Journal of Clinical Investigation
Vol. 43, No. 11, 1964

Lipoproteins of Synovial Fluid as Studied by Analytical Ultracentrifugation *

DONALD M. SMALL, ALAN S. COHEN, AND KARL SCHMID

(From the Robert Dawson Evans Memorial Department of Clinical Research, Massachusetts Memorial Hospitals; the Fifth and Sixth Medical Services, Boston City Hospital; and the Departments of Medicine and Biochemistry, Boston University School of Medicine, Boston, Mass.)

Studies on the synovial fluid lipoproteins were initiated several years ago. In 1953 Ropes and Bauer (1) reported on the cholesterol content of joint fluid from patients with rheumatoid arthritis. Subsequently Schmid and MacNair (2, 3) noted that when referred to the same amount of protein rather than volume of fluid, the lipoprotein content of pathological and pooled postmortem synovial fluids was very similar and amounted to approximately two-thirds of the corresponding value of normal human serum. The ratio of β- to α-lipoproteins of certain fluids from patients with rheumatoid arthritis, however, was essentially the same as that of the patient's serum. Recently Chung, Shanahan, and Brown (4) and Bole (5) studied the lipid content of the different classes of synovial fluids, as did Schur and Sandson (6), who stated that β-lipoprotein was markedly decreased as compared to normal serum.

Since most of the fractionation procedures used separated the serum and synovial fluid lipoproteins into only two groups, the reported results led to a very rough description of these substances. The use of ultracentrifugal techniques would permit characterization of essentially all classes of lipoproteins of serum and synovial fluid and the detection of small differences that might be found in various lipoprotein classes. The present study therefore was undertaken *a*) to assess analytical ultracentrifugation methodology in the analysis of synovial fluid lipoproteins, *b*) to compare synovial fluid and serum lipoprotein levels in a given patient, and *c*) to determine whether different lipoprotein patterns might be found in various classes of synovial fluids.

Methods

I) Collection and analysis of synovial fluid and blood specimens

Blood was obtained by venipuncture under sterile conditions with a no. 20 needle simultaneously with joint fluid from fasting patients at Boston City Hospital or Massachusetts Memorial Hospitals. The blood obtained was transferred to a sterile tube, allowed to clot, and stored for 2 hours at 0° C. It was then centrifuged at 3,000 rpm at 4° C for 30 minutes. The resultant serum was pipetted into a sterile tube and stored at 2 to 4° C. If preparative ultracentrifugation was not initiated on the same day, 0.1 mg Merthiolate per ml of serum was added.

The synovial fluid was obtained from patients by arthrocentesis under sterile conditions and was centrifuged at 3,000 rpm at 4° C for 30 minutes to remove the cells and clot. The synovial fluid was pipetted into a sterile tube and stored at 2 to 4° C. A sample of fluid, usually 5 ml, was incubated with 0.1 mg of hyaluronidase [1] per ml fluid for 3 hours at 37° C. One milligram of this enzyme preparation contained 1,550 turbidity reducing U. The hyaluronidase caused rapid reduction of the viscosity to that of the serum and facilitated pipetting (7). If preparative ultracentrifugation was not to be initiated on the same day or if synovial fluid was drawn from a patient with infectious arthritis, 0.05 mg Merthiolate per ml of synovial fluid was added. A portion of each synovial fluid was set aside for total and differential cell count, mucin test, culture, paper electrophoresis, determination of total protein, albumin, and globulin, and in some cases, lipid analysis.

The paper electrophoresis was carried out by the Spinco procedure B, using pH 8.6, ionic strength 0.075, barbiturate buffer, and Schleicher and Schuell 2043A paper. The strips were stained with bromphenol blue and analyzed in a Beckman RB analytical densitometer

* Submitted for publication February 3, 1964; accepted July 9, 1964.

Grants in support of these investigations have been received from the National Institutes of Health (grants S AM 04599, TI AM 5285, and GM 10374).

[1] The gift of hyaluronidase from the Wyeth Institute for Medical Research, Philadelphia, Pa., is gratefully acknowledged.

TABLE I

Clinical data on patients studied

Patient	Age	Sex	Diagnosis	Joint	Differential PMN*				
					Mucin	Leukocytes	Round cells	Westergren ESR†	Latex fixation test
A.O.	47	F	Definite rheumatoid arthritis, very active	Right knee	Fair	15,400	80/20	35	Positive
H.C.W.	66	M	Definite rheumatoid arthritis, very active	Left knee	Poor	25,000	74/26	55	Positive
R.C.	34	M	Definite rheumatoid arthritis, with chronic effusions, inactive‡	Right knee Left knee	Good Good	4,000 3,400	56/74 47/53	40	Negative Negative
F.Par.	86	F	Probable rheumatoid arthritis, active	Left shoulder	Fair—poor	15,000	82/18		Negative
R.R.	58	F	Degenerative joint disease	Left knee	Good	900	13/87	19	Negative
L.B.	32	F	Noninflammatory effusion§	Left knee	Good	500	20/80	19	Negative
L.S.‖	50	M	Acute gout, chronic effusion of left knee	Left knee	Good	2,500	57/43		Negative
A.L.‖	53	M	Gout with chronic effusion of right knee	Right knee	Good	750	34/66	27	Negative
F.Pin‖¶	67	M	Chronic tophaceous gout with effusions	Right knee	Good	200	90/10	50	Negative
J.O.	38	M	Monoarticular arthritis of unknown etiology	Left knee	Fair—good	9,450	93/7	48	Negative
J.B.	18	M	Polyarticular arthritis of unknown etiology, probable sarcoidosis	Left knee	Fair	3,750	71/29	50	Negative
W.N.	43	M	Infectious arthritis, possible Reiter's syndrome	Left knee	Poor	53,000	85/15		Negative
G.R.	50	M	Infectious arthritis	Left knee	Fair	49,300	88/12	13	Negative

