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SERUM LIPOPROTEINS OF KILLER WHALES

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Abstract—1. Concentration distributions of serum lipoproteins of captive killer whales, *Orcinus orca* (four males and three females), have been characterized by analytic ultracentrifugation. High density lipoproteins (HDL) comprise the major portion (50–70 per cent) of their serum lipoproteins. The concentration of serum low density lipoproteins (LDL) was equal to or greater than normal human values.

2. Lipid compositions of the ultracentrifugal classes (total very low density lipoprotein, VLDL, LDL and HDL) were determined. The lipid composition of LDL was very similar to that reported for humans.

3. Electrophoretic lipoprotein patterns were also determined. Two distinct alpha bands were detected. The presence of alpha lipoproteins with $d < 1{\cdot}063$ g/ml is discussed in terms of VLDL metabolism.

4. In the electron microscope the killer whale HDL seen "face on" are 70–130 Å in diameter with a subunit configuration similar to human HDL. Individual subunits are approximately 40Å across and 75Å long. Killer whale LDL are similar to those from human serum and are approximately spherical particles 200–220Å in diameter.

INTRODUCTION

IN THIS study, we characterized the distribution of serum lipoproteins of captive killer whales, *Orcinus orca* (four males and three females). Lipid composition and concentration data for three major classes* of serum lipoproteins are presented together with their ultracentrifugal distributions. Paper electrophoretic studies were also performed on whole serum. Electron micrographs of LDL and HDL fractions are also presented.

*The lipoprotein classes and their abbreviations as used in this text are defined as follows: (a) Total very low density lipoproteins, VLDL, a combination of the chylomicron class ($d < 0{\cdot}94$ g/ml; $S_f^0 > 400$) and very low density lipoprotein class (d: 0·94—1·006 g/ml; S_f^0:20–400); (b) low density lipoproteins, LDL (d:1·006—1–063 g/ml; S_f^0: 0–20); and (c) high density lipoproteins, HDL (d: 1·063—1·21 g/ml; $F_{1\cdot 30}$: 0–20). S_f rate is defined as Svedbergs of flotation, measured at 26°C in a medium of 1·745 molal NaCl (d: 1·0630 g/ml). Flotation rates corrected for the effects associated with concentration dependence are indicated by the symbol S_f^0. F rate denotes a flotation rate measured at any other density, signified by a subscript, e.g. $F_{1\cdot 30}$.

DISCLAIMER

MATERIALS AND METHODS

The animals were fed fish each day in amounts approximately equivalent to 5 per cent of their body weight. The weights of these animals are given in Table 1. All of the animals with the exception of Male IV are performers in public oceanariums. In general, when the

TABLE 1—WEIGHTS OF THE KILLER WHALES

Males	Weight (lb)	Females	Weight (lb)
I	4200	I	3100
II	5800	II	3700
III	1500	III	10000
IV	5500		

blood was drawn, the animals had not been fed for 15–20 hr. Female II and Male III had been fed 4 hr prior to bleeding. Female III was under treatment for respiratory infection when the blood was drawn. Blood samples were obtained from either the fluke or flipper veins.

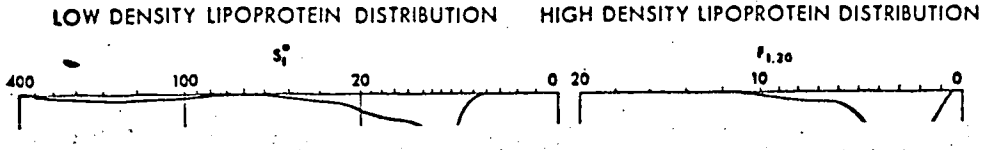
Because killer whales are lacking clotting factor XII, the Hagemann factor (Robinson *et al.*, 1969) the blood was centrifuged before a clot had formed. The supernatant was refrigerated under nitrogen for 24 hr, and the fibrin, which had formed during this time, was removed with a glass stirring rod. The procedures used for analytical ultracentrifugation of serum lipoproteins and the computer programs for graphic representation of fully corrected schlieren patterns were those described by Lindgren *et al.* (1967), and Jensen *et al.* (1970). Computer analysis of the schlieren patterns was also used to obtain flotation rates (Lindgren *et al.*, 1967). Procedures for the paper electrophoresis (Hatch & Lees, 1968) and the electron microscopic studies (Forte *et al.*, 1968) have been described elsewhere. Electron microscopic studies were done on the *d*: 1·02–1·03 g/ml and the *d*: 1·063–1·21 g/ml fractions isolated by preparative ultracentrifugation. The procedures of Freeman *et al.* (1963), were employed for the isolation of the three major lipoprotein classes, for the extraction and chromatography of the lipids, and for the quantification of the lipids by infrared spectrophotometry.

