CHARACTERIZATION OF THE PROTEINS OF HUMAN SYNOVIAL FLUID IN CERTAIN DISEASE STATES [1]

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In search of the similarities and differences between blood plasma and synovial fluid, on one hand, and normal and pathological joint fluid on the other, it appeared important to investigate systematically the protein components of joint fluid. Investigations of the synovial fluid proteins by electrophoresis have been carried out by several authors (1–4). The identification of these proteins has been initiated (1, 5). Fractionation with ammonium sulfate to obtain the albumin-globulin ratio (6) and demonstration of definite properties of certain proteins by immunochemical methods and enzymatic reactions have been reported (1, 5).

This publication presents a study on the characterization of the proteins in pathological synovial fluids.

MATERIALS AND METHODS

The collection of synovial fluid and plasma as well as the procedure for the fractionation of their proteins have been described in a previous publication (7). All fluids were drawn from knee joints of patients with rheumatoid or traumatic arthritis or neuroarthropathy, and were obtained through the courtesy of the clinical staff of the Arthritis Unit. Most of the fluids deposited a precipitate within 20 hours although acid-citrate-dextrose (ACD) solution had been added. Prior to fractionation the joint fluids were treated with hyaluronidase (7).

The *electrophoretic and ultracentrifugal analyses* were carried out in a Perkin-Elmer electrophoresis apparatus and a Spinco Model E ultracentrifuge, respectively. *Paper electrophoresis* was performed essentially according to the method of Grassmann and Hannig (8, 9). After being heated at 110° for 10 minutes, the paper strips were stained with Amidoschwarz 10B.[2] The intensity of the color was read in an "Elphor" densitometer.[2] The relative percentage of the separated proteins was determined planimetrically from the curve obtained by plotting the optical density as a function of distance on the paper strip. A correction factor of 1.5 was applied for orosomucoid (10, 11), of which the polypeptide moiety was 66 per cent.

Immunochemical methods: For the *immunoelectrophoresis* described by Grabar and Williams (12) a plate glass (25 × 25 cm.) was used as support. Plasticin (1.2 cm. high) was mounted approximately 2.5 cm. from the edges of the plate. The plate glass was carefully warmed and 20 ml. of pH 8.6, $\Gamma/2$ 0.07 borate buffer containing 2 per cent agar were added so as to obtain an even layer, (to 1 volume of 4 per cent aqueous agar solution, Bacto Agar, Difco, extracted with distilled water for 24 hours prior to heating, 1 volume of borate buffer was added; the borate buffer consisted of 1 part of 0.07 M $Na_2B_4O_7$ and 2 parts 0.07 M H_3BO_3; borate buffer was preferred because of its inhibitory effect on the growth of molds). After cooling to room temperature, 6 plastic pieces (0.6 × 1.2 × 15 cm.) were arranged on the agar parallel to each other and to one edge of the plate. Their ends were 5 cm. away from the next edge, thus forming 5 spaces of 3 cm. width and 15 cm. length. Next, 200 ml. of the same agar solution were added giving a thickness of 0.8 to 1.0 cm. After the plate had again been cooled to room temperature, the plastic pieces were loosened with a spatula and carefully removed. The resulting 6 troughs were used after electrophoresis to receive the antiserum. In the center of each section (3 × 15 cm.) which was separated by the troughs, a small reservoir (1.2 cm. long, 0.25 cm. wide and 0.9 cm. deep) was cut out. The main axis of the reservoirs was perpendicular to the troughs·and one or two drops of liquid borate-agar was added to each to seal any breaks. Then, the protein solutions (serum, synovial fluid digested with hyaluronidase, 4 per cent human serum albumin, and 1.2 per cent human γ-globulin), previously dialyzed against the same borate buffer, were mixed at 52°C with an equal volume of 4 per cent aqueous agar solution. The individual reservoirs were filled exactly to the level with one of the protein solutions. The agar plate was then placed in the electrophoresis box (outer dimensions: 38 × 26 × 12 cm.; buffer chambers 26 × 6.7 × 3.4 cm.; center compartment 26 × 26 × 5.5) and transferred to a 2°C room. Four layers of

