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Effects of Hypophysectomy and Corticosterone Acetate Treatment on Hepatic Lipid Composition in the Chick (*Gallus domesticus*) Embryo

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Lipid is transferred from yolk to liver and to other tissues at an accelerated rate after 16 days of incubation in the intact chicken embryo. Hypophysectomy (partial decapitation) at 36 hr of incubation reduced liver lipid content on Days 16 and 18. The greatest losses on Day 18 were among cholesterol esters but decreased unesterified cholesterol and phospholipid were also observed. Treatment of hypophysectomized embryos with corticosterone acetate (300 μg per day on Days 13, 14, 15) partially restored total hepatic lipid content to control values on Day 16, with an even greater effect on 18 days of incubation. Most of the increase in lipid occurred in the triglyceride fraction. Liver cholesterol content also increased in response to hormone but neither cholesterol esters nor phospholipids were elevated by corticosterone acetate. Hypophysectomy interferes with the absorption of lipids from yolk and with fat deposition in embryonic liver. However, treatment of hypophysectomized embryos with corticosterone acetate did not correct the major defects in hepatic lipid content. Therefore, fat metabolism and transport of yolk lipids to the embryo during the last week of incubation does not depend exclusively on adrenal glucocorticoids.

Lipid metabolism in the avian embryo differs markedly from that observed in birds after hatching. This difference stems from the large amount of lipid stored in the yolk. Some yolk lipid components can be readily utilized by the embryo with only modest transformations in fatty acid composition (Noble and Moore, 1964). Lipogenesis, therefore, can be low in embryonic liver without loss of substrate required for lipid metabolism or for biosynthesis of cellular membranes (Goodridge, 1968a,b; Donaldson *et al.*, 1971; Joshi and Sidbury, 1975; Freeman, 1978). The embryonic liver, however, can remodel fatty acid composition of phospholipids derived from yolk (Noble and Moore, 1967). Similar to reduced lipogenesis, lipolytic pathways which degrade triglycerides to fatty acids in liver and in adipose tissue are also poorly developed in the embryo (Goodridge, 1968c; Langslow, 1972). Though β-oxidative pathways are active in the embryonic liver, fatty acid substrate can be obtained directly from yolk (Pugh and Sidbury, 1971). Lipid is transferred from yolk to embryo by the yolk sac membrane. The rate of transport increases after 14 days of incubation and exceeds 1 g/day in the final 2 days of incubation in the chick (Noble and Moore, 1964).

In contrast to our better understanding of the endocrine regulation of lipid metabolism in the postnatal or adult bird (Fisher and Goodridge, 1978; Goodridge and Ball, 1967; Goodridge, 1978; Joshi and Aranda, 1979a, b; Langslow *et al.*, 1979), hormones that regulate embryonic lipid metabolism or its major controlling step, the transport of lipid from yolk, have not yet been identified. A few experiments suggest important regulatory roles for some pituitary hormones and for adrenal glucocorticoids in embryonic lipid metabolism or transport. Hypophysectomy (partial decapitation) reduced hepatic lipid content after 16 days of incubation (Sandra and Thommes, 1977). Liver lipid content was partially restored in hypophysectomized embryos receiving pituitary trans-

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plants (Sandra and Thommes, 1977). Partial decapitation of chick embryos likewise depressed plasma cholesterol early in development (prior to 16 days), and this deficiency was corrected in part by a single treatment with cortisone acetate (Thommes and Shulman, 1967).

The purposes of this paper are first, to quantitatively characterize the ontogeny of some major hepatic lipid classes which are derived from yolk and deposited in the liver of the embryonic chick; and second, to observe the effects of hypophysectomy and corticosterone acetate treatment on liver lipid content and composition during the last week of incubation. These studies can decide if pituitary hormones and adrenal glucocorticoids are required for transfer of particular yolk lipids to the embryonic liver.

METHODS

Embryos. Fertilized eggs (Rhode Island Reds × White Leghorn hybrids) were obtained locally from a commercial supplier. To begin the timed incubation period, eggs were transferred from 12° to a humidified (60%) stationary incubator (37.5 ± 0.5°). Livers were examined in intact embryos on alternate days from Day 12 to Day 20. Livers were also examined in unfed chickens 12–24 hr after hatching (Day 21). Hypophysectomized embryos were produced by partial decapitation according to Fugo (1940) between 36 and 40 hr of incubation and eggs resealed with transparent tape. In some eggs the shell was opened and resealed but the embryo was not operated upon (windowed controls).