RESULTS

Ultracentrifugal analysis of serum lipoprotein distributions

The ultracentrifugal distributions obtained from flotation analyses of the serum lipoproteins of the killer whales are shown in Figs. 1 and 2. In both sexes, a major portion (50–70 per cent) of the serum lipoproteins are present in the HDL class. $F_{1.20}$ values for the major HDL component of the killer whales are within the range of values reported for human females (Lindgren *et al.*, 1967). Minor HDL components with $F_{1.20}$ values in the range between 8·0 and 11·3 are also present. HDL with $F_{1.20}$ values greater than 9·0 are normally not observed in humans (Fredrickson *et al.*, 1968).

In the low density distribution, a single major component is present in five of the animals; however, Female I has two components and Female III has three. The major components of all but one of the killer whales tend to have S_f^0 values higher than those of normal humans. These flotation rate data are summarized in Table 2. The serum concentrations of killer whale LDL are approximately equal



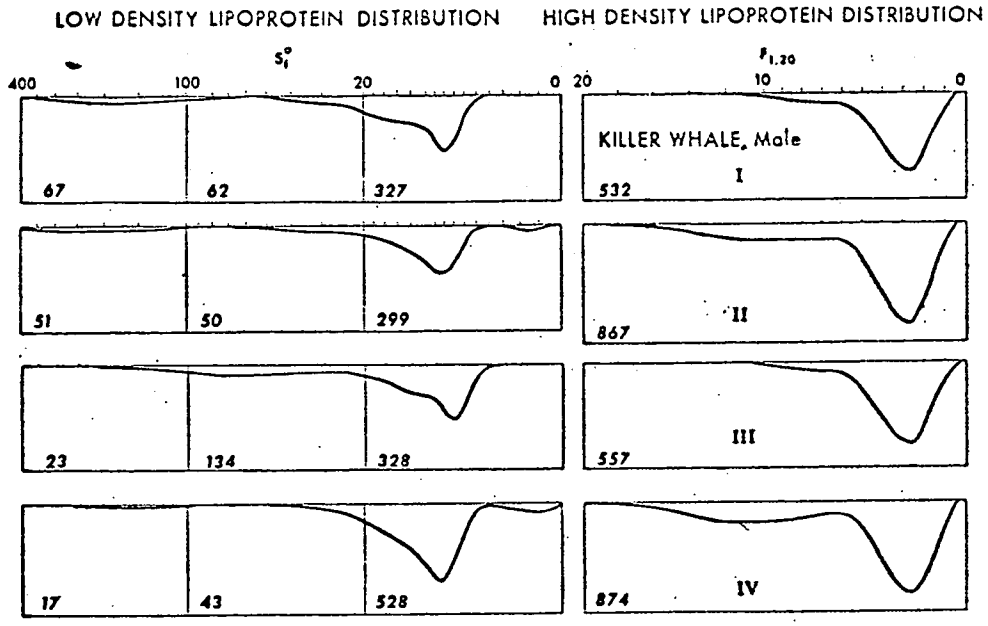


FIG. 1. The ultracentrifugal distribution of serum lipoproteins in male killer whales.