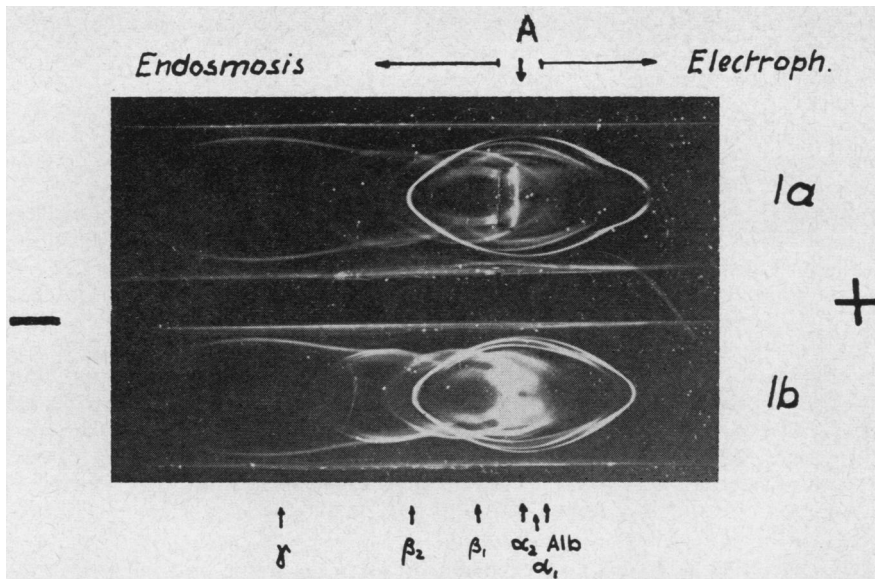[2] Bender and Hobein, Munich, Germany.

FIG. 1. IMMUNOELECTROPHORESIS OF TRAUMATIC SYNOVIAL FLUID (a) AND SERUM (b)
FROM THE SAME INDIVIDUAL

Joint fluid had been treated with hyaluronidase prior to the experiment. A indicates
the position of application. Electrophoresis in borate buffer, pH 8.6, $\Gamma/2$ 0.07, contain-
ing 2 per cent agar, for 17 hours at 180 V and 40 mA. The arrow "Electroph." indi-
cates the direction of the electrophoretic movement of the proteins, and the arrow
"Endosmosis" indicates the direction of the electroendosmosis. After the electrophore-
sis was completed, the troughs between the agar sections were filled with horse serum
against human serum. Therefore, the precipitin zones formed symmetrical patterns.
The positions of albumin and γ-globulin were found by applying these proteins as refer-
ences on adjoining sections. The precipitin zones of synovial fluid (a) appeared less
intense than those of the serum because of its lower protein concentration. The line
corresponding to albumin, however, was very distinct due to the relatively high albumin
concentration in this joint fluid.

Whatman No. 1 paper (24 cm. wide) soaked in the borate
buffer were used as electric connection between the ends
of the agar plate and the adjoining borate buffer reser-
voirs. The buffer chambers, in turn, were again con-
nected to holes filled with glass wool with a second set
of buffer chambers in which the electrodes were placed.
The electrodes were made by winding 120 cm. of stain-
less steel wire (0.05 cm.) around a glass tube with diam-
eter of 1.5 cm. To secure an even level of the buffer in
all 4 compartments, the two inner reservoirs were con-
nected with each other by a thin rubber tubing. After
half an hour of temperature equilibration, 180 volts were
applied giving a current of 40 mA. The electrophoresis
was continued for 17 hours. Owing to strong electro-
endosmosis, acting in the direction opposite to the elec-
trophoretic movement, albumin remained almost exactly
at the position of application, and γ-globulin appeared to
have moved against the direction indicated by the elec-
trophoretic mobility (Figure 1). Between the albumin
and γ-globulin, the α_1-, α_2-, β_1-, and β_2-globulins were
distributed according to their electrophoretic mobilities.
During electrophoresis, diffusion occurred so that each
group of globulins would appear as a band, broader than

the reservoir where the protein solution was originally
applied. Under these circumstances, the albumin, α_1- and
α_2-globulin did not appear to be well separated. After
electrophoresis, the plate was brought to room tempera-
ture, the troughs filled with the antiserum, and the sys-
tem kept in an air-tight box to prevent dehydration. The
proteins of the antiserum diffused into the two adjoining
agar sections at approximately the same rate, at each
point along a line parallel to the troughs. The proteins
of the analysis, now separated according to their electro-
phoretic mobilities, diffused further toward the troughs,
as well as in the direction of the main axis of the agar
sections. At the point where the corresponding anti-
bodies and antigens met (point of equivalence), a com-
plex of these two components was formed and precipitated
as lines. Such complexes are known to be soluble in ex-
cess of either antibody or antigen. Thus, depending on
their concentration, different amounts were transported
by diffusion toward the precipitin zones, which were dis-
solved, and new ones formed (assuming that the precipi-
tin zones did not present an obstacle to diffusion). Con-
sequently, one observed that these zones moved either
toward or away from the troughs. The precipitin zones

