(9) A. S. Michaels and J. C. Bolger, *Ind. Eng. Chem. Fundam.*, **1,** 24(1962).
(10) A. M. Gaudin and M. C. Fuerstenau, *Eng. Mining J.*, **159,** 110(1958).
(11) A. M. Gaudin and M. C. Fuerstenau, *Int. Mining Proc. Congr.*, London, England, Apr. 1960.
(12) A. M. Gaudin, M. C. Fuerstenau, and S. R. Mitchell, *Mining Eng. (London)*, **11,** 613(1959).
(13) C. B. Egolf and W. L. McCabe, *Trans. Amer. Inst. Chem. Eng.*, **33,** 620(1937).
(14) J. deBoer, *Advan. Colloid Interface Sci.*, **3,** 21(1950).
(15) F. London, *Z. Phys.*, **63,** 245(1930).
(16) B. Deryagin, A. Titijevskaia, I. Abriscova, and E. Lifshitz, *Discuss. Faraday Soc.*, **18,** 24(1954).
(17) B. Deryagin, I. Abriscova, and E. Lifshitz, *Quart. Rev. (London)*, **10,** 295(1956).
(18) E. Verway and J. Overbeek, "Theory of the Stability of Lyophobic Colloids," Elsevier, Amsterdam, The Netherlands, 1948.
(19) V. Vand, *J. Phys. Colloid Chem.*, **52,** 277(1948).
(20) C. R. Wylie, Jr., "Advanced Engineering Mathematics," McGraw-Hill, New York, N. Y., 1960, p. 177.
(21) C. M. Metzler, APhA Academy of Pharmaceutical Sciences, 5th National Meeting, Washington, D. C., November 20, 1968.
(22) J. T. Carstensen, J. B. Johnson, D. C. Spera, and M. J. Frank, *J. Pharm. Sci.*, **57,** 23(1968).
(23) G. A. Ratcliff, D. A. Blackader, and D. N. Sutherland, *Chem. Eng. Sci.*, **22,** 201(1967).
(24) G. A. Ratcliff, *J. Colloid Interface Sci.*, **25,** 586(1967).
(25) W. Higuchi and R. G. Stehle, *J. Pharm. Sci.*, **54,** 265(1965).
(26) T. Gillespie, *J. Phys. Chem.*, **66,** 1077(1962).
(27) T. Gillespie, *J. Colloid Sci.*, **15,** 219(1960).
(28) C. F. Goodeve, *Trans. Faraday Soc.*, **35,** 342(1939).

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Cholesterol Biosynthesis and Lipid Biochemistry in the Scolex of *Echinococcus granulosus*

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Abstract □ When live scolices of *Echinococcus granulosus* were incubated with labeled mevalonate *in vitro*, no radioactivity was incorporated into the parasites' cholesterol fraction. However, labeled cholesterol was recovered after a scolices' homogenate was incubated with 4-^{14}C-cholesteryl acetate. It is suggested that the scolex of *E. granulosus* is unable to biosynthesize cholesterol and that it obtains cholesterol in an esterified form from the host, which is subsequently hydrolyzed within the scolex to free cholesterol. The fatty acid content of *E. granulosus* was examined by gas-liquid chromatography.

Keyphrases □ *Echinococcus granulosus* scolices—cholesterol biosynthesis □ Cholesterol biosynthesis—*E. granulosus* scolices □ Lipid biochemistry—*E. granulosus* scolices □ Hydrolytic activity, *E. granulosus* scolices—4-^{14}C-cholesteryl acetate □ TLC—separation □ GLC—identity □ Scintillometry—analysis

It is a well-known fact that, in vertebrates, cholesterol is biosynthesized from acetate or mevalonate. In many insects, however, radioactive acetate or mevalonate is not incorporated into cholesterol; some insects do not even synthesize squalene, the noncyclic precursor of sterols (1, 2).