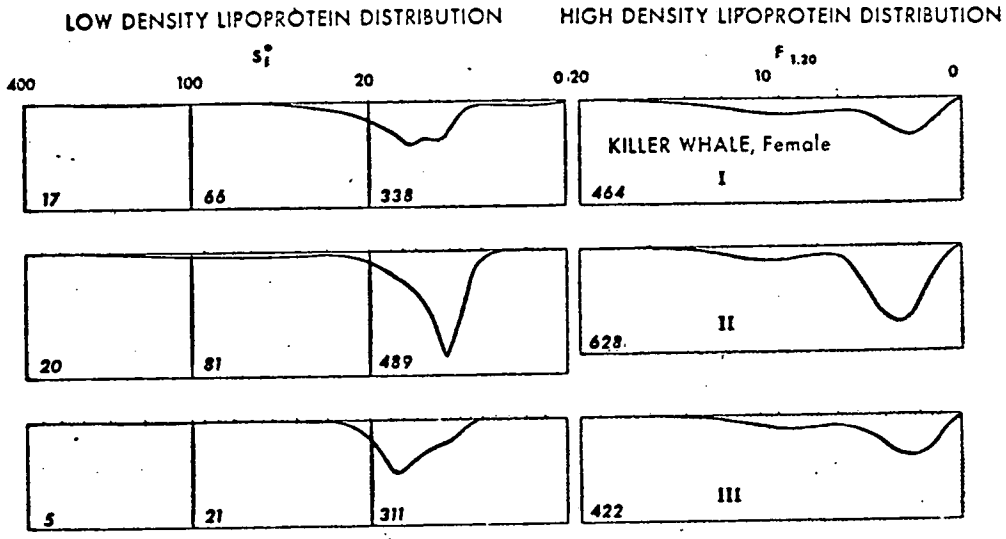


FIG. 2. The ultracentrifugal distribution of serum lipoproteins in female killer whales.

TABLE 2—FLOTATION RATE DATA

Killer whales	S_f^0 values of LDL components			$F_{1.20}$ values of HDL components	
Males					
I	7·4			2·9	8·2
II	7·6			2·8	11·3
III	6·9			2·9	8·0
IV	7·8			2·6	10·8
Females					
I	8·2	11·7		2·5	8·7
II	6·9			3·1	10·0
III*	*ca.* 7·0	*ca.* 10·0	13·7	2·6	9·3
Human mean values (Lindgren *et al.*, 1967)					
Male (16 cases)	6·3			2·0	
Females (16 cases)	7·3			2·9	

* In a standard ultracentrifugal analysis (d: 1·20 g/ml), three LDL components were detected in this animal. Flotation rate of the major component is given above. Approximate values for the other two components are also given in S_f^0 units.

to or greater than normal human serum values (409 mg/100 ml in males and 323 mg/100 ml in females, Fredrickson *et al.*, 1968). It is interesting to note the presence of detectable amounts of serum lipoproteins in the S_f^0 interval 0–3 (22 mg/100 ml in Female I, 18 mg/100 ml in Male II and 41 mg/100 ml in Male IV). Lipoproteins within this S_f^0 interval were noted only in those animals in which lipoproteins were also detected within the $F_{1.20}$ interval 16–20. The possible significance of these lipoproteins will be treated in the discussion.

Lipid concentrations and compositions of the three density classes and of whole serum

The data in Table 2 and 3 indicate that in each of the killer whales cholesteryl esters are the principal lipids of LDL (37–47 per cent), HDL (54–64 per cent) and whole serum (32–43 per cent). Interestingly the lipid composition of killer whale LDL is similar to that reported for humans (Lindgren *et al.*, 1967; Hatch & Lees, 1968). The amount of extracted lipid in the total VLDL class was not sufficient for accurate cholesterol measurements. However, the data suggest that more unesterified than esterified cholesterol is present in the total VLDL class of killer whales.

Electrophoretic analysis of the serum lipoproteins

The electrophoretic patterns of the whale serum of killer whales (Figs. 3 and 4) clearly show the presence of both alpha and beta bands. The mobility of the alpha bands are faster in Males I and II (Fig. 3) and Females I and III (Fig. 4) than in the other animals. Two distinct alpha bands can be seen in the electrophoretic pattern of Female II. In the other animals, although two alpha bands also appear

TABLE 3—CONCENTRATIONS AND COMPOSITIONS OF LIPIDS AND LIPOPROTEINS IN THE SERA OF CAPTIVE MALE KILLER WHALES

Animal	Concentration (mg lipid/100 ml serum)	Lipid composition (%) CE	PL	TG	UC	UFA
	Total VLDL (d: 1·006 g/cm³)					
I	142	10·4	18·1	63·8	8·0	—
II	128	14·3	21·2	55·9	8·7	—
III	155	6·5	20·2	65·8	7·2	—
IV	92*	10	24	58	8	—
	LDL (d: 1·006–1·063 g/cm³)					
I	252	37·2	24·6	24·0	13·8	0·4
II	320	45·8	27·1	11·4	14·3	0·5
III	267	40·1	26·3	19·6	13·8	0·3
IV	455	39·8	30·9	17·0	11·9	0·4
	HDL (d: 1·063–1·21 g/cm³)					
I	286	57·7	33·3	2·1	5·5	1·4
II	451	57·5	33·1	1·3	6·4	1·7
III	287	53·8	37·8	2·6	5·2	0·7
IV	371	58·6	33·1	1·7	5·7	0·9
	Whole serum					
I	853	39·9	26·5	20·9	9·9	2·8
II	1050	43·2	29·1	14·5	10·5	2·8
III	838	35·4	31·7	22·8	9·3	0·9
IV	1110	41·0	36·4	12·9	8·1	1·6

