The mother liquor, removed the above-mentioned first crop from the crude hydroxylation product, was diluted with AcOEt and washed with H_2O. The organic layer was dried on Na_2SO_4 and evaporated to give a resinous residue, which was chromatographed over silica gel. Elution with benzene-AcOEt (3:1) yielded crystals, which was recrystallized from EtOH and the precipitated crystals was removed by filtration. The filtrate was evaporated and the residue was recrystallized twice from MeOH yielding plates of fairly pure IIb, which sinters at 116~117° and melts at 140~148°. (reported[5] m.p. 125~126° after moisten at 112°). IR ν_{max} cm^{-1}: 3470, 1725.

The foregoing IIb (37 mg.) was acetylated with Ac_2O (1 ml.) and pryidine (1.5 ml.) at room temp. in the usual manner. The acetylated product (41 mg.) was subjected to preparative TLC developing with benzene-AcOEt (3:1) on silica gel G plates. The more mobile fraction (3 mg.), m.p. 225~228°, was identified with the above-mentioned IVb-acetate by IR spectra. The less mobile fraction (36 mg.) on recrystallization from EtOH gave needles of IIb-acetate, m.p. 194~196°. (reported[5] m.p. 185~186°). This compound was identical with a sample, obtained from hydrogenation of Ib-acetate, in all respects.

Hydroxylation of Testosterone Propionate (IIIc)——a) With OsO_4 and H_2O_2 in ether. A solution of IIIc (1.6 g.) in ether was treated with OsO_4 and H_2O_2, in the manner described in the preceding experiment. Crystallization of the crude product from MeOH gave crystals (150 mg.), m.p. 188~200°, which was recrystallized twice from MeOH yielding IVc (51 mg.) as plates, m.p. 199~200°, $[\alpha]_D$ +17.8° (c=0.99). ORD (c=0.2634): $[\varphi]_{297}$ +4598°, $[\varphi]_{257}$ −5245°. IR ν_{max} cm^{-1}: 3440, 1730, 1190. *Anal.* Calcd. for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05. Found: C, 70.00; H, 9.20.

The mother liquor of the first crystallization was evaporated and the resinous residue (1.7 g.) was chromatographed on silica gel. The fractions (252 mg.) eluted with $CHCl_3$, was recrystallized from aq. MeOH yielding crystals, m.p. 144~145°. Although this fraction was supposed to be a mixture of IIc and IVc, attempts to resolve into respective components were unsuccessful.

b) With OsO_4 in pyridine. A solution of IIIc (1.23 g.) and OsO_4 (1.0 g.) in pyridine (13 ml.) was allowed to stand in a dark place at room temp. for 3 days. To the reaction mixture was added petr. ether (140 ml.) and the precipitated osmate was separated by decantation, washed with petr. ether and dissolved in dioxane (70 ml.). A stream of H_2S was bubbled through the solution kept in an ice bath. The mixture was filtered and the filtrate was evaporated to give a dark crystalline residue (1.225 g.). Recrystallization from EtOH gave needles (324 mg.), m.p. 171~173°, which was identical with a sample of IIc obtained from catalytic reduction of Ic. Concentration of the mother liquor afforded a second crop (88 mg.) of IIc, m.p. 165~170°.

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Tatsuzo Fujii*1 and Tomio Fujii*2 : Aggregation of High-density Lipoproteins from Egg Yolk.

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In the course of preparation of lipovitellin, a high-density lipoprotein of hen egg yolk, Joubert and Cook[1] reported the occurrence of a minor component which sedimented faster than lipovitellin upon ultracentrifugation. A similar component was also found by one of the present authors[2] in lipovitellin preparations from the eggs of frog, trout and dog-fish, having a sedimentation constant of 14~15 S as compared with 10 S of lipovitellin. Later Radomski and Wallace[3] and Wallace[4] investigated on such

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a faster sedimenting component (called a X component) in more details and they concluded that this is an autoxidation product of lipovitellin.

The present note reports the formation of a component or components similar to this X component after ether treatment or incubation of lipovitellin solution prepared from trout and frog eggs.

Egg yolk lipovitellin of a trout (*Salmo irideus*) and of a frog (*Rana esculenta*) was prepared as described previously.[2] It was subjected to one of the following treatments and then analyzed by ultracentrifugation.

A) It was dissolved in 10% NaCl solution and diluted 10 times with water to form precipitate. This procedure was repeated 5 times and the final precipitate was, after being dialyzed against water, lyophilized and redissolved in carbonate-bicarbonate buffer (pH 9.8, I0.2).*3

B) It was dissolved in the same buffer as above and extracted with diethly ether at room temperature for 10 minutes, and then diluted with water to precipitate the lipovitellin, which was then redissolved in the same buffer.

C) It was dissolved in the same buffer and incubated at 37° for 48 hours under toluene.

The Fig. 1 shows the sedimentation patterns*4 of lipovitellins before (Fig. 1-O) and after (1-A through 1-C) treatment. The $S_{20,w}$ of each component is indicated on Table I, together with the ratio of S value of the formed component (X) *vs.* that of the original lipovitellin (LV), as well as the ratio of molecular weight of X/LV, calculated by means of an equation cited below the Table, using the values of physicochemical constants reported previously.[2]

Repeated precipitations followed by lyophilization of the trout and frog lipovitellin similarly yielded a faster sedimenting compound (X_1 of Fig. 1-A). Its S value is 1.41~1.48 times as large as that of LV.