Hormone treatment. Corticosterone 21-acetate (Sigma) was dissolved in a small volume of 95% ethanol and then finely suspended in sterile 0.9% saline (final ethanol concentration, 2%). Treated embryos received 300 μg hormone each day on Days 13, 14, and 15 of incubation by application of 50 μl hormone suspension to chlorioallantoic membrane. Vehicle-treated embryos received 50 μl 2% ethanol in 0.9% saline.

Lipid isolation, separation, and measurement. Livers were dissected from embryos, weighed within 1 min, and frozen in dry ice–ethanol. Livers from 12-day embryos were pooled in groups of two. Measurements on older embryos used individual livers. Livers were homogenized in chloroform:methanol (2:1) and lipid extracted according to Folch *et al.* (1957). Resuspended homogenate (10 μl) was taken for measurement of protein using the method of Lowry *et al.* (1951) with bovine serum albumin as standard. The residue which remained after lipid extraction was dried and weighed. Residue weight was added to lipid weight determined later to yield the total dry weight. Lipid extracts were collected and solvent evaporated in pretared tubes. Lipid (1.0 to 1.5 mg) was applied in 2:1 chloroform:methanol to Whatman LK5D silica gel thin-layer chromatography (tlc) plates and neutral lipids separated using a one-dimensional, two-solvent system (*n*-hexane:diethyl ether:acetic acid, 90:10:1, followed by the same components, 70:30:2) (Mangold, 1964). The following neutral lipid standards (Supelco, Inc.) were used: cholesterol, tripalmitin for triglycerides, palmitic acid for fatty acids, and cholesterol oleate for cholesterol esters. These compounds were located on tlc plates by spraying with phosphomolybdic acid in 5% propanol (Pierce Chemical Co.). Neutral lipids in extracts, standards, and blanks, were measured spectrophotometrically after reaction of gels with acid dichromate (Amenta, 1964). Polar lipids were separated on the same tlc plates using chloroform:methanol:water (65:35:3) as solvent (LePage, 1964). Lipid extract (0.5–0.7 mg) was applied to the tlc plate. Phosphatidylcholine and phosphatidylethanolamine (Supelco, Inc.) were used as standards and were located by phosphomolybdic acid staining. Gels were eluted as described (Bienzenski, 1964) and phosphorus determined in 70% perchloric acid digests (Bartlett, 1959). Total phospholipid phosphorus was also measured following perchloric acid digestion of 0.1–0.3 mg lipid extract (Bartlett, 1959). Control experiments showed that extraction and recovery of all lipid classes analyzed exceeded 95%.

Statistics. In experiments involving hypophysectomy and treatment with hormone, data were analyzed by the Student's *t* test (one-sided).

RESULTS

Mean hepatic wet weight increased steadily from 83 mg on 12 days of incubation to 1155 mg on Day 21. Liver dry weight increased from 15% of the wet weight on Day 12 to 36% on Day 21. The rate of increase in hepatic dry weight rose sharply after 16 days compared with a slower rise on Days 12–16. The accelerated accretion of lipid after 16 days is shown in Fig. 1. Also illustrated is the somewhat lower rate of increase in liver protein during this same period, and a steady decline in tissue hydration from 83.5% water on Day 12 to 69.5% on Days 20 and 21. In this report, liver contents of particular lipid classes are

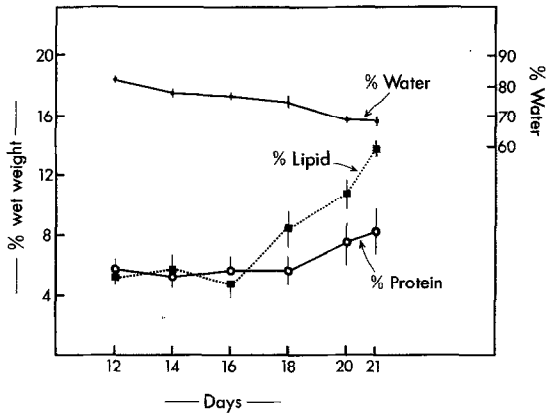

FIG. 1. Increased lipid and protein contents and decreased water content in embryonic chick liver from Day 12 to 21 of incubation.

