Lipid Distribution in Lipoproteins Separated by Polyanion Precipitation. (26991)

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The isolation of human β-lipoproteins by polyanion precipitation methods has been the subject of several recent reports(1,2,3). Since the technique offers the advantages of speed and simplicity, and does not require specialized equipment, it has considerable potential as a research tool.

The completeness of separation of β-lipoproteins by polyanion precipitation methods has been evaluated by paper electrophoresis (4) with human sera and by ultracentrifugation with both human and animal sera(5).

Since the chick is widely employed as an experimental animal for study of lipid metabolism, data are presented concerning the effectiveness of polyanion precipitation methods in this species. This report also presents data on the lipid content of plasma and of the supernatant fraction (α-lipoprotein) following polyanion precipitation for the chick, and for other animal species.

Methods. Blood samples were obtained from experimental animals by cardiac puncture. Unless otherwise noted, the animals were fed a stock diet. Human blood samples were obtained from volunteers by venipuncture.

The polyanion precipitation method described by Florsheim and Gonzales(5) was employed. This procedure uses Mepesulfate, the sodium salt of sulfated polygalacturonic acid methyl ester methyl glycoside (Hoffmann-LaRoche), as the polyanion. The completeness of β-lipoprotein precipitation was evaluated by paper electrophoresis and by ultracentrifugation. Electrophoretic separations were made of the plasma and the supernatant after Mepesulfate precipitation; a Durrum-type cell was employed with a barbiturate buffer (pH 8.6; ionic strength 0.075). The strips were stained with oil red O to locate the lipoprotein bands. The plasma and supernatant obtained after Mepesulfate precipitation were subjected to ultracentrifugation by the method of Havel *et al.*(6) after initial adjustment to a density of 1.063 with sodium chloride. Completeness of separation was estimated by the cholesterol content of the α-(D>1.063) and β-(D<1.063) lipoprotein fractions.

Lipid distribution in the α- and β-lipoproteins was estimated by determining lipid content of the plasma and the supernatant resulting from Mepesulfate precipitation, values for β-lipoprotein lipid being obtained by difference. Lipids were extracted with chloroform:methanol (2:1). In some cases, the extracted lipids were fractionated by silicic acid chromatography by a modification of the method of Hirsch and Ahrens(7) previously described(8). Cholesterol and lipid phosphorus were determined by the method of Searcy and Bergquist(9) and Fiske and Subbarow(10), respectively. Glycerides were determined by a modification of the method of Van Handel and Zilversmit(11) previously described(12).

Results and discussion. Chick plasma was subjected to paper electrophoretic analysis before and after Mepesulfate precipitation. Mepesulfate precipitation resulted in disappearance of the β-lipoprotein band. Total cholesterol determinations demonstrated that 70-80% of the total plasma level of cholesterol was present in the supernatant after Mepesulfate precipitation (α-lipoprotein fraction). This finding is in contrast to values reported for the chicken. Orma(13) reported 50-70% of the total cholesterol to be in the β-fraction as separated by paper electrophoresis.

To verify the results obtained, cholesterol was determined in high (D>1.063) and low (D<1.063) density lipoproteins separated by ultracentrifugation. The level of total cholesterol in the high density fraction of plasma and the supernatant fraction obtained after Mepesulfate precipitation were identical (104 vs. 105 mg/100 ml). The amount of cholesterol remaining in the low density lipo-

TABLE I. Plasma and α-Lipoprotein Cholesterol in Chicks Fed Different Diets.

Dietary treatment	No. of samples	Plasma cholesterol, mg/100 ml	
		Total	α-Lipoprotein fraction
20% protein	8	102 ± 7*	73 ± 7*
20% " + 2% cholesterol	7	523 ± 51	94 ± 10
18% " (stock diet)	23	147 ± 4	118 ± 6

* Mean ± standard error.

protein fraction after Mepesulfate precipitation was only 22% of that present before precipitation (9 vs. 41 mg/100 ml). This finding confirms the observation made concerning cholesterol distribution in α- and β-lipoproteins of chick plasma. The chick, thus, differs from the human, but is similar to the dog in that a greater amount of cholesterol is bound to the α-lipoproteins than to the β-fraction. The results obtained from the ultracentrifugal analysis confirm the observation of Florsheim and Gonzales(5) concerning the completeness of Mepesulfate precipitation of β-lipoprotein from chicken plasma.

