Further Studies on the Lipid Metabolism of the Normal and Vitamin B₁₂-Deficient Chick Embryo

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1. The triglyceride, cholesterol ester and total phospholipid fractions were isolated from the livers and yolk sacs of normal and vitamin B₁₂-deficient chick embryos after 13, 15, 17, 19 and 21 days of incubation, and the fatty acid compositions were determined. 2. At all stages of incubation, the concentration of cholesterol ester in the livers of the normal embryos were greater, and on days 15 and 17 the concentrations of triglyceride were considerably less, than the corresponding concentrations in the livers of the deficient embryos. 3. Between day 13 and day 21 of incubation the concentration of oleic acid in the liver triglycerides of the normal embryos increased, whereas the concentrations of palmitic acid and docosahexaenoic acid decreased. Vitamin B₁₂ deficiency resulted in higher concentrations of palmitic acid in the liver triglycerides on days 15, 17 and 19, higher concentrations of C₁₈ polyunsaturated acids on days 13 and 15 and lower concentrations of oleic acid on days 13, 15, 17 and 19. 4. At all stages of development, cholesterol oleate accounted for almost 80% of the total liver cholesterol esters in both normal and deficient embryos. 5. As development of the normal embryos progressed, the concentrations of palmitic acid and arachidonic acid in the liver phospholipid decreased, whereas the concentrations of stearic acid and docosahexaenoic acid increased. Vitamin B₁₂ deficiency resulted in markedly higher concentrations of stearic acid and palmitic acid and markedly lower concentrations of arachidonic acid and docosahexaenoic acid in the liver phospholipids. 6. Vitamin B₁₂ deficiency did not influence the fatty acid composition of the triglyceride, cholesterol ester and phospholipid fractions either in the yolks of fertile unincubated eggs or in the yolks obtained from eggs that had been incubated for 13, 15, 17, 19 and 21 days.

Moore & Doran (1962) found that the development of the normal chick embryo between day 15 and day 19 of incubation was accompanied by a progressive accumulation of esterified cholesterol in the liver. This accumulation occurred to such an extent that in the 19-day embryo cholesterol ester accounted for about 70% of the total lipid and about 30% of the total dry matter of the liver. Moore & Doran (1961, 1962) also found that this characteristic pattern of lipid distribution in the liver of the developing chick embryo was markedly deranged by a deficiency of vitamin B₁₂, which resulted in a pronounced decrease in the proportion of cholesterol ester and a pronounced increase in the proportion of triglyceride. Although considerable progress has been made in the elucidation of the function of vitamin B₁₂ in intermediary metabolism (White, 1962), the role, whether direct or indirect, of vitamin B₁₂ in lipid metabolism as yet remains obscure. It seemed possible that an investigation into the nature of the fatty acids

present in the major lipid fractions contained in the livers of normal and deficient chick embryos might contribute to an understanding of the part played by vitamin B₁₂ in the metabolism of lipids. The results of such an investigation are now reported.

EXPERIMENTAL

Embryos. As described fully by Moore & Doran (1962), vitamin B₁₂-deficient embryos were obtained from the eggs of hens that had been given for 15 months the all-vegetable-protein diet used by Coates, Harrison & Kon (1951). Normal embryos were obtained from the eggs of hens that had been given for a similar period the same diet supplemented with 27.5 µg. of vitamin B₁₂/kg. Embryos were taken from the eggs after 13, 15, 17, 19 and 21 days of incubation and, after removal of the yolk sac, the liver was excised from each embryo. (On day 21 of incubation some chicks had in fact emerged from the shell, but for convenience they are referred to as embryos.) To obtain sufficient liver lipid for analysis, the livers obtained from the embryos at each stage of incubation were pooled as shown in Table 1. The yolk sacs plus contents were pooled in the same manner

as were the livers. In addition, the yolks were separated from six fertile unincubated eggs that had been obtained from the normal hens and from six fertile unincubated eggs that had been obtained from the deficient hens.

