drought-fed or food and/or water deprived) sheep, the drug had at least a 7–8-fold therapeutic index at the 25 mg/kg level; at least a 3–4-fold index at the 40 mg/kg level.

The safety and efficacy of SYD-230 was confirmed in the field. During trials carried out under a variety of climatic and geographic conditions in Australia, 12,755 sheep were drenched at 25 mg/kg. Of 22 deaths, only 2 were judged to be drug-related. The other deaths were attributed to enterotoxemia, other clostridial toxins, the consequences of the parasitic infections or drought conditions encountered in Australia during the period of testing the drug. Among 1572 sheep dosed at 50 mg/kg, there were 12 deaths of which 3 were probably drug-related. The high efficacy of the drug against immature and mature *F. hepatica* was confirmed in these statistically designed field trials. The superiority of SYD-230 over carbon tetrachloride has been demonstrated in outbreaks of acute fascioliasis. Details of these experiments will be published at a later date.

Zusammenfassung. 2-Acetoxy-4'-chlor-3,5-dijodbenzanilid zeigte eine hohe Wirksamkeit gegen junge und gereifte *F. hepatica* and *H. contortus* in Schafen bei einer oralen Dosis von 25 und 40 mg/kg. Die gute Verträglichkeit der Verbindung in Schafen ergab sich aus Feldversuchen in Australien.

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The Lipid Composition of Frog Retinal Rod Outer Segments

Retinal rod outer segments are derived from the plasma membrane of a neuron, the rod cell[1]. They may, therefore, provide a relevant macromolecular model for neuron plasma membranes, just as myelin may for oligodendrocyte plasma membranes. The paracrystalline structure of outer segments makes them amenable to examination by X-ray diffraction as well as electron microscopy; consequently, circumstances are especially favorable for determining their biochemical architecture by correlated chemical techniques[2]. This article describes studies of the lipid composition of chloroform-methanol extracts of intact dark-adapted outer segments of the leopard frog.

Materials and methods. All operations were done under dark-room conditions and retinas and outer segments were kept at 0 °C as much as possible. Retinas dissected from dark-adapted large male frogs (*Rana pipiens*) were suspended in frog Ringer's solution in a 3 ml tube (1 or 2 retinas/1 ml). The tube was held flexibly by its top while the lower end was stroked repeatedly with a finger or wet piece of rubber tubing, to cause the retinas to spin rapidly in the saline. After 3 min, another 1 ml of saline was added and the agitation continued for 2 min. This simple technique effectively freed outer segments from rod inner segments and produced fewer free nuclei and less debris than any other method of detachment tried[3]. The retinas and retinal fragments were allowed to settle for 10 min. The outer segments in the supernatant fluid were pipetted off, combined, and passed through Nitex mesh 25 μ in diameter (Tobler, Ernst and Traber, Inc., New York). In some experiments 10 μ Nitex was also used. The outer segments were sedimented in 2 min at half speed on an International Clinical Centrifuge (1500 rpm, 350 *g*) and washed with saline. The red pellet consisted predominantly of intact rod outer segments, the chief contaminant being large nuclei. Ten retinas yielded 2–3 million outer segments (hemacytometer count). A series of 19 pellets, totalling 30 million outer segments, provided 7 mg of lipids.

The pellets were extracted with chloroform-methanol (2:1, v/v) in the dark for several days at 4 °C. The extracts were combined and washed to remove non-lipid contaminants[4,5]. The residues from the pellets were then extracted with chloroform-methanol-concentrated. HCl (200:100:1, v/v) to solubilize any polyphosphoinositides present[6,7] and these acid extracts were combined.

Analyses for protein and lipid hexose were performed as previously described[5]; cholesterol was assayed in the non-saponifiable fraction by the method of Glick et al.[8]. Individual phospholipids were determined on the weighed solids of the washed chloroform-methanol extract by successive chemical hydrolyses and separation of the hydrolysis products by paper chromatography and electrophoresis according to Dawson et al.[9]; for comparison, lipids were also extracted from frog retinas and the phospholipids and cholesterol similarly analyzed.

Results and discussion. The outer segment preparations contained a high percentage of lipid, two-thirds of which was phospholipid (Table I). Lesser amounts of glycolipid and cholesterol were present. The molar ratio of phospholipid-glycolipid-cholesterol was 1:0.33:0.13. Examination of the non-saponifiable fraction by thin layer chromatography (silica gel G, $CHCl_3$ solvent) confirmed the presence of cholesterol and revealed 4 additional spots as yet unidentified[10]. Chemical evidence for glycolipids in rod outer segments has not been reported before, but their presence was suggested by Lillie[11] on the basis of histochemical staining reactions. The outer segments resembled mito chondria, chloroplasts and myelin in containing appreci-

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able amounts of proteolipid protein. These proteins appear to be an integral part of the compound membrane structure of such organelles[5].

