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PROTEIN CONCENTRATION OF OEDEMA FLUID IN RHEUMATOID ARTHRITIS*

PART I

BY

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The protein content of oedema fluid depends on several factors, including vascular permeability, serum protein level, lymphatic clearance, venous pressure, and tissue tension. Recently, Park and Swinburne (1964) reported that oedema fluid obtained from the feet and ankles of patients with rheumatoid arthritis contained less than 1 g. per cent. protein, and they suggested that it was a transudate. This was at variance with the experience of Bywaters (1948), who found a high protein content in the neighbourhood of the swollen and inflamed joints of rheumatoid arthritis; the tissue fluid clotted and contained over 2 g. per cent. protein compared with less than 1 g. per cent. in cardiac or renal oedema. Some of his cases showed an inflammatory oedema for some months before the joints became obviously involved.

The present investigation was, therefore, undertaken with particular reference to cases showing an active arthritis as judged by local heat and soft tissue swelling. Inferences about the nature of the oedema in active arthritis may be drawn more easily in the hand than in the foot, because of the higher hydrostatic pressure present in the latter: fluid from both hands and feet was therefore studied.

Material and Methods

Oedema fluid was obtained from 22 patients (Cases 1-18, 21-23, 31) suffering from classical or definite rheumatoid arthritis (American Rheumatism Association criteria; Ropes, Bennett, Cobb, Jacox, and Jessar, 1958), and two (Cases 19 and 20) from Still's disease (criteria as cited, Ansell and Bywaters, 1959). Nine patients with oedema from a variety of causes served as a control group, making a total of 33 subjects. Before sampling the oedema fluid, an independent assessment of the nature of the case and the state of activity of the underlying joints was made by one of us (B.M.A.).

Specimens were collected from dependent limbs whenever possible, and preferably after the patient had been up and walking about for several hours. To obtain fluid, an area of oedematous limb was cleaned with methylated spirit and dried with sterile wool. A No. 1 needle attached to a 2-ml. syringe was inserted into the subcutaneous tissues and by a combination of gentle expression and aspiration, small quantities (up to 0·5 ml.) of oedema fluid were transferred to a plastic bottle containing solid EDTA or to a plain glass bottle. In some cases, further expression samples were taken through the channel left on withdrawing the needle and beads of fluid were transferred to a bottle by a needle and syringe.

Samples macroscopically contaminated with blood were rejected. Red blood cell counts were made on all specimens which were analysed; they were usually less than 1,000 $mm.^{-3}$, although a few were as high as 5,000 $mm.^{-3}$. A tiny cobweb clot was observed in almost every specimen. The protein content was estimated by a Biuret method (Wolfson, Cohn, Calvary, and Ichiba, 1948), giving values correct to $\pm$ 0·2 g. per cent. Serum protein levels were also determined and the urine was examined for protein.

Results

The protein content of the thirteen samples of oedema fluid obtained from the feet of nine patients (Cases 1 to 9) considered to have active arthritis of the feet and ankles varied from 0·9 to 2·8 g. per cent. (mean 1·6), while that from the same site in seventeen samples from twelve patients (Cases 10 to 20 and 31) in whom the arthritis was considered to be quiescent, varied from 0·2 to 1·2 g. per cent. (mean 0·7).

Table I (opposite) shows that twelve out of thirteen samples from the former group were above 1 g. per cent., whereas sixteen out of seventeen of the inactive group were 1 g. per cent. or less. Among the nine considered to have active arthritis, six were receiving salicylate, two phenylbutazone, two corticosteroids, and two nothing. Of the twelve whose arthritis was considered to be inactive

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TABLE I

PROTEIN CONTENT OF OEDEMA FLUID FROM THE LOWER EXTREMITIES IN ACTIVE AND INACTIVE RHEUMATOID ARTHRITIS OF THE FEET AND ANKLES

