PROTEIN CONCENTRATION OF CANTHARIDES BLISTERS IN RHEUMATOID ARTHRITIS

BY

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Previous investigation of the electrophoretic protein patterns of oedema fluids (Consdén and Smith, 1965) showed that, in a high proportion of fluids from patients with rheumatoid arthritis, the beta globulin was altered. The purpose of this investigation was to ascertain if a similar change occurred also in the fluids of blisters produced in patients with rheumatoid arthritis and also to compare the differential protein patterns of the blisters with those of the corresponding sera.

Material and Methods

Blisters were produced on the anterior abdominal areas of five adult healthy volunteers (Cases 1 to 5) and of five adult patients (Cases 6 to 10) with active rheumatoid arthritis by application of liquor epispasticus (*cf.* Ansell, Antonini, and Glynn, 1953) on three areas approximately 1 inch square on each person. After the evaporation of the solvent the area was covered with oiled silk and gauze. Fluid sufficient for analysis was withdrawn at intervals from one or more of the resulting blisters by means of a syringe and transferred to plain bottles. The blisters appeared on each site within 6 to 12 hours, but in one case a small blister from which it was possible to collect a small amount of fluid was present at 6 hours. Some blisters were allowed to remain as long as possible before withdrawing their contents. These blisters reached a maximum size at about 24 hours and thereafter became smaller; in no case, however, did any of the blisters survive longer than 48 hours after application of cantharides, owing either to bursting or to reabsorption. After collection, the fluids were stored in the refrigerator for 1 to 3 hours and were then separated from fibrin clots and cell debris by centrifugation. The supernatants were stored in the refrigerator until required. One specimen of blood was taken from each volunteer just before the application of the cantharides liquor.

All methods of analysis were as described by Consdén and Smith (1965). Total protein was determined by the biuret method on all specimens, except for the fluid from the 6-hour blister which was less than 0.1 ml. In this case the total protein was derived from the sum of the extinctions of the individual protein bands on the electro-

phoretic strip. Electrophoresis and staining with Ponceau S was carried out on cellulose acetate membranes. For qualitative examination, sera and fluids were freshly prepared. For quantitative electrophoresis none of the fluids was stored longer than 48 hours.

Results

Total protein concentrations are recorded in the Table. Owing to fragility of the blisters and bursting, it was not always possible to obtain both 12- and 24-hour blisters from each case. Reabsorption after 24 hours as well as fragility prevented survival of all but a few of the blisters at 48 hours.

TABLE
PROTEIN CONTENT OF BLISTER FLUIDS AND SERA

Group	Case No.	Protein Content (g./100 ml.)				
		Serum	Blister Fluid (hrs)*			
			6	12	24	48
Normal Subjects	1	7.1	—	6.0	4.7	6.7
	2	7.6	—	—	5.5	—
	3	6.9	—	—	5.3	5.7
	4	6.5	—	5.3	5.1	—
	5	7.3	6.1†	6.1	5.5	—
Rheumatoid Arthritis	6	6.2	—	5.2	4.7	—
	7	7.6	—	6.7	6.1	—
	8	6.7	—	—	5.2	5.7
	9	7.1	—	6.5	—	—
	10	7.3	—	—	5.7	—

*The times refer to hours after application of cantharides.

†Estimated from the electrophoretic strip.

The differential protein patterns of sera and blisters are shown diagrammatically in the Figure (opposite). In each individual the serum histograms are followed by the histograms of the blisters occurring in the same order as in the Table. Thus for Case 1, there are three blister fluid histograms for each protein, the first, second, and third corresponding to the 12-, 24-, and 48-hour blisters respectively.

The 24-hour blisters of the healthy group were much larger than and yielded at least twice as much fluid as those of the rheumatoid group. The patients

Figure.—Differential Protein Diagrams of Sera (solid bars) and Blister Fluids (open bars) (a) Normal subjects (b) Rheumatoid arthritis. Where there is more than one blister fluid, they are shown in the same order as that in the Table.

of this latter group were all on salicylate therapy. This drug was shown by Ansell and others (1953), in their studies of rheumatic fever cases, not to have a diminishing effect on the size of the blisters.

Discussion

In all the freshly isolated blister fluids the β globulin pattern was a sharp doublet as in the sera. This could have been due to failure of any of the blisters to survive long enough to allow changes to take place in the labile β_2 globulin. However, some of the blisters were about 40 hours old, which is long enough to destroy much of the β_2 globulin, since this protein in isolated body fluids or sera does not survive 2 to 3 days incubation at 37° C. That there was no observable diminution of the β_2 globulin band in the blister fluid as compared with that of the sera suggests that there is a rapid turnover of serum proteins within the blister. The reabsorption of blister fluids after some 40 hours would also be in keeping with this postulation.

