talline hydrochloride separated from the solution. Recrystallization from acetone gave white needles, melting 244–247° with decomposition.[27]

Anal. Calcd. for $C_6H_5N_2Cl$: C, 51.26; H, 3.59. Found: C, 51.52; H, 3.86.

(b) **From the Salt II.**—A solution of 17.4 g. (0.03 mole) of salt II dissolved in 50 ml. of water was added slowly to a solution of 6.6 g. (0.1 mole) of potassium cyanide dissolved in 50 ml. of water at room temperature. The yellow color of the iodide solution was discharged upon addition to the cyanide solution and a white solid separated from the solution. When addition was completed, the solid was separated from the solution by filtration and then recrystallized from a water–ethanol mixture. This yielded 4.3 g. (82%) of 1,10-decanediol, melting 72–74°.[28]

Diacetate.—A solution of the diol in 25 ml. of acetic anhydride was heated on a steam-bath overnight. The solution was evaporated under reduced pressure and the dark residue taken up in a hot water–ethanol mixture. Cooling the solution in an ice-bath gave the diacetate as colorless plates, melting 23–26°.[29]

The aqueous phase was extracted with two 25-ml. portions of ether. The combined extract was dried over magnesium sulfate and evaporated. There remained 2.3 g. (37%) of crude 4-cyanopyridine. A pure sample of the cyanopyridine was obtained by recrystallization from water, melting 79–81°.

(c) **From 1-Methoxypyridinium Methyl Sulfate.**—To a solution of 74 g. (1.5 mole) of sodium cyanide dissolved in 250 ml. of water and cooled to −5° was added slowly a solution of 110 g. (0.5 mole) of 1-methoxypyridinium methylsulfate dissolved in 125 ml. of water over a period of 1 hour. The solution was held between −5 and 0° during the addition and then for 1 hour after the addition was complete. The solution was then allowed to warm slowly to 20° and stirred for 3 hours at which time 250 ml. of chloroform was added. The layers were separated and the aqueous layer was allowed to stand at room temperature overnight and then extracted again with 250 ml. of chloroform. The extracts were dried and the solvent removed giving 37 g. and 10.5 g., respectively, of high boiling residues. The combined residue was distilled at atmospheric pressure and the fraction boiling 200 to 230° collected giving 34 g. (65%) of crude cyanopyridines. A vapor chromatogram showed this mixture contained 20–25% (16% over-all) 4-cyanopyridine and 75–80% (49% over-all) 2-cyanopyridine. Fractional distillation of the mixture gave 10.2 g. of 4-cyanopyridine boiling 208–212° and 19.5 g. of 2-cyanopyridine boiling 220–225°. The first fraction after recrystallization from dilute ethanol gave a sample of 4-cyanopyridine melting 80–82°. A portion of the second fraction was recrystallized from ether and gave a sample of 2-cyanopyridine melting 22–25°.

Cyano-pyridines and -Quinolines.—In general, these nitriles were prepared by the method described above (c) for 4-cyanopyridine. Three examples are described below in detail where there are variations from this procedure. Our initial experiments, described here, were performed in the presence of air (except in the case of 4-cyano-2,6-dimethylpyridine). In all cases, a two- to threefold excess of potassium or sodium cyanide was employed.

4-Cyano-2,6-dimethylpyridine.—To a solution of 23.3 g. (0.1 mole) of 1-methoxy-2,6-dimethylpyridinium methyl sulfate dissolved in 50 ml. of water, and under purified nitrogen, was added a solution of 19.8 g. (0.3 mole) of potassium cyanide dissolved in 75 ml. of water. The solution was allowed to stand at 20° for 2 days at which time the nitrile, which had separated from the solution as long white needles, was removed by filtration, yielding 3.8 g. (29%), melting 80–82°. The filtrate was extracted with two 50-ml. portions of ether which, after drying and evaporating, gave an additional 1.5 g. (11%) of nitrile, melting 77–81°. Sublimation at 50° (760 mm.) gave white needles, melting 83–85°.