Extraction of lipids and methods of analysis. The lipids were extracted from the livers and yolks by the method of Folch, Lees & Sloane-Stanley (1957). Portions of the purified lipid extracts were then chromatographed on 3g. columns of silicic acid as described in detail by Moore & Doran (1962); only the cholesterol ester, triglyceride and total phospholipid fractions were retained for analysis. The purity of each of the fractions was checked by thin-layer chromatography (Mangold, 1961). Cholesterol, glyceride glycerol and lipid phosphorus were determined by the methods used by Moore (1962) and Moore & Doran (1962). Weights of cholesterol ester and triglyceride were arbitrarily calculated as cholesterol oleate and triolein respectively. Weights of phospholipid were arbitrarily obtained by multiplying the lipid phosphorus values by 25 (Wittcoff, 1951). The fatty acids of the cholesterol ester, triglyceride and phospholipid fractions were converted into the corresponding methyl esters by the transesterification procedure of Stoffel, Chu & Ahrens (1959). The methyl esters were then analysed by gas-liquid chromatography as described in detail by Moore & Williams (1963, 1964).

The significance of the differences between the means for the normal and deficient embryos was determined by Student's *t* test, the 1% level of significance being accepted as indicative of a real biological difference.

RESULTS

Livers. Only on day 19 of incubation was there any significant difference between the dry weight of the normal and deficient embryonic livers (Table 1). This difference was entirely accounted for by the higher lipid content of the normal embryonic livers, since the mean lipid-free dry weights of the normal and deficient livers on day 19 of incubation were 94.1 and 92.3mg. respectively. The total lipid content of the normal livers increased substantially between day 13 and day 19 of incubation, but subsequently remained constant. On day 15 the concentration of total lipid in the livers of the deficient embryos was about 40% greater than that in the livers of the normal embryos, but on day 19 the concentration of total lipid in the livers of the deficient embryos was about 15% less than in the livers of the normal embryos. As with the normal embryos, no change in the total lipid content of the livers of the deficient embryos occurred between day 19 and day 21. The concentration of triglyceride in the normal livers did not change appreciably between day 13 and day 19 of incubation, but decreased somewhat between day 19 and day 21. In marked contrast, the triglyceride content of the deficient livers increased to the extent that, on day 17, triglyceride accounted for about 45% of the total liver lipid. After day 17 of incubation, the triglyceride content of the deficient livers decreased until on day 21 it was little different from

Table 1. *Lipid compositions of the livers of normal and vitamin B₁₂-deficient chick embryos*

Experimental details are given in the text. Mean values $\pm$ S.E.M. are given.											
Age of embryos (days)	...	Normal embryos					Vitamin B ₁₂ -deficient embryos				
		13	15	17	19	21	13	15	17	19	21
No. of pooled groups of livers	...	4	4	4	4	4	4	4	4	4	4
Total no. of embryos	...	68	36	36	24	16	68	60	40	32	16
Mean dry wt. of livers (mg./embryo)	...	33.3 $\pm$ 0.83	75.1 $\pm$ 0.61	128 $\pm$ 7.01	195 $\pm$ 5.33	296 $\pm$ 6.36	37.8 $\pm$ 1.35	91.8 $\pm$ 7.68	131 $\pm$ 5.65	155 $\pm$ 8.49*	315 $\pm$ 14.7
Component lipids (g./100 g. of dry matter)											
Total lipid		21.2 $\pm$ 0.45	27.3 $\pm$ 0.58	37.4 $\pm$ 0.57	51.5 $\pm$ 0.62	51.4 $\pm$ 1.28	25.0 $\pm$ 0.53*	37.9 $\pm$ 2.01*	40.5 $\pm$ 2.02	44.1 $\pm$ 1.54*	43.7 $\pm$ 0.44*
Triglyceride		3.20 $\pm$ 0.06	2.88 $\pm$ 0.13	3.06 $\pm$ 0.17	3.20 $\pm$ 0.18	1.82 $\pm$ 0.29	6.08 $\pm$ 0.81	17.0 $\pm$ 1.90*	18.1 $\pm$ 2.67*	11.8 $\pm$ 4.68	2.32 $\pm$ 0.50
Cholesterol ester		4.93 $\pm$ 0.37	9.70 $\pm$ 0.53	23.1 $\pm$ 0.40	37.9 $\pm$ 0.71	41.2 $\pm$ 1.35	1.90 $\pm$ 0.22*	2.48 $\pm$ 0.28*	5.32 $\pm$ 0.90*	16.1 $\pm$ 0.43*	26.6 $\pm$ 1.15*
Phospholipid		10.9 $\pm$ 0.08	10.3 $\pm$ 0.24	8.51 $\pm$ 0.17	7.48 $\pm$ 0.31	7.28 $\pm$ 0.17	9.85 $\pm$ 0.49	8.52 $\pm$ 0.54	7.51 $\pm$ 0.20*	7.03 $\pm$ 0.45	6.77 $\pm$ 0.30

* Significantly different ($P < 0.01$) from that in the normal embryos.