In 1954, Butenandt and Karlson (3) first isolated 25 mg. of ecdysone, the molting hormone, from 500 kg. of silkworm pupae. The compound [shown later to be a steroid (4)] was effective in accelerating molting, even in quantities below 7.5×10^{-3} mcg., when injected into ligated fly abdomens. In 1963, Karlson and Hoffmeister (5) demonstrated the incorporation of radioactivity from tritium-labeled cholesterol into ecdysone in *Calliphora* larvae, indicating that cholesterol is a precursor to ecdysone. Clark and Bloch (6) showed that 95% of the dietary cholesterol in the beetle *Dermestes vulpinus* could be replaced by β-sitosterol for structural utilization, but 5% of it remained essential for metabolic function; Clayton (7), working with the cockroach *Blattella germanica*, obtained similar results. According to Karlson (8), only a small part of the cholesterol injected into insects was converted to ecdysone; the majority still fulfilled cellular functions. Smissman *et al.* (9) isolated dehydroepiandrosterone, pregnenolone, and progesterone as the products of normal insect metabolism of dietary sterols in the confused flour beetle, *Tribolium confusum*. Horn *et al.* (4) isolated crustecdysone from crayfish, a compound which proved to be both chemically and biologically similar to ecdysone. Carlisle (10) demonstrated the biological activity of extracts from crabs, copepods, and locusts on the immature shore crab *Carcinus maenas*, indicating the presence of a common set of interactive ecdysones in crustaceans and insects.

The hormone that has an effect opposite to that of ecdysone on the molting stages of insects was first isolated from the abdomens of *Cecropia* males by Williams (11). Karlson and Schmialek (12) injected extracts of the excretions of the beetle *Tenebrio molitor* into mature larvae of the same species and noted the retardation of pupation in 88% of the treated larvae. In

1961, Schmialek (13) isolated 60 mg. of an oil from 80 kg. of *Tenebrio* feces, which was identified as farnesol and its oxidation product farnesal. Later it was shown that insects can biosynthesize labeled farnesol and farnesal from tagged mevalonic acid (14). Using the farnesol isolated by Schmialek (13), as well as the commercial compound, Wigglesworth (15) showed that farnesol reproduces all the effects of a juvenile hormone when applied to the surface of the cuticle of the insect *Rhodnius prolixus*. The main effects were retention of larval characters and partial reversal of metamorphosis in the molting adult. Other active compounds also have been isolated from the microsporidian *Tribolium castaneum* (16), various other microorganisms (17), and even from the balsam fir tree (18). It appears from these studies that in insects the pathway of mevalonate is directed toward the synthesis of specific hormones and not cholesterol, whereas in vertebrates part of the mevalonate is incorporated into cholesterol.[1]

The work on cholesterol biosynthesis in cestodes is still in its infancy. Frayha (20) observed that 1-^{14}C-acetate did not incorporate into the cholesterol fraction of *E. granulosus* scolices; but when 26-^{14}C-cholesterol was fed to mice infected with the parasite, the label did appear in the cholesterol isolated from the scolices. Indeed, Meyer *et al.* (21) reported the *Spirometra mansonoides* lacks the mechanisms required for the synthesis *de novo* of its sterols.[2]

The authors of this article (23) have recently shown that lipid extracts of *Echinococcus granulosus*, a cestode, contained substances with both stimulatory and depressive effects on the growth of excysted cysticercoids of *Hymenolepis diminuta* in *in vitro* cultures. Since mevalonate has been shown to be a precursor of farnesol and farnesal in insects (14), an attempt was made to correlate the biological activity of the different lipid fractions of *E. granulosus* scolices with their biosynthesis from mevalonate.

MATERIALS AND METHODS

Collection and Preparation of Scolices—The livers and lungs of the sheep and cattle infected with cysts of *E. granulosus* were obtained within 2 hr. of slaughter. The hydatid fluid and scolices[3] were collected aseptically from the cysts according to the previously published methods (23) to be used in both the authors' biosynthetic studies and hydrolytic studies.

