THE PROTEINS IN PSORIATIC SCALES

NONEXISTENCE OF A METACHROMATIC GLYCOPROTEIN REPORTED IN EXTRACTS OF PSORIATIC SCALES

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SUMMARY

A description is given of attempts to isolate a metachromatic glycoprotein from alkaline extracts of psoriatic scales. Salt fractionation and isoelectric precipitation of the proteins in these scales produces a number of complex mixtures which are contaminated with serum proteins. Electrophoretic and immunological studies have been conducted on the fraction reported to contain the specific glycoprotein. The results indicate that no such compound exists and that the metachromasia in this special protein fraction, if detectable, must be attributed to the chondroitin sulphate coprecipitating with human serum albumin.

INTRODUCTION

In 1959 ROE[1] published the results of a study by paper electrophoresis of the proteins extractable from psoriatic scales with an alkaline borate buffer. The three proteins detected on the stained strips were tentatively identified as a keratin precursor, a glycoprotein and a nucleoprotein respectively. The glycoprotein, precipitated with ammonium sulphate between 30–60% saturation, dialyzed and purified by isolectric precipitation at pH 4.2, proved to be especially interesting because it exhibited marked metachromasia with buffered thionin, contained free sulphydryl, and released N-acetylglucosamine and hexoses upon hydrolysis. On the basis of these findings ROE suggested the compound to be a protein–chondroitin sulphate complex.

MATOLSKY AND MATOLSKY[2] have recently discovered eight proteins in psoriatic scales by applying small samples of nondefatted material to polyacrylamide gel for subsequent disc electrophoresis. This seems an astonishingly good result, taking into account that these scales are notoriously water repellent.

FLESCH[3] extracted a substance which he claimed to be a glycolipoprotein, although no evidence was presented for the homogeneity of the compound.

Psoriatic scales invariably contain appreciable quantities of normal serum proteins[4]. This contamination with serum exudate seriously complicates the reliable estimation of the abnormality of the proteins in psoriatic scales. This difficulty was especially encountered in attempts to isolate ROE's