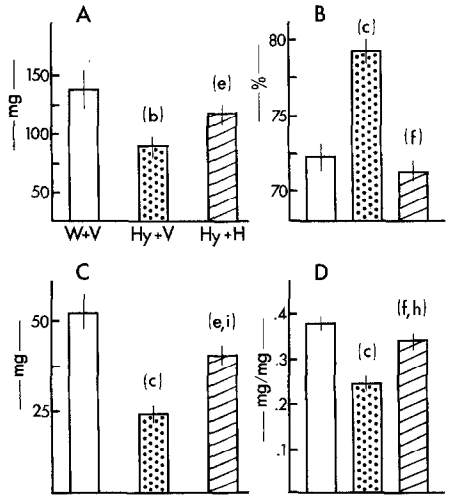

FIG. 2. Effects of hypophysectomy and 3 days treatment with vehicle or corticosterone acetate on liver composition in the 18-day embryo. (A) Liver dry weight (mg). (B) Hepatic percentage water. (C) Total liver lipid (mg). (D) Ratio of liver lipid content to liver dry weight (mg/mg). *N* in each group: W + V, 10; Hy + V, 9; Hy + H, 8. Other symbols defined in Table 2.

normalized with respect to dry or to lipid weights rather than to wet weight. This is especially important in operated embryos as hypophysectomy elevated hepatic water content independently of its effects on liver biochemical composition (Fig. 2 below; cf. Sandra and Thommes, 1977).

Table 1 documents increased contents of certain hepatic lipid components, cholesterol, total phospholipid, and the major phospholipid, phosphatidylcholine, from Days 12 to 21. When normalized to hepatic dry weight (which also increased during the same interval), the neutral lipid and phospholipids showed dissimilar ontogenetic patterns. Cholesterol content (per milligram of dry weight) increased from Days 12 to 18, but decreased on Day 20 and further at hatching. In contrast, fractional dry weight contributed by phospholipids declined between Days 12 and 14 but showed little further change to hatching. Fractional phosphatidylcholine content also varied little from Days 12 to 20, but declined significantly ($P < 0.05$) at hatching (Table 1).

Effects of hypophysectomy and corticosterone acetate treatment were observed on Days 16 and 18 of incubation. Results ob-

TABLE 1
ONTOGENY OF LIVER CHOLESTEROL, PHOSPHOLIPID, AND PHOSPHATIDYLCHOLINE: TOTAL CONTENT AND CONTENT NORMALIZED WITH RESPECT TO DRY WEIGHT

Incubation time (days)	*N*	Cholesterol		Phospholipid		Phosphatidylcholine	
		mg	mg/mg dry wt ($\times 10^3$)	μmol	μmol/mg dry wt	μmol	μmol/mg dry wt
12	5	0.12 ± 0.02	9.19 ± 1.32	2.02 ± 0.15	22.5 ± 3.2	0.83 ± 0.15	4.61 ± 0.70
14	7	0.27 ± 0.04	8.69 ± 0.64	4.75 ± 0.49	12.2 ± 1.0	1.58 ± 0.18	5.18 ± 0.71
16	7	0.62 ± 0.12	15.04 ± 1.70	6.46 ± 0.88	14.6 ± 1.2	1.93 ± 0.17	4.49 ± 0.92
18	8	2.54 ± 0.41	27.01 ± 2.08	11.05 ± 1.62	11.8 ± 0.9	5.06 ± 0.46	5.99 ± 0.35
20	5	4.41 ± 0.77	16.19 ± 0.81	12.87 ± 0.95	11.1 ± 1.1	8.91 ± 0.72	7.05 ± 0.43
21	5	6.82 ± 0.46	14.45 ± 1.88	41.94 ± 4.39	10.8 ± 2.1	11.65 ± 0.68	2.63 ± 0.73

tained using 16-day livers are summarized in Table 2. Hypophysectomized embryos showed minor (and statistically insignificant) reductions in total dry weight, lipid weight, and in cholesterol and phospholipid contents when compared with windowed controls. Hormone-treated hypophysectomized embryos displayed modestly increased hepatic dry weight, lipid weight, and cholesterol content, when compared with the hypophysectomized + vehicle group. Of these, only the elevation in total lipid weight was statistically significant ($P < 0.05$). Livers of hypophysectomized hormone-treated embryos also displayed minor reductions in total phospholipid and phosphatidylcholine contents when compared with livers of hypophysectomized + vehicle-treated embryos.