* PMN = polymorphonuclear leukocytes.
† ESR = erythrocyte sedimentation rate.
‡ Associated with ulcerative colitis.
§ Rheumatic heart disease, congestive heart failure with peripheral edema, and hypoalbuminemia.
‖ Hyperuricemia.
¶ Uric acid crystals in synovial fluid.

equipped with a B-5 cam.[2] The total cholesterol was measured by the method of Schoenheimer and Sperry (8).[2] The clinical characteristics of the patients studied and the results of the analyses are presented in Tables I and II.

II) Ultracentrifugation of lipoproteins

The method of Del Gatto, Lindgren, and Nichols for serum lipoprotein analysis (9) utilizing a medium of NaBr of 1.20 density was adapted (see below) to the study of lipoproteins of synovial fluid. An analytical ultracentrifuge, Spinco model E, equipped with an R.T.I.C. type temperature controller and Schlieren optics, was used for these studies.

A) Preparative ultracentrifugation. 1) Serum. Exactly 2 ml of serum (density of background solution, 1.006, 20° C) was pipetted into a 12-ml lusteroid preparative-ultracentrifuge tube to which was added 8.00 ml of aqueous solution of NaBr, density 1.248[3] (20° C), to make a final volume of 10.00 ml with a density of 1.200. The rotor cap was secured and the tube inverted several times to insure proper mixing. The tube was then placed in a no. 40 preparative ultracentrifuge rotor and spun at 40,000 rpm for 24 hours at 20° C in a model L Spinco ultracentrifuge. Before spinning, a small amount of water was added to the holes of the rotor. (In the course of the experiments 150 preparative runs were performed without breakage or leakage from any of the plastic tubes.) Then the tubes were removed from the rotor, care being taken not to shake or jar the contents. By careful use of an eyedropper bulb connected to a micropipette, the top ½ ml containing all lipoproteins (less dense than 1.20) could be removed completely and placed in a 1.00-ml volumetric flask. The rotor cap, top of the tube, and pipette were washed with clear solution remaining at the uppermost level of the infranatant fluid, and these washings were added to the lipoprotein concentrate to give a final volume of 1.00 ml. This lipoprotein concentrate was stored at 4° C. The second milliliter of the subnatant fluid was found to be essentially as free of protein as is obtained with the 40.3 rotor.

2) Synovial fluid. Exactly 4 ml of synovial fluid (density[4] 1.008, 20° C) was pipetted into a 12-ml lusteroid tube. To obtain a final density of 1.200, 6.00 ml of an aqueous solution of NaBr, density 1.328[5] (20° C),

[2] Courtesy of Mr. Joseph Annino, Chief of Chemistry, Massachusetts Memorial Hospitals.

[3] The density of the 8.00 ml of aqueous NaBr (1.248) added to 2 ml of serum and the density of the 6.00 ml aqueous NaBr (1.328) added to 4 ml of synovial fluid were calculated from the equation (10), $(A)(Y) + (B)(Z) = (A+B)X$, where A = volume of protein-free background solution of serum or synovial fluid, B = volume of aqueous solution NaBr, Y = assumed density of protein-free background solution, Z = density of NaBr solution, and X = density of the protein-free background solution of the mixture.

[4] The background solution was assumed to have a density of 1.008, a value slightly higher than that of serum because of the presence of soluble hyaluronic acid breakdown products.

[5] Refer to footnote 3.

was added. Subsequent operations were the same for synovial fluid and serum.

B) Analytical ultracentrifugation. A sample of the lipoprotein concentrate described above was placed in an analytical cell with a plus-one degree prismatic window and spun in the An-D analytical rotor of the ultracentrifuge at 42,040 rpm for 64 minutes at 26° C. The time taken to reach full speed was 420 seconds. One-third of this time (140 seconds) was taken as time of equivalent force at full speed and used for the calculation of the flotation constants [$S_{f(1.20)}$]. Pictures were taken at 0, 2, 12, 14, 24, 44, and 64 minutes after full speed had been obtained. The same analytical wedge cell was used throughout the entire study. Base-line solutions were also run in the same cell before analysis of lipoprotein. Base-line solutions for serum and synovial fluid were made up by mixing the same mock solutions for serum and synovial fluid (aqueous NaCl with a density of 1.006) with the corresponding NaBr solution in volume ratios as indicated above to give a final density of 1.20 each. No corrections for the sedimentation of salt during preparative ultracentrifugation were made. As Del Gatto and co-workers (9) showed, this introduces a very minute error which is comparable to that of the procedure. In addition, it should be emphasized that the serum of each patient was run in parallel as a control for the synovial fluid.