Abbreviations used in Tables 3 and 4: CE, cholesteryl esters; PL, phospholipid; TG, tryglycerides; UC, unesterified cholesterol; UFA, unesterified fatty acids.
* Amounts analyzed were to the limits of detection of the i.r. measurements. In these cases, the amounts of extracted lipids were low, and the data were measured to two significant figures.

to be present, the band with the faster electrophoretic mobility stains more intensely. Pre-beta bands are also present in each of the animals except Female III.

Electron microscopy of HDL and LDL

The serum high density lipoproteins of the killer whale visualized after negative staining are seen in Fig. 5. This micrograph indicates that the HDL particles are non-homogeneous in size; their diameters range from 70 to 130Å and are comparable to those observed for human HDL (Forte *et al.*, 1968, 1969). The lipoprotein structures in many instances appear to be composed of several smaller subunits: such details are more readily seen in Fig. 6 which is an enlarged micrograph of closely packed HDL particles. When seen "face on", many of the particles have irregular or scalloped edges (single arrows) which are highly suggestive of a subunit configuration similar to that described for human HDL (Forte *et al.*, 1968). Moreover, one of the particles appears to be tilted (double arrows), and when seen

in this plane, it appears that the subunits may be elongated. Although the diameter and the length of the subunits can only be approximated, they are estimated at 40 Å and 75 Å respectively.

A representative micrograph of a subfraction of the serum low density lipoproteins (d: 1·02–1·03 g/ml) is seen in Fig. 7. Many of the freestanding particles are approximately spherical and measure 210 Å in diameter. Upon contact with one another, the particles assume more angular shapes as a result of deformation. These low density lipoproteins do not appear to have an obvious substructure as observed for the HDL fraction.

TABLE 4—CONCENTRATIONS AND COMPOSITIONS OF LIPIDS AND LIPOPROTEINS IN THE SERA OF CAPTIVE FEMALE KILLER WHALES

Animal	Concentration (g lipid/100 ml serum)	Lipid composition (%) CE	PL	TG	UC	UFA
	Total VLDL (d < 1·006 g/cm³)					
I	79*	23	21	45	10	—
II	110	5·7	18·9	62·9	12·7	—
III	52*	10	20	56	14	1
	LDL (d: 1·006–1·063 g/cm³)					
I	264	40·2	24·4	18·0	16·9	0·5
II	431	38·5	25·5	20·3	15·5	0·2
III	268	36·7	24·2	22·8	16·0	0·3
	HDL (d: 1·063–1·21 g/cm³)					
I	222	61·0	29·2	1·7	6·9	1·3
II	302	55·0	36·3	2·7	5·6	0·5
III	204	63·9	27·2	1·7	5·7	1·6
	Whole serum					
I	589	32·2	32·1	17·3	14·8	3·6
II	1010	36·8	31·1	19·9	11·6	0·7
III	592	42·3	27·2	16·5	9·0	5·0

* Amounts analyzed were to the limits of detection of the i.r. measurements. In these cases, the amounts of extracted lipids were low, and the data were measured to two significant figures.

DISCUSSION

Our present study indicates that in the sera of killer whales, as in most other mammals (Lewis *et al.*, 1952; Hillyard *et al.*, 1955; Evans *et al.*, 1961; Evans, 1964; Fried *et al.*, 1968; Puppione & Nichols, 1970a), the predominant lipoprotein class is HDL. The high content of cholesteryl esters, noted in killer whale HDL, has also been reported for the HDL of bisons (Evans, 1964), dairy cows (Evans *et al.*, 1961), and bottlenose dolphins (Puppione & Nichols, 1970a).

A major portion (40–60 per cent) of killer whale HDL have $F_{1\cdot20}$ values greater than 3·5. In humans, the percentage of HDL exhibiting flotation rates within the

$F_{1\cdot20}$ 3·5–9 interval is less (approximately 40 per cent in females and 20 per cent in males) (Lindgren *et al.*, 1967). Serum lipoproteins with $F_{1\cdot20}$ values between 9 and 20 have been reported in dogs and rats (Lewis *et al.*, 1952). We also have observed these lipoproteins in the high density distribution of different species of pinnipeds and cetaceans, in a dairy cow, and in a human female subject (Puppione & Nichols, 1970a; Puppione *et al.*, 1970b).