appeared as elliptical curves because the concentration of the antigens varied from zero over a maximum to zero when observed along a line parallel to the troughs. For the exact interpretation of the precipitate lines see references 12–14. After 6 hours, the first faint line corresponding to albumin became visible, and within 6 days all lines had formed. The antiserum which diffused into the agar sections cleared the turbidity of the agar plate and facilitated the taking of pictures. The number of precipitin zones indicated the minimal number of antigen components. Using this particular antiserum, 12 lines were observed. The pictures were taken in the following way: A beam of light illuminating the agar section with the precipitin lines, was projected from an angle beneath the plate. In this way the precipitate lines showed up against the dark background. Care was taken to avoid dust. The agar plate was washed with soft paper previously soaked in saline.

For the *Ouchterlony* test (15), 10 ml. of the above mentioned agar-borate solution was poured into each petri dish and allowed to gel. After prolonged cooling, rubber-serum stoppers (1.4 cm. outer diameter) perforated with 21 gauge hypodermic needles were placed on each plate, one in the center and usually four more at distances of 1.6 cm. from the center and at equal distance from each other. Next, 25 to 30 ml. more agar solution was added. When thoroughly cooled, the stoppers were removed and 2 to 3 drops of agar were added to each reservoir to seal off any breaks. In our experiments, the antiserum was placed in the center reservoir and the test solutions in the others. The formation of the zones is governed by the same principle as described in the method of Grabar. The exact interpretation, particularly of the complications, has been further studied by Burtin (13) and Wilson and Pringle (14). It was concluded from the precipitin zones which formed closed systems (fusion of the precipitate lines), that the corresponding proteins were identical.

Furthermore, the technique described by *Oudin* (16) was applied to certain protein fractions to identify the γ-globulins. For the titration of the *antistreptolysin-O* content and for the determination of the *diphtheria antitoxin* content (rabbit skin test), the standard methods (17, 18) were used.

Ceruloplasmin (19) was determined as follows: Fraction III was dialyzed at 2°C against distilled water for 14 hours to remove the ethanol. Insoluble material was centrifuged off. The optical density of the supernatant solution was read against water between 580 and 630 mμ at intervals of 10 mμ, in a Beckman DU spectrophotometer using a 1-cm. cell. An aliquot of 3.0 ml. of the supernatant solution was mixed with 0.2 ml. of a 1 per cent KCN solution, and its optical density read in the same way after standing for 5 hours at room temperature. For the calculation of the percentage of ceruloplasmin, the difference in optical densities at 610 mμ, representing the maximal absorption of this protein in the visible spectrum, was divided by the extinction coefficient of 0.68 ($= E_{1 cm}^{1\%}$). Appropriate corrections for dilution occurring during fractionation, dialysis, and addition of the

KCN solution were applied. The corresponding fraction derived from normal human plasma was measured simultaneously. This procedure can obviously be simplified, when sufficient material is available, by reading the dialyzed and centrifuged Fraction III against an aliquot of the same solution which had previously been treated with KCN.

Lipoproteins expressed as cholesterol were measured by the method of Bloor, Pelkan, and Allen (20).

The *β₁-metal-combining protein* determinations (21, 22) were carried out as follows: 500 ml. of citrate barbiturate buffer, pH 8.6, Γ/2 0.1, commonly employed in electrophoresis, were added to 37.5 ml. of acetate buffer, pH 4.0, Γ/2 0.1, to obtain a pH of 7.4. After standing over night in the cold room, the precipitated barbituric acid was separated, and the supernatant solution was mixed with 20 ml. of freshly prepared 20 per cent $Fe(NH_4)_2$ $(SO_4)_2$-solution. Aliquots of Fractions IV and V were dialyzed for two days against this buffer. The intensity of the developed pink color ($E_{1 cm}^{1\%} = 0.57$) was measured at 465 mμ, part of the same protein solutions acidified with 0.1 ml of acetic acid per ml. being used as a blank.

Albumin was measured by the method of Rutstein, Ingenito, and Reynolds (23) which involved the determination of the capacity of albumin to bind 2-(4′-hydroxy-benzeneazo) benzoic acid (HBABA).

RESULTS

For the characterization of the synovial fluid proteins, two fundamentally different approaches were chosen. First, the proteins were identified by precipitin reactions after separation by electrophoresis in agar (method of Grabar). Second, the proteins were separated into six fractions by means of solubility differences (7), and were then characterized by chemical and physico-chemical methods and by the measurement of definite properties of proteins known to occur in blood plasma. In contrast to the first method that permits qualitative measurements only, the second makes possible qualitative and quantitative determinations.

