CHARACTERIZATION OF THE PROTEINS OF CERTAIN POST-MORTEM HUMAN SYNOVIAL FLUIDS^{1, 2}

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In a recent publication (1), the first part of our study on the characterization of the synovial fluid proteins, evidence was presented that the protein components of pathological joint fluid are identical with those of blood plasma. In the present communication the proteins of postmortem synovial fluid from patients who had no signs or symptoms of joint disease are described.

This study was carried out in three sections. First, postmortem synovial fluid was analyzed electrophoretically. Secondly, immunoelectrophoresis was performed to gain information concerning the nature of the proteins of postmortem joint fluid. Thirdly, to quantitate the latter results the proteins were separated into six solubility groups by the low temperature-low salt-ethanol procedure, Fractions I-VI, and further characterized.

MATERIAL AND METHODS

The synovial fluid specimens were aspirated within 5 to 10 minutes after death from knee joints of patients clinically free of joint disease. The specimens were collected without anticoagulant; they appeared clear, *i.e.*, no clot was observed. They were kept at -30° C. until enough material had been obtained to start investigation. The volume, protein concentration and cholesterol content of individual and pooled samples were measured (1). No attempt was made to determine the exact degree of edema, although the largest volumes of fluids were obtained from patients with probable edema.

To render possible the characterization on the synovial fluid proteins, prior to analysis all joint fluid samples were incubated at 37° C. for a two hour period with 0.2 ml. of bovine testicular hyaluronidase solution per 1 ml. of fluid (3.0 mg. of enzyme dissolved in 1.2 ml. of 0.15 M NaCl solution; the enzyme preparation contained

1,050 turbidity reducing units per mg.) (1, 2). For the extremely viscous samples,³ designated as gel-like in Tables I and II, the incubation time had to be extended to three hours because the viscosity was reduced at a much lower rate. As shown earlier (2), hyaluronidase does not alter the plasma or synovial fluid proteins and does not influence their analyses.

Immunoelectrophoresis has recently been described in detail (1, 5-7). The synovial fluid specimens used for the present analysis and listed in Table I were digested separately with hyaluronidase, combined and lyophilized. The residue was dissolved in 2.5 ml. of distilled water, dialyzed against water at 2° C. for 8 hours to remove most of the inorganic salt and further dialyzed against pH 8.6, $\Gamma/2$ 0.07 borate buffer for 17 hours. The resulting solution which had a volume of 4.0 ml. and a protein concentration of 7.5 per cent was then employed for immunoelectrophoresis.

The procedure for the fractionation of the proteins, based on the low temperature-low salt-ethanol method in conjunction with the use of heavy metal ions, has also been described in detail (2). The joint fluid samples employed for these investigations had been aspirated from 40 knee joints and were digested individually with a total of 17 mg. of hyaluronidase dissolved in 15 ml. of 0.15 M NaCl solution. They were pooled with a resulting volume of 114 ml. corresponding to 2.4 ml. per knee joint. This series showed a large variation in the individual fluid volumes (0.5 to 6.0 ml. per joint) and in the color (from almost colorless to dark brown). Most of the samples had a high viscosity as judged by their gel-like appearance. Two samples were of medium viscosity and only one exhibited a low viscosity. For the fractionation of the proteins 80 ml. of the digested fluid was used. The methods employed for the subsequent characterization of the synovial fluid proteins were the same as those applied previously (1). Normal human plasma was fractionated simultaneously to make possible a comparison of the proteins of these two body fluids.

RESULTS

I. General observations

The frozen synovial fluid specimens appeared turbid when thawed and, following enzymatic di-

³ The viscosity of the postmortem synovial fluid specimens was estimated roughly. The exact viscosity of such fluids had been described earlier (3, 4).