* Significantly different (*P* < 0.01) from that in the normal embryos.

that found in the livers of the normal embryos. Throughout the whole period of incubation studied, pronounced increases occurred in the concentration of cholesterol ester in the normal livers, and on day 21 cholesterol ester accounted for about 80% of the total liver lipid. The concentration of cholesterol ester in the livers of the deficient embryos also increased during the last week of incubation, but the increase was much less pronounced than that observed in the livers of the normal embryos. At each stage of incubation studied, the concentration of cholesterol ester in the deficient livers was significantly less than that in the normal livers. From day 13 to day 21 the concentration of phospholipid in the livers of both normal and deficient embryos declined somewhat. The concentration of phospholipid in the livers of the deficient embryos was significantly less than that in the livers of the normal embryos only on day 17. These observations therefore confirm and extend those made over a shorter incubation period by Moore & Doran (1962). That the effects of vitamin B₁₂ deficiency on the liver lipids were apparently greater on day 17 than on day 21 (Table 1) may be explained by the fact that maximum mortality due to vitamin B₁₂ deficiency occurs at day 17 of incubation, and embryos surviving beyond this stage have greater reserves of the vitamin (Moore & Doran, 1962, and unpublished work).

In the normal embryos, the concentration of oleic acid in the liver triglycerides increased, whereas the concentrations of palmitic acid and docosahexaenoic acid decreased somewhat as development proceeded (Table 2). [In Table 2 and elsewhere in this paper, the shorthand designation suggested by Farquhar, Insull, Rosen, Stoffel & Ahrens (1959) is used to denote the individual fatty acids.] The concentrations of the other fatty acids present in the liver triglycerides of the normal embryos did not alter appreciably between day 13 and day 21 of the incubation period. Attention is drawn to the relatively high concentration of docosahexaenoic acid in the liver triglycerides of the 13-day and 15-day embryos. Although this C₂₂ polyunsaturated acid is characteristically found in fairly high concentrations in certain phospholipid classes, the concentration of docosahexaenoic acid in the liver triglycerides of the 13-day and 15-day embryos was almost twice that found in the corresponding phospholipid fractions (Table 4). Between day 13 and day 19 of incubation, the concentration of oleic acid in the liver triglycerides of the deficient embryos remained relatively constant, and at all stages during this period was about 28% lower than that in the triglycerides of the normal embryonic livers. After day 19 of incubation, the concentration of oleic acid in the liver triglycerides of the deficient embryos rose

Table 2. *Fatty acid compositions of the triglyceride fractions isolated from the livers of normal and vitamin B₁₂-deficient chick embryos*

Age of embryos (days)...	Fatty acid content (molar percentage of the total)									
	Normal embryos					Vitamin B ₁₂ -deficient embryos				
	13	15	17	19	21	13	15	17	19	21
Acid										
23:3	1.38	2.33	2.21	2.11	1.90	29.2	31.4	33.0	30.8	22.6
16:0	1.72	1.33	1.26	1.22	1.35	1.20	1.45	1.32	1.12	1.42
18:1	9.39	9.59	9.63	10.1	9.36	8.28	7.73	8.18	9.65	10.3
18:0	33.2	35.4	37.9	39.1	41.0	26.2	26.7	24.7	27.1	36.9
18:2	12.5	12.5	13.8	13.6	13.8	15.8	17.6	16.6	15.5	15.0
20:4	4.95	4.84	4.01	4.34	4.80	4.67	3.81	4.24	3.87	4.01
22:5	1.45	0.89	0.82	1.22	1.46	1.30	1.29	1.06	1.71	1.60
22:6	11.7	11.8	9.24	8.12	7.51	11.2	7.74	7.61	7.52	4.99

* Significantly different ($P < 0.01$) from that in the normal embryos.

abruptly and on day 21 was little different from that found in the liver triglycerides of the normal embryos. The results given in Table 2 also show that on days 15, 17 and 19 the concentration of palmitic acid, and on days 13 and 15 the concentration of the C₁₈ polyunsaturated acids, in the liver triglycerides of the deficient embryos were considerably greater than the corresponding concentrations in the liver triglycerides of the normal embryos. Vitamin B₁₂ deficiency did not result in any marked changes in the concentrations of stearic acid and the C₂₀ and C₂₂ polyunsaturated acid in the liver triglycerides of the developing chick embryo.