4-Cyano-2-methylquinoline.—A solution of 143 g. (0.5 mole) of 1-methoxy-2-methylquinolinium methyl sulfate dissolved in 200 ml. of water was added slowly to a solution of 49 g. (1.0 mole) of sodium cyanide dissolved in 200 ml. of water. The temperature of the reaction mixture was maintained between 25 and 30° throughout the addition. When the addition was complete (1.5 hours), the solution was stirred overnight at room temperature. The dark solid was suspended in 200 ml. of water and steam distilled. A white solid crystallized from the distillate. Recrystallization of the solid gave 6 g. (7.2%) of 4-cyano-2-methylquinoline as white silky needles, melting 105–106°.

2,4-Dicyanopyridine.—To 26 g. (0.25 mole) of 4-cyanopyridine-1-oxide was added slowly 31.5 g. (0.25 mole) of dimethyl sulfate. The solution was heated at 100° for 3 hours and the resulting 1-methoxy-4-cyanopyridinium methyl sulfate was obtained as a thick red oil. This oil was taken up in 100 ml. of water, whereupon 8 g. (0.077 mole) of 4-cyanopyridine-1-oxide separated from the solution. The oxide was removed by filtration and the filtrate, containing 0.173 mole of the methoxy salt, was added slowly to a solution of 33 g. (0.5 mole) of potassium cyanide dissolved in 100 ml. of water. The addition was made at such a rate that the temperature of the reaction mixture varied between 0 and 5°. When the addition was complete, the solution was allowed to stand at room temperature for one hour, and the solid which had separated was filtered and recrystallized from water. There was obtained 11.8 g. (54%) of 2,4-dicyanopyridine, melting 88–91°.

PHILADELPHIA 37, PENNA.

(27) H. Camps, *Arch. Pharm.*, **240**, 361 (1902); *Chem. Zentr.*, **73**, II, 649 (1902), reports that this hydrochloride melted at 199° with decomposition.

(28) R. H. Manske, *Org. Syntheses*, **14**, 20 (1934), reports 72–74°.

(29) R. Scheuble, *Monatsh.*, **24**, 630 (1903), reports 25.5°.

[CONTRIBUTION FROM THE MERCK, SHARP & DOHME RESEARCH LABORATORIES, DIVISION OF MERCK & CO., INC.]

Coenzyme Q. VII. Isolation and Distribution of Coenzyme Q_{10} in Animal Tissues

BY BRUCE O. LINN, ATWOOD C. PAGE, JR., EMILY L. WONG, PAUL H. GALE, CLIFFORD H. SHUNK AND KARL FOLKERS

RECEIVED JANUARY 23, 1959

The isolation of coenzyme Q_{10} from beef hearts has been confirmed and extended. Crystalline Q_{10} has been isolated from human hearts and beef striated muscle. Q_{10}, identified by spectra and R_f's, has also been found in the mouse carcass, in the kidney tissue of steers and lambs and in the heart tissue of swine, rats, chicks, turkeys and rabbits.

The isolation of a crystalline substance melting at 48–49° was described first in 1957 by Crane, *et al.*;[1] it was then recognized as a quinone (Q-275)[2] and is now designated coenzyme Q_{10}.[1-4] The original source used by these investigators was mitochondria from beef heart, but later work omitted the preparation of mitochondria.

(1) F. L. Crane, Y. Hatefi, R. L. Lester and C. Widmer, *Biochim. et Biophys. Acta*, **25**, 220 (1957).

(2) F. L. Crane, R. L. Lester, C. Widmer and Y. Hatefi, *ibid.*, **32**, 73 (1959).

(3) R. L. Lester, F. L. Crane and Y. Hatefi, THIS JOURNAL, **80**, 4751 (1958).

(4) Coenzymes Q_6, Q_7, Q_8, Q_9 and Q_{10}, members of the coenzyme Q family, are homologs which differ only in the number of isoprenoid units in the side chain. The number of these units is indicated by the subscript; see ref. 3.