Biosynthetic Studies—For the biosynthetic studies, the live[3] scolices were washed four times with an aqueous buffer solution (pH 7.4) of disodium monohydrogen phosphate and potassium dihydrogen phosphate (25).

The washed scolices were then incubated in a water bath shaker for 5 hr. at 37° with 4 ml. of phosphate buffer (pH 7.0), 1 ml. of antibiotic solution,[4] and 1 ml. of 2-^{14}C-DL-mevalolactone[5] (specific activity 3.11×10^5 c.p.m.[6]/0.04 μM/ml.). At the end of the incubation period, 2 ml. of 3 N H_2SO_4 was added to the flask.[7] The scolices were separated by low-speed centrifugation, washed several times with distilled water, and then transferred to a 125-ml. conical flask. A quantitative sample taken from a combination of the incubation supernatant fluid and the scolices washings was analyzed for radioactivity. From this figure the amount of the label actually taken up by the scolices was determined to be 1.33×10^5 c.p.m.

Isolation and Fractionation of the Lipids—The total lipids were extracted from the scolices and fractionated by thin-layer chromatography (TLC); the cholesterol was isolated according to the previously published methods (23).

Saponification of Neutral Fats—The lipids of Band III were saponified by refluxing them in 5 ml. of 5% ethanolic KOH for 24 hr. under nitrogen. At the end of the time, 5 ml. of distilled water was added to the mixture, and the ethanol was evaporated by bubbling nitrogen into the gently warmed (ambient 45°) flask. The solution was then extracted with three separate 5-ml. portions of ether. The ether extracts were dried over sodium sulfate, and the solvent was evaporated under nitrogen. The residue was taken up in a minimal amount of chloroform which was transferred into a liquid scintillation vial, evaporated, and assayed for ^{14}C.

The aqueous layer, containing the saponifiable fraction of the lipids, was acidified to pH 2.0 with 2 N H_2SO_4 and then extracted four times with ether. The ether fractions were dried overnight over anhydrous Na_2SO_4 and, subsequently, concentrated to a volume of about 5 ml. An aliquot from the solution was assayed for ^{14}C.

In another experiment the ether solution of the saponifiable fraction of lipids from scolices (not treated with radioactive mevalonate) was prepared for GLC analysis by prior treatment with diazomethane according to the method of Beames (26).

Gas-Liquid Chromatography—Gas-liquid chromatography (GLC) of the fatty acids was performed by a Pye argon chromatography apparatus equipped with a 1.21-m. (4-ft.) glass column (4 mm. in diameter) packed with 10% ethylene glycol adipate on diatomaceous earth (Celite 545, 80/100 mesh). Ten-microliter samples were injected. The gas-flow rate was 30 ml./min. and the column temperature was 180°. The calibration of the GLC instrument, as well as the quantitation and identification of chromatograms, was performed according to previously published procedures (27).

Enzyme Activity of a Scolex Homogenate—The hydrolytic action of homogenates from *E. granulosus* scolices on cholesteryl acetate was investigated as follows: 1 g. of live scolices[3] was homogenized in a Potter-Elvehjem homogenizer for 3 min. The homogenate was incubated with 1 ml. of antibiotic solution,[4] 4 ml. of phosphate buffer pH 7.0, and 6.0 mg. of radioactive 4-^{14}C-cholesteryl acetate[8] in a water bath at 37° for 24 hr. with vigorous and continuous shaking. At the end of the incubation period, the lipids were extracted from the mixture with chloroform. The residue, obtained by evaporation of the chloroform extracts, was subjected to TLC, and its cholesterol fraction was isolated as described before (23) and analyzed for its radioactivity content.