1. Characterization of the synovial fluid proteins by immunoelectrophoresis

Synovial fluids from patients with rheumatoid arthritis or knee injuries together with the plasma from the same individuals were investigated simultaneously. The results obtained were consistent: The characteristics, number and distribution of precipitin zones were the same for each set of fluids, and suggested that the proteins of plasma also occur in synovial fluid as judged by these characteristic tests. The precipitin zones were

grouped according to the electrophoretic mobilities of albumin, α_1-, α_2-, β_1-, β_2, and γ-globulin, but only the zones corresponding to albumin and γ-globulin were identified. It is important, however, to note that the immunoelectrophoretic patterns obtained from joint fluid and plasma of the same patient were identical. A typical experiment is illustrated in Figure 1.

2. Characterization of the synovial fluid proteins after separation by the low temperature—low salt—ethanol procedure

Joint fluids and plasma from the same individuals were fractionated simultaneously. A proper comparison could thus be obtained as well as a test of the reliability of the method, for which the results from normal human plasma were used as references (7). Two characteristic experiments are reported as examples.

a. *Synovial fluid from a patient with rheumatoid arthritis:* Synovial fluid obtained from a patient (W. W. 745536) with rheumatoid arthritis was centrifuged at approximately 12,000 r.p.m. for one hour, yielding a straw-yellow, viscous fluid. Since fractionation was not carried out immediately, the fluid was kept at $-30°$ for two months. After being thawed the clear fluid was digested with hyaluronidase. A clot which formed within 14 hours was centrifuged off. ACD-plasma from the same individual was also kept at $-30°$ for the same time. The electrophoretic distribution of the proteins of the original fluid and plasma was identical.

For the fractionation, 28 ml. of ACD-synovial fluid and 20 ml. of ACD-plasma were used so that each contained approximately equal amounts of protein. Essentially the same results (Table I) were obtained from both joint fluid and plasma: The protein content of each of the six fractions (except Fraction I of synovial fluid because of its previous clotting), the content of protein-bound hexose, the distribution of the cholesterol, the electrophoretic distribution and the mobilities of the proteins and the distribution in the ultracentrifuge, as well as the calculated sedimentation constants, were within experimental errors found to be alike for both sets of fractions.[3] The percentages of

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[3] These sedimentation constants were identical to those reported for the corresponding fractions from normal human plasma.

γ-globulin in Fraction I and III were very similar to those of the corresponding fractions from normal human plasma (7).

In addition to these investigations, during which the proteins were studied from the point of view of general characteristics, except for the cholesterol and in part the carbohydrate content, experiments were carried out for the determination of definite properties of certain protein components known to occur in plasma. Again, these experiments were performed simultaneously with the fractions from both synovial fluid and plasma. The clotting of *Fraction I* indicated the presence of fibrinogen. The clear supernatant solution was kept at $+2°C$ for 20 hours resulting in the formation of a precipitate. The solubility of the precipitated material at room temperature proved the presence of "cold-insoluble globulin" (24). *Fraction II,* in which the γ-globulins were concentrated, was analyzed for specific antibodies. The antistreptolysin-O content, of which normal human plasma contains between 50 and 250 standard units per ml. amounted to 45 U per ml. of ACD-synovial fluid. However, the protein concentration of the latter fluid was only 5.7 per cent. The antistreptolysin-O titre in both Fractions II were almost equal. Diphtheria antitoxin showed the same distribution, namely, the activity of the proteins in each of the two Fractions II was approximately half of the total. The presence of ceruloplasmin precipitated in *Fractions III* was evidenced by its blue color after incubation of an aliquot for 10 minutes at 70°C. Fraction III of synovial fluid and of plasma exhibited the same isoagglutinin titre. The content of the β_1- and α_1-lipoproteins, judged by the cholesterol content of Fraction III and *Fraction IV,* was almost equal for both fluids. Fraction IV contained approximately 20 per cent of the α_2-globulins; the rest had previously been precipitated in Fraction III. The estimation of the α_2-globulins in Fraction III from the electrophoretic pattern might be erroneous owing to the change in the mobility of the β_1-lipoproteins due to autoxidation (25). The amount of β_1-metal-binding protein concentrated in *Fraction V* was the same for both fluids, amounting to 3 per cent, while Fraction IV was free of this component. The albumin of Fraction V, which was marked by a distinct yellow color and its low content of bound hexose, exhibited the same capacity to bind