To delineate the boundaries of the five major classes of lipoproteins, the $S_{f(1.20)}$ boundaries limiting each class were calculated from the integrated Svedberg equation for ultracentrifugation (11). The calculated distances of the $S_{f(1.20)}$ boundaries to the base of the cell were multiplied by the combined linear magnification of the Schlieren optical system and the enlarger (4.7) and the values plotted on an enlarged template.

The four major classes of serum lipoproteins in NaCl

TABLE II

Paper electrophoretic analysis of serum and synovial fluid (SF) proteins

Patient	Fluid	Total protein	Albumin	Globulin				
				Total	α_1	α_2	β	γ
		g/100 ml	*g/100 ml*			*g/100 ml*		
A.O.	Serum	6.6	3.3	3.3	0.33	0.65	0.98	1.34
	SF	4.4	2.3	2.1	0.20	0.40	0.47	1.02
H.C.W.	Serum	7.3	3.2	4.1	0.43	0.97	1.17	1.53
	SF	7.2	3.3	3.9	0.42	0.78	0.99	1.76
R.C.	Serum	8.6	3.5	5.1	0.42	1.03	1.07	2.57
	SF (L)	5.2	2.2	3.0	0.17	0.27	0.53	2.00
	SF (R)	5.3	2.3	3.0	0.19	0.33	0.49	1.94
F.Par.	Serum	6.3	2.8	3.5	0.44	1.02	0.95	1.10
	SF	3.9	2.0	1.9	0.28	0.44	0.53	0.68
R.R.	Serum	7.2	3.8	3.2	0.31	0.86	1.03	1.20
	SF	2.8	1.6	1.2	0.22	0.32	0.25	0.45
L.B.	Serum	7.0	1.9	5.1	0.50	0.86	0.94	2.74
	SF	1.8	0.5	1.3	0.20	0.25	0.27	0.60
L.S.	SF	4.8	2.2	2.6	0.16	0.28	0.47	1.68
A.L.	Serum	7.6	3.3	4.3	0.46	0.68	0.95	2.20
	SF	3.5	1.8	1.7	0.21	0.31	0.41	0.81
F.Pin.	Serum	7.2	4.0	3.2	0.34	0.64	0.82	1.42
	SF	3.5	2.0	1.5	0.16	0.21	0.40	0.73
J.O.	Serum	6.3	2.6	3.7	0.49	0.78	0.99	1.38
	SF	4.6	2.6	2.0	0.34	0.50	0.50	0.64
J.B.	Serum	9.2	4.1	5.1	0.45	0.76	1.38	2.51
	SF	4.0	2.1	1.9	0.27	0.29	0.36	0.96
W.N.	Serum	7.2	2.2	5.0	0.49	0.90	1.12	2.54
	SF	5.3	2.1	3.2	0.31	0.52	0.69	1.69
G.R.	Serum	7.1	2.3	4.8	0.65	1.34	1.07	1.73
	SF	2.3	0.9	1.4	0.19	0.28	0.32	0.60
	Mean	7.3	3.1 (42%)	4.2 (58%)	0.44 (6%)	0.87 (12%)	1.04 (14%)	1.86 (26%)
Serum	Range	6.3–9.2	1.9–4.1	3.2–5.1	0.31–0.65	0.64–1.34	0.83–1.38	1.10–2.74
	SD	0.82	0.89	0.88	0.89	0.21	0.14	0.18
Synovial	Mean	4.2	2.0 (48%)	2.2 (52%)	0.23 (5%)	0.37 (9%)	0.51 (12%)	1.04 (25%)
fluid	Range	1.8–7.2	0.5–3.3	1.2–3.9	0.16–0.42	0.21–0.78	0.25–0.99	0.45–2.00
	SD	1.48	0.67	0.81	0.08	0.14	0.23	0.17

TABLE III

*Effect of hyaluronidase incubation on lipoproteins of synovial fluid**

Fluid no.	Hyaluronidase treated	Lipoprotein classes† I (802–185)	II (61–185)	III (44–61)	IV (16–44)	V_a (6–16)	V_b (0–6)	Total
					mg/100 ml			
31	No	1	41	15	137	45	64	303
	Yes	1	40	16	133	45	57	292
5	No	0	25	26	215	47	71	384
	Yes	0	33	30	218	46	68	395

* 0.1 mg hyaluronidase per ml synovial fluid, 3 hours at 37° C.
† S_f boundaries in parentheses.

with a density of 1.063 (S_f 100 to 400, S_f 20 to 100, S_f 12 to 20, S_f 0 to 12) and high density lipoproteins ($HDL_2 + HDL_3$) have been shown to correspond to $S_{f(1.20)}$ 185 to 800, $S_{f(1.20)}$ 61 to 185, $S_{f(1.20)}$ 44 to 61, $S_{f(1.20)}$ 16 to 44, and $S_{f(1.20)}$ 0 to 6, respectively, in the NaBr system with a density of 1.20 (9) and are designated as I, II, III, IV, and V_b. The class $S_{f(1.20)}$ 6 to 16, which was designated in this study as V_a, was not mentioned by Del Gatto and associates (9).