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TABLE I
*Synovial fluid specimens used for immunoelectrophoresis **

Case no.	Age yrs.	Diagnosis	Volume of fluid from one knee† ml.	Fluid taken
1	82	Myocardial infarct	0.9	Premortem
2	70	Cancer of lung	1.2	Postmortem
3	81	Thrombosis	1.2‡	Premortem
4	58	Myocardial infarct	0.2	Two mins. after death
5	68	Multiple cerebral vascular accident	1.0	Premortem
6	65	Septicemia	1.5	Premortem

* All fluids were straw-yellow and of medium viscosity.

† Although the fluid from both knees was used for this experiment, the average volume of the fluid from both knees is given here in order to compare these values with those reported by others (3, 4).

‡ This sample included the fluid from one elbow joint.

gestion, yielded insoluble material. The joint fluid samples listed in Table II were divided in six groups arranged according to increasing estimated viscosity. It appears that with decrease of fluid volume, there was an increase in protein concentration ranging from 0.9 to 2.9 per cent. A similar observation was made with respect to the cholesterol content inasmuch as the latter value increased with increasing protein content as judged by the pooled samples. The cholesterol content of the specimens listed in Table II, which was found to be significantly lower than that of pathological or traumatic joint fluid (1), varied from 5 to 14 mg. per 100 ml. of fluid. In addition, it should be noted that the fluid samples listed in Table II showed a relatively constant cholesterol-protein ratio of only 6 mg. per gram as compared with a value of 23 to 32 mg. per gram of normal human plasma proteins.

For comparative purposes the cholesterol content of pooled bovine synovial fluid which was taken from the astrogalotibial joints of young steers⁴ immediately after their death was also studied. The protein concentration was 1.2 per cent, and the cholesterol content was found to be 16 mg. per 100 ml., or 13 mg. cholesterol per gram of protein.

II. Specific results

A. Electrophoretic investigation. The electrophoretic distribution of the proteins of postmortem

synovial fluid, as observed under standard conditions (pH 8.6, 1/2 0.1 diethyl-barbiturate buffer), indicated that of proteins present, the relative proportion of albumin was higher in joint fluid than in normal plasma (Table II). The relative increase was most pronounced with fluids of patients from whose joints only a small volume of fluid could be aspirated (Group 6, Table II). Furthermore, the presence of α_1 - and α_2 -globulins as well as the other electrophoretic groups of globulins corresponding to those of plasma were observed consistently (8, 9). The relative concentration of these proteins could easily be determined since the analyses were performed on specimens which had been digested with hyaluronidase (Tables II and III). In undigested specimens the high viscosity produced by hyaluronic acid blurred the electrophoretic patterns sufficiently so as to make quantitative measurements difficult.

Typical of all fluids reported in Tables II and III was the relatively low concentration of the α_2 -globulins which was consistently lower than that of the α_1 -globulins (1, 10) in contrast to the distribution of the proteins of normal plasma. The electrophoretic albumin-globulin ratio ranged from 1.4 to 2.1, as compared with a value of 1.2 for normal human plasma. Electrophoresis at pH 4.5 suggested the presence of orosomucoid (11, 12) and certain α_2 -glycoproteins distinguished by their low molecular weights (13).

B. Immunoelectrophoretic investigation. The average protein concentration of the synovial fluid specimens employed for the immunoelectrophoresis was 2.7 per cent and the average volume 1.0 ml. per knee joint (Table I). The immunoelec-

⁴ These animals had been used for a study on growth at the Iowa State College for Agriculture and Mechanic Arts, Ames, Iowa.