In the three killer whales which had lipoproteins within the $F_{1\cdot20}$ 16–20 interval, serum lipoproteins were also present in the S_f^0 0–3 interval. The S_f^0 0–3 lipoproteins exhibit hydrated densities in the range 1·04–1·06 g/ml. In certain mammalian systems, lipoproteins within this density fraction have alpha mobility (Windmueller & Levy, 1967; Puppione *et al.*, 1970b).

Our paper electrophoretic studies indicate the presence of beta as well as small amounts of alpha lipoproteins in the $d < 1{\cdot}063$ g/ml fraction of Male II. However, alpha lipoproteins were not detected in the $d < 1{\cdot}063$ g/ml fraction of Female I. Recent peptide analyses (Shore & Shore, 1969; La Rosa *et al.*, 1969) as well as statistical studies (Nichols, 1967) suggest that the S_f^0 0–3 lipoproteins are perhaps involved in VLDL metabolism. Although we have never observed these lipoproteins in fasting bottlenose dolphins, they were detected, in sera obtained postprandially from two bottlenose dolphins (a young male, which was nursing, and an adult female) (Puppione, 1970c).

Our data on the LDL of killer whales (concentration and chemical composition of the lipid moiety, flotation rate, and size) interestingly are very similar to those for humans. The electron microscopic structure of the subfraction of killer whale LDL like that of humans (Forte *et al.*, 1968; Gotto *et al.*, 1968) show more or less spherical shape with diameter ranging between 200–220 Å. In our studies no apparent subunit structure was observed in the LDL of either killer whales or humans. A recent report by Pollard *et al.* (1969) indicates that human LDL may contain subunits consisting of twenty globular protein subunits arranged in a dodecahedral structure.

Each of the male killer whales and one of the females had a major LDL component with flotation rates within the range of values observed in humans. The higher flotation rates, exhibited by the LDL components in two of the females, have also been observed in the low density distribution of humans with biliary cirrhosis (McGinley *et al.*, 1952; Mills *et al.*, 1969). We have reported on the presence of LDL components with flotation rates within the range of human LDL in other cetaceans (Puppione, 1969; Puppione & Nichols, 1970a). However, the serum concentrations of LDL in these cetaceans were approximately one half those observed in killer whales. Lower serum concentrations (48 mg/100 ml) of LDL have been noted in a female killer whale (Hashimoto *et al.*, 1967); however, the blood for the analysis was drawn from the animal following its death. At the time of capture the animal was sick, and it died after thirty-six hours in captivity. Interestingly, the autopsy indicated that this animal (approximate age, thirty years) had severe atherosclerosis (Roberts & Straus, 1965).

Our paper electrophoretic analyses indicated the presence of two alpha bands

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in the serum of killer whales. The reason for the intense staining seen in the alpha band with the faster mobility is not apparent at this time. Both of the alpha bands had higher mobility in those animals which also had a high concentration of unesterified fatty acid. The binding of UFA to serum lipoproteins has been shown to increase their mobility (Gordon, 1955).

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Key Word Index—Serum lipoproteins; *Orcinus orca*; killer whales; lipoprotein; analytical ultracentrifuge.