A crystalline product, m.p. 48.5°, from pig heart was described by Fahmy, *et al.*,[5] in 1958, and a crystalline product, m.p. 48–49°, from horse heart was also reported in 1958 by Bouman, *et al.*[6]; these products and coenzyme Q_{10} are presumably identical. Morton, *et al.*, in 1955–1957, described lower melting products (33–34°, 36°, 41°, all sharp)[7] from different sources, considered identical, and named ubiquinone. These lower and sharp melting products have been compared with the ethoxy homologs of coenzyme Q_{10}.[8] This group[9] recently cited material, estimated spectrophotometrically, from human kidney, medulla and cortex.

We have confirmed and extended the isolation technique[2] for the production of coenzyme Q_{10} from beef heart. Our modified procedure is described herein together with new isolation data on the existence of Q_{10} in human and other animal tissues.

A spectrophotometric assay[2] and the yellow color of the quinone were used as guides for the isolation. The assay, which is based upon the characteristic differential in ultraviolet absorption for the quinone and hydroquinone forms, was also used for calculating the purity of coenzyme Q. A radial paper chromatographic method (reverse-phase) with a water–dimethylformamide–Vaseline system of these lipid-like quinones has been very useful for identifications. This papergram technique can readily differentiate between coenzyme Q_{10} and the closely related Q_9, and, of course, lower analogs. The coenzyme Q spots on the papergram may be observed by ultraviolet absorption if so much as 10 mcg. per square cm. is present. If less than that amount is present, it is convenient to demonstrate coenzyme Q with a leucomethylene blue spray.

Fresh beef myocardial tissue was hydrolyzed in ethanolic sodium hydroxide solution in the presence of pyrogallol. The hydrolysate was extracted with hexane. The solvent extracts were freed of alkali, ethanol and fatty insolubles, and then were chromatographed over Florisil in hexane solution. Eluates containing Q_{10} were combined and concentrated. The residue was crystallized from ethanol, yielding the crystalline Q_{10}, m.p. 49.5–50.5°; maximum yield, 94 mg./kg. of moist tissue. A second Florisil column was used on occasion. The product showed only one spot on paper chromatography and was used for further characterization and structure elucidation.[10]

After the existence of coenzyme Q_6, Q_7, Q_8 and Q_9 was demonstrated,[3] it became important to determine the identity of the Q or Q's present in human tissue. Human hearts were obtained at autopsy and stored under alcohol at 3° until seven hearts had been collected—*ca.* two weeks. This tissue was processed by the procedure described above. Spectrophotometric assay of the hexane extracts before column chromatography showed a content of 70 mg. of Q/kg. of moist tissue. This is comparable to the value found for beef heart. Further purification yielded 20 mg. of crystalline coenzyme Q_{10}/kg. of moist tissue, m.p. 49–50°. Only one Q was found by paper chromatographic analysis of this product, and it was indistinguishable from the beef heart Q_{10}. The identity was further established by infrared and nuclear magnetic resonance analyses.

In the interest of possible studies of Q_{10} in muscle dystrophies, the isolation process was performed on beef striated muscle, which was obtained from the neck muscle of steers. Again, a crystalline product was obtained which was indistinguishable in paper chromatographic behavior and infrared analysis from coenzyme Q_{10}. The yield of Q_{10} from neck tissue was 1.2 mg./kg. of moist tissue or approximately 1% of that found in beef heart tissue.

Although evidence for the presence of Q in the tissues of certain animals is appearing from several laboratories, we have determined the identity of the specific Q present in those animals which are common for research studies and in those farm animals which are most commonly used for food.

Small samples, 100 g. or less, of representative heart tissue from swine, rabbits, rats, turkeys and chicks, mouse carcass and kidney tissue of steers and lambs were examined for coenzyme Q content. The methods used for initial purification were minor variants of our isolation method described for beef heart and skeletal muscle. Since only a few milligrams of coenzyme Q were present in the concentrates, we did not attempt to isolate crystalline products. Instead, the concentrates obtained after column chromatography were examined directly. The criteria applied in these cases were the characteristic ultraviolet absorption before and after reduction with borohydride and behavior on reverse-phase papergrams. The coenzyme Q concentrates from the tissues all gave papergram spots corresponding well with authentic Q_{10} control samples. In addition, a second spot was present on the papergrams derived from rats and mice. Although this Q was not fully characterized, it does correspond to coenzyme Q_9 in this system.