Although large increases occurred in the concentrations of cholesterol ester in the dry matter of the normal embryonic livers during development (Table 1), the fatty acid composition of the cholesterol ester fraction remained relatively constant (Table 3). At each stage of incubation, cholesterol oleate accounted for almost 80% of the total cholesterol esters present in the embryonic livers. Similar findings have been reported by Schjeide (1963) and Feldman & Grantham (1964). In spite of the pronounced effect of vitamin B₁₂ deficiency on the cholesterol ester content of the embryonic livers (Table 1), a deficiency of this vitamin did not appear to influence to any great extent the composition of the fatty acids esterified with cholesterol. However, the results given in Table 3 show that, in the early stages of the incubation period, the concentrations of palmitoleic acid, docosahexaenoic acid and the C₁₈ polyunsaturated acids in the cholesterol esters in the livers of the deficient embryos were somewhat different from the corresponding concentrations of these acids in the cholesterol esters of the normal embryonic livers.

During the last week of development, the concentrations of palmitic acid and arachidonic acid in the phospholipids of the normal embryonic livers decreased, the concentration of oleic acid remained fairly constant and the concentrations of stearic acid, docosahexaenoic acid and the C₁₈ polyunsaturated acids increased (Table 4). Analogous changes occurred with development in the concentrations of palmitic acid, stearic acid and docosahexaenoic acid in the liver phospholipids of the deficient embryos. Although the effect of vitamin B₁₂ deficiency on the concentrations of phospholipid in the embryonic liver was not very marked (Table 1), the effect on the concentrations of certain of the fatty acids present in the liver phospholipids was, on the other hand, particularly striking. In the liver phospholipids of the deficient embryos the concentrations of stearic acid and palmitic acid were consistently greater, and the concentrations of arachidonic acid and docosahexaenoic acid consistently less, than the corresponding concentrations of these acids in the

Table 3. *Fatty acid compositions of the cholesterol ester fractions isolated from the livers of normal and vitamin B₁₂-deficient chick embryos*

Age of embryos (days)...	Fatty acid content (molar percentage of the total)											
	Normal embryo						Vitamin B ₁₂ -deficient embryos					
	13	15	17	19	21		13	15	17	19	21	
Acid												
16:0	2.31 ± 0.04	2.66 ± 0.06	2.42 ± 0.31	2.56 ± 0.20	1.95 ± 0.09		3.86 ± 0.51	3.42 ± 0.39	2.41 ± 0.30	2.21 ± 0.25	1.76 ± 0.09	
18:0	2.04 ± 0.20	2.67 ± 0.36	2.83 ± 0.36	3.26 ± 0.13	2.74 ± 0.18		2.82 ± 0.24	3.12 ± 0.37	2.58 ± 0.31	2.38 ± 0.32	2.48 ± 0.10	
16:1	0.91 ± 0.05	0.92 ± 0.03	0.99 ± 0.03	0.98 ± 0.08	0.97 ± 0.02		1.24 ± 0.05*	1.14 ± 0.03*	1.04 ± 0.12	0.97 ± 0.06	1.17 ± 0.05	
18:1	77.2 ± 1.13	76.8 ± 2.60	77.3 ± 1.44	77.1 ± 1.63	79.6 ± 1.19		76.5 ± 1.20	79.0 ± 1.50	79.9 ± 1.27	78.4 ± 1.08	81.7 ± 0.70	
18:2+18:3	12.7 ± 0.06	13.8 ± 0.57	13.7 ± 0.23	12.4 ± 1.03	12.5 ± 0.84		9.05 ± 0.17*	9.29 ± 0.72*	10.8 ± 0.71*	10.7 ± 0.74	9.81 ± 0.62	
20:4	2.24 ± 0.19	1.56 ± 0.09	1.59 ± 0.04	2.14 ± 0.12	1.47 ± 0.13		1.92 ± 0.11	1.38 ± 0.17	2.14 ± 0.33	3.57 ± 0.39	1.80 ± 0.10	
22:6	1.91 ± 0.12	1.31 ± 0.06	0.92 ± 0.21	1.11 ± 0.34	0.42 ± 0.08		3.40 ± 0.19*	1.54 ± 0.76	0.85 ± 0.11	0.97 ± 0.24	0.58 ± 0.12	

* Significantly different ($P < 0.01$) from that in the normal embryos.