Vertical alignment of the pattern on the template was accomplished by superimposition of the Schlieren representation of the air space and horizontal alignment by superimposition of the reference edge (11). The area of each class of lipoproteins was calculated from the enlarged tracing by planimetry. After the area had been corrected for the effects of radial dilution in a sector-shaped cell, it was converted to mg per 100 ml by deLalla and Gofman's modification of Pickel's equation (11). In these calculations, for the specific refractive increment of the $S_{f(1.20)}$ 16 to 802 and 0 to 6 lipoproteins, values given in the addendum to the paper of Del Gatto and associates (9) were used, i.e., 0.00130 and 0.00142 (Δn per g per 100 ml), respectively. For the $S_{f(1.20)}$ 6 to 16 lipoproteins a value of 0.00130 (Δn per g per 100 ml) was employed. As suggested by previous workers (9) the correction for the Johnston-Ogston effect is not necessary in this procedure and therefore has not been carried out. Since the same workers (9) precisely delineated the relationship between flotation rate and concentrations of the lipoproteins, this relationship was not further studied.

Results

I) Control experiments. The effect of incubation with hyaluronidase and storage of synovial fluid on lipoproteins were studied separately. Incubation with hyaluronidase for 3 hours at 37° C produced no significant difference in quantity or distribution of the five major classes of lipoproteins when compared to the nonincubated control (Table III). There are several checks on the effects of hyaluronidase and incubation at 37° C in Table III. It had been shown before that lipoproteins of serum do not change after 28 days (11) of storage at 0 to 4° C. All samples of serum or synovial fluid were run within 28 days of collection. Thus, the data in Table IV substantiate that synovial fluid acts like serum and does not change during the first 33 days of storage. Storage of synovial fluid for 33 days at 4° C also did not change significantly the quantity or distribution of the five major classes of lipoproteins when compared to fresh, unstored synovial fluid (Table IV). Finally, the serum lipoproteins of three normal young male volunteers were analyzed, and the quantity of lipoproteins of our classes I to V was found to agree with published reports for normal young males (12). For these com-

TABLE IV

Effect of storage at 4° C on lipoproteins of synovial fluid

Fluid no.	No. of days stored	Lipoprotein classes* I (885–802)	II (61–185)	III (44–61)	IV (16–44)	V_a (6–16)	V_b (0–6)	Total
					mg/100 ml			
5	0	0	28	30	212	47	69	386
5	33	0	33	30	218	46	68	395

* S_f boundaries in parentheses.

TABLE V

Lipoproteins of the five major classes of serum and synovial fluid (SF)

		Lipoprotein classes*												
		I (185–802)		II (61–185)		III (44–61)		IV (16–44)		V_a (6–16)		V_b (0–6)		Total lipoprotein
Patient	Fluid	*mg/100 ml*	*% of total lipoprotein*	*mg/100 ml*	*% of total lipoprotein*	*mg/100 ml*	*% of total lipoprotein*	*mg/100 ml*	*% of total lipoprotein*	*mg/100 ml*	*% of total lipoprotein*	*mg/100 ml*	*% of total lipoprotein*	*mg/100 ml*
A.O.	Serum	101	10.9	144	15.6	70	7.6	451	48.8	22	2.4	136	14.7	924
	SF	0		33	8.4	30	7.6	218	55.2	46	11.6	68	17.2	395
H.C.W.	Serum	49	6.2	130	16.5	35	4.4	339	42.9	21	2.7	216	27.3	790
	SF	1	.3	40	13.7	16	5.5	133	45.5	45	15.4	57	19.5	292
R.C.	Serum	5	.8	40	6.3	24	3.8	311	48.9	17	2.7	239	37.6	636
	SF left	0		18	6.7	4	1.5	118	43.9	42	15.6	87	32.3	269
	SF right	0		18	6.9	5	1.9	108	41.2	44	16.8	87	33.2	262
F.Par.	Serum	5	.7	14	1.9	16	2.3	499	70.3	22	3.1	154	21.7	710
	SF	7	1.7	8	1.9	13	3.1	231	55.8	79	19.1	76	18.4	414
R.R.	Serum	34	3.6	54	5.7	35	3.7	499	52.7	16	1.7	309	32.6	947
	SF	0		12	6.7	10	5.6	80	44.7	46	25.7	31	17.3	179
L.B.	Serum	29	6.3	23	5.0	16	3.5	246	53.6	5	1.1	140	30.5	459
	SF	0		1	.6	12	7.4	76	46.9	25	15.4	48	29.6	162
L.S.	Serum							Contaminated						
	SF	4	1.3	45	15.4	12	4.1	168	57.3	31	10.6	33	11.3	293
A.L.	Serum	29	5.1	23	4.0	27	4.7	346	60.5	32	5.6	115	20.1	572
	SF	7	2.6	6	2.2	7	2.6	142	53.2	53	19.9	52	19.5	267
F.Pin.	Serum	21	2.5	147	17.4	3	.4	260	30.7	24	2.8	391	46.2	846
	SF	0		16	5.3	12	4.0	84	27.9	88	29.2	101	33.6	301
J.O.	Serum	16	3.3	36	7.5	27	5.6	268	55.7	19	4.0	115	23.9	481
	SF	0		4	1.7	11	3.9	180	64.1	38	13.5	48	17.1	281
J.B.	Serum	8	1.1	105	14.7	59	8.2	169	23.6	24	3.4	351	49.0	716
	SF	0		14	7.9	19	10.7	43	24.2	54	30.3	48	27.0	178
W.N.	Serum	0		20	6.2	8	2.5	209	64.9	14	4.3	71	22.0	322
	SF	0		7	2.4	9	3.1	174	59.8	20	6.9	81	27.8	291
G.R.	Serum	0		14	2.8	0		139	27.5	126	25.0	226	44.8	505
	SF	0		6	5.4	0		20	8.0	37	33.3	48	43.2	111
Serum	Mean	25	3.8	62	9.4	27	4.1	312	47.3	28	4.3†	206	31.3	652
	Range	0–101		14–147		0–70		139–499		5–126		71–391		322–947
	SD	27.3		16.1		23.4		115.8		32.8		97.0		188.7
SF	Mean	1	0.6	16	6.2	11	4.3	127	48.0	46	175	62	23.4	268
	Range	0–7		4–45		0–30		20–231		25–88		31–101		111–414
	SD			13.2		6.9		60.6		17.8		20.8		

