contain *N*-sulfated functional groups. By contrast the hyaluronic acids and chondroitin sulfates have only *N*-acetylated functional groups[2,3]. The observation that different acid mucopolysaccharides have different staining intensities may be of some value in distinguishing among acid mucopolysaccharides which have similar R_F values, particularly after thin-layer chromatography.

The blue color reaction has remained stable on paper strips for at least 1 year during which the strips were protected from direct light. The blue color may also be preserved on thin-layer chromatograms if the thin layer is first treated with a plastic coating (Brinkmann Instruments, New York), then removed with transparent plastic tape, and finally stored out of direct light. Studies are now in progress to determine whether the color intensity of various sphingolipids can be related to molar quantities.

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Comparative chromatographic and dissociative behaviour of avian and amphibian lipovitellin*

The sedimenting granules in hen's egg yolk and the platelets of frog's eggs both contain a phosphoprotein (phosvitin) and a lipoprotein (lipovitellin). The corresponding proteins from both species are similar in several respects but they may not be identical[1]. Lipovitellin from avian egg yolk has been separated into two components, α- and β-lipovitellin, by column chromatography on hydroxyapatite, and both have been shown to dissociate into two subunits in alkaline solvents[2,3]. Apart from their differential affinity for hydroxyapatite (β- eluted at a phosphate buffer concentration of 0.6 M, α- at 1.1 M), these two avian lipovitellins can be distinguished by their protein phosphorus content (α- has 0.6%, β- 0.3%) and the pH required for equivalent dissociation (α- requires pH 10.5 and β- pH 7.8 to produce 50% dis-

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sociation)[4,5]. As neither the fractionation nor dissociation of lipovitellin from amphibian yolk platelets has been reported, such a study was undertaken and a preliminary comparison with avian lipovitellin is given here.

Frog (*Rana pipiens*) yolk platelets (without their superficial layers) were isolated in a sucrose-EDTA medium[1] and subsequently examined by methods developed for avian yolk granules[3,5]. Chromatography in 0.7 M sodium acetate on Dowex-1 columns yielded a lipovitellin with 0.45% protein phosphorus similar to the value obtained by WALLACE[1] for phosvitin-free amphibian lipovitellin. Ultracentrifugal examination in 0.5 M NaCl (Fig. 1A) confirmed the removal of phosvitin[3] from the lipovitellin by Dowex-1. Aged lipovitellin solutions often contain a faster sedimenting X-component[6,7] and this can also be removed by Dowex-1 columns (Fig. 1B).

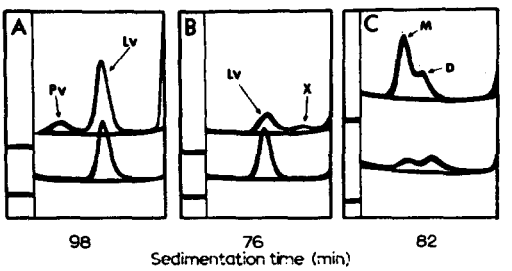

Fig. 1. Sedimentation patterns showing efficacy of chromatographic separation and dissociation of amphibian lipovitellin (Lv): A, Amphibian platelet proteins in 0.5 M NaCl before (upper) and after (lower) Dowex-1, showing removal of phosvitin (Pv). B, Aged sample of amphibian lipovitellin in 0.5 M NaCl before (upper) and after (lower) Dowex-1, showing removal of X- component. C, Dissociation of avian α-lipovitellin (upper) and amphibian lipovitellin (lower) obtained from hydroxyapatite in carbonate buffer at pH 11.0 and *I* 0.2. M, monomer; D, dimer.

Following the removal of phosvitin, the frog lipovitellin was added to a hydroxyapatite column. Gradient elution with phosphate buffer (pH 6.8) yielded a single component at buffer concentrations between 1.0 and 1.5 M with a protein phosphorus content of 0.93%. The absence of any component eluted at concentrations below 1.0 M is presumptive evidence that there is nothing equivalent to avian β-lipovitellin in the frog platelet. Frog lipovitellin evidently more closely resembles avian α-lipovitellin (elution concentration 0.7–1.1 M; protein phosphorus 0.60–0.70%). Moreover, the recovery of frog lipovitellin from hydroxyapatite columns was comparable with that reported for avian α-lipovitellin[3]. The non-lipid phosphorus content of the frog lipovitellin, however, was increased by passage through these columns and differs from the avian type in this respect. Whether this increase is due to an exchange of phosphorus through protein-binding or otherwise has not been investigated.

Amphibian lipovitellin and a sample of avian α-lipovitellin, for comparison, were dialyzed into carbonate buffer at pH 11.0 (*I* 0.2) and examined in the ultracentrifuge. The dissociative behaviour of both lipovitellins is shown by their sedimentation patterns in Fig. 1C. In the solvent employed, amphibian lipovitellin was 40% dissociated and the monomer and dimer components had sedimentation rates ($s^\circ_{20,w}$) of 6.8 and 10.7 S respectively. The corresponding figures for avian α-lipovitellin were 72% dissociation and sedimentation rates of 7.0 and 11.0 S. Quantitatively, these observations are subject to confirmation in work now under way but the qualitative aspects clearly indicate that avian α-lipovitellin and amphibian lipovitellin are closely

related lipoproteins. An interesting difference between the "granular" yolk components of the hen and frog egg is the apparent absence of β-lipovitellin from yolk platelets.

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