two closely opposed lipid bilayers, a probable morphological and functional adaptation to parasitism. This membrane complex provides an effective tool for defeating the host's immune system. The authors presented an overview of lipid metabolism in *S. mansoni* adults of interest because schistosome adults and other helminths cannot synthesize fatty acids or cholesterol and must obtain these lipids from the host.

IV. SAMPLE PREPARATION

Lipid analysis should be done as soon as possible after samples are obtained from plant and animal tissues. If analysis is delayed, samples should be refrigerated overnight at 4°C or for longer periods in a freezer at −20°C or colder. Formalin or other fixatives used in histology laboratories should not be added to tissues, because they may produce spurious results during subsequent TLC analysis. Reddy et al. (67b) tested the effects of freezing and thawing and of formalin and ethanol fixation on HPTLC analysis of neutral lipids in *Biomphalaria glabrata* snails. Their study showed that these procedures produced spurious results in subsequent TLC analysis of neutral lipids in snail tissues. For lipid work, glassware should be chemically cleaned in dichromate or sulfuric acid and then further cleaned in chloroform–methanol (2:1) prior to use. Glass vials, jars, and bottles are recommended for lipid analysis, along with aluminum foil or Teflon-lined lids.

Because lipids are easily auto-oxidized, samples should be handled and processed under nitrogen whenever possible. Samples should not be stored dry but preferably in a small volume of suitable solvent, e.g., chloroform or hexane, under nitrogen and maintained at −20°C. The presence of significant amounts of methyl esters in animal tissues may indicate a preparation artifact, because this lipid is usually not a signficant component of animal tissue (68). The neutral lipid mixture 18-4A supplied by Nu-Chek (Elysian, MN) contains methyl oleate. A similar lipid mixture containing methyl oleate along with other neutral lipids is supplied by Matreya (Pleasant Gap, PA) under the name Non-Polar Lipid Mix-B. The use of either standard during TLC, along with the sample, allows for a check on the presence of methyl esters in the sample. An extract of a blank can be used to determine the validity of a TLC observation. For example, if saline is supplemented with human serum and the mixture is extracted with chloroform and spotted on a TLC plate, neutral lipid TLC procedures should detect mainly cholesterol and cholesteryl esters along with lesser amounts of free fatty acids and triacylglycerols. If the saline alone is treated in an identical manner, it should be lipid-negative.

If the lipids of interest are major constituents, the sample may be applied directly to the plate with no further cleanup (see first method in Table 4). Samples used may be blood, urine, saliva, or tissue homogenates. Kupke and Zeugner (69) directly applied a 0.5 μL sample of plasma to an HPTLC silica gel plate. The sample was applied to the origin over a 15 μL spot of methanol, and the spot was covered immediately with an additional 1–3 μL of methanol. The plate was air-dried and then developed with chloroform–methanol–water (65:30:5) twice, each time for a distance of 3.7 cm, then developed in hexane–diethyl ether–acetic acid (80:20:1:5) to within 1 cm of the top of the plate. The plate was treated with NH_4HCO_3, then heated for 10 min at 150°C, and sharp fluorescent lipid spots were detected. Excellent separations of the major neutral lipid and phospholipid fractions were obtained.

If the lipids of interest are minor constituents of the sample or present in relatively low concentrations in a complex biological sample, extraction, isolation, and concentration steps usually precede TLC. Numerous sample preparation methods are available, and personal choice often dictates which one will be used. Of all the procedures, that of Folch et al. (70), or a modification of the original procedure, is used more frequently than any other. Table 4 lists numerous procedures used for the sample preparation of lipids.

Reviews on sample preparation include that of Christie (77a) on obtaining lipid extracts from tissues and that of Fried (77b) on obtaining and handling biological materials and prefractionating extracts for lipid analyses. Chapter 2 in Hammond (1a) also provides detailed descriptions on lipid extraction of photosynthetic tissue, oilseeds, tiger prawns (crustaceans), coffee whitener, wheat flour, spores, and volatile fatty acids from cells grown in culture media.