TABLE I

Fractionation of synovial fluid from a patient with rheumatoid arthritis (W. W. 745536)

Fraction	Total protein* mg.	Total hexose mg.	Total hexose %	Total cholesterol mg.	Alb.	α1	α2	β1	β2+Fibr.	γ	S20,w†	Total albumin HBABA mg.	Total β1-metal-combining protein mg. (%)	Total anti-streptolysin-O	Total diphtheria antitoxin	Titer of isoagglutinins‡	Electrophoresis at pH 4.5 (Relative percentage)
Synovial Fluid — 28 ml. ACD-																	
Fluid	1,610(5.8%)			28	55	5	7	15	5	13		860		1,260 U	2.8 U		α1 1%, α2 3%, Rest 96%
I	64	1.9	1.1		clotted												
II	170			17	1			3	20	76							
III	340	12	3.7		5		25	39	20	11	6.8			400 U	0.6–2.0 U		
IV	94	1.1	1.2	2.8	44	24	15	10	2	5	4.6§	720	0			inactive	
V	900	6.4	0.9		92	1	1	6	5		4.1					Anti-A 1:64	
VI	27										2.9§		24(3)			Anti-B 1:8	
Plasma — 20 ml. ACD-																	
Plasma	1,550(7.7%)			24	55	4	8	14	7	12		640		560 U	0.2–0.6 U		α1 1%, α2 3%, Rest 96%
I	110	2.4	1.7		clotted												
II	140			15	4			18		78							
III	300	8.6	2.9		4	7	24	37	16	12	6.9						
IV	110	4.1	3.6	3	31	37	22	6		4	4.6§	450‖	0			inactive	
V	730	5.3	0.7		92	2	1	5	5		4.0					Anti-A 1:64	
VI	27										2.9§		21(3)			Anti-B 1:8	

* Biuret.
† In 0.15 M NaCl at a protein concentration of approx. 1 per cent.
‡ Both Fractions III adjusted to 20 ml.
§ Sedimentation constant of heavier component not indicated.
‖ The value of 450 mg. albumin determined by the HBABA-method seems to be erroneous, because 670 mg. were expected from measurements of the protein content of Fraction V by the biuret method and electrophoresis. We feel that this discrepancy is not significant in view of the other measurements (solubility, electrophoretic distribution and mobility, ultracentrifugal distribution and sedimentation constants, immunochemical analyses, and carbohydrate content) carried out on the two Fractions V. In addition, the albumin content of other plasma and their Fractions V found by the HBABA-method agreed with that obtained from protein content and electrophoresis.
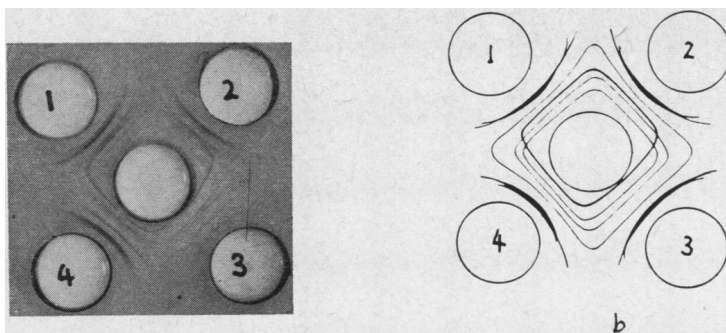
FIG. 2. OUCHTERLONY PLATE (2a) OF FRACTION III OBTAINED FROM ARTHRITIC
SYNOVIAL FLUID

Figure 2b represents a sketch of Figure 2a. Reservoir 1 contained Fraction III of this synovial fluid, reservoir 2 Fraction III of the same patient's plasma, reservoir 3 normal human plasma, reservoir 4 Fraction II + III of normal human plasma and center reservoir horse serum against human serum.

HBABA as normal human serum albumin is known to have (23). The presence of α_1- and α_2-glycoproteins in *Fraction VI* was shown by their low sedimentation constant. The acid isoelectric points (10), indicated by electrophoresis of the original fluids at pH 4.5, conform with this view.

Fractions II, III, IV, and V were also investigated for identity of the proteins by the method of Ouchterlony. The corresponding fractions of synovial fluid and plasma from the same patient as well as that of normal human plasma were put on the same plate. The concavities for these four solutions were arranged on the plate at equal distances from the center one in which horse serum active against human serum was placed. It was again concluded that most of the proteins in the corresponding fractions were identical, as judged by the precipitin zones which formed closed systems (Figure 2a). The γ-globulin content of Fractions III and Fractions IV, amounting to a small percentage of the total as judged by electrophoretic analysis, was identified with a specific rabbit serum against human γ-globulin with use of the technique of Oudin.