TABLE II
*Electrophoretic study of postmortem synovial fluid **

Group	Case	Age yrs.	Diagnosis	Volume of fluid from one knee ml.	Color of the fluid	Rough estimate of the viscosity of the fluid	Protein concentr. (Biuret) %	Choles- terol content mg./ml. fluid	Ratio of cholesterol: protein mg./Gm.					Protein conc. %	A/G ratio (electro- phoretic)	Electrophoresis at pH 4.5 (relative percentage)			
									Alb.	α_1	α_2	β_1	β_2						
1	Av	74	Carcinoma of the prostate, metastases	3.7	Colorless	Low	1.0	0.04†	61	8	5	11	4	11	1.6	3	4	93	
2	Da	48	Hepatic coma (alcoholic)	5.4	Yellow	Medium	0.9	0.03†	62	4	2	9	4	19	1.6	1	4	95	
3	Yo	70	Myocardial infarct	3.9	Light yellow	Medium	1.0	0.05	62	6	5	15	3	9	1.6	4	3	93	
4	Lo Co Ba Si	37 75 17	Hypertension Broncho- pneumonia Septicemia Cerebral vascular accident (C.V.A.)	1.9 0.8 1.6 1.4	Colorless Light yellow Bright yellow Colorless	Medium	1.4	0.09	58	10	5	11	3	13	1.4	5§ 4 91			
5	Mc	48	Hepatic coma, cirrhosis	3.0	Yellow	Almost gel-like	0.9		62	6	5	15	3	9	1.6				
6	Le Wa Er Fo Mw	85 66 52 50 55	Carotid occlusion C.V.A. C.V.A. C.V.A. Uremia	1.7 ¶ 0.2 0.6 0.3 ¶ 0.2	Yellow	All gel-like	2.9	0.14	68	4	2	13	4	9	2.1	2	4	94	

* All values are corrected for the dilution due to the addition of hyaluronidase solution.

† Again the average fluid volume of both knees is quoted, although for the experiments the total fluid is used. Some of the fluids from different patients were pooled as indicated by the brackets.

‡ Reading on Coleman below 0.09.

§ After electrophoresis at pH 8.6, combined and rerun at pH 4.5.

|| Contained bilirubin and was taken 3.5 hours after death.

¶ Taken premortem.

TABLE III
*Fractionation of pooled postmortem synovial fluid from patients clinically free of joint disease**

Fraction	Total protein† mg.	Total hexose		Electrophoresis at pH 8.6 (expressed in relative percentage)						Electrophoresis at pH 4.5 (relative percentage)	S ₂₀ , w†	Total albumin-HBABA‡ mg.	Total β-metal binding protein mg. %	Total ceruloplasmin mg.	Total anti-streptolysin-O units	Total diphtheria anti-toxin units	Titer of isoagglutinins
		mg.	%	Alb.	α ₁	α ₂	β ₁	β ₂	Fibr.	γ							
80 ml. pooled postmortem synovial fluid	1,200 (1.5%)			57	8	4	18	3	10			880			960-1,280	24-80	{ anti-A 1:8 anti-B 1:4
I	69	0.6	1.7														
II	0.1																
III	430	13.4	3.1	11	7	15	32		35		8.2; 6.6						
IV	272	5.9	2.2	83	11	2	4				14; 6.8	230	10	15	1,280	8-40	{ anti-A 1:16 anti-B 1:16
V	450	5.8	1.2	91	4	2	3				4.6	253					
											4.3 ¶						
VI	110	8.6	7.8	27	46	8	18	1			3.2 ¶, **						
20 ml. acid-citrate-dextrose-normal plasma	1,400 (7.0%)			53	5	10	15	7	10			680					
I	70	2.0	2.8	8	2	5	79	6			16; 6.4						
II	170	3.2	1.9	3	2	8	5	82			6.8 ¶						
III	234	8.7	3.7	3	8	41	31	9	8		13.2; 7.0			14			
IV	88	3.3	3.7	49	21	15	12	3			4.6	390	1.0				
V	628	2.5	0.4	93	2	1	4				4.1 ¶		18				
VI	22	2.0	9.1	38	31	15	12	4			3.3 ¶						

* Obtained from patients with various diseases. The relatively large volume of some of the joint fluids indicated varying degree of edema. For the determination of the biological activities an aliquot of Fraction III was dialyzed at 2° C. against 0.15 M NaCl to remove the ethanol. The insoluble material was then centrifuged off and 1 ml. of the supernatant solution adjusted to the original fluid volume was used for each test. Electrophoresis of Fraction VI at pH 8.6 and 4.5 was carried out on paper.