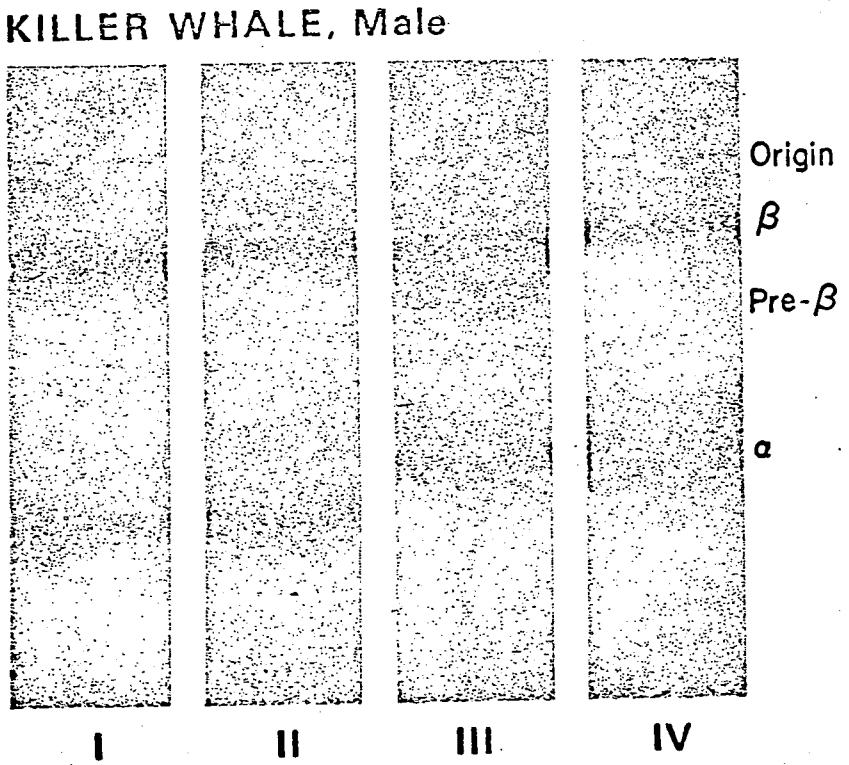

FIG. 3. The electrophoretic distribution of serum lipoproteins of male killer whales.

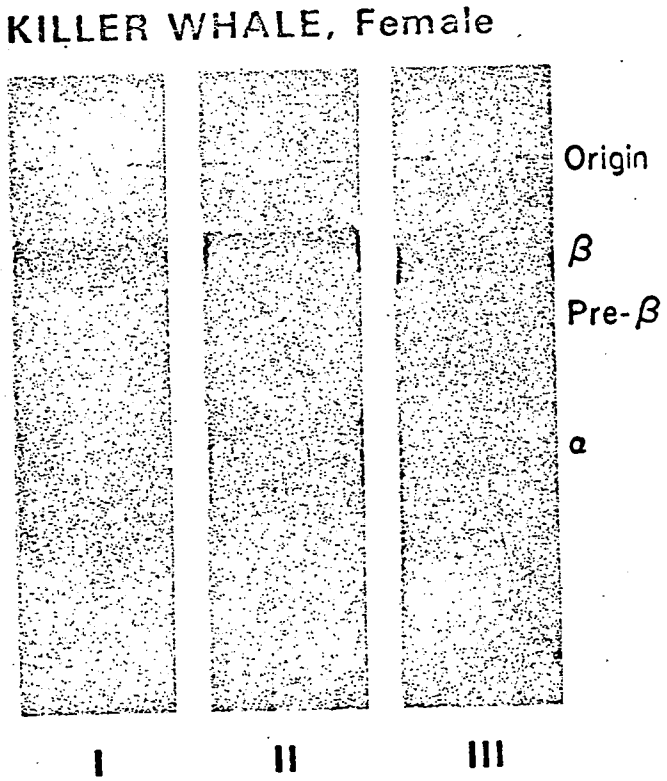

FIG. 4. The electrophoretic distribution of serum lipoproteins of female killer whales.