Experimental details are given in the text. Mean values ± S.E.M. are given. Numbers of pooled groups and total numbers of embryos are as given in Table 1.

Table 4. *Fatty acid compositions of the phospholipid fractions isolated from the livers of normal and vitamin B₁₂-deficient chick embryos*Experimental details are given in the text. Mean values $\pm$ S.E.M. are given. Numbers of pooled groups and total numbers of embryos are as given in Table 1.

Age of embryos (days)...	Fatty acid content (molar percentage of the total)									
	Normal embryos					Vitamin B ₁₂ -deficient embryos				
	15	17	19	21		13	15	17	19	21
Acid										
16:0	22.4 $\pm$ 0.54	19.7 $\pm$ 0.26	18.4 $\pm$ 0.45	16.9 $\pm$ 0.85	17.8 $\pm$ 0.88	28.8 $\pm$ 0.42*	25.5 $\pm$ 0.29*	24.4 $\pm$ 0.39*	25.3 $\pm$ 0.84*	23.9 $\pm$ 0.37*
18:0	24.6 $\pm$ 0.17	25.0 $\pm$ 0.25	26.9 $\pm$ 0.2	27.5 $\pm$ 0.29	28.4 $\pm$ 0.55	32.5 $\pm$ 0.48*	33.3 $\pm$ 0.67*	33.8 $\pm$ 0.40*	34.4 $\pm$ 1.78*	35.1 $\pm$ 0.29*
18:1	7.29 $\pm$ 0.13	8.38 $\pm$ 0.27	7.86 $\pm$ 0.41	7.46 $\pm$ 0.23	7.97 $\pm$ 0.64	9.60 $\pm$ 0.17*	10.4 $\pm$ 0.56	9.44 $\pm$ 0.11*	8.28 $\pm$ 0.17	9.60 $\pm$ 0.80
18:2 + 18:3	9.94 $\pm$ 0.50	13.0 $\pm$ 0.23	15.0 $\pm$ 0.16	14.1 $\pm$ 0.28	13.8 $\pm$ 0.30	11.0 $\pm$ 0.27	14.2 $\pm$ 0.61	13.8 $\pm$ 0.40	13.1 $\pm$ 0.50	13.5 $\pm$ 0.33
20:4	26.2 $\pm$ 0.32	24.3 $\pm$ 0.08	22.1 $\pm$ 0.26	22.3 $\pm$ 0.39	20.0 $\pm$ 0.50	12.6 $\pm$ 0.14*	11.0 $\pm$ 0.33*	12.4 $\pm$ 0.33*	11.2 $\pm$ 0.70*	10.2 $\pm$ 0.51*
22:6	6.45 $\pm$ 0.17	6.49 $\pm$ 0.19	7.11 $\pm$ 0.18	8.20 $\pm$ 0.30	9.12 $\pm$ 0.63	2.34 $\pm$ 0.18*	1.98 $\pm$ 0.16*	2.97 $\pm$ 0.18*	4.18 $\pm$ 0.73*	4.18 $\pm$ 0.35*
Unsatd.:saturd. fatty acid ratio	1.06 $\pm$ 0.026	1.17 $\pm$ 0.018	1.15 $\pm$ 0.010	1.18 $\pm$ 0.030	1.11 $\pm$ 0.039	0.580 $\pm$ 0.005*	0.642 $\pm$ 0.024*	0.670 $\pm$ 0.011*	0.618 $\pm$ 0.031*	0.637 $\pm$ 0.022*

* Significantly different ($P < 0.01$) from that in the normal embryos.

phospholipids of the normal embryonic livers. On days 13 and 17 the concentrations of oleic acid in the liver phospholipids of the deficient embryos were somewhat higher than in the liver phospholipids of the normal embryos. Attention is drawn to the fact that the unsaturated:saturated fatty acid ratios in the phospholipids of the deficient embryonic livers were little more than half the corresponding ratios in the phospholipids of the normal embryonic livers.