The total protein content of all the blister fluids was high in both groups, ranging from about 70 to 90 per cent. of the serum protein. Most of these figures are higher than we have hitherto observed

in any oedema fluid, and are to be expected from an acute inflammatory process uncomplicated by any other mechanism. However, there are significant differences in protein content between blisters of different ages in the same individual (Cases 1, 5, 6, and 7 in the Table). The data also indicate that a fall in protein content occurs until the blister attains its maximum size (*i.e.* up to 24 hours) and then rises during its diminution. This would be indicative of selective passage of water into the compartment of the blister after its establishment and of selective reabsorption of water after a further period.

In a transudate, differences between its differential pattern and that of the serum are to be expected on account of differences in the size and shape of the individual serum proteins. Thus, in a transudate, the albumin content is raised, and if the serum is rich in α_2 globulin, this protein is markedly lowered. On the other hand, an inflammatory exudate, because of increased capillary permeability, should exhibit a differential pattern more closely resembling that of the serum. This was found to be the case for oedema fluids (Consden and Smith, 1965). In the blisters, especially those having a total protein content approaching that of the corresponding sera, one might

expect the fluid and serum patterns to be almost identical. However although this appears to be true for some of the normal subjects (Figure), there is a tendency in most of their blisters for the albumin to be raised and the α_2 globulin to be lowered. These differences are greatly enhanced in some of the rheumatoid cases, especially those which had a low serum albumin (Cases 6, 8, and 7, Figure), but even in the same individual (Cases 6 and 8, Figure) one blister fluid has a pattern characteristic of an inflammatory exudate whereas another fluid shows more resemblance to a transudate. There appears to be no correlation between the type of pattern and the age of the blister or its protein content. However, it may be concluded that increased capillary permeability is not necessarily the only mechanism operating in the formation of cantharides blisters and that a selective effect can also occur on some of the serum proteins when they are transferred across the vascular barrier. A few quantitative studies of cantharides blisters in disease other than rheumatoid arthritis have been recorded by Wuhrmann and Wunderly (1953; 1957) and Antonini and Piva (1952). Some of the data of these authors confirm that relatively large differences between serum and fluid patterns do occur.

The high protein contents of the blister fluids in rheumatoid arthritis, which are at least as high relative to the corresponding sera as are those of the blister fluids of the normal group, are indicative of marked increased capillary permeability. The smallness of the blisters of the rheumatoid group is therefore to be ascribed to increased diffusion of fluid away from the blister as compared with normal subjects. This is strongly suggestive of a generalized alteration in properties of connective tissue in active rheumatoid arthritis, a conclusion which parallels that of Ansell and others (1953) in their studies of cantharides blisters in active rheumatic fever.

Summary

Cantharides blisters were produced in healthy adults and in patients with active rheumatoid arthritis. The blisters were analysed for total protein and their electrophoretic patterns compared with those of the corresponding sera. The results are indicative of a rapid turnover of serum proteins in the blisters and of selective passage of water into the freshly-formed blister and selective reabsorption of water from the older blisters. Total protein in the blister fluids was high, but their differential pattern in some of the rheumatoid cases was markedly different from that of the corresponding serum, showing that a selective mechanism could operate in

addition to increased capillary permeability in the passage of serum proteins into the blisters. The relative smallness of the blisters of the rheumatoid patients are indicative of a generalized effect on connective tissue in this disease.

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La concentration de la protéine dans les vésicules provoquées par la cantharide dans l'arthrite rhumatismale

RÉSUMÉ

On provoqua par la cantharide des vésicules chez des sujets sains et des malades atteints d'arthrite rhumatismale active. On détermina dans ces vésicules le taux de la protéine totale et son tableau électrophorétique que l'on compara avec les sérums correspondants. Les résultats obtenus indiquent un déplacement rapide des protéines sériques dans les vésicules, un passage sélectif de l'eau dans les vésicules fraîches et sa re-absorption sélective dans les vésicules plus anciennes. La protéine totale dans le liquide vésiculaire fut élevée, mais son tableau différent de celui observé dans le sérum correspondant; il peut bien s'agir ici d'un mécanisme sélectif qui, en plus de la perméabilité capillaire augmentée, facilite le passage des protéines sériques dans les vésicules. La petitesse relative des vésicules chez les malades atteints d'arthrite rhumatismale indique un effet général sur le tissu conjonctif dans cette maladie.

La concentración de la proteína en vesículas producidas con la cantárida en la artritis reumatoide

SUMARIO

Se produjeron con la cantárida vesículas experimentales en sujetos sanos y afectados con artritis reumatoide. En estas vesículas se determinó la proteína total con su cuadro electroforético que se comparó con los sueros correspondientes. Los resultados obtenidos indican un giro rápido de las proteínas séricas en las vesículas, un paso selectivo del agua en vesículas recientes y su re-absorción selectiva de las vesículas más viejas. La proteína total en el líquido vesicular fué alta, pero su cuadro diferencial en algunos casos reumatoides fué muy diferente del observado en el suero correspondiente; se puede tratar aquí de un mecanismo selectivo que, paralelamente con la permeabilidad capilar aumentada, facilita la entrada de proteínas séricas en las vesículas. La pequeñez relativa de las vesículas en enfermos con artritis reumatoide indica un efecto general sobre el tejido conectivo en esta enfermedad.