Scintillation Counting—All radioactivity measurements of the cholesterol samples were performed in a Packard Tricarb scintillation spectrometer model 3003 with an efficiency of 70% and a background count of 8 c.p.m. The samples were counted in liquid scintillation glass vials (20-ml. capacity) with 18 ml. of liquid scintillation cocktail containing 4 g. of 2,5-diphenyloxazol (PPO) as primary scintillator and 50 mg. of dimethyl-1,4-bis[2(5-phenyloxazol)benzene] (POPOP) as secondary scintillator per liter of toluene. Samples that were insoluble in the cocktail were first solubilized in 3

[1] Durr (19) recently provided evidence for the incorporation of mevalonic acid into the nonsaponifiable lipids of omental and subcutaneous adipose tissue of man, epididymal fat pad of rat, and the fat tail of the Syrian sheep. His stoichiometric studies showed that about one-half of the incorporated radioactivity in the nonsaponifiable lipids was in squalene, 20% in lanosterol and cholesterol, and the remainder in unidentified substances.

[2] However, Ginger and Fairbairn (22) working with *Hymenolepis diminuta* demonstrated the incorporation of 1-^{14}C-acetate into the cestode's cholesterol fraction.

[3] The viability of the collected scolices was determined by their motility and staining properties (1:1000 eosin solution) according to the procedure described by Meymerian *et al.* (24).

[4] The antibiotic solution was prepared by diluting with distilled water, 200,000 units of penicillin G and 200 mg. of streptomycin to a final volume of 400 ml.

[5] Purchased from the Radiochemical Centre, Amersham, Bucks, England.

[6] This abbreviation will be used throughout this paper to denote the number of counts per minute.

[7] Microscopic examination (24) of a sample of the scolices showed them to be totally killed by the sulfuric acid treatment.

[8] Radioactive cholesteryl acetate with a specific activity of 1.55×10^4 c.p.m./mg. was synthesized by treating 4-^{14}C-cholesterol with acetic anhydride in the presence of anhydrous pyridine (28). The ester was shown to be pure by mixed melting point (115–116°) with authentic cholesteryl acetate and by TLC in two solvents (29). Subsequent radioautography of the chromatograms exhibited only one radioactive spot.

Table I—Incorporation of 2-^{14}C-DL-Mevalolactone into Various Lipid Fractions of *E. granulosus* Scolices

Band	c.p.m.	Incorporation, %
I	1096	0.82
I*a*	5	Negligible
I*b*	115	0.08
I*c*	148	0.20
I*d*	502	0.37
II	171	0.13
II*a*	Negligible	Negligible
II*b* (ecdysone level)	8	0.01
II*c*	34	0.02
II*d*	93	0.07
II*e*	9	0.01
III	826	0.62
Unsaponifiable fraction of Band III	Negligible	Negligible
Saponifiable fraction of Band III	827	0.62

ml. of dioxane and then added to 15 ml. of the cocktail. In the case of samples that were too small to be eluted from the TLC plates, the silica gel was scraped off, mixed with the cocktail, and counted according to the method of Snyder and Stephens (30). All samples were counted twice for at least 10 min.

RESULTS

In a duplicate determination, 3.0 g. of live scolices of *E. granulosus* was incubated at 37° with 1 ml. of 2-^{14}C-mevalolactone (specific activity 3.11×10^5 c.p.m./0.04 μM/ml.). At the end of the incubation period (5 hr.), the lipids[9] were extracted from the scolices and fractionated by TLC using 0.25-mm. thick layers of silica gel G with methylene chloride–acetone (92:8, v/v) as a developer. The three zones obtained[10] were designated Bands I, II, and III (23), Bands II and III being the origin and front, respectively.

It was observed that the eluate from Band I inhibited growth markedly (length 0.5 to 1.0 mm.) as compared with the growth of worms under the influence of Band III or in a control (9.5 to 10 mm.) (23). Band I was subjected to further chromatography on a 20 × 20-cm. silica gel[11] plate with chloroform–acetone (90:10, v/v) as eluant. Four different zones were observed which were designated Band I*a* (origin), I*b* (R_f = 0.36), I*c* (R_f = 0.74), and I*d* (R_f = 0.93). The extent of the incorporation of 2-^{14}C-mevalonate into the various lipid fractions of *E. granulosus* scolices is presented in Table I.