* $S_{f(1.2)}$ boundaries in the NaBr system in parentheses.
† 19.6 mg per 100 ml or 2.9% of total lipoprotein excluding G.R.

parisons it seemed reasonable to assume, as noted above, that our $S_{f(1.20)}$ classes I to V were equivalent to $S_{f(1.063)}$ 100 to 400, $S_{f(1.063)}$ 20 to 100, $S_{f(1.063)}$ 12 to 20, $S_{f(1.063)}$ 0 to 12, and $HDL_2 + HDL_3$, respectively.

II) Lipoproteins in synovial fluids and serum of patients with various articular diseases. The results of analytical ultracentrifugation of the lipoproteins of serum and synovial fluid of 13 patients are tabulated in Table V, with standard deviations for serum and synovial fluid lipoproteins of the five main classes listed at the bottom. A typical set of serum and synovial fluid analytical films with corresponding salt base lines is reproduced in Figure 1. As seen in this figure there is a minor difference in the sedimentation rate of the major peak of the $S_{f(1.20)}$ 16 to 44 of the serum lipoproteins and the synovial fluid. This was observed to a lesser degree in several other cases but was not unexpected in view of the reported concentration dependence of this class of proteins (9). Although the individual values for serum in this heterogeneous group of patients are quite variable, the mean for each class of serum lipoproteins and the total lipoproteins, as expected, differ from the normal serum values published by Lindgren and Nichols (12) only in their lower concentrations.

Synovial fluid lipoproteins showed definite differences from those of paired serums. The total lipoprotein was less, i.e., 40% that of serum. There was only a minute amount of class I low density lipoprotein in the synovial fluids (0.6% versus 3.8%). Class II low density lipoprotein was present but considerably reduced in concentration when compared to serum (6.2% versus 9.4%). Classes III and IV were present in the two body fluids in comparable relative percentages of the total lipoproteins (4.4% versus 4.3% for class III; 47.3% versus 48% for class IV).