Active Arthritis (9 cases)						Inactive Arthritis (12 cases)					
Case No.	Protein (g. per cent.)	Therapy* A	B	C	Associated Features	Case No.	Protein (g. per cent.)	Therapy A	B	C	Associated Features
1	2·0	−	−	−	—	10	1·0	−	+	−	Impaired mobility
2	R. 1·4 L. 1·2	+	−	−	—	11	0·6	−	−	−	Impaired mobility
3	R. 1·5 L. 1·6	+	−	−	—	12	0·5	+	−	+	Impaired mobility
4	1·2	+	−	−	Treated congestive heart failure	13	R. 0·5 L. 0·7	+	−	+	Impaired mobility
5	R. 1·1 L. 1·6	−	−	−	Impaired mobility	14	R. 0·8 L. 0·8	+	−	−	Impaired mobility
6	R. 2·2 L. 1·9	−	+	−	—	15	0·8	−	+	−	Impaired mobility
7	2·8	+	−	−	Old poliomyelitis	16	1·0	−	+	−	Congestive heart failure
8	0·9	+	+	+	Diuretics	17	R. 0·9 L. 0·9	+	−	−	Congestive heart failure
9	1·3	+	−	+	Recent fracture	18	1·2	−	+	−	Vasculitis
						19	R. 0·2 L. 0·2	+	−	+	Amyloidosis
						20	R. 0·3 L. 0·2	+	−	+	Amyloidosis
						31	R. 0·8	−	+	−	Varicose veins
Mean	1·6 (0·9-2·8)					Mean	0·7 (0·2-1·2)				

*A = Salicylate B = Phenylbutazone C = Corticosteroid

locally, six were receiving salicylate, five phenylbutazone, four corticosteroids, and one nothing.

The nine controls (Cases 24-30, 32, 33) consisted of four patients (24, 25, 32, 33) with congestive cardiac failure, two (26 and 27) with degenerative joint disease complicated by impaired mobility and varicose veins respectively, one (28) with oedema of the foot following phenylbutazone given for a mild traumatic lesion, one (29) with inactive ankylosing spondylitis and varicose veins, and one (30) with chronic bilateral oedema of the legs of unknown origin with a superimposed inflammatory skin lesion over the left calf. Twelve oedema fluid samples were obtained from the feet in this group (Table II, overleaf) and the protein content was found to range from 0·2 to 1·9 g. per cent. (mean 0·8).

Severe oedema of the hand in active rheumatoid arthritis is well recognized (Fig. 1, overleaf). The protein content of such oedema fluid in four cases (1, 21, 22, 23) was found to be very much higher than that from the legs, varying from 3·4 to 6·5 g. per cent. (mean 4·5). (Table III, overleaf.)

In five patients (Cases 1, 5, 14, 15, 20), serial samples taken through the same puncture wound showed no significant difference (Table IV, overleaf). In Case 1 it was possible to obtain several samples of oedema fluid from the dorsum of a foot of a patient who was taking phenylbutazone, which had caused an apparent increase in oedema. Despite therapy, there was continued activity of the arthritis and the fluids had a lower protein content than previously (Fig. 2, overleaf), presumably because of fluid retention secondary to therapy.

Serum protein levels were normal in every patient except in Cases 19 and 20, who had proteinuria and hypoproteinaemia due to renal amyloidosis; these two patients alone had proteinuria.

Discussion

Crockett (1956) divided oedema fluids due to single causes into those of low protein content containing less than 1 g. per cent., and those containing protein above this figure. Low protein fluids result from disturbances in the balance of the Starling cycle of

TABLE II

PROTEIN CONTENT OF OEDEMA FLUID FROM THE LOWER EXTREMITIES IN INACTIVE RHEUMATOID ARTHRITIS COMPARED WITH THAT FROM OTHER CAUSES

Inactive Arthritis (12 cases)						Other Diseases (9 cases)			
Case No.	Protein (g. per cent.)	Therapy*			Associated Features	Case No.	Diagnosis	Protein (g. per cent.)	Associated Features
		A	B	C					
10	1·0	−	+	−	Impaired mobility	24	Congestive heart failure	0·4	Untreated
11	0·6	−	−	−	Impaired mobility	25	Congestive heart failure	0·9	Diuretics
12	0·5	+	−	+	Impaired mobility	26	Degenerative joint disease	R. 0·5 L. 0·6	Impaired mobility
13	R. 0·5 L. 0·7	+	−	+	Impaired mobility	27	Degenerative joint disease	L. 0·6	Varicose veins
14	R. 0·8 L. 0·8	+	−	−	Impaired mobility	28	Post-traumatic	0·4	Phenylbutazone
15	0·8	−	+	−	Impaired mobility	29	Inactive ankylosing spondylitis	R. 1·0 L. 0·6	Varicose veins
16	1·0	−	+	−	Congestive heart failure	30	Inflammation of leg	R. 1·6 L. 1·9	Bilateral chronic oedema
17	R. 0·9 L. 0·9	+	−	−	Congestive heart failure	32	Congestive heart failure	1·2	Diuretics
18	1·2	−	+	−	Vasculitis	33	Congestive heart failure	0·2	Diuretics
19	R. 0·2 L. 0·2	+	−	+	Amyloidosis (Still's disease)				
20	R. 0·3 L. 0·2	+	−	+	Amyloidosis (Still's disease)				
31	0·8	−	+	−	Varicose veins				
Mean	0·7 (0·2-1·2)					Mean	0·8 (0·2-1·9)		