In 18-day embryos, hypophysectomy produced statistically significant depressions in liver dry weight, total lipids, and in the ratio of lipid to dry weight when compared with windowed embryos (Fig. 2). Hormone treatment restored, or partially restored, each of these characteristics to near control values (Fig. 2). Hypophysectomy and glucocorticoid replacement had different effects on the hepatic contents of particular lipid components at 18 days. Cholesterol was significantly elevated above the hypophysectomized level by hormone treatment (Table 3). Liver phospholipid content, in contrast, was reduced by hypophysectomy, but was not restored by hormone. Instead, the two major phospholipid components, phosphatidylcholine and phosphatidylethanolamine, were further depressed by hormone treatment (Table 4).

Data in Table 5 summarize hepatic contents of cholesterol esters, triglycerides, and free fatty acids observed in two unoperated groups of embryos (18- and 21-day) and in 18-day control, hypophysectomized, and hormone-treated embryos. Liver cholesterol ester content was markedly reduced by hypophysectomy, but was not restored by hormone treatment. Hormone-treated hypophysectomized embryos, in fact, showed a further reduction in hepatic cholesterol esters below the comparatively low level produced by pituitary ablation. Hepatic triglycerides were sharply in-

TABLE 2

EFFECTS OF HYPOPHYSECTOMY AND TREATMENT WITH VEHICLE OR CORTICOSTERONE ACETATE ON LIVER LIPID COMPOSITION AT 16 DAYS OF INCUBATION

Treatment	N	Lipid weight (mg)	Cholesterol (mg)	Phospholipid (μmol)	Phosphatidylcholine (μmol)
W + V	5	14.7 ± 1.8	0.73 ± 0.10	7.82 ± 0.92	3.02 ± 0.33
Hy + V	4	12.3 ± 0.9	0.51 ± 0.08	6.05 ± 0.54	4.17 ± 0.47
Hy + H	5	19.6 ± 3.4*,[d]	0.66 ± 0.07	5.85 ± 0.61	3.68 ± 0.43

Note. W + V, Windowed controls; Hy + V, hypophysectomized controls; Hy + H, hypophysectomized and hormone treated.

* Statistics: In Tables 2–6 and in Fig. 2, statistical differences (t test, one-sided) are summarized according to the following code:

[a] Hy + V significantly different from W + V (control), $P < 0.05$.
[b] Hy + V significantly different from W + V (control), $P < 0.01$.
[c] Hy + V significantly different from W + V (control), $P < 0.001$.
[d] Hy + H significantly different from Hy + V (control), $P < 0.05$.
[e] Hy + H significantly different from Hy + V (control), $P < 0.01$.
[f] Hy + H significantly different from Hy + V (control), $P < 0.001$.
[g] Hy + H significantly different from W + V (control), $P < 0.05$.
[h] Hy + H significantly different from W + V (control), $P < 0.01$.
[i] Hy + H significantly different from W + V (control), $P < 0.001$.

Unlabeled values are not significantly different ($P < 0.05$) from controls.

TABLE 3
EFFECTS OF HYPOPHYSECTOMY AND TREATMENT WITH VEHICLE OR CORTICOSTERONE ACETATE ON LIVER CHOLESTEROL CONTENT AT 18 DAYS OF INCUBATION

		Cholesterol	
Treatment	N	mg	mg/mg lipid ($\times 10^2$)
W + V	10	3.37 ± 0.38	4.60 ± 0.26
Hy + V	9	0.81 ± 0.07[c]	3.46 ± 0.38[b]
Hy + H	8	1.76 ± 0.18[f,a]	4.30 ± 0.15[e]

creased in hypophysectomized embryos compared with control and unoperated 18-day embryos. Hepatic triglyceride concentration remained elevated in the corticosterone acetate-treated group. Most of the increase in lipid obtained in response to hormone could be attributed to gains in the triglycerides fraction. A modest (nonsignificant) rise in liver free fatty acids in hypophysectomized embryos was also not altered by hormone treatment.