When Band I*c*, containing the free cholesterol,[12,13] was eluted from the TLC chromatogram, it yielded 4 mg. of residue which was dissolved in 10.0 ml. of benzene. When 2.5 ml. from this solution was mixed with scintillation cocktail and its radioactivity counted, it was found to have a specific activity of 37 c.p.m./mg. To show whether or not the label was incorporated into cholesterol, 7.5 ml. from the cholesterol solution was mixed with 10 ml. of benzene containing 0.047 g. of pure, unlabeled cholesterol. The benzene was evaporated under a stream of nitrogen and the residue recrystallized once from 95% ethanol. No radioactivity was detected in the recrystallized cholesterol. In contrast, however, the supernatant solution was found to contain all the radioactivity (110 c.p.m.), indicating that I*c* contained compound(s) other than cholesterol[14] to which the label was incorporated.

Band III, which was shown to contain the neutral fat fraction of the lipids (31), was eluted and saponified. Aliquots from the saponifiable and nonsaponifiable fractions were counted for radioactivity. The results in Table I demonstrate that the entire radioactivity of Band III from the 2-^{14}C-mevalonate was incorporated into the saponifiable fraction.

With the significance of the saponifiable fraction in mind, the authors proceeded to determine by GLC the type and quantity of the fatty acids in this fraction. The results are shown in Table II.

The presence of hydrolytic enzymes in the scolices was indicated when a homogenate from 1 g. of live scolices was incubated with 6.0 mg. of radioactive cholesteryl acetate[8] at 37°. After incubation, free cholesterol was isolated from the mixture, and it was found to be radioactive (1056 c.p.m.). No radioactive cholesterol was detected in a control mixture[15] without the scolex homogenate.

DISCUSSION

With the recent findings on insect lipid biochemistry in mind, the authors have attempted to elucidate the mevalonate–cholesterol biosynthetic pathway of *E. granulosus*. First, however, a very brief description of the parasite's life cycle is necessary. The adult worm discharges eggs in the feces of the primary host, often a dog. When the eggs are ingested by an intermediate host, such as cow, sheep, or man, the eggs hatch in the duodenum. The released oncospheres then travel *via* the lymphatics and bloodstream to various parts of the body, usually the liver or lungs, where they become hydatid cysts. In man these cysts may grow to 15–20 cm. in diameter after several years. Within the cysts are found scolices which, if ingested by a suitable host, become adult worms within 7 weeks.

The only effective[16] treatment of the hydatid disease (echinococcosis), surgical removal of the cyst, suffers from both (*a*) a relatively high risk if secondary cyst infection by the viable hydatid scolices within the cyst and (*b*) the possibility of a violent and sometimes fatal anaphylactic reaction by the patient to the cyst fluid.

It seems to the authors that a more knowledgeable approach to the treatment and (or) control of echinococcosis could be made if more was known about the biochemistry of the parasite. Also, the possibility of finding an endocrine system in parasites similar to the one producing ecdysis in insects presents itself.

Cholesterol appears to be the major sterol in helminths. Fairbairn and Jones (34) showed that 75% of the unsaturated sterols of *Ascaris lumbricoides* was cholesterol. Thompson *et al.* (35) reported that 98 and 85% of the unsaponifiable matter of adult *Taenia taeniaeformis* and *Moniezia* sp. (respectively) were cholesterol. The authors' studies recently showed that in *E. granulosus* scolices, free cholesterol per dry weight constituted a high 3.03% (23, 32) as compared to 1.4% for *Taenia taeniaeformis* reported by von Brand *et al.* (36).