*A = Salicylate B = Phenylbutazone C = Corticosteroid

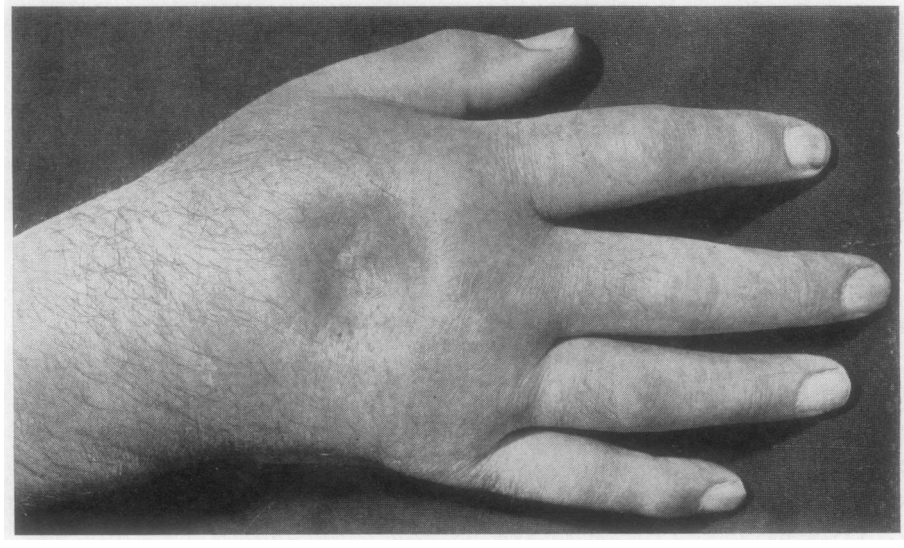

Fig. 1.—Pitting oedema of the hand in acute rheumatoid arthritis (Case 1).

TABLE III

ROTEIN CONTENT OF OEDEMA FLUID IN FOUR CASES OF ACTIVE ARTHRITIS OF THE HANDS

Case No.	Protein (g. per cent.)	Associated Features
1	3·4	—
21	4·6	—
22	4·0	—
23	6·5	Impaired mobility
Mean	4·6 (3·4-6·5)	

capillary changes. In hypoproteinaemia, oedema fluid proteins range from 0·1 to 0·3 g. per cent. A rise in intracapillary pressure, as in congestive cardiac failure, produces a more concentrated fluid from 0·3 to 0·5 g. per cent., and in venous oedema associated with venous thrombosis, in which there is also an element of increased capillary permeability, values of from 0·6 to 0·9 g. per cent. occur. High protein fluids may result from impaired lymphatic clearance (1 to 4 g. per cent.), the actual value depending on whether lymphatic insufficiency be due to interrupted pathways, functional failure due to muscle disuse, or the congenital abnormalities of idiopathic lymphoedema. The value increases with the duration of the oedema. Inflammatory lesions, burns, and allergic reactions give the highest levels, which in severe cases may reach 4 to 6 g. per cent. In the erect subject interstitial fluid protein concentration diminishes from above downwards. Accordingly, when oedematous legs are elevated, the protein concentration therein rises because of the redistribution of water.

From our results, clinically active arthritis in the feet is associated with oedema of higher protein concentration (mean 1·6 g. per cent.) than inactive arthritis (mean 0·7 g. per cent.). In the only patient considered active in which where the level was less than 1 g. per cent., four drugs, namely salicylate, phenylbutazone, corticosteroid, and a diuretic, all of which might affect the quantity and/or concentration of the oedema, were being taken: in all other Cases the levels were above 1 g. per cent.