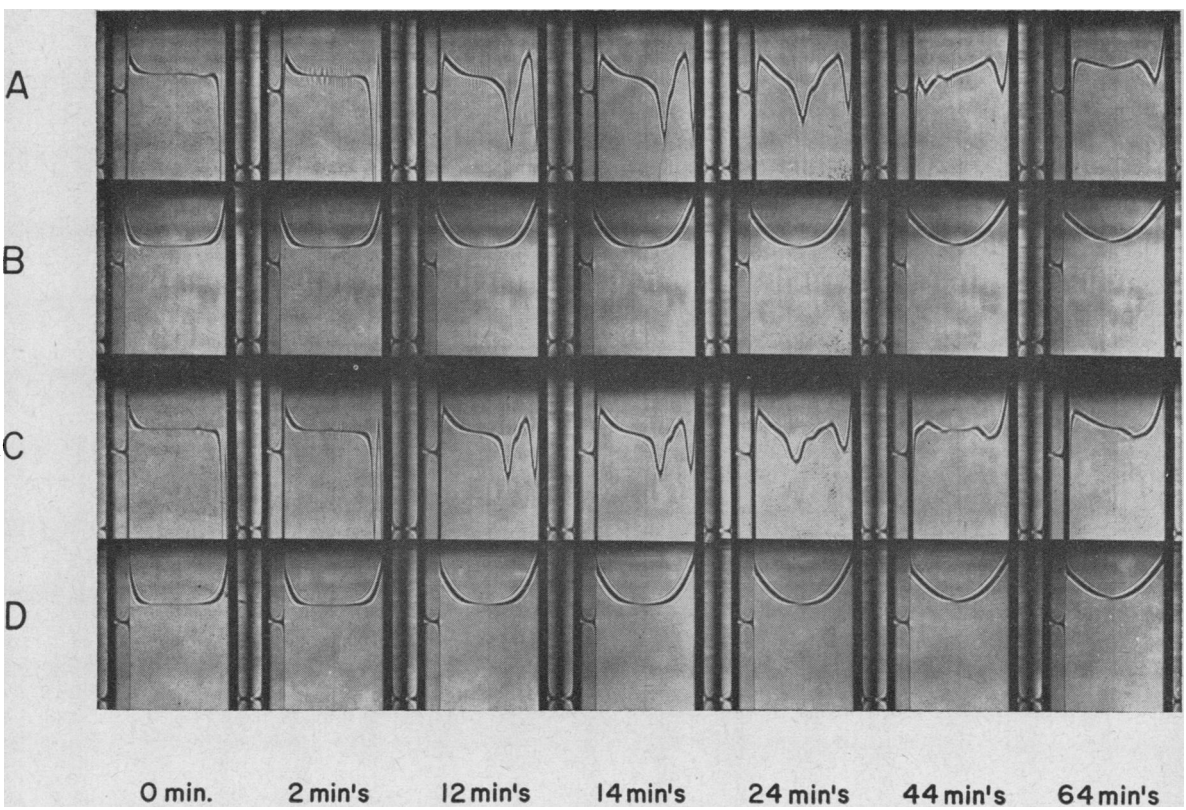

FIG. 1. ANALYTICAL ULTRACENTRIFUGAL FILM RECORDS DEMONSTRATING THE MIGRATION OF LIPOPROTEINS IN THE SERUM AND SYNOVIAL FLUID OF PATIENT R.C. IN A SOLUTION WITH A DENSITY OF 1.20 AT 26° C. Numbers on the base of the diagram represent time in minutes at up to speed. *A*) Serum. *B*) Base-line NaCl/NaBr solution for serum. *C*) Synovial fluid. *D*) Base-line NaCl/NaBr solution for synovial fluid.

TABLE VI

A comparison of the distribution of lipoproteins in three groups of patients

Group (no. of patients)			Lipoprotein classes* I (185–802)		II (61–185)		III (44-61)		IV (16–44)		V_a (6–16)		V_b (0–6)		Total
			mg/100 ml	*% of total lipoprotein*	*mg/100 ml*	*% of total lipoprotein*	*mg/100 ml*	*% of total lipoprotein*	*mg/100 ml*	*% of total lipoprotein*	*mg/100 ml*	*% of total lipoprotein*	*mg/100 ml*	*% of total lipoprotein*	*mg/100 ml*
Active rheumatoid arthritis (3)	Mean lipoprotein	Serum	51.6	6.4	95.6	11.8	40.2	5.0	430	53.2	21.6	2.7	169	20.9	808
		SF	2.7	0.7	27	7.1	19.6	5.4	194	53.0	56.7	15.4	67.0	18.4	367
	Synovial fluid lipoprotein as % serum lipoprotein		5%		28%		49%		45%		262%		40%		45.5%
Nonin-flamed effusions (2)	Mean lipoprotein	Serum	31.5	4.7	38.5	5.5	25.5	3.6	373	52.9	10.5	1.4	225	31.9	704
		SF	0	0	6.5	3.8	11	6.4	78	45.7	35.5	20.8	39.5	23.3	170
	Synovial fluid lipoprotein as % serum lipoprotein		10%		17%		42%		21%		338%		17%		24%
Gout (3)	Mean lipoprotein	Serum	25	3.5	85	12.0	15	2.1	303	42.8	28	3.9	253	35.7	709
		SF	3.7	1.3	22.3	7.8	10.3	3.6	131	45.6	57.3	20.0	62.0	21.7	287
	Synovial fluid lipoprotein as % serum lipoprotein		15%		26%		69%		43%		204%		25%		41%

*$S_{f(1.2)}$ boundaries in the NaBr system in parentheses.

A significant finding appeared in the class V synovial fluid high density lipoproteins. All synovial fluids contained greatly increased quantities in the $S_{f(1.20)}$ 6 to 16 boundary (class V_a), i.e., 17.5% of the total lipoprotein, whereas only 4.3% of the total serum lipoprotein fell in this area. The components of class V_b were present in comparable relative percentages.

III) The total lipoprotein to total globulin ratio. Serum contained considerably more total lipoprotein per gram of total globulin (157 mg per g) than synovial fluid (120 mg per g). This indicates that even when the level of total lipoprotein is adjusted to a comparable level of globulin, a difference still remains.

IV) Lipoproteins of three clinical groups: active rheumatoid arthritis, noninflammatory effusions, and gout. The synovial fluid data were grouped according to clinical and diagnostic criteria into *1*) active rheumatoid arthritis (R.A.), *2*) noninflammatory effusions, and *3*) gout.[6] The following differences were found to exist (Table VI). The total lipoprotein was twice as high in active rheumatoid arthritis (367 mg per 100 ml) as in noninflammatory effusions (170 mg per 100 ml), although it was still lower than that of the patient's serum. Gouty effusions [6] had intermediate levels (287 mg per 100 ml). The increased level of synovial fluid lipoproteins in group 1 (R.A.) was primarily the result of increases in class II, class IV, and class V_b lipoproteins.

Discussion

In the present study the lipoproteins of certain pathological synovial fluids and parallel serums were investigated by the flotation technique at a density of 1.20. This method effects a high resolution of these proteins of both joint fluid and serum, permitting a comparison of the lipoprotein spectra with each other. The results obtained indicate that apparently all lipoproteins with flotation constants corresponding to those of serum are present in synovial fluid. However, the relative concentration of certain classes of lipoproteins differs markedly in these two body fluids.