Yolks. The fatty acid compositions of the triglyceride, phospholipid and cholesterol ester fractions isolated from the yolks obtained from the normal and deficient embryos at each stage of incubation are not given in detail, since at no stage in the incubation process did vitamin B₁₂ deficiency appear to exert any effect on the constituent fatty acids of the three yolk lipids. Moreover, the fatty acid compositions of the various yolk lipids at each stage of incubation were almost identical with those found in an investigation with normal embryos reported by Noble & Moore (1964). In the fertile unincubated eggs obtained from the normal hens, the mean fatty acid composition of the yolk triglyceride was: 16:0, 26%; 18:0, 5.0%; 16:1, 3.0%; 18:1, 49%; 18:2 + 18:3, 16%; 20:4, 0.3%; 22:6, < 0.2%. On day 21 of incubation, the concentration of oleic acid in the yolk triglycerides of the normal embryos had risen to 54% and the concentration of the C₁₈ polyunsaturated acids had decreased to 11%; no changes occurred in the concentrations of the other fatty acids of the yolk triglycerides during incubation. The mean fatty acid composition of the cholesterol esters in the yolks of the fertile unincubated eggs obtained from the normal hens was: 16:0, 17%; 18:0, 5.5%; 16:1, 2.5%; 18:1, 43%; 18:2 + 18:3, 28%; 20:4, 1.5%; 22:6, 1.9%. During incubation, the concentration of oleic acid in the yolk cholesterol esters increased progressively until on day 21 oleic acid accounted for 74% of the total acids esterified with cholesterol; the concentration of all of the other acids decreased proportionately. The mean fatty acid composition of the phospholipids in the yolks of the fertile unincubated eggs obtained from the normal hens was: 16:0, 31%; 18:0, 18%; 16:1, 0.8%; 18:1, 24%; 18:2 + 18:3, 14%; 20:4, 6.7%; 22:6, 4.0%. On day 21 of incubation, the concentration of oleic acid in the yolk phospholipids had increased to 28% whereas the concentration of docosahexaenoic acid had decreased to 1%; the concentrations of the other fatty acids in the yolk phospholipids remained constant during incubation. No significant differences were observed either before incubation had begun or at the various stages of the incubation process between normal and deficient yolks with respect to the fatty acid compositions of the three lipid fractions.

DISCUSSION

The findings of the present investigation show that vitamin B₁₂ deficiency exerts a pronounced influence not only on the concentrations of cholesterol ester and triglyceride in the liver of the developing chick embryo but also on the constituent fatty acids of the liver triglycerides and phospholipids. On the other hand, vitamin B₁₂ deficiency did not appear to interfere in any way with the synthesis of the yolk lipids by the parent hen.

In view of the findings of Budowski, Bottino & Reiser (1961), Moore & Doran (1962) assumed that the triglyceride in the liver of the normal embryo was derived directly from the triglyceride of the yolk and concluded that the higher concentrations of triglyceride in the livers of the deficient embryos were simply due to a decreased utilization of yolk triglyceride. However, the validity of this conclusion must now be doubted in view of the marked dissimilarity between the fatty acid composition of the yolk triglycerides and that of the liver triglycerides in the deficient embryos. It is known that cholesterol is actively esterified in the yolk of the chick embryo during incubation (Tsuji, Brin & Williams, 1955; Moore & Doran, 1962), and recent evidence (Noble & Moore, 1964) seems to indicate that the cholesterol ester that accumulates in the embryonic liver during development is derived entirely from that synthesized in the yolk. Since vitamin B₁₂ deficiency did not appear to affect the fatty acid pattern of the various lipid fractions in the yolk during incubation it was perhaps not surprising to find that the composition of the liver cholesterol esters in the deficient embryos was little different from that of the liver cholesterol esters in the normal embryos (Table 3). Until a solution is found to the problem of why such large quantities of cholesterol oleate accumulate in the liver of the normal chick embryo during development, there seems little prospect of explaining why this characteristic accumulation of cholesterol oleate is so markedly decreased by a deficiency of vitamin B₁₂. By far the most striking and consistent effect of vitamin B₁₂ deficiency on the constituent fatty acids of the various liver lipids of the chick embryo was that observed on the fatty acids of the total phospholipid fraction. This is perhaps all the more surprising when it is remembered that a deficiency of the vitamin appeared to

influence the concentration of phospholipid in the embryonic liver only on day 17 of incubation. The reason for the lower concentrations of the C₂₀ and C₂₂ polyunsaturated acids and the higher concentrations of palmitic acid and stearic acid in the liver phospholipids of the deficient embryos is not clear. Nevertheless, it was calculated that the amount of the C₂₀ and C₂₂ polyunsaturated acids contained in the additional triglyceride that accumulated in the deficient embryonic livers would have been sufficient to account for the lower concentrations of these polyunsaturated acids in the liver phospholipids of the deficient embryos.

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