The amount of cholesterol bound to the α-lipoproteins was determined for chicks fed a 20% sesame oil meal semi-purified diet, the same diet with 2% added cholesterol or a stock diet. The results obtained are presented in Table I. The chicks fed the 20% protein diet or the stock diet had 72 and 73% of the total cholesterol bound to the α-lipoprotein fraction, respectively. Chicks in which the plasma cholesterol had been elevated by feeding cholesterol showed little change in the absolute amount of cholesterol bound to the α-lipoproteins; however, this accounted for only approximately 23% of the total plasma cholesterol. These data demonstrate that the cholesterol increase took place in the β-lipoprotein fraction. This is in agreement with observations in the human(14,15), but not with values reported for cholesterol-fed cockerels(13). Since the values reported by Orma(13) were determined on eluates of samples separated by paper electrophoresis, differences in methods may account for the variations noted.

In Table II are presented data on lipid distribution in plasma and α-lipoproteins for various species. Definite species differences in percentage distribution of the various lipid classes exist. The rabbit and guinea pig are similar to man in that less than 50% of the total cholesterol is bound to the α-lipoproteins. In the rat, dog, mouse and chick, however, more than 50% of the cholesterol is bound to the α-lipoproteins. A greater species variability is evident for lipid phosphorus and glyceride levels. In most species there is less lipid phosphorus bound to the α-lipoproteins than is the case in the human. The amount of glycerides bound to the α-lipoproteins is similar for man, guinea pig, dog and rat, with approximately 20% of the total glycerides found in the α-lipoprotein fraction. The rabbit has only 10% of the total plasma glycerides bound to the α-lipoproteins while for the mouse, 39%, and the chick

TABLE II. Lipid Distribution in Lipoproteins of Various Species Separated by Polyanion Precipitation.

Species	No. of animals	Cholesterol mg/100 ml		Lipid phosphorus mg/100 ml		Glycerides mM/L	
		Total	α	Total	α	Total	α
Man (male)	2	198	58	9.02	4.52	1.48	.28
Rabbit	4	40	9	3.33	.42	1.18	.12
Guinea pig	4	30	6	.68	.14	.43	.10
Rat	4	82	54	1.67	.84	.81	.17
Dog	5	186	156	11.49	4.33	.71	.15
Mouse	8	87	83	4.24	1.80	.49	.19
Chick	8	102	73	6.41	5.18	.41	.14

34% of the glycerides are found in the α-lipoprotein fraction.

The values reported demonstrate definite species differences in the percentage distribution of lipids bound to α- and β-lipoproteins. The method employed, because of its simplicity and accuracy, enables further studies of the role of the lipoproteins in lipid metabolism in both human and animal studies. The probable significance of the blood lipids and, consequently, lipoproteins in atherogenesis make such studies of considerable importance.

Summary. Paper electrophoresis and ultracentrifugation have been employed to demonstrate the completeness of β-lipoprotein isolation from chick plasma by a polyanion precipitation technique employing Mepesulfate as the precipitating agent. Data are presented demonstrating that in the chick made hypercholesterolemic by cholesterol feeding, the increase in plasma cholesterol occurs almost entirely in the β-lipoprotein fraction. Lipid distribution in the α-lipoprotein fraction of plasma has been determined for chick, rabbit, guinea pig, rat, mouse and dog as well as for the human. The chick, rat, mouse and dog were found to have more than 50% of the total plasma cholesterol bound to the α-lipoproteins in contrast to man, the rabbit and guinea pig. Values are also presented for distribution of lipid phosphorus and glycerides.

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Unilateral Renal Ischemia and Erythropoietin.* (26992)

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The presence of a circulating erythropoietic stimulating factor (erythropoietin) in the plasma of anemic animals has been demonstrated by a large number of investigators (1). Hypoxia is considered to be the fundamental stimulus for erythropoietin production and therefore erythropoiesis(2). The concept has been developed by Jacobson and his associates that rate of erythropoiesis is determined by the relationship between oxygen tension in the blood and oxygen demand by the tissues (including the site of formation of erythropoietin)(3). The failure of nephrectomized rats, but not ureter-ligated rats, to respond to a single massive hemorrhage or injection of cobalt with an elevation of plasma erythropoietin has led this group to

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