FOLIA PARASITOLOGICA (PRAHA) 27: 59—61, 1980.

UNSAPONIFIABLE LIPIDS OF COTUGNIA DIGONOPORA AND RAILLIETINA FUHRMANNI (CESTODA: CYCLOPHYLLIDEA)

S. C. NIGAM and G. PREMVATI

Department of Zoology, University of Lucknow, Lucknow

Abstract. Unsaponifiable substance in *Cotugnia digonopora* and *Raillietina fuhrmanni* (Cestoda: Cyclophyllidea) was 6.3 % and 7.5 % respectively of the total lipid content. On characterization of the unsaponifiable fraction, cholesterol was found to be 90 % and 93 % respectively in the two cestodes. A search for friedelin was negative.

The unsaponifiable lipid fraction of cestodes studied so far contains a larger or smaller portion of sterols, having predominantly cholesterol. The synthesis of cholesterol does not occur in cestodes and is due to absorbing it directly from the host intestine (Frayha and Fairbrain 1968, Bailey and Fairbairn 1968). The present communication deals with the unsaponifiable fraction of the lipids of two bird cestodes, not so far studied: *Cotugnia digonopora* Pasquale, 1890 from *Gallus gallus* and *Raillietina fuhrmanni* Southwell, 1922 from *Columbia livia*. Besides, the method for the detection of the position of friedelin, a pentacyclic triterpenoid has been described, although it was found to be absent in these cestodes.

METHODS

The experimental procedure is similar for both species of cestodes. Here the description pertains to *C. digonopora*.

The parasites were collected from freshly killed host, wiped on Whatman filter paper No. 1, and then dried under vacuum till the weight became constant. Dried material was pulverized in a morter; 25 m of this was percolated with 500 ml of 95 % ethyl alcohol. This eliminated free fatty acids, free amino acids and free steroids, if any, and ensured that the steroids are coming from lipid fraction alone (Nigam et al. 1963).

The residual matter left after alcoholic extraction was dried at room temperature and was subjected to soxhlet extraction with 200 ml of hexane. The hexane was distilled off under vacuum and the resultant residue was macerated with excess of acetone (200 ml). This was then centrifuged to eliminated phospholipids and the decanted acetone was removed under vacuum to yield total lipids amounting to 1.9 g (7.6 %). This lipid was then taken up in 100 ml of 0.5 N alcoholic KOH and subjected to saponification under reflux for five hours. The reaction mixture was cooled and 15 ml distilled water added. It was then transferred to a separating funnel and shaken with ether. The separated ethereal layer containing the unsaponifiable matter was washed with distilled water to remove the lingering alkali and dehydrated over anhydrous sodium sulphate. Ether was removed under vacuum resulting in 300 g of total unsaponifiable material.

The unsaponifiable matter responded to colour reaction (Liebermann—Burchard, pink-violet-blue-green and Solkowski, blood red; Fieser and Fieser, 1959) indicating the presence of steroids. This was further fractionated by TLC solvent system. The solvent used was a mixture of benzene and ethyl acetate in the ratio of 9 : 1, followed by 10 % (v/v) sulphuric acid spray. This revealed three spots, out of which one corresponded to cholesterol on co-TLC (Fig. 1). The cholesterol was further characterised as follows:

a) The material was taken up in 10 ml of petrol ether and subjected to column chromatography over neutral alumina. A number of fractions were collected using hexane, hexane-benzene in increasing sequential proportions and benzene as eluent. The residues obtained from the six fractions, only from hexane-benzene (50 : 50) elutions responded to colour reactions for steroids and each showed

similar behaviour on TLC. All the six fractions were combined to yield 280 mg of total product. It was purified through crystallisation from a mixture of ether-alcohol (1 : 9) and left overnight to furnish clusters of colourless needles melting at 150—151 °C, showing a solitary spot on TLC corresponding to cholesterol.

b) The purified product was further subjected to co-argentation TLC using the same solvent system so as to ensure the compound to be a single entity.

c) The identity of cholesterol was finally confirmed by mixed m.p. and superposable infrared spectra using an authentic sample of cholesterol.