The authors' results showing the lack of incorporation of 2-^{14}C-mevalonate into cholesterol by the scolices suggest that the terpenic pathway is directed towards compounds other than cholesterol. The scolices' apparent "inability to synthesize" their own cholesterol is substantiated by the results of Frayha (20) who demonstrated that 1-^{14}C-acetate did not incorporate into the cholesterol fraction of *E. granulosus* scolices. He also demonstrated the passage of 26-^{14}C-

[9] The classic saponification of the lipids prior to fractionation by TLC was avoided because a correlation between the biological activity of the various lipid fractions and their biosynthesis from mevalonate was sought.

[10] The visualization and the elution of the substances from the zones obtained by TLC fractionation were performed as described previously (23).

[11] Chromagram, Eastman Kodak, Co., Inc., Rochester, N. Y.

[12] It was previously shown (23, 32) that when the lipid fraction of 0.495 g. of lyophilized scolices[13] was subjected to a similar TLC fractionation, it yielded 0.022 g. of residue from Band I. Further purification of the residue showed that 0.015 g. of it was cholesterol in its free form. Only trace amounts of cholesterol were shown to exist in their esterified form in Band III.

[13] One gram of lyophilized scolices was found equivalent to 14.5 g. of wet live scolices (32).

[14] Preliminary examination of I*c* by combined GLC–mass spectroscopy revealed that this fraction consisted of cholesterol and other compounds, the identities of which are under investigation.

[15] The control mixture consisted of 6.0 mg. of radioactive cholesteryl acetate, 1 ml. of antibiotic solution, and 4 ml. of phosphate buffer (pH 7.0).

[16] Chemotherapy appears to be difficult since the permeability of the membrane (particularly the internal germinal membrane) surrounding the hydatid cyst is known to be highly selective (33).

Table II—Major Fatty Acid Composition of the Scolex of *E. granulosus*

Fatty Acid	Shortened Designation	Methyl Esters as Percentage of Total Esters
n-Dodecanoic	12:0[a]	0.35
Tetradecanoic	14:0	1.67
	14:1	1.34
Hexadecanoic	16:0	8.17
	16:1	2.95
Heptadecanoic	17:0	0.63
	17:1	1.78
Octadecanoic	18:0	23.5
	18:1	27.7
	18:2	7.44
	18 un[b]	1.86
Eicosanoic	20:0	6.03
	20:1	2.4
	20:2	0.67
	20:3	0.74
	20:4	5.21
	20 un[b]	2.60

[a] The numerals following the colon refer to the number of double bonds in the molecule, whereas the numerals before the colon refer to the chain length (number of carbon atoms) of the fatty acid. [b] This abbreviation denotes unknown number of unsaturations.

cholesterol from the host (infected mice) to the scolices of *E. granulosus* (20). Furthermore, Meyer *et al.* (21) reported that the cestode *Spirometra mansonoides* lacked mechanisms required for the *de novo* synthesis of sterols.

The fact that free radioactive cholesterol was isolated from the scolices upon incubation with radioactive cholesteryl acetate indicates the presence of an enzyme system within the cyst responsible for the hydrolysis of the cholesteryl ester. This finding was consistent with the results of Lee *et al.* (37) who reported the presence of nonspecific esterases in various tapeworms.

Based on these recent findings, it seems reasonable to assume that cholesterol passes, probably in an esterified[17] form, from the host through the cyst membrane into the scolices where the cholesteryl ester is hydrolyzed to free cholesterol by an esterase system within the scolices.

REFERENCES

(1) A. J. Clark and K. Bloch, *J. Biol. Chem.*, **234,** 2578(1959).

(2) W. E. Robbins, J. N. Kaplanis, S. J. Louloudes, and R. E. Monroe, *Ann. Entomol. Soc. Amer.*, **53,** 128(1960).

(3) A. von Butenandt and P. Karlson, *Z. Naturforsch.*, **9b,** 389 (1954).

(4) D. H. S. Horn, E. J. Middleton, J. A. Wunderlich, and F. Hampshire, *Chem. Commun.*, **11,** 339(1966).

(5) P. Karlson and H. Hoffmeister, *Hoppe-Seylers Z. Physiol. Chem.*, **331,** 298(1963).