The knowledge that Q_{10} of the coenzyme Q group (five members to date) exists widespread in animal species is basic to further studies on the significance of coenzyme Q_{10} in human and animal metabolism.

Experimental[11]

Spectrophotometric Analysis.—The following spectrophotometric analysis was used for estimating the purity of the coenzyme Q_{10} preparations obtained during the various stages of isolation. Coenzyme Q_{10} shows a characteristic change in the ultraviolet absorption for the quinone and hydroquinone forms at the wave length where the quinone absorbs maximally; $\Delta E^{1\%}_{1cm}$ ($E^{1\%}_{1cm}$ quinone minus $E^{1\%}_{1cm}$ hydroquinone) is 142 (169 minus 27) at λ_{EtOH} 275 mμ. Comparison of the observed $\Delta E^{1\%}_{1cm}$ value with 142 allows calculation of the quantity and the percentage of coenzyme Q_{10} in a particular preparation.

The solutions for this determination were prepared by dissolving an amount of concentrate in absolute ethanol which corresponds to *ca.* 4 mg. of coenzyme Q_{10} per 100 ml. of solution. The hydroquinone form was prepared by adding an excess of sodium borohydride, *ca.* 10 mg., to a 10

(5) N. I. Fahmy, F. W. Hemming, R. A. Morton, J. Y. F. Paterson and J. F. Pennock, *Biochem. J.*, **70**, 1P (1958).

(6) J. Bouman, E. C. Slater, H. Rudney and J. Links, *Biochim. et Biophys. Acta*, **29**, 456 (1958).

(7) R. A. Morton, G. M. Wilson, J. S. Lowe and W. M. F. Leat, *Biochem. J.*, **68**, 16P (1958); *Chemistry & Industry*, 1649 (1957).

(8) B. O. Linn, N. R. Trenner, C. H. Shunk and K. Folkers, THIS JOURNAL, **81**, 1263 (1959).

(9) F. W. Hemming, J. F. Pennock and R. A. Morton, *Biochem. J.*, **68**, 29P (1958).

(10) D. E. Wolf, C. H. Hoffman, N. R. Trenner, B. H. Arison, C. H. Shunk, B. O. Linn, J. F. McPherson and K. Folkers, THIS JOURNAL, **80**, 4752 (1958).

(11) Melting points were taken on a Kofler micro hot-stage using a stage-calibrated thermometer.

ml. portion of this solution. The resulting mixture was shaken briefly and then the solids removed by centrifugation. The absorption of the solutions at 275 mμ was determined rapidly using a Beckman quartz spectrophotometer model DU. Complete quartz ultraviolet absorption spectra were obtained using a Cary recording spectrophotometer, model 11.

Isolation of Coenzyme Q_{10} from Beef Myocardial Tissue.—The fat and connective tissue were removed from fresh beef hearts and the myocardial tissue was passed through a grinder. An ethanolic 10% sodium hydroxide solution (1.3 l./kg. of tissue) was prepared by dissolving 9.0 kg. of sodium hydroxide pellets in 90.0 l. of 95% ethanol. To this stirred solution, which was under a nitrogen atmosphere, was added 4.5 kg. of pyrogallol (66 g./kg. of tissue) and 68 kg. of ground moist tissue. The mixture was refluxed and stirred for 30 minutes and then rapidly cooled; 15 l. of water was added and the resulting solution extracted with four portions (one 50-l. portion and three 20-l. portions) of *n*-hexane (Skellysolve B). The *n*-hexane extract was washed with 80-l. portions of water until free of alkali. Four washings were usually sufficient. The hexane solution was dried over anhydrous sodium sulfate and then concentrated under reduced pressure. The residue, 200–500 g., was taken up in *n*-hexane, *ca.* 150 ml./10 mg. of residue. Fatty insoluble material was removed by filtering through a mat containing 100 g. of Supercel. The mat was washed with three 1-l. portions of *n*-hexane. The *n*-hexane filtrate and washings were combined and evaporated under reduced pressure in order to remove residual ethanol. The residue was dissolved in 4 l. of *n*-hexane and the solution applied to a chromatographic column, 8.5 × 120 cm., containing 2.0 kg. of 60/100 mesh magnesium silicate (Florisil) in *n*-hexane. The column was eluted with 5% ethyl ether in *n*-hexane. The colorless eluates, *ca.* 20 l., were discarded. The yellow to orange colored eluates were collected and aliquots taken for spectrophotometric analysis. Usually two orange bands developed on the column. The faster moving band, which appeared to contain the carotenes, was eluted in about 6 l. of solution. The slower moving band contained coenzyme Q_{10} and was eluted in about 17 l. of solution. The volumes of eluant varied from batch to batch as did the quantity of the non-saponifiable fraction. A description of the fractions collected during one such chromatographic purification is given in Table I.