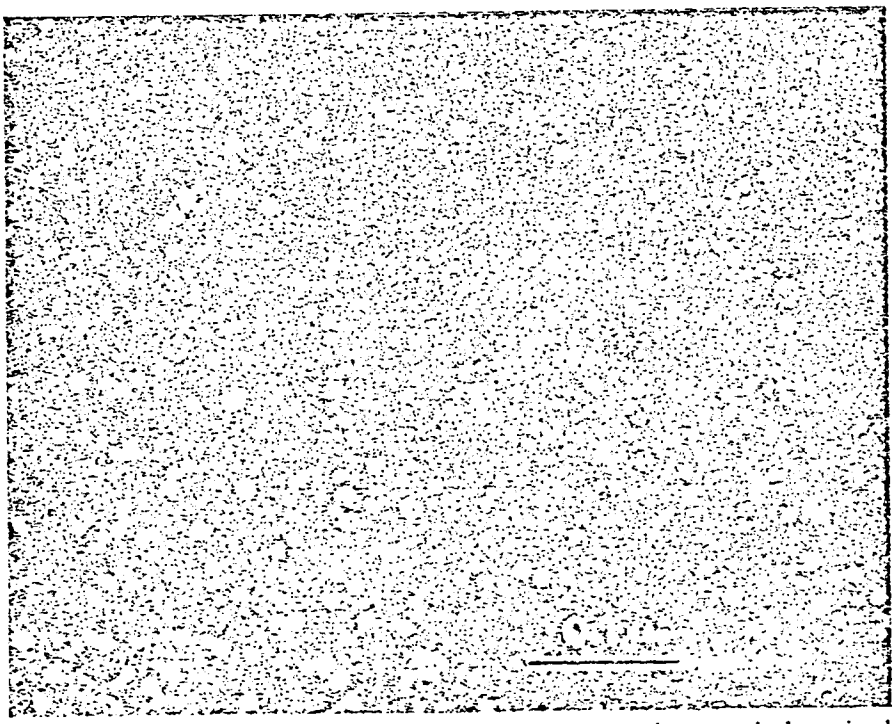

FIG. 5. An electron micrograph of high density lipoproteins negatively stained with 1% sodium phosphotungstate, pH 7·4.

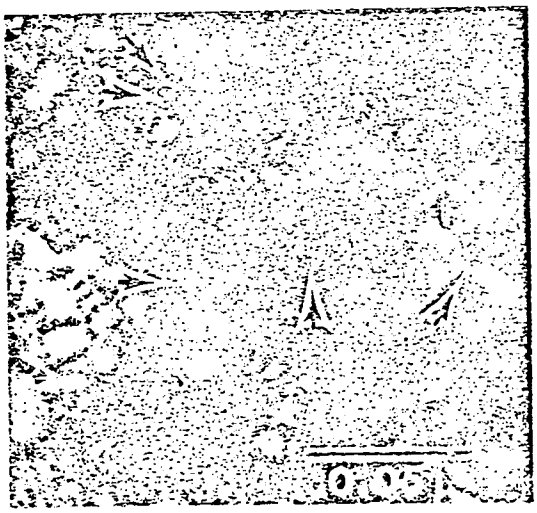

FIG. 6. High magnification micrograph showing detail of negatively stained high density lipoproteins. Single arrows indicate HDL seen "face on" while the double arrow indicates a tilted particle revealing the cylindrical nature of the HDL subunits.

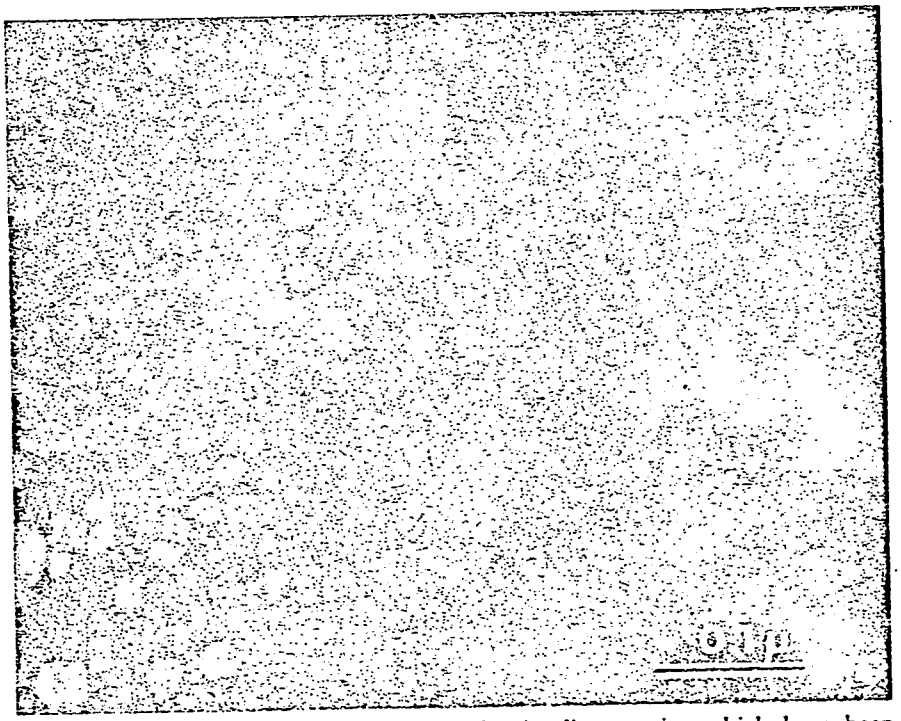

FIG. 7. An electron micrograph of low density lipoproteins which have been negatively stained. Single standing particles appear to be spherical while contiguous ones become flattened at the point of contact.