The other two spots developed on preparative layer chromatography plate were scraped separately, extracted with ether and filtered but no crystalline compound was obtained. The presence of three similar spots of TLC in the unsaponifiable fraction of both *C. digonopora* and *R. fuhrmanni* is unique. The presence of friedelin in cork contaminated unsaponifiable material of both species of cestodes was also observed on co-TLC (Fig. 1) with an authentic sample. The positive detection of friedelin only in the cork contaminated unsaponifiable fractions lends support to Thompson et al. (1960).

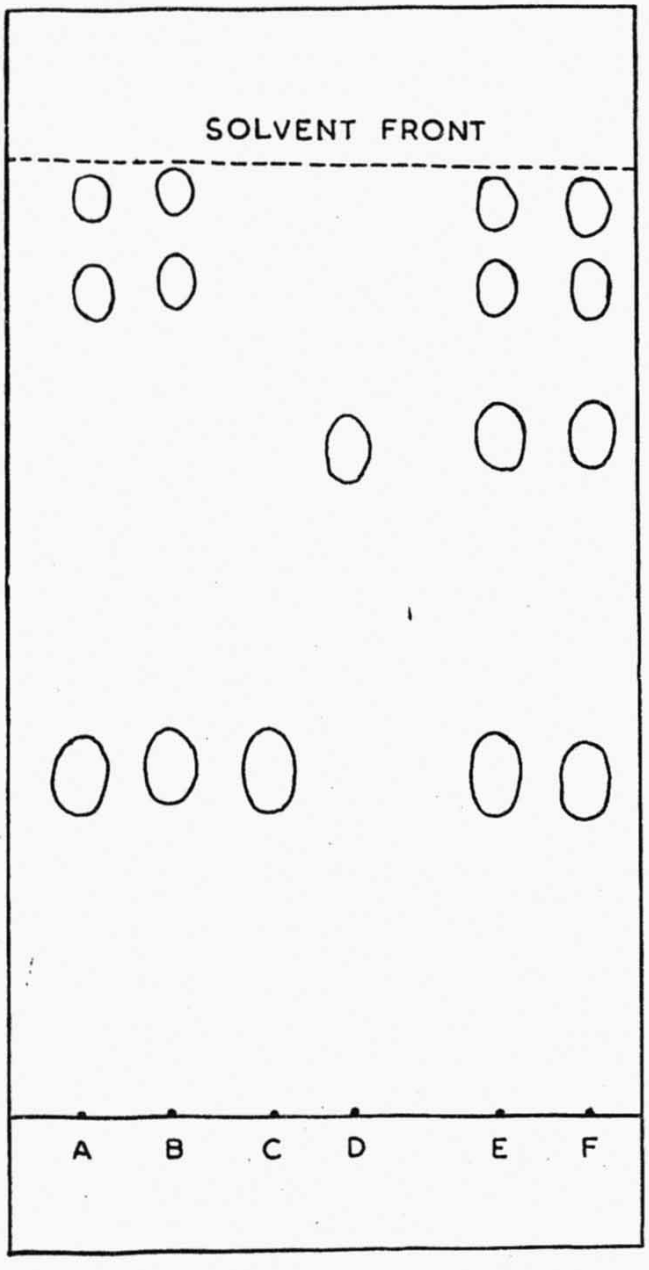

Fig. 1. TLC replica showing the presence of cholesterol in *Cotugnia digonopora* and *Raillietina fuhrmanni*, and friedelin in cork contaminated material. A — unsaponifiable fraction of *C. digonopora*, B — unsaponifiable fraction of *R. fuhrmanni*, C — cholesterol, D — friedelin, E — cork contaminated unsaponifiable material of *C. digonopora*, F — cork contaminated unsaponifiable material of *R. fuhrmanni*.