For the experiment described in section 2a, a patient with rheumatoid arthritis was chosen who showed severe knee involvement, and whose synovial fluid and plasma revealed essentially the same patterns upon electrophoresis. During this study, joint fluids from six other patients with rheumatoid arthritis or neuroarthropathy were also fractionated. They were characterized by

their low content of the α_2-globulins and increased content of γ-globulins, compared with the plasma of the same individuals (26). The results obtained from investigating the protein fractions of these joint fluids were essentially the same as those reported in the previous experiment. If the γ-globulin content was 20 per cent or higher, only part of it could be extracted from Fraction II + III into Fraction II.

b. *Synovial fluid from a patient with traumatic arthritis:* Synovial fluid and serum obtained from a healthy individual (RBJE) 2 days after an injury to his knee were centrifuged and stored at − 30°C until fractionation was carried out. Since the volume of the serum was too small to perform a complete analysis, particularly fractionation, plasma from a different healthy individual was fractionated simultaneously. Prior to the fractionation, the synovial fluid was digested with hyaluronidase. The fluid clotted within 20 hours. The electrophoretic distribution of the proteins of the original synovial fluid, distinguished by the high content of albumin and γ-globulin and low content of α_2-globulin, is typical of traumatic joint fluid (26). The electrophoretic pattern of the serum was normal.

The characterization of the synovial fluid proteins after fractionation showed the following results (Table II): The relative amount of protein in the six fractions was essentially the same as that reported for normal human plasma. The low protein content of Fraction I was due to the decreased concentration of fibrinogen, and that

TABLE II

Fractionation of traumatic synovial fluid (R. B. J.)

Fraction	Total protein* mg.	Total cholesterol mg.	Electrophoresis at pH 8.6, Γ/2 0.1 (Expressed in relative percentage)						$S_{20,w}$†	Total albumin HBABA mg.	Total β_1-metal-combining protein mg. (% protein)	Total ceruloplasmin mg.	Total anti-streptolysin-O	Total diphtheria antitoxin	Titer of isoagglutinins‡	Electrophoresis at pH 4.5 (Relative percentage)
			Alb.	α_1	α_2	β_3	β_2+Fibr.	γ								
Fluid	910(3.2%)	16	68	3	3	14	3	9		640			930 U	29–90 U	Anti-A 1:8 / Anti-B 1:2	α_1 2% / α_2 5% / Rest 93%
I	22§															
II	37			3	4	7	17	65								
III	220	14	4	17	4	45	4	30	6.4			4.2			Anti-A 1:16 / Anti-B 1:1	
IV	65	1.0	88	6	2	4			4.0¶							
V	450	<0.3	97	1	1	1			3.8	400	23(2.4)					
VI	12‖		30	37	18	11	4	4	2.6¶							α_1 30% / α_2 16% / Rest 54%
12 ml. serum	960(8%)	22	55	4	7	19	2	13		580			1,200 U	36–120 U	Anti-A 1:64 / Anti-B 1:16	α_1 3% / α_2 4% / Rest 93%

29 ml. Joint Fluid

* Biuret.
† In 0.10 M NaCl at a protein concentration of approx. 1 per cent.
‡ Solution of Fraction III adjusted to 29 ml.
§ Six mg. of fibrin clot.
‖ Determined by weight after lyophilization.
¶ Sedimentation constant of heavier component not indicated.
Electrophoreses of Fraction VI, both at pH 8.6 and 4.5, were carried out on paper; ceruloplasmin content of normal human serum is 0.32 mg. per ml. as found by Scheinberg and Gitlin, Science, 1952, 116, 484.
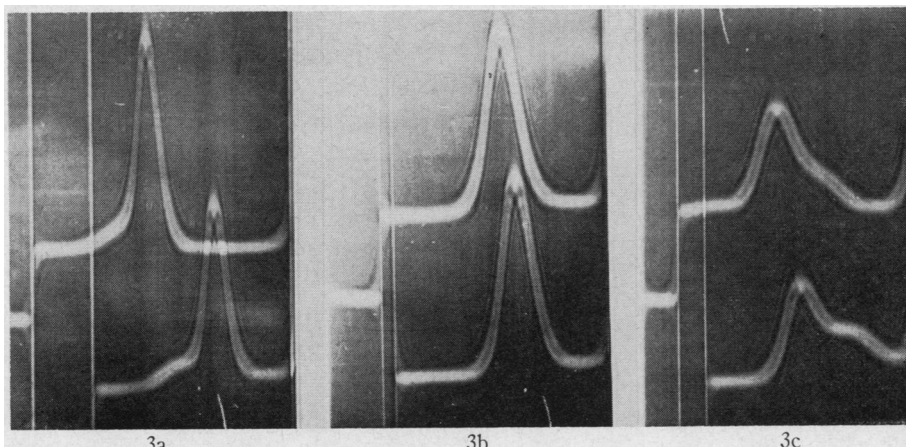
FIG. 3. ULTRACENTRIFUGAL ANALYSES OF PROTEIN FRACTIONS FROM TRAUMATIC JOINT
FLUID