(6) A. J. Clark and K. Bloch, *J. Biol. Chem.*, **234,** 2583(1959).

(7) R. B. Clayton, *ibid.*, **235,** 3421(1960).

(8) P. Karlson, *Angew. Chem., Intern. Ed.*, **2,** 175(1963).

(9) E. E. Smissman, N. A. Jenny, and S. D. Beck, *J. Pharm. Sci.*, **53,** 1515(1964).

(10) D. B. Carlisle, *Gen. Comp. Endocrinol.*, **5,** 366(1965).

(11) C. M. Williams, *Nature*, **178,** 212(1956).

(12) P. Karlson and H. Schmialek, *Z. Naturforsch.*, **14b,** 821 (1959).

(13) H. Schmialek, *ibid.*, **16b,** 461(1961).

(14) *Ibid.*, **18b,** 462(1963).

(15) V. B. Wigglesworth, *J. Insect Physiol.*, **7,** 73(1961).

(16) F. M. Fisher and R. C. Sanborn, *Nature*, **194,** 1193(1962).

(17) H. A. Schneiderman, L. I. Gilbert, and M. Weinstein, *ibid.*, **188,** 1041(1960).

(18) W. S. Bowers, H. M. Fales, M. J. Thompson, and E. C. Uebel, *Science*, **154,** 1020(1966).

(19) I. F. Durr, *Biochem. J.*, **98,** 317(1966).

(20) G. J. Frayha, *Comp. Biochem. Physiol.*, **27,** 875(1968).

(21) F. Meyer, S. Kimura, and J. F. Mueller, *J. Biol. Chem.*, **241,** 4224(1966).

(22) C. D. Ginger and D. Fairbairn, *J. Parasitol.*, **52,** 1097(1966).

(23) R. E. Thorson, G. A. Digenis, A. Berntzen, and A. Konyalian, *ibid.*, **54,** 970(1968).

(24) E. Meymerian, G. W. Luttermoser, G. J. Frayha, C. W. Schwabe, and B. Prescott, *Ann. Surg.*, **158,** 211(1963).

(25) W. M. Clark, "The Determination of Hydrogen Ions," Williams and Wilkins, Baltimore, Md., 1920, p. 144.

(26) C. G. Beames, Jr., *Exp. Parasitol.*, **16,** 291(1965).

(27) J. W. Farguhar, W. Insull, Jr., P. Rosen, W. Stoffel, and E. H. Ahrens, *Nutr. Rev.*, **17,** Part II, 1, Supplement (Aug. 1959).

(28) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 4th ed., Wiley, New York, N. Y., 1959, p. 212.

(29) D. Waldi, in "Thin-Layer Chromatography," E. Stahl, Ed., Academic, New York, N. Y., 1965, p. 252.

(30) F. Snyder and N. Stephens, *Anal. Biochem.*, **4,** 128(1962).

(31) G. A. Digenis and B. A. Faraj, unpublished data.

(32) A. K. Konyalian, M.S. dissertation, American University of Beirut, Beirut, Lebanon, 1967.

(33) C. W. Schwabe, *Amer. J. Trop. Med. Hyg.*, **8,** 20(1959).

(34) D. Fairbairn and R. N. Jones, *Can. J. Chem.*, **34,** 182(1956).

(35) M. J. Thompson, E. Mosettig, and T. von Brand, *Exp. Parasitol.*, **9,** 127(1960).

(36) T. von Brand, Y. Sato, and R. Overton, *J. Parasitol.*, **51,** 483 (1965).

(37) D. L. Lee, A. H. Rothman, and J. B. Senturia, *Exp. Parasitol.*, **14,** 285(1963).

(38) D. Waldi, in "Thin-Layer Chromatography," E. Stahl, Ed., Academic, New York, N. Y., 1965, p. 254.

[17] 70–75% of human serum cholesterol is found in an esterified form (38).

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