Ether treatment brought about apparently drastic influence on the trout lipovitellin (Fig. 1-B, the left), whereas it gave only slight action on the frog lipovitellin (Fig. 1-B, the right). In the

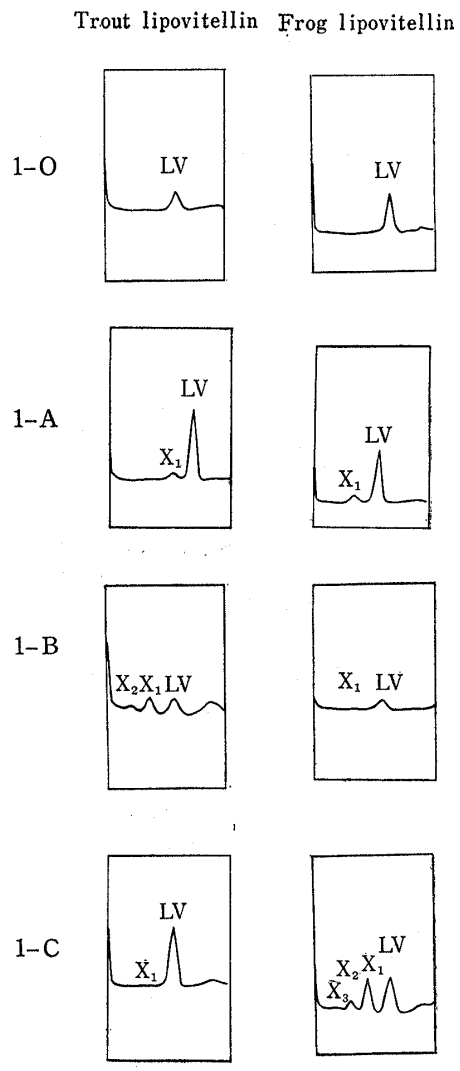

Fig. 1. Sedimentation Patterns of Trout and Frog Lipovitellins before and after Certain Treatment

1-O : before treatment (control)
1-A : after repeated precipitations followed by lyophilization
1-B : after ether extraction at room temperature for 10 minutes
1-C : after incubation at 37° for 48 hours
Buffer employed : Carbonate-bicarbonate buffer (pH 9.8, I 0.2).
Ultracentrifugation : At 60,000 r.p.m. (227,000×g); photographs taken at 8 minutes intervals. Sedimentation from right to left. The LV denotes original lipovitellin, and the X aggregation product.

*3 At this pH, the trout and frog lipovitellins did not show any dissociation as observed on the avian lipovitellins.[1,2,5]

*4 A slowly sedimenting peak at the right extremities of the photographs is due to a contaminating protein (phosvitin) in the lipovitellin preparations.

5) H. Sugano, K. Watanabe : J. Biochem., **50**, 473 (1961).

TABLE I. Ratio of Sedimentation Constant and of Molecular Weight of Aggregation Product (X) *vs.* the Original Lipovitellin (LV)

Treatment	Component	Trout			Frog		
		$S_{20,w}$	$S_{20,w}$ ratio	$M^{a)}$ ratio	$S_{20,w}$	$S_{20,w}$ ratio	$M^{a)}$ ratio
A) precipitations and lyophilization	LV	10.0	1	1	11.1	1	1
	X_1	14.1	1.41	1.70	16.4	1.48	1.80
B) ether extraction	LV	10.3	1	1	10.9	1	1
	X_1	15.0	1.46	1.78	15.9	1.46	1.73
	X_2	18.1	1.76	2.34			
C) incubation	LV	10.0			11.4	1	1
	X_1				16.8	1.47	1.74
	X_2				21.4	1.88	2.58
	X_3				26.2	2.30	3.43

a) M=molecular weight, calculated according to the following equation :

$$M=\frac{4690(S)^{8/2}[\eta]^{1/2}}{(1-\bar{V}\rho)^{8/2}}$$

where S : sedimentation constant at 20° in water; $[\eta]$: intrinsic viscosity; $\bar{V}$: partial specific volume; ρ : density of water at 20°

$$M \text{ of trout LV}=\frac{4690(S)^{8/2}(0.046)^{1/2}}{(1-0.787\times0.998)^{8/2}} \quad \log M=\log(S)+4.0052$$

$$M \text{ of frog LV}=\frac{4690(S)^{8/2}(0.062)^{1/2}}{(1-0.777\times0.998)^{8/2}} \quad \log M=\log(S)+4.0403$$

former case, large proportions of X_1 and X_2 components were observed, having a S value 1.46 and 1.76 times as large as that of LV. In the latter case, only a trace of X_1 component of a S value 1.46 times that of LV was detected.

Prolonged incubation in an alkaline medium gave practically no influence on the trout lipovitellin (Fig. 1-C, the left), but gave a strong action on the frog lipovitellin to form three faster sedimenting components, X_1, X_2 and X_3 (Fig. 1-C, the right), having a S value which is 1.47, 1.88 and 2.30 times as large as that of the original LV, respectively.

Thus, though there seems to be a difference in its sensitivity, it was disclosed that both trout and frog lipovitellins tend to form faster sedimenting X component or components by certain kinds of treatments. Furthermore, an inspection of the Table I will reveal a fact that, between the molecular weights of these X components and the LV, a close correlationship exists, the molecular weight of X_1 is 1.7~1.8 times, that of X_2 2.3~2.6 times and that of X_3 3.4 times the molecular weight of respective LV If we assume, after such a treatment, a formation of a slightly smaller lipoprotein unit of which molecular weight is about 0.85 times that of the original lipovitellin molecule, for example, resulting from a kind of delipidation, we can consider a kind of irreversible aggregation (or association) taking place to form a dimer, trimer and tetramer of such an assumed unit.

Though the identities of these X components obtained by different kind of treatments, as well as the mechanism of their formation, should be ultimately determined by isolation of the individual component followed by chemical and physicochemical analyses, the ease with which such an aggregation of lipovitellins from various sources occurs may suggest a characteristic property of the high-density lipoproteins of egg yolk in general which tends to form larger aggregates.

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