† Biuret.

‡ In 0.15 M NaCl at a protein concentration of approximately 1 per cent.

§ HBABA refers to determination of albumin by the "2-(4'-hydroxy-benzene-azo)-benzoic acid" method (22).

¶ Includes 22 mg. of insoluble material obtained prior to fractionation.

** Sedimentation constant of heavier component not indicated.

** A component with smaller sedimentation constant was observed.

trophoretic analysis revealed the following results: The number, intensity and relative position of the precipitin lines which were essentially the same for both synovial fluid and normal human plasma suggested that most of the proteins of postmortem synovial fluid are, with respect to many properties, very similar to those of plasma. This similarity of the γ -globulins of joint fluid to those of plasma was further proven by the closing of the corresponding precipitin zones, which is indicated in Figure 1 by the arrow on the left. The blood serum analysed simultaneously with the synovial fluid showed an additional unidentified component in the β_2 -globulin region. Most of the postmortem as well as the traumatic synovial fluids showed a

prealbumin component which is probably identical with that of serum (14, 15).

This method, of course, would only identify proteins present in plasma and any proteins not present in plasma would remain undetectable. However, if an antiserum against normal human joint fluid is used in future studies, possible components produced solely by the synovial tissue might be detected.

C. Fractionation and characterization of the proteins of postmortem synovial fluid. The protein concentration of the pooled joint fluid used for these experiments (Table III) was 1.5 per cent and averaged 1.7 per cent with respect to the undigested fluids. This finding suggests again

FIG. 1. IMMUNOELECTROPHORESIS OF POSTMORTEM HUMAN SYNOVIAL FLUID (a) AND NORMAL HUMAN SERUM (b)

The pooled postmortem joint fluid specimens (Table I) were treated with hyaluronidase prior to electrophoresis. "A" points to the position of application. Electrophoresis was carried out in borate buffer, pH 8.6, $\Gamma/2$ 0.07, containing 2 per cent agar, for 17 hours at 180 V. and 40 ma. The arrow on the bottom indicates the direction of the electrophoretic movement of the proteins towards the anode. After electrophoresis the troughs between the agar sections indicated by arrows on the right were filled with horse serum against human serum. Therefore, the precipitin zones formed symmetrical patterns. According to this analysis the joint fluid is devoid of a strongly antigenic protein of the β_2 -globulins which is clearly visible in the picture of the serum. The γ -globulins of synovial fluid and normal serum produced precipitin lines which formed a closed system (not visible), the position of which is indicated by the arrow on the left.

that some of the specimens had been obtained from patients with edema. The ultraviolet absorption curve showing a maximum at 280 $m\mu$ and a minimum at 255 $m\mu$ was characteristic of proteins. There was no evidence of nucleic acid in the absorption spectrum. Electrophoretic analysis indicated a relative albumin content of 57 per cent and an albumin-globulin ratio of 1.3. The cholesterol content was 0.24 mg. per ml. or 16 mg. per gram of protein. The discrepancy between the cholesterol content of this series and that of the specimens used for the electrophoretic investigation can perhaps be explained by a relatively increased permeability of the lipoproteins through the synovial tissue due to edema. Further studies on normal synovial fluid are required to corroborate these findings.

The insoluble material of the pooled fluids obtained after enzymatic digestion and unavoidable repeated freezing was centrifuged and washed. It amounted to 34 mg. or 2 per cent with respect to the total amount of protein and contained 5.4 per cent of protein-bound hexose as determined by the orcinol method of Sørensen and Haugaard (16), 1.0 per cent of glucosamine measured by the method of Rimington (17), and less than 0.2 per cent of uronic acid determined by the method of Fishman and co-workers (18). It was concluded that this material did not contain significant amounts of hyaluronic acid breakdown products and represented, at least in part, denatured protein.