Class I lipoproteins, which are characterized by very high molecular weights, are greatly decreased in their relative content, as expected from the relative concentration of the 19 S nonlipoproteins of synovial fluid in rheumatoid arthritis (13). The class II is decreased, but class III and IV lipoproteins occur in the two fluids at about the same relative percentages.

The major finding of this study is the greatly increased amount of class V_a lipoproteins of the synovial fluid. It is interesting that the $S_{f(1.20)}$ 6 to 16 (i.e., V_a) space has been given scant attention in the previous literature. One reason is that in serum it indeed constitutes a very small proportion of the total lipoprotein (less than 3%). Although V_a is a previously unreported entity in the $S_{f(1.20)}$ NaBr system, there is evidence that alludes to its presence. For example, several investigators (14–16) noted serum components that floated somewhat faster than HDL_2 and had estimated hydrated densities of 1.081. Although the total lipoprotein content of the investigated joint fluids averages approximately 40% that of serum, this class (V_a) of constituents is increased to such a level that its absolute amount is almost double that of serum. Due to the high content of these constituents in joint fluid, the synovial fluid class V lipoproteins revealed *a*) a refractive index gradient curve that is unlike the spectrum of the corresponding serum lipoproteins and *b*) a flotation rate of the mid-area position (Figure 2) of the total class V components of 4.8 $S_{f(1.20)}$ while the corresponding value of the same patient's serum was 2.3 $S_{f(1.20)}$. The latter value obtained in this study is quite comparable to those reported by Del Gatto and associates (9).

Since the lipoproteins were isolated from both synovial fluid and serum by exactly the same procedure, it is unlikely that V_a is an artifact due to the technique employed. Further, the major peak of the $S_{f(1.20)}$ 16 to 44 class of both body fluids floated at essentially the same rate (with due allowance for the concentration difference), thus making unlikely the possibility that the increased flotation rate of the class V lipoproteins of synovial fluid is due to a difference in density of the background solutions. It should be emphasized that we used paired serum controls in all cases to facilitate interpretation of the ultracentrifugal patterns.

Although it is possible that the V_a components

[6] In the three patients with gout, synovial fluid was taken from chronic inactive effusions.

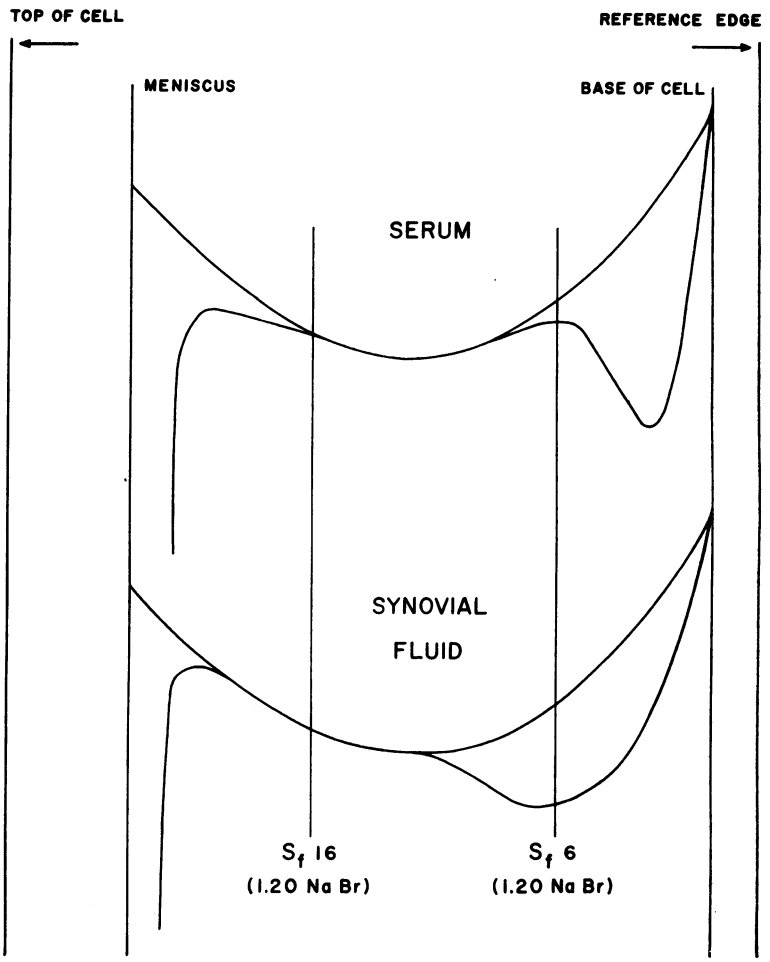

FIG. 2. AN ENLARGED PLANOGRAPHIC TRACING OF THE 64-MINUTE ANALYTICAL ULTRACENTRIFUGAL FILMS SHOWN IN FIGURE 1. The corresponding base lines have been superimposed on the lipoprotein pattern. Lines corresponding to the $S_{f(1.20)}$ boundaries 6 and 16 have been drawn to demonstrate that although very little serum high density lipoprotein falls in the 6 to 16 area, nearly 40% of the synovial fluid high density lipoprotein has migrated to this area. This finding is typical of the difference in migration of high density serum and synovial lipoproteins.