TABLE I

FRACTIONS OBTAINED FROM THE FLORISIL COLUMN

Fraction	Volume, l.	Color	Weight of residue, mg./25 ml.	Purity, % coenzyme Q_{10}
1	2.5	Lt. yellow	20.2	0
2	1.1	Dk. orange	182	0
3	2.8	Lt. yellow	19.2	0
4	1.6	Lt. yellow	25.0	25
5	4.6	Dk. yellow	28.6	83
6	1.9	Yellow	16.4	98
7	5.2	Lt. yellow	6.6	95
8	3.6	Lt. yellow	9.2	40
9	3.6	Lt. yellow	7.5	0

The eluates which contained coenzyme Q_{10} of less than 40% purity were held for further purification on a second Florisil column, and the eluates which contained Q_{10} of over 40% purity were combined and evaporated under reduced pressure. The residue, a red oil, was crystallized from absolute ethanol, *ca.* 40 ml./g. of residue. The warm ethanol was filtered through a sintered glass funnel and allowed to cool slowly in the dark at room temperature. The orange crystals were collected, washed with three portions of absolute ethanol and dried *in vacuo*. Fractions 5–7 (Table I) yielded 5.74 g. (84 mg./kg. of tissue) of orange crystalline coenzyme Q_{10} melting at 49–50°.

In this manner, most of the batches gave an average of 4.5 g. (66 mg./kg. of moist myocardial tissue) of coenzyme Q_{10} melting over a one degree range from 48–50°; maximum yield, 94 mg./kg. These preparations showed only one spot on papergrams, see Table III, and were found to be 95–100% pure based upon spectrophotometric analysis ($\Delta E^{1\%}_{1cm}$ 135 to 142).

The best preparations of coenzyme Q_{10}, m.p. 49.5–50.5°, have the following absorption characteristics: quinone in isoöctane, max. at 271 mμ, $E^{1\%}_{1cm}$ 169; min. at 235 mμ, $E^{1\%}_{1cm}$ 31; quinone in ethanol, max. at 275 mμ, $E^{1\%}_{1cm}$ 169; min. at 236 mμ, $E^{1\%}_{1cm}$ 31; hydroquinone in ethanol, max. at 290 mμ, $E^{1\%}_{1cm}$ 48; min. at 251 mμ, $E^{1\%}_{1cm}$ 10; $\Delta E^{1\%}_{1cm}$ at 275 mμ (quinone minus hydroquinone) 142. The infrared spectrum in carbon disulfide showed bands characteristic of the 2,3-dimethoxy-1,4-benzoquinone functionality at 6.03, 6.18 and 7.90 μ and other bands at 3.45, 7.24, 7.50, 7.76, 7.90, 8.30, 8.67, 9.05, 9.76, 10.55, 11.97 and 13.45 μ.

Anal. Calcd. for $C_{59}H_{90}O_4$ (863.31): C, 82.08; H, 10.51; CH_3O, 7.19. Found: C, 81.98, 82.05; H, 10.38, 10.31; CH_3O, 7.16, 7.07.