As judged by the characteristics of the protein fractions [the distribution of the cholesterol between Fractions III and IV; amount, purity and electrophoretic mobility of the γ -globulin in Fraction II; low content of albumin in Fraction IV and high purity of the albumin in Fraction V (1, 2)] derived from normal human plasma that had been fractionated simultaneously with the synovial fluid, it must be concluded that the deviations observed with these synovial fluid protein fractions (γ -globulins not extractable as Fraction II, relatively large amount of Fraction VI) did not stem from the fractionation process, but were inherent in the synovial fluid itself.

The characterization of the synovial fluid proteins based upon the investigations of the six protein fractions revealed the following results: The distribution of the proteins among the fractions was similar to that of normal human plasma. The

hexose content of the corresponding fractions was the same within the error of the method. The hexose content of Fraction V was low and that of Fraction VI high as found previously in the corresponding fractions of pathological fluids (1).

Fraction I dissolved readily at room temperature in the appropriate salt solution (1) and remained clear for at least 14 hours, *i.e.*, no clot was formed. Upon prolonged cooling (17 hours) at 2° C. insoluble material separated which dissolved easily when the suspension was warmed to room temperature. This solubility property is characteristic of the so-called "cold insoluble" globulin (19). The γ -globulins which usually represent the major component of *Fraction II* could not be extracted from Fraction II plus III probably because of the presence of only a relatively small amount of β_1 -lipoprotein which, in addition, had perhaps been partially denatured by the process of repeated freezing and thawing. Indeed, the γ -globulins remained in *Fraction III* as judged by electrophoretic analysis and from the results of the determinations of the antistreptolysin-O and diphtheria antitoxin content. The antistreptolysin-O content amounted to 12 to 16 units per ml. of the digested pooled synovial fluid, a value which, after correction for the protein concentration, is essentially the same as that found for pooled, normal human plasma (76 to 80 units per ml.) collected in the New England area. The isoagglutinin titer of Fraction III suggested that these protein components were precipitated completely in Fraction III. As expected, ceruloplasmin and the larger part of the cholesterol content were also rendered insoluble in this fraction. Recent investigations on the distribution of cholesterol between α_1 - and β_1 -lipoproteins in pathological, traumatic and post-mortem synovial fluid, involving the separation of the two groups of lipoproteins by flotation technique (20) and by paper electrophoresis (21), suggested a ratio of the α_1 - to the β_1 -lipoproteins of approximately 1:3, which is similar to that found in blood plasma. Thus, the cholesterol distribution reported in Table III is affected by the fact that some of the α_1 -lipoproteins, usually rendered insoluble in Fraction IV, had probably been denatured and were, at least partly, precipitated earlier in Fraction III. Immunochemical analysis of Fraction III using the Ouchterlony technique revealed precipitin zones very similar to

FIG. 2. OUCHTERLONY PLATES OF THE PROTEIN FRACTIONS FROM POSTMORTEM SYNOVIAL FLUID

In all plates Reservoir 1 contained the synovial fluid protein fraction to be analysed. Reservoir 2 was filled with the corresponding fraction derived from normal human plasma. Reservoir 3 contained β_1 -metal combining protein. Reservoir 4 normal human plasma and the center reservoir horse serum against human serum. For the sake of clarity a sketch of each figure is given on the right hand side.

Fig. 2a—Fraction IV. After a diffusion time of two days a picture similar to Figure 2b was observed and is, therefore, not included here. After a diffusion time of nine days, Figure 2a was obtained.

Fig. 2b—Fraction V. Picture taken after a diffusion time of two days. The heavy line close to the center is due to serum albumin.

Fig. 2c—Fraction V. Picture taken after a diffusion time of nine days. The precipitin zone due to albumin in the center reservoir and no longer visible. Additional precipitin lines partly seen in Figure 2a formed now closed systems.