RESULTS AND DISCUSSION

The amount of total lipids in dry tissue of adult cestodes has been extremely variable, ranging from 6.5 in *Taenia taeniaeformis* to 34.6 in *Hymenolepis diminuta* (reviewed by von Brand 1973). Cain et al. (1977) reported 37 % and 22.1 % lipids of the dry weight of brush borders and vesicle rich fractions separated by differential centrifugation of isolated *H. diminuta* tegument. In the present study the total lipid in dry weight tissue was found to be 7.6 % in *C. digonopora* and 8.5 % in *R. fuhrmanni*.

The unsaponifiable lipid fraction of all parasites studied so far contains a larger or smaller proportion of sterols, with predominant cholesterol. Most of this work has been reviewed by von Brand (1973). In the case of *C. digonopora* and *R. fuhrmanni* the unsaponifiable fraction was found to be 6.3 % and 7.5 % respectively, out of which 90 % and 93 % respectively was cholesterol. Fairbairn (1970) has stated that adult helminths are unable to synthesize fatty acids de novo or to desaturate existing fatty acids. One of the explanations he gave was that it is less troublesome for a parasite to convert excess fatty acids to triglycerides than to control their absorption and excretion. Meyer and Meyer (1972) after having experimented with a number of platyhelminthes including free-living acoels, were of the opinion that platyhelminthes lack the ability to synthesize fatty acids and sterols and depend on an exogenous supply of these compounds for the biosynthesis of their complex lipids.

Acknowledgement. The authors thank Dr. S. K. Nigam, National Botanic Gardens, Lucknow, for kindly supplying the authentic samples of cholesterol and friedelin, and gratefully recognise his helpful suggestions.

НЕОМЫЛЯЕМЫЕ ЛИПИДЫ У *COTUGNIA DIGONOPORA* И *RAILLIETINA FUHRMANNI* (CESTODA: CYCLOPHYLLIDEA)

С. Ц. Нигам и Г. Премвати

Резюме. Обнаружено, что неомыляемые вещества у *Cotugnia digonopora* и *Raillietina fuhrmanni* (Cestoda: Cyclophyllidea) 6,3 % и 7,5 % общего содержания липидов. При характеризовании неомыляемых фракций у этих цестод содержание холестерина было 90 % и 93 %. Поиски фриделина были отрицательны.

REFERENCES

BAILEY H. H., FAIRBAIRN D., Lipid metabolism in helminth parasites. V. Absorption of fatty acids and monoglycerides from micellar solution by *Hymenolepis diminuta* (Cestoda). Comp. Biochem. Physiol. 26: 819—836, 1968.

BRAND T. von., Biochemistry of parasites. Academic Press, New York, 3rd Edition,

CAIN G. D., JOHNSON W. J., OAKS J. A., Lipids from subcellular fractions of the tegument of *Hymenolepis diminuta*. J. Parasitol. 63: 486—491, 1977.

FAIRBAIRN D., Biochemical adaptation and loss of genetic capacity in helminth parasites. Cambridge Philosophical Society (Biological Review) 45: 29—72, 1970.

FIESER L. F., FIESER M., Steroids. Reinhold Publications, New York, 1959.

FRAYHA G. J., FAIRBAIRN D., Lipid metabolism in helminth parasites. VII. Absorption of cholesterol by *Hymenolepis diminuta* (Cestoda). J. Parasitol. 54: 1144—1146, 1968.

MEYER F., MEYER H., Loss of fatty acid biosynthesis in flatworms. In: H. Van den Bossche (Ed.): Comparative Biochemistry of Parasites. Academic Press, New York and London: 383—393, 1972.

NIGAM S. K., MITRA C. R., KAUL K. N., Chemical examination of *Cosmos bipinnatus* Seed. Ind. J. Appl. Chem. 26: 197, 1963.

THOMPSON M. J., MOSETTIG E., von BRAND T., Unsaponifiable lipids of *Taenia taeniaeformis* and *Moniezia* sp. Exp. Parasitol. 2: 127—130, 1960.

Received 24 November 1978.

S.C.N., Department of Zoology, University of Lucknow, Lucknow, India