Isolation of Coenzyme Q_{10} from Human Myocardial Tissue.—Human myocardial tissue, 2.0 kg., was processed as described for beef heart tissue. Concentration of the *n*-heptane (Skellysolve C) extracts gave 7.0 g. of dark residue. The residue was taken up in 300 ml. of *n*-heptane, and the solution was filtered through a mat of 3 g. of Supercel. The Supercel was washed with 150 ml. of *n*-heptane and the washings were combined with the filtrate. The *n*-heptane solution, 450 ml., contained 2.5 g. of solids. A spectrophotometric analysis of the residue showed that it contained 5.6% of coenzyme Q_{10} which corresponds to a total of 140 mg. (70 mg./kg. of moist tissue) of coenzyme Q_{10}. The *n*-heptane solution was applied to a chromatographic column containing 250 g. of 60/100 mesh Florisil in *n*-heptane. An orange band formed which was washed with more *n*-heptane and then eluted with a 5% solution of ethyl ether in *n*-heptane. A description of the fractions obtained with 5% ether elution is given in Table II.

TABLE II

FRACTIONS OBTAINED FROM THE FLORISIL COLUMN

Fraction	Color	Weight of residue, mg.	Purity % coenzyme Q_{10}
1–9	Colorless		
10	Lt. yellow		
11	Yellow	9.2	44
12–16	Yellow		
17	Yellow	11.5	71
18–19	Yellow		
20	Yellow	9.8	42
21–22	Lt. yellow		
23	Colorless		

Fractions 10 to 22 were combined and concentrated under reduced pressure. The red liquid residue, 112 mg., was crystallized from absolute ethanol giving 64.6 mg. of coenzyme Q_{10}, m.p. 46–49°. One more crystallization from ethanol gave 41.0 mg. (20 mg./kg. of moist tissue) of coenzyme Q_{10}, m.p. 49–50°. The infrared spectrum in carbon disulfide was indistinguishable from that of coenzyme Q_{10} obtained from beef myocardial tissue. The identity of this material was further established by paper chromatography as described below.

Isolation of Coenzyme Q_{10} from Beef Striated Muscle Tissue.—The fat tissue was removed from the neck muscle of freshly slaughtered steers, and the resulting moist striated muscle tissue, 3.4 kg., was saponified and extracted as described for beef heart tissue. Concentration of the *n*-heptane extracts gave 1.62 g. of a yellow solid residue. A spectrophotometric assay of this material showed $\Delta E^{1\%}_{1cm}$ 7.4 which corresponds to *ca.* 84 mg. of coenzyme Q_{10}. The residue contained cholesterol which was partially removed by crystallization from 75 ml. of 95% ethanol. The filtrate was concentrated under reduced pressure, and the residue was dissolved in *n*-heptane. This solution was applied to a chromatographic column containing 38 g. of 60/100 mesh Florisil in *n*-heptane. The orange band which formed was eluted with *n*-heptane. Yellow colored eluates obtained from this band were collected and found to have $\Delta E^{1\%}_{1cm}$'s 10 to 56. These eluates were combined and evaporated under reduced pressure giving 50 mg. of an orange oil. Further purification was effected by a second chromatographic column containing 3.0 g. of Florisil. The orange liquid, 15.7 mg., which was obtained from the second column, was dissolved in 1 ml. of warm absolute ethanol.

When the solution was cooled, a colorless flocculent precipitate formed which was removed by centrifugation. The clear solution was kept at 0° for 3 days. The crystalline Q_{10} was collected and dried *in vacuo*, 4.0 mg., m.p. 43–46°. The infrared spectra of this sample and of beef heart coenzyme Q_{10} in potassium bromide pellets were indistinguishable. The identity was further established by paper chromatographic analysis.

Paper Chromatographic Analysis.—The circular paper type of chromatographic analysis using a reverse phase system was employed for identity and purity studies. Whatman No. 1 filter paper was impregnated with Vaseline by passing the paper through a 5% (w./v.) solution of Vaseline in petroleum ether. The paper was air-dried. The mobile phase was prepared by saturating a 3% (v./v.) solution of water in N,N-dimethylformamide with Vaseline.

Ultraviolet absorbance or a leucomethylene blue indicator spray was used for the detection of the compounds on the paper. The leucomethylene blue indicator solution was prepared as follows: To 100 mg. of methylene blue in 100 ml. of ethanol was added 1 ml. of glacial acetic acid and 1 g. of zinc dust. The mixture was swirled gently until the blue color disappeared. When a strip is sprayed with this mixture, the Q spots appear as blue zones with white or light blue background. The entire sheet becomes blue from air oxidation after five minutes, but there is ample time to mark the Q spots. The lower limit of detection for this spray is about 1 mcg. per sq. cm.