Fraction III was analyzed by the same method. After nine days six closed systems of precipitin lines were noted. The picture appeared similar to that of Fraction III of arthritic synovial fluid which has been reproduced in the previous publication (1).

those of the corresponding fraction of certain pathological joint fluids (1) and suggested the identity of the synovial fluid proteins with those of normal human plasma. *Fraction IV* was analyzed by the same technique. The formation of closed systems of precipitin lines (Figure 2a) again strongly suggested the identity of these proteins with those of normal human plasma. The ca-

capacity of the albumin present to bind 2-(4' hydroxybenzeneazo-) benzoic acid (HBABA) (22) supported the latter finding (Table III). On the basis of the results obtained by fractionating postmortem and traumatic synovial fluid and normal and pathological human plasma, no indication could be obtained as to whether a particular α_2 -globulin present in Fraction III or IV of plasma

FIG. 3. PAPER ELECTROPHORESIS OF FRACTION VI DERIVED FROM POSTMORTEM HUMAN SYNOVIAL FLUID

Dark lines indicate the position of application. Electrophoretic movement of the proteins was from left to right towards the anode. Staining was done with Amidoschwarz 10B. For comparison Fraction VI of normal human plasma designated as "Plasma" was analyzed simultaneously. Top pictures represent the analysis at pH 8.6 in diethyl-barbiturate buffer, $\Gamma/2$ 0.1. Bottom pictures represent the analysis in pH 4.5 acetate buffer, $\Gamma/2$ 0.1. Due to the flow of buffer in the direction of the electric movement the albumin which is essentially immobile under the latter conditions was displaced to the right. Fraction VI contains albumin, low molecular weight acidic α_1 - and α_2 -glycoproteins and β_1 - and β_2 -globulins (11-13). The designations on the top and bottom pictures, Alb, α_1 , α_2 , β_1 , and β_2 , refer to the same proteins.

may have preferentially been excluded during the passage of the blood proteins through the synovial tissue. *Fraction V* was of yellow color, in contrast to the colorless quality of the corresponding plasma fraction, and consisted for the most part of albumin as judged by its solubility, sedimentation constant, electrophoretic mobility and relatively low content of protein-bound hexose. The relative capacity of the albumin present in this fraction to bind HBABA was 0.7. The corresponding value of *Fraction IV* was 1.0 and that of the pooled digested synovial fluid 1.3 as compared with a value of 1.0 for pure human serum albumin. The found deviations in the albumin determination obtained by this dye-binding method have already been reported (1) and were not significant as could be shown with known artificial protein mixtures. Immunochemical analysis of this fraction by the Ouchterlony method (Figures 2b and 2c) strongly supported the finding of the identity of the synovial fluid proteins with those of normal plasma. *Fraction VI* was composed of 110 mg. and was approximately fivefold the weight of the corresponding fraction derived from the same amount of normal plasma proteins. In addition paper electrophoresis at pH 8.6 and 4.5 (Figure 3) indicated a relatively increased amount of orosomucoid (11) and certain β_1 -globulins and a relatively low content of the low molecular weight α_2 -glycoproteins (13). Again a yellow color was noted in this fraction—thus, by the method of fractionation the proteins of postmortem joint fluid proved to be identical with those of blood plasma.

DISCUSSION

The characterization of the proteins of postmortem synovial fluid was carried out essentially in the same manner as presented in an earlier paper on the characterization of the proteins of pathological joint fluid (1). It was based to a large extent on methods which measure general and specific properties of blood plasma proteins. From the results obtained during this study it was concluded that essentially the total amount of the synovial fluid proteins is derived from blood plasma as judged by the employed criteria.

In the present investigation certain properties of postmortem synovial fluid reported by others were confirmed. The volume of fluid which could