The analyses were carried out under the following conditions: 0.15 M NaCl, 56100
r.p.m., temperature 19°C to 20°C, protein concentration approximately 1 per cent, direc-
tion of sedimentation from left to right. Each picture shows two curves; two samples
were run simultaneously by means of a wedge window cell. The upper curve, the menis-
cus of which is displaced to left due to slightly larger volume of the sample, is that of
the sample in the wedge window. The bottom curves represent the synovial fluid frac-
tions and the top curves the corresponding fractions of normal human plasma. Figure 3a,
Fraction II (γ-globulin) ; 73 minutes, wire angle 50° ; Figure 3b, Fraction V (albumin),
116 minutes, wire angle 50° ; Figure 3c, Fraction VI (glycoproteins), 137 minutes, wire
angle 50°.

of Fraction IV perhaps due to the low α_2-globulin
concentration in the original fluid. The electro-
phoretic distribution and the mobilities of the pro-
teins of the six fractions as well as their ultra-
centrifugal distribution (Figure 3) and sedimen-
tation constants were essentially identical with
those reported for normal human plasma (27).

Again, additional specific tests were carried out
to prove the existence in traumatic joint fluid of
protein components present in blood plasma.
Fibrinogen was detected in the synovial fluid and
in Fraction I by the formation of a clot. *Cold
insoluble globulin* concentrated in Fraction I was
recognized by its extreme insolubility at + 2°C and
its relatively high solubility at room temperature.
The titres of *diphtheria antitoxin* and *antistrepto-
lysin-O* of both synovial fluid and serum, corrected
for protein concentration, were found to be com-
parable to those reported for average normal hu-
man plasma. The content of *ceruloplasmin,* ex-
pressed as percentage of the total protein concen-
tration, ranged within the values reported for
normal human plasma. The total *cholesterol* con-
tent was approximately one-half that of normal

human plasma, if calculated on the basis of the
total amount of protein. It is important to point
out that the cholesterol content of Fractions IV
and V, representing the α_1-lipoproteins, was
about 12 per cent of the total as compared with
25 per cent of the same fractions of normal hu-
man plasma. Similar observations were made by
Noble, Boucek, and Kao (28) in their study on
the lipoproteins of connective tissue. Further in-
vestigation by the flotation technique might yield
more information about the distribution of the
lipoproteins. The content of β_1-*metal-combining
protein* was 6 per cent of the protein in Fraction
V or 3 per cent of the total protein, which was
equal to the value reported for normal human
plasma (29). The content of *albumin,* measured
by its capacity to bind HBABA, was essentially
that found by electrophoresis. The presence of
orosomucoid and other *glycoproteins* of low mo-
lecular weights and acid isoelectric points was
proven by paper electrophoresis of Fraction VI
(Figure 4) and by free electrophoresis of the
original joint fluid, both at pH 4.5. Furthermore,
the high content of protein-bound hexose of this

fractioi., amounting to 10 per cent, equaled the corresponding value of Fraction VI of normal human plasma. The identity of the proteins in traumatic synovial fluid was further confirmed by immunochemical analyses using the Ouchterlony technique described in section 1.

DISCUSSION

For the characterization of a protein, it has become clear that the results obtained from one method only can never establish its identity. Because of the complexity of the protein molecule, a series of methods based on different principles should be included in such a study.

For the characterization of the synovial fluid proteins, the proteins of joint fluid were separated from each other by means of differences in their electrostatic charges as well as in their solubilities. The method of Grabar combined electrophoresis with immunochemical reactions, of which the specificity is extremely high. The procedure for the separation of the proteins into solubility groups, referred to as fractions in Tables I and II,

made possible the recovery and further investigation of the proteins. The conditions of precipitation of the proteins represent a precise measure for their solubilities, which are some of their characteristics.