DISCUSSION

Ontogeny of Hepatic Lipids

These data confirm that hepatic lipid content increases during ontogeny with the most marked rise occurring after 16 days of incubation (Fig. 1; Noble and Moore, 1964, 1967; Romanoff, 1960). In terms of particular lipid components, cholesterol and phospholipid (including phosphatidylcholine) content increased rapidly from 16 days on. When normalized to hepatic lipid content or dry weight, however, the proportion contributed by each component to the total liver lipid changed during ontogenesis in nonparallel fashion (Table 1). The period after 16 days of incubation corresponds with the incubation interval during which lipid transport from yolk to embryo is greatest (Noble and Moore, 1964) and also coincides with maturation of the pituitary–adrenal axis (Wise and Frye, 1973; Kalliecharan and Hall, 1974).

Effects of Hypophysectomy on Hepatic Lipid Composition

Most differences observed in livers of control and hypophysectomized 16-day embryos were minor (Table 2). The lack of clear differences in lipid content or composition in experimental and control groups may indicate that embryonic lipid metabolism or transport of fat from yolk functions without an important pituitary hormone requirement before 16 days. In contrast, hypophysectomy produced several changes in lipid composition of the liver of the 18-day chick embryo. Compared with intact-windowed controls, livers in the hypophysectomized group displayed reductions in dry weight, in total lipid, and in lipid per unit dry weight; hypophysectomy also increased hepatic water content (Fig. 2; Sandra and Thommes, 1977) and produced a decline in liver protein content (data not shown). Hypophysectomy reduced total liver cholesterol and also lowered its relative abundance in hepatic lipids (Table 3). This result seems consistent with the depressed serum cholesterol concentration observed in hypophysectomized embryos (Thommes and Shulman, 1967). Pituitary ablation also reduced total hepatic

TABLE 4
EFFECTS OF HYPOPHYSECTOMY AND TREATMENT WITH VEHICLE OR CORTICOSTERONE ACETATE ON LIVER PHOSPHOLIPID CONTENT AND COMPOSITION AT 18 DAYS OF INCUBATION

		Phospholipid (μmol)		Phosphatidylcholine (μmol)		Phosphatidylethanolamine (μmol)	
Treatment	N	Total	mg lipid^{-1}	Total	mg lipid^{-1}	Total	mg lipid^{-1}
W + V	10	14.35 ± 1.90	0.40 ± 0.02	7.34 ± 0.36	0.19 ± 0.01	2.13 ± 0.25	0.04 ± 0.01
Hy + V	9	9.95 ± 0.88[c]	0.39 ± 0.03	5.42 ± 0.63[c]	0.27 ± 0.03[c]	1.45 ± 0.19[c]	0.08 ± 0.01[c]
Hy + H	8	9.77 ± 0.76[i]	0.29 ± 0.05[e,h]	4.61 ± 0.52[i]	0.14 ± 0.02[f,h]	1.04 ± 0.13[e,i]	0.03 ± 0.01[f]

TABLE 5
EFFECTS OF HYPOPHYSECTOMY AND TREATMENT WITH VEHICLE OR CORTICOSTERONE ACETATE ON HEPATIC CONTENTS OF CHOLESTEROL ESTERS, TRIGLYCERIDES, AND FREE FATTY ACIDS AT 18 DAYS OF INCUBATION

Treatment	N	mg/mg lipid ($\times 10^2$)		
		Cholesterol esters	Triglycerides	Free fatty acids
21-Day intact	4	45.06 ± 3.81	4.1 ± 1.2	0.49 ± 0.15
18-Day intact	4	37.18 ± 2.51	5.9 ± 1.5	0.49 ± 0.13
18-Day W + V	5	29.77 ± 2.25	9.1 ± 1.8	0.71 ± 0.20
18-Day Hy + V	5	9.19 ± 0.83[c]	27.4 ± 2.3[c]	1.17 ± 0.32
18-Day Hy + H	8	3.38 ± 0.34[e,i]	32.1 ± 2.7[e,i]	1.18 ± 0.24

phospholipids, phosphatidylcholine, and phosphatidylethanolamine (Table 4). However, after normalization to lipid content the fractional contribution of phosphatidylcholine and phosphatidylethanolamine to total lipid actually increased. That is, the decrease in these two phospholipid components was relatively less than the decline in lipid content generally. Therefore, one effect of hypophysectomy was to selectively alter the proportions of particular lipid classes.