The only previous report on the lipid composition of frog outer segments is that of COLLINS et al.[12]. These authors found that phospholipid P accounted for 61% of total P in the outer segments, but only 38% in the frog retina. However, several investigators have studied lipids in outer segment preparations from cattle[12-14]. In terms of dry weight, the lipid content for frog outer segments (40.6%) corresponded with the result of SJÖSTRAND (38.8%)[13]; and the phospholipid content (26.6%) agreed with the value of COLLINS et al. (27.7%)[12], but was lower than that of SJÖSTRAND (31.5%), who reported 81% of the lipids as phospholipids. Our value of 27 μg P/mg lipid for intact frog outer segments was likewise lower than that for bovine outer segment disks (32 μg P/mg lipid) given by FLEISCHER and MCCONNELL[14].

Frog outer segments were characterized by a relatively simple phospholipid composition (Table II). Phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine constituted the highest percentage (83.5%) of the lipid P yet found in any tissue or subcellular fraction. Small amounts of acidic phospholipids and sphingomyelin were present. Polyphosphoinositides were not detected on analysis of the acidified chloroform-methanol extract[7]. In comparison with frog retina, the outer segments were enriched in phosphatidyl ethanolamine and relatively deficient in plasmalogens as well as sphingomyelin + alkyl ether phospholipids. In all brain subcellular fractions so far analyzed[15], the amounts of plasmalogen (9.4–25.8% of total lipid P) and sphingomyelin (3.7–12.4%) are far higher than in outer segments. The lipid composition of frog outer segments was thus simpler than that of myelin. A total of 54% of the lipid weight was made up by phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine; 11% by other phospholipids; 23% by glycolipids; and only 4% by cholesterol.

The low cholesterol content of frog outer segments is in agreement with results on cattle preparation[13,14]. The molar ratio of cholesterol to phospholipid fell at the low end of the range found in a series of membranes in which the ratio varied from 1 : 4 (muscle sarcolemma) to approximately 1 : 1 (myelin)[16,17]. Since outer segments and myelin both arise from plasma membranes and represent compound membrane structures, they might be expected to resemble one another in having a high ratio if either an origin from surface membranes or eventual membrane contact relationships were of critical importance. The low ratio in outer segments, however, would tend to support the conclusion[16] that there are no characteristic proportions of sterol to phospholipid in plasma membranes generally. Neuronal plasma membranes free of glial contamination have not yet been prepared. Hence, it is impossible to know whether the low ratio of cholesterol to phospholipid in outer segments reflects their origin specifically from the neuronal cell surface, but this seems a reasonable hypothesis[18,19].

Table I. Chemical composition of frog retinal rod outer segments

Constituent	% of WTLE solids[a]	% of lipids	% of dry weight[d]
Lipids	86.0	100.0	40.6
Cholesterol	3.7	4.3	1.7
Phospholipids (lipid P × 25)	56.3	65.5	26.6
Glycolipids (hexose × 4.6)	20.0	23.3	9.5
Proteolipid protein[b]	14.0		6.6
Residue protein[c]			52.8
Total protein			59.4

The outer segments were extracted with chloroform-methanol (2:1, v/v). The lipid extract was partitioned[4]. [a] WTLE, washed total lipid extract (lower phase). [b] Protein extractable by chloroform-methanol. [c] Protein not extractable by the solvent. [d] Dry weight, WTLE solids + residue protein.

Table II. Phospholipids of frog rod outer segments and retina

Substance	% of total lipid P	
	Rod outer segments	Retina
Phosphatidyl choline	49.4	51.7
Phosphatidyl ethanolamine	24.6	18.8
Phosphatidyl serine	9.5	9.6
Phosphatidyl inositol	1.4	1.6
Phosphatidic acid	3.0	0.9
Cardiolipin	1.3	2.4
Ethanolamine plasmalogen	0.6	3.0
Choline plasmalogen	trace	not detected
Sphingomyelin	1.8	12.7
Alkyl ether phospholipid	3.5	
Recovery	96.5[a]	100.7

Substance	Rod outer segments	Retina
WTLE solids[b] (mg)	7.38	13.2
Phospholipid (% of WTLE solids)	56.3	67.9
Cholesterol/lipid P (molar ratio)	0.13	0.33

[a] Includes 1.4% of a phospholipid tentatively identified as diphosphoinositide. [b] These solids include chloroform-methanol soluble protein (proteolipid protein).

Résumé. Les lipides des segments extérieures des bâtonnets de la rétine de la grenouille sont principalement composés de phospholipides (66%), glycolipides (23%) et cholestérol (4%). Le phosphatidyle choline, le phosphatidyle éthanolamine et le phosphatidyle sérine constituent le 84% des phospholipides. Les résultats ne s'accordent pas avec le concept qu'un rapport élevé du cholestérol aux phospholipides caractérise les membranes du plasma et leurs dérivés.

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[19] Supported by National Science Foundation Grant No. GB 3128 and United States Public Health Service Grant No. NB-130 from the National Institute of Neurological Diseases and Blindness.