be aspirated from the knee joints of patients free from joint disease and edema, was found to vary within the range reported earlier by Ropes, Rossmesl, and Bauer (3) and Coggeshall, Warren, and Bauer (4). The protein concentration of the fluid samples listed in Group 6 of Table II agrees with the corresponding value reported by Ropes and co-workers for postmortem (3) and Platt, Pigman, Holley, and Patton for normal human synovial fluid (8). For comparison it is of great interest to observe that the protein content of the interstitial fluid of muscle (23) and tissue fluid (24) is approximately the same. Most synovial fluid samples investigated during this study showed that the relative proportion of albumin was increased over that of certain pathological joint fluids (1, 10) and that of normal human plasma. A similar increase has previously been noted in traumatic synovial fluid (1, 10). However, the fluid volume found in traumatic arthritis is severalfold larger and the viscosity considerably lower than the corresponding values of postmortem joint fluid from patients free of joint disease. Further, the significantly elevated albumin-globulin ratio, particularly of the specimens of Group 6 in Table II, agrees with that of normal human synovial fluid (8), although it is difficult to evaluate exactly the significance of the percentage of albumin since some of our cases included patients with diseases often characterized by a lowering of the percentage of albumin in the blood. However, it is known that albumin which has a molecular weight lower than that of the average globulins, but approximately the same shape, permeates into the tissue about one and one-half times faster than the latter (24). The much more pronounced relative increase of α_1 -acid glycoprotein (orosomucoid) concentrated in *Fraction VI* and characterized by an even lower molecular weight (45,000) but the same shape, is expected on the basis of these considerations. Furthermore, it is interesting to note that such fluids showed the calculated content of γ -globulin of 9 per cent (2).

The demonstration of hitherto unknown properties of postmortem synovial fluid, the consistent presence of the α_1 - and α_2 -globulins, the relative concentration of the α_2 -globulins being consistently lower than that of the α_1 -globulins, and of the α_1 - and β_1 -lipoproteins and of the severalfold increased amount of *Fraction VI*, are significant for

the characterization of joint fluid. Postmortem joint fluid is further characterized by the small quantity which can be aspirated from one knee joint (average, 1.0 ml), its gel-like appearance, the high protein concentration (3 per cent), and the low cholesterol-protein ratio (6 to 16 mg. per Gm.) and by the normal content of γ -globulin (9 per cent).

The results of our study further support the general finding that considerable amounts of plasma proteins of the body are in the extravascular system. Gitlin, Landing, and Wipple (25) recently demonstrated the presence of plasma proteins in various organs and tissues and, lately, Humphrey, Neuberger, and Perkins (26) reported on the presence of these proteins in skin and tendon.

SUMMARY

The proteins of postmortem synovial fluid from patients clinically free of signs or symptoms of joint disease have been characterized. As far as investigated they were found to be identical with those of blood plasma. The total amount of synovial fluid protein was essentially accounted for. This conclusion was based on the results obtained by the following methods: electrophoresis, immunoelectrophoresis, low temperature-low salt-ethanol fractionation and investigation of the obtained protein fractions by ultracentrifugation, electrophoresis, immunochemical analysis and for ion-binding capacity and other biological activities. Components produced solely by the synovial tissue are not excluded.

Taking normal human plasma as baseline, post-mortem synovial fluid was further characterized by the following differences: relatively low protein concentration, decreased but distinct content of α_1 - and β_2 -lipoproteins, relatively increased content of albumin and orosomucoid, consistent presence of the α_1 - and α_2 -globulins, and the concentration of α_2 -globulins being consistently lower than that of the α_1 -globulins. Moreover, post-mortem joint fluid contains a prealbumin component.

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ADDENDUM

The low molecular weight components noted in Fraction VI of postmortem synovial fluid have been further studied. On ultracentrifugal analysis a single peak with a sedimentation constant of approximately 1S was observed. As judged by the estimated, relatively high diffusion constant, the molecular weights of these constituents are probably below 15,000. Electrophoretic analysis (Tiselius apparatus) in pH 8.6, $\Gamma/2$ 0.1 barbiturate buffer of Fraction VI derived from hyaluronidase digested synovial fluid of young steers revealed four prealbumin components. Their respective electrophoretic mobilities were 13, 10, 9 and 8×10^{-5} cm.²/Volt·sec. These substances were resistant to hyaluronidase, were not metachromatic, and did not bind protein stains. It is important to emphasize that these compounds did not occur in pathological synovial fluid.

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