The R_f values for the samples of crystalline coenzyme Q_{10} are given in Table III. The R_f values shown for experiment 1 were obtained on one papergram, the values given for experiment 2 on another.

TABLE III

PAPERGRAM ANALYSIS OF CRYSTALLINE COENZYME Q_{10}

Samples coenzyme	Standard added	R_f values Expt. 1[a]	Expt. 2[b]
Q_{10} (beef heart)		0.40	0.27
Q_{10} (human heart)		.38	.26
Q_{10} (beef striated muscle)		.40	.30
Q_9[4]		.49	
Q_{10} (human heart)	Q_{10}	.38	.25
Q_{10} (human heart)	Q_9	.53, 0.40	
Q_{10} (beef striated muscle)	Q_{10}	.40	.27
Q_{10} (beef striated muscle)	Q_9	.49, 0.39	
Q_{10} (human heart and beef muscle)			.25

[a] This chromatogram was run for 200 min. at 26°. [b] This chromatogram was run for 2.5 hr. at 26°.

Identification of Coenzyme Q_{10} in Certain Animal Tissue Samples.—A number of different species have been examined for coenzyme Q. A typical example follows: eighty-five grams of chick hearts, ground in a Waring Blendor to a paste, was added to 6 g. of pyrogallol, 120 ml. of ethanol and 12 g. of sodium hydroxide. The mixture was refluxed for 0.5 hour and extracted three times with 100-ml. volumes of Skellysolve C. The combined extracts were partially dried over anhydrous sodium sulfate. Removal of the solvent *in vacuo* yielded 124 mg. of oil. This residue was dissolved in 4.0 ml. of isoöctane. White crystals, mostly steroids, that separated after an hour at room temperature were removed. A second crop of similar impurities was removed from the mother liquor at 0°. A column containing 1.5 g. of Decalso, 50 mesh and finer, in a 5-mm. diameter glass tube was prepared. The isoöctane mother liquors were percolated slowly through the column. The adsorbent was washed with Skellysolve B, and developed with ether–Skellysolve B mixtures (Table IV).

TABLE IV

FRACTIONS OBTAINED FROM THE DECALSO COLUMN

Solvent	Volume of fraction, ml.	Color
Skellysolve B	40	Colorless
5% ether in skellysolve B	25	Pale yellow
50% ether in skellysolve B	40	Deep yellow

Fractions 2 and 3 were concentrated *in vacuo* to oily residues. The oil from fraction 3 was dissolved in 3.0 ml. of isoöctane, and a white crystalline impurity that formed at 0° was removed. The mother liquor was evaporated to dryness *in vacuo*. Aliquots of this type of oily residue were used for spectrophotometric assay and papergrams. Results are reported in Table V.

TABLE V

CHARACTERISTICS OF COENZYME Q FROM TISSUE CONCENTRATES

Species	Organ	Ultraviolet Oxidized $\lambda_{max.}$, mμ	Ultraviolet Reduced $\lambda_{max.}$, mμ	Amt. 100 g. wet tissue, mg.	R_f of Q spots Tissue concn.	Q_{10} reference
Man	Heart	275	287	6.3	0.38	0.36
Swine	Heart	275	290	8.5	.50	.49
Lamb	Kidney	275	290	4.7	.48 (0.42)[a]	.50
Beef	Kidney	275	290	4.1	.50 (0.41)[a]	.49
Rabbit	Heart	275	290	9.5	.42	.43
Rat	Heart	275	292	14	.52, .62	.51
Mouse	Carcass	275	290	2.3	.55, .63[b]	.50
Chick	Heart	275	287	4.5	.54 (0.46)[a]	.52
Turkey	Heart	275	290	5.5	.43	.43

[a] Apparent traces of Q-like substances. [b] Major component.

Acknowledgment.—We are indebted to Mr. Arthur W. Ritchie for technical assistance, Mr. Richard N. Boos for microanalytical data and Mr. Robert W. Walker for infrared absorption measurements.

RAHWAY, N. J.