. (1967, 1969). Percentage recoveries of the pesticide residues in the egg yolk fractions varied from 83 to 98% and were, in general, equal to or greater than the percentage recoveries obtained for yolk solids.

Table II summarizes the pesticide concentrations expressed as ppm based on solids for the albumen, yolk, and yolk fractions. The distribution of lindane, dieldrin, and DDT compounds in albumen is 0.1, 0.05, and 0.2 ppm, respectively, on a liquid basis; and in lipid-free phosvitin it is 0.27, 0.96, and 0.82 ppm, respectively. The amount of dieldrin associated with phosvitin exceeds that which would be soluble in water associated with the protein. Therefore, protein pesticide interactions may contribute to their occurrence. Moss and Hathaway (1964) have previously reported that protein-dieldrin complexes are important in the transport of dieldrin in plasma. These interactions may also contribute to the occurrence of dieldrin in the phosvitin fraction.

Analyses of variance established highly significant differences among the pesticide residues (ppm based on solids) of the egg yolk and egg yolk fractions. Duncan’s (1957) multiple range was used to sort out these differences. The lindane and p,p'-DDT contents of lipovitellenin were significantly higher than those in the yolk, whereas the lipovitellenin content of dieldrin and p,p'-DDT values equaled those of the unfractionated yolk. The values for these pesticides in the three other egg yolk fractions were significantly less than those in the unfractionated yolk (Table II).

When the amount of lipid material was compensated for by calculating the residues as ppm based on lipid, lipovitellenin contained the greatest amount of pesticide residues, as shown in Table III. None of these differences were significant, however. These data are similar to the earlier findings of Rumsey et al. (1967) who reported that total DDT isomers were evenly distributed throughout beef tissues when expressed as a function of the lipid content.

Association of pesticides in fat has been reported to be influenced by both neutral (Hayes, 1965) and phospholipids (Hugunin and Bradley, 1969). Based on solids, the high lipid lipovitellenin fraction possessed the highest pesticide
residues (Figure 2). However, when the pesticides were expressed as a function of the amount of lipid present (Figure 3), lipovitellin, which had 25% lipid of which 40% was phospholipid, contained the greatest residue values. Correlating the residue values, expressed as ppm based on the lipid content, with the percentage of neutral and phospholipids established positive correlations with phospholipid and negative correlations with neutral lipid. Nevertheless, only the correlations between lindane and phospholipid ($r = 0.659$) and neutral lipid ($r = 0.659$) were significant and then only at the 5% level of probability. The other correlation coefficients ranged from 0.3 to 0.4.

In this study, the amount of lipid present is the most important determinant of chlorinated hydrocarbon pesticide content.
Secondarily, the type of lipid and the type of chlorinated hydrocarbon pesticides play a role in the pesticide’s distribution since the location of lindane in the egg yolk lipids appeared to be related to the phospholipid content.

LITERATURE CITED


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