as a source of Co Q in view of the recent attempts to produce Co Q by bacterial fermentation of certain synthetic media[8]). The detailed paper on these and related aspects will be published elsewhere.

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Coenzyme Q (Ubiquinone) in Avian Egg and Embryo

Consistent with the view of WARBURG[1]) that an embryo is an aerobic organism several oxidative enzymes[2]),[3]),[4]) and respiratory pigments[5]) have been shown to be present and to increase in quantity during development in chick embryo. Further, particulate material isolated from chick embryonic liver homogenates has been shown to contain coenzyme Q (Co Q) and evidence has been deduced for its participation in electron transport activities similar to its role in animal tissue mitochondria[5]). The chick embryo inside the shell, being a "closed unit" and free from microbial contamination, should have all its supply of Co Q deposited in the egg by the hen or should synthesize it during development, if absent in the egg. The data presented here show that Co Q is present in eggs and it is progressively incorporated into the embryo during development.

In eggs, Co Q was found entirely in yolks and the amounts present in three batches are given in the Table. The mean values

Table. *Coenzyme Q content of Hens' Eggs and Chick Embryos.* Wet weight (in gm.) and Co Q (μmole)

	Yolks from fresh eggs*)					
Batch 1: wet weight .	18·0	16·5	17·0	18·5	17·5	18·0
Co Q	0·131	0·162	0·120	0·180	0·172	0·166
Batch 2: wet weight .	14·0	15·0	16·0	15·5	20·0	
Co Q	0·138	0·143	0·119	0·163	0·133	
Batch 3: wet weight .	17·0	16·5	16·0	16·0		
Co Q	0·088	0·087	0·088	0·104		
Embryos	6—7th	13—14th	15th	16th	17-18th day	
wet weight . .	0·66	10·00	12·50	16·00	21·00	
Co Q	0·064	0·124	0·139	0·164	0·152	

*) Because of the high lipid content of yolks, a high concentration of alkali (0·5 gm. NaOH per gm. wet weight) was used and the saponification time was reduced to 20 min. to minimize destruction of Co Q.

of Co Q content in the three batches are 0·155 (± 0·024), 0·147 (± 0·038) and 0·092 (± 0·008) μmole per egg yolk. The variation in batch 3 suggests that Co Q content of eggs might depend on the hen's feed as in the case of vitamins A and E[6]). Progressively increasing amounts of Co Q were found in the developing embryo and by about the 15th day of development negligible amounts of Co Q were left in the residual material in the egg.

The procedure used in the isolation of Co Q from eggs and embryos of White Leghorn variety hens consisted of saponification in presence of pyrogallol, extraction of the unsaponifiable matter with hexane, removal of carotenoids by washing hexane layer with 80% ethanol, freezing out most of sterols from petroleum ether (40 to 60°) at 0°, and fractionation on silicic acid column. The 50% $CHCl_3$ in petroleum ether fraction showed small but definite peaks at 275 mμ and on reduction with $NaBH_4$ maximum decrease in absorption was found at 275 mμ, indicating the presence of Co Q, from which the amount of Co Q was calculated[7]). The reduced spectra, however, showed absorption peaks at 280 mμ for egg fractions and 272 and 280 mμ for embryo fractions. In all cases the presence of Co Q was confirmed by reverse phase paper chromatography[8]) and Q_{10} was the only form found. However, egg yolk unsaponifiable matter contained another compound having R_f 0·90 in 80% n-propanol system, giving positive test with *leuco*methylene blue spray and showing an absorption peak at 265 mμ which was not reduced with $NaBH_4$. [The *leuco*-methylene blue spray developed by FOLKER's group[4]) for detection of quinones was, however, found to be not specific for quinones since some ring ketones, particularly unsaturated ones, also gave a positive test]. Further, infrared data (peak at 5·82 μ) suggested that this compound possibly is a saturated ring ketone or a α-diketone, but not a quinone.

The unsaponifiable matter of egg yolk and chick embryo also contained reducing compounds (α,α'-dipyridyl-$FeCl_3$ positive) having R_f 0·90 in 80% n-propanol system which are different from ubichromenol (R_f, 0·42) and α-tocopherol (R_f, 0·85) but appear to be similar to the compound described by MAHLER[5]). A preparation of this compound from egg yolk showed absorption peaks at 272 and 310 mμ.

Further work following the changes in Co Q, vitamin E and this new non-vitamin E reducing compound during embryonic development is in progress.

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The Localization of Copper in Agar Gel Electrophoretic Patterns of Crustacean Blood

Recently WHITTACKER[1]) was able to demonstrate that the two protein bands, obtained by starch gel electrophoresis after SMITHIES[2]) from blood of two Orconectes species, both contain copper. Therefore he sectioned the gels horizontally and while one half was stained for protein with amidoblack 10B dye, the other half was placed for 24 hours in a solution of 50 ml of 10% aqueous sodium acetate and 3 ml of alcoholic 0·1% rubeanic acid. In these circumstances the two protein bands of Orconectes blood stained a light greenish-black.

With this method however, we were unable to demonstrate copper in electropherograms of crustacean blood obtained by micro agar gel electrophoresis after WIEME[3]). Moreover the time required in the method of WHITTACKER is too long because diffusion phenomena are very important in agar gels and bring about a broadening of the protein bands, preventing a sharp separation. Therefore the protein mixture is applied as a narrow band (4 mm long and $^1/_4$ mm wide) and electrophoresis is completed in a very short time, the protein fractions being fixed immediately afterwards.

In order to overcome all these difficulties we established a more sensitive method for copper localization by complexation with rubeanic acid. The best results were obtained by incubating the gels, after electrophoresis, in a mixture of: glacial acetic acid 5 ml; alcohol 94% 70 ml; alcoholic 0·2% rubeanic acid 25 ml.

In these circumstances the proteins are fixed by the acetic acid-alcohol mixture and the Cu^{2+}-ions set free in this medium are immediately precipitated in situ by the rubeanic acid. A greenish colour develops within 20 minutes and after 1 hour the positive fraction turns dark green. Copper estimation showed that the minimum amount of copper, demonstrable with our method is 0·132 μg Cu^{2+}, put into the narrow slit on the application line.

In our experiments electrophoresis was carried out in 0·9 per cent special agar-Noble(Difco)gels of $p_H = 8·4$. The tension between the electrodes is kept at 130 volts, the current