In characterizing the synovial fluid proteins, it was found that the proteins of the corresponding fractions, derived from synovial fluid and plasma of the same individual, exhibited the same solubility properties, the same electrophoretic distribution and mobilities of the respective components, and the same sedimentation constants. Yet, the possibility existed that these proteins were not identical. In order to exclude such a possibility, further independent methods were employed which allowed the measurement of very distinct properties of the proteins such as titres of isoagglutinin, diphtheria antitoxin, and antistreptolysin-O, reaction of ceruloplasmin with cyanide ions, and ion-binding capacity of albumin and β_1-metal-combining protein. It was concluded from the results of the different methods used that, for the most part, the synovial fluid proteins are identical
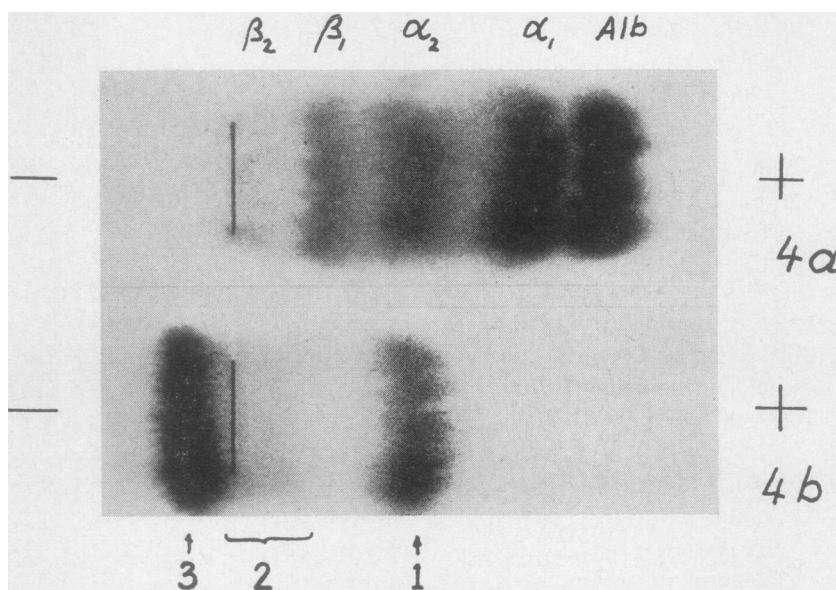

FIG. 4. PAPER ELECTROPHORESIS OF FRACTION VI, DERIVED FROM TRAUMATIC JOINT FLUID, IN DIETHYL-BARBITURATE BUFFER pH 8.6, $\Gamma/2$ 0.1 (4a) AND IN ACETATE BUFFER pH 4.5, $\Gamma/2$ 0.1 (4b)

The dark lines indicate the position of application. Staining with Amidoschwarz 10B. Area 1 in Figure 4b is representative of orosomucoid identical with α_1-globulin in Figure 4a; area 2 is identical with most of the α_2-globulin in Figure 4a, and area 3 representing the proteins which were positively charged at pH 4.5, includes albumin, β-globulin, and a minute part of the α_2-globulin.

with those of plasma. It is emphasized that the findings described do not relate to normal synovial fluid which may present a different pattern. The existence of special protein components present solely in synovial fluid is not excluded.

Although the synovial fluid proteins exhibited the same characteristics as the plasma proteins, the following differences were found: First, the differences in total protein concentration and in the relative content, especially of albumin, α_2- and γ-globulins, reported earlier (26), were confirmed. Second, the ratio of β_1- to α_1-lipoprotein, measured as cholesterol, appeared higher than that of normal human plasma.

It should be pointed out that it is a difficult task to establish the identity of two proteins (30). The results obtained from these investigations led obviously to the question of the fine structure of the "identical" proteins, in other words, as to whether or not one individual always synthesizes a certain protein by exactly the same process.

SUMMARY

The proteins of synovial fluid from patients with rheumatoid arthritis, neuroarthropathy, or traumatic injury have been characterized. They are identical with those of plasma as judged by the results obtained from several different methods including electrophoresis, ultracentrifugal analyses, separation by solubility, immunochemical methods, and ion-binding measurements. The following proteins were identified: Fibrinogen, cold insoluble globulin, diphtheria antitoxin, antistreptolysin-O, isoagglutinins, ceruloplasmin, β_1-metal-combining protein, α_1- and β_1-lipoproteins. Albumin, γ-globulin, and glycoproteins of low molecular weights and acid isoelectric points were isolated. Known differences between synovial fluid and plasma were confirmed.

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Addendum

The designation "orosomucoid" is used for the human glycoprotein previously named "MP-1" by Winzler (11) and "acid glycoprotein" by Schmid (10).

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