In patients with inactive arthritis, in which aetiological factors such as impaired mobility, congestive

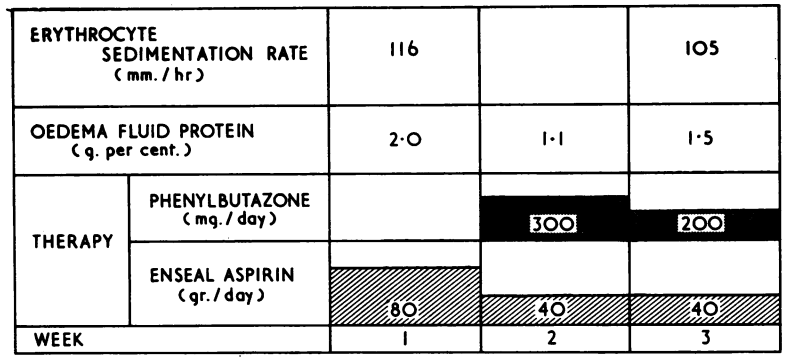

Fig. 2.—Variation in protein content of oedema fluid when phenyl-butazone therapy was followed by an increase in oedema of the feet (Case 1).

TABLE IV

PROTEIN CONTENT IN SERIAL SAMPLES OF OEDEMA FLUID FROM THE LOWER EXTREMITIES IN TWO CASES OF ACTIVE AND THREE CASES OF INACTIVE ARTHRITIS

Case No.	Sample No.	Protein (g. per cent.)	Arthritis	Associated Features
1	I II III	1·5 1·5 1·5	Active	Phenylbutazone
5	I II	1·0 1·2	Active	Impaired mobility
14	I II	1·1 0·8	Inactive	Impaired mobility
15	I II	0·8 0·7	Inactive	Impaired mobility
20	I II	0·2 0·3	Inactive	Amyloidosis

cardiac failure, amyloidosis, etc. were considered important, the mean protein content (0·7 g. per cent.) closely resembled that in the controls (0·8 g. per cent.). The protein content of the two patients in which rheumatoid arthritis was complicated by a nephrotic syndrome due to amyloidosis was similar to that reported by Freeman and Joekes (1957) in uncomplicated nephrosis, while the findings in our cases considered to have congestive cardiac failure are similar to those reported by Hammond and Ross (1960). Two patients, one with inactive arthritis and one control (Cases 18 and 30) had a protein content greater than 1 g. per cent.; the patient with rheumatoid arthritis (Case 18) had chronic bilateral oedema and had been confined to bed for several days before sampling because of gangrene of the other foot, while the control patient (Case 30) had chronic bilateral leg oedema of undetermined origin and had been confined to bed because of an inflammatory lesion over the left calf; the measured protein level could therefore have been raised by preferential resorption of salt and water.

It is, however, in the hand that more relevant information about the nature of the oedema fluid associated with underlying acute joint inflammation due to the rheumatoid process may be gained. Here, the protein levels are unequivocally of the order seen in an acute inflammatory exudate. The only other cause of such high protein concentrations is chronic lymphatic insufficiency, of which there was no evidence in any of these patients.

No general conclusions may be drawn about the effects of drug therapy in the above series of patients. However, in Case 1, a fall in protein concentration was observed in oedema fluid from the foot after phenylbutazone therapy (see Fig. 2), which had caused an increase in the peripheral oedema, the arthritis remaining extremely active throughout the period of observation.

Oedema may arise from a single cause or there may be multiple factors. In our experience it is almost always of high protein content in the hand, and is due to inflammatory joint disease, unless there are such rare complicating circumstances as hysterical non-use or surgical interruption of the lymphatic pathways. As would be expected the protein content is lower in the foot, even in active cases. In these, a protein concentration above 1 g. per cent. was usual, suggesting an inflammatory origin, or the oedema might be due to water and salt retention from renal or cardiac disease, to immobility, or to venous or lymphatic obstruction by, *e.g.* knee effusion, or to a combination of such factors: these latter factors may also be operative in patients with inactive arthritis of the feet and ankles. Contrary to the opinion of Park and Swinburne (1964) that oedema fluid in the feet and ankles of patients with rheumatoid arthritis is a transudate, we believe that this oedema fluid may be of inflammatory type and due to the rheumatoid arthritic process. Nor can we agree with the more recent suggestion by Swinburne (1964) that oedema of the feet and ankles in rheumatoid arthritis is always venous in origin. While in some cases there may well be an element of increased venous pressure due to obstruction, other factors already cited are usually of greater importance.

Summary

(1) The protein content of 34 samples of oedema fluid from 24 patients with adult and juvenile rheumatoid arthritis of varying degrees of activity and with various complications were analysed. In four the fluid was obtained from hands which were the site of acute arthritis and in these the mean protein concentration was 4·6 g. per cent. In oedema fluid from the feet, the mean protein level of the thirteen samples from those patients whose arthritis was considered locally active was 1·6 g. per cent., only one being below 1 g. per cent., while that of the seventeen samples from patients with inactive arthritis was 0·7 g. per cent., only one being above 1 g. per cent. This closely resembled the findings in the controls, in which twelve samples showed a mean of 0·8 g. per cent.