Cholesterol esters are quantitatively the most important lipid class in the avian embryonic liver, accounting for about 30% of the dry weight in the 19-day embryo (Moore and Doran, 1962; Noble and Moore, 1964). Cholesterol ester content was especially sensitive to hypophysectomy. Hepatic lipid in the hypophysectomized group possessed only 30% of the esterified cholesterol contained in windowed controls, but displayed increased hepatic triglyceride content (Table 5). Increased triglyceride and decreased cholesterol ester concentrations have also been observed in the livers of vitamin B_{12}-deficient embryos (Noble and Moore, 1964) and may signal severe metabolic disturbance.

Effects of Treatment with Corticosterone Acetate

At issue is whether liver lipid content or composition is controlled by adrenal glucocorticoids. Treatment with corticosterone acetate (300 μg/day on Days 13, 14, and 15) elevated liver lipid content above that seen in hypophysectomized livers in 16- and 18-day embryos (Table 2, Fig. 2). Hormone treatment also raised liver protein content (data not shown) and reduced the tissue hydration typically observed after hypophysectomy (Fig. 2). Alterations in the liver content of particular types of lipids in response to hormonal treatment was highly lipid specific. Cholesterol content in 18-day livers was elevated by glucocorticoid to twice the hypophysectomized level, but did not reach the higher content observed in the intact group (Table 3). In contrast, total hepatic phospholipid and its two major components, phosphatidylcholine and phosphatidylethanolamine, were not increased above the depressed hypophysectomized value in response to hormone. Glucocorticoid treatment actually caused a further decline in the relative proportions of the major hepatic phospholipids (Table 4). Corticosterone acetate treatment neither restored the loss of hepatic cholesterol esters observed after hypophysectomy, nor did this hormone lower elevated liver triglycerides and free fatty acids measured in hypophysectomized embryos (Table 5). An important conclusion can be drawn with respect to the types of hepatic lipids lost (after hypophysectomy) and gained (after corticosterone treatment of hypophysectomized embryos). The major constituents lost were cholesterol esters, whereas the

greatest gain in response to hormone was in triglycerides.

The experiments reported in this paper demonstrate an important role for pituitary hormones in the regulation of hepatic lipid content in the chick embryo. One or more pituitary hormones are required for normal development of liver lipid composition, and this requirement is most stringent after 16 days of incubation. The failure of corticosterone acetate to elevate individual and total phospholipids and cholesterol esters, as well as its inability to lower triglyceride concentration to levels seen in intact embryos, argues that control of these important liver lipids may depend on other hormones which are also reduced or eliminated by hypophysectomy. One possible agent in thyroid hormone, the secretion of which is controlled by pituitary TSH in the chick embryo from Day 12.5 (Thommes *et al.*, 1977). T_3 is lipogenic in hepatocytes isolated from the 19-day chick and acts with insulin to increase acetyl-CoA carboxylase, fatty acid synthetase (Fisher and Goodridge, 1978), and malic enzyme (Goodridge, 1978; Joshi and Aranda, 1979b). With respect to yolk sac development and function per se, T_3 and T_4 appear to promote retraction of this membrane during the period of maximal fat absorption on Days 19–20 (Wishart *et al.*, 1977). Further investigations are required to establish the roles of thyroid hormone and also of insulin in embryonic lipid metabolism and transport.

Sites of pituitary and adrenal hormone actions cannot be located from the results of these studies. As the metabolic consequences of hypophysectomy are widespread and severe (Thommes and Shulman, 1967), many metabolic pathways which are directly or only indirectly concerned with lipid metabolism or transport will also be disturbed by partial decapitation. However, the notable decline of hepatic cholesterol esters and phospholipids after hypophysectomy suggest direct or indirect effects of pituitary hormones on yolk absorption. Both kinds of lipids originate in the yolk and are modified by the yolk sac membrane during absorption (Noble and Moore, 1964, 1967). Cholesterol esters are especially important for the assembly of lipoproteins which move lipid from yolk to embryo (Freeman, 1978). These esters appear to be synthesized by blood and yolk sac lecithin–cholesterol acyl transferases which use cholesterol transported from yolk by very low density lipoproteins as substrate (Noble and Moore, 1967; Bengtsson *et al.*, 1977). Cholesterol ester so formed are then deposited and stored in the embryonic liver. Hypophysectomy interferes at some point with this transport sequence which normally translocates yolk lipids to the liver.

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