represent a high density lipoprotein with a flotation rate faster than HDL_2 and slower than HDL_1 called "$HDL_{1.5}$" by Hazelwood (17), the ultimate explanation of the observed difference between the high density lipoproteins of the two fluids awaits further isolation and physicochemical characterization. Further reasons for the preferential increase of the V_a lipoproteins must be sought. Whereas synovial fluid contains low molecular weight dialyzable components in concentrations equivalent to those of the serum, the total protein content of pathological synovial fluids varies between 0.3 and 0.7 times that of normal serum. Although the relative concentrations of the protein molecules are often related to their size, it has become clear that many other factors play a role in normal and pathological states. Altered permeability, altered synovial membrane metabolism, and viscosity changes due to hyaluronate degradation must all be considered. Local synthesis of lipids by the synovial membrane, once considered to be unlikely, now has been demonstrated to occur in at least one unusual situation (18).

Attempts to relate specific lipoprotein patterns to specific types of pathological fluids were hindered by the small number of cases in each subgroup (Table VI). It would appear, however, that the increases in classes I and III are somewhat greater in the gouty synovial fluids, whereas class V_a is not so markedly elevated. Until further studies are obtained on larger groups of fluids, no significance can be attributed to these clinical correlations.

Summary

1) The lipoproteins of synovial fluid and serum obtained from 13 patients with various rheumatic diseases were studied by analytical ultracentrifugation at a density of 1.20 in NaBr.

2) The total synovial fluid lipoprotein was 40% that of serum. Class I low density lipoprotein was present in only minute amounts; class II was considerably decreased when compared to paired serums. Relative percentages of classes III and IV were comparable in the two body fluids.

3) The class V lipoproteins of synovial fluid, which were arbitrarily divided into V_a [6 to 16 $S_{f(1.20)}$] and V_b [0 to 6 $S_{f(1.20)}$], demonstrated a significant increase in the V_a class lipoproteins.

Acknowledgment

The authors would like to express their gratitude to Miss Alise Polis for her expert technical assistance.

References

1. Ropes, M. W., and W. Bauer. Synovial Fluid Changes in Joint Disease. Cambridge, Harvard University Press, 1953.
2. Schmid, K., and M. B. MacNair. Characterizations of the proteins of human synovial fluid in certain disease states. J. clin. Invest. 1956, **35**, 814.
3. Schmid, K., and M. B. MacNair. Characterization of the proteins of certain post-mortem human synovial fluids. J. clin. Invest. 1958, **37**, 708.
4. Chung, A. C., J. R. Shanahan, and E. M. Brown, Jr. Synovial fluid lipids in rheumatoid and osteoarthritis. Arth. and Rheum. 1962, **5**, 176.
5. Bole, G. G. Synovial fluid lipids in normal individuals and patients with rheumatoid arthritis. Arth. and Rheum. 1962, **5**, 589.
6. Schur, P. H., and J. Sandson. Immunologic studies of the proteins of human synovial fluid. Arth. and Rheum. 1963, **6**, 115.
7. Schmid, K., E. C. Rosa, and M. B. MacNair. Fractionation of the proteins of human synovial fluid and plasma. J. biol. Chem. 1956, **219**, 769.
8. Schoenheimer, R., and W. M. Sperry. A micromethod for the determination of free and combined cholesterol. J. biol. Chem. 1934, **106**, 745.
9. Del Gatto, L., F. T. Lindgren, and A. V. Nichols. Ultracentrifugal method for the determination of serum lipoproteins. Analyt. Chem. 1959, **31**, 1397.
10. Havel, R. J., H. A. Eder, and J. H. Bragdon. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J. clin. Invest. 1955, **34**, 1345.
11. DeLalla, O. F., and J. W. Gofman. Ultracentrifugal analysis of serum lipoproteins *in* Methods of Biochemical Analysis, D. Glick, Ed. New York, Interscience, 1954, vol. 1, p. 459.
12. Lindgren, F. T., and A. V. Nichols. Structure and function of human serum lipoproteins *in* The Plasma Proteins, F. W. Putnam, Ed. New York, Academic Press 1960. vol. 2, pp. 1–58.
13. Binette, J. P., and K. Schmid. Studies on synovial fluid proteins: ratio of alpha-1 to alpha-2 globulins. Arth. and Rheum., in press.
14. Shore, B. C- and N-terminal amino acids of human serum lipoproteins. Arch. Biochem. 1957, **71**, 1.
15. Barclay, M., R. K. Barclay, O. Terebus-Kekish, E. B. Shah, and V. P. Skipski. Disclosure and characterization of new high-density lipoproteins in human serum. Clin. chim. Acta 1963, **8**, 721.
16. DeGennes, J. L., J. Polonovski, M. Ayrault-Jarrier, D. Bard, and G. Levy. Étude analytique des lipoproteines par ultracentrifugation préparatrice dans 21 cas d'hyperlipidemies majeures. Rev. franç. Étud. clin. biol. 1964, **9**, 273.
17. Hazelwood, R. N. The molecular weights and dimensions of some high-density human serum lipoproteins. J. Amer. chem. Soc. 1958, **80**, 2152.
18. Newcombe, D. S., and A. S. Cohen. Chylous joint effusion: a study of intra-articular lipid synthesis (abstract). J. clin. Invest. 1963, **42**, 960.