(2) In any individual patient with rheumatoid arthritis and oedema, a number of factors may be operative. Unless there are rare complicating circumstances such as hysterical non-use or surgical interruption of the lymphatic pathways, oedema in the hand is almost always due to inflammatory joint disease and the oedema fluid is of high protein content. In the foot, the protein content is lower and, although in active cases the oedema is frequently inflammatory with a protein content above 1 g. per cent., even in these cases other factors which assume more importance in inactive arthritis may be operative, *i.e.* immobility, venous or lymphatic obstruction (*e.g.* by a knee effusion), salt and water retention from cardiac or renal disease, or therapy. The actual concentration measured is the combined effect of varying contributions from these different aetiological factors; inflammation, producing altered capillary permeability, is probably the most important in determining the protein content.

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PART II

BY

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The differential protein patterns in sera and oedema fluids have been examined by quantitative electrophoresis on cellulose acetate.

Material and Methods

Specimens.—The oedema fluids were taken from thirteen of the patients and controls studied in Part I, but were not necessarily the same samples. Details of those concerned are given in Table V. Serum was obtained at the same time as the oedema fluid. Specimens were examined as soon as possible after collection; if not tested on the same day they were stored frozen until required. As described in Part I, red blood cell counts were carried out on all oedema fluid specimens to ensure that blood contamination had not occurred.

Protein Estimations.—The total protein concentration of sera and oedema fluids was determined by the biuret method, which was scaled down to deal with the oedema fluids. Fluid (20-100 μl.), water to 0·5 ml., and biuret reagent (0·5 ml.) were mixed, and extinctions were measured at 540 mμ. in the Uvispek Spectrophotometer, employing microcells of 1 cm. path length and capacity about 0·2 ml. Standard curves were derived from human albumin (Armour and Crookes), range 0·14-2·76 mg./ml. final solution. In three cases in which the amount of oedema fluid was only about 50 μl. the total protein was derived from the electrophoresis strips as described below.

Total Protein from Electrophoresis Strips.—This method was used for those fluids which were too small in amount for analysis by the biuret method. Measured volumes of serum of known protein concentration and of oedema fluid were applied to the membranes and electrophoresis and differential determinations carried out as described below. The oedema fluid protein concentration, C_o, was then calculated from the following expressions:

$$C_o = \left(\frac{\frac{E_{OA}}{1\cdot6} + E_{OG}}{\frac{E_{SA}}{1\cdot6} + E_{SG}} \right) \frac{V_o \cdot C_s}{V_s}$$

where E_{OA}, E_{SA}, are the extinctions of oedema fluid albumin and serum albumin, respectively,
E_{OG}, E_{SG}, the sum of the extinctions of oedema fluid and serum globulins respectively,
V_o, V_s, the volumes of oedema fluid and serum,
C_s the serum protein concentration.

It was found, under the experimental conditions employed, that the amount of dye taken up by albumin was 1·6 times that taken up by the same amount of gammaglobulin. The above expression assumes that each individual globulin takes up the same proportion of dyestuff as the others.

Concentration of Oedema Fluids.—If the total protein content was less than about 1·5 g. per cent., the fluid was concentrated before electrophoresis. This was carried out by placing 0·1-0·15 ml. from a capillary

TABLE V
DETAILS OF THIRTEEN PATIENTS TESTED

Group I			Group II			Group III		
Active Arthritis at Site of Oedema			Inactive Arthritis at Site of Oedema			Other Diseases		
Patient No.	Site	Associated Feature	Patient No.	Site	Associated Feature	Patient No.	Site	Cause of Oedema
22 (Figs 3, 4)	Hand	—	19 (Fig. 4)	Feet	Amyloidosis (Still's disease)	25	Foot	Congestive heart failure On diuretics
21 (Fig. 3)	Hand	—	20	Feet	Amyloidosis (Still's disease)	24	Foot and calf	Congestive heart failure
3 (Fig. 3)	Feet	—	17 (Fig. 3)	Feet	Congestive heart failure	28 (Fig. 4)	Foot	Post-traumatic On phenylbutazone
6 (Fig. 4)	Feet	On phenylbutazone	31	Foot	Varicose veins			
7	Foot	Impaired mobility						
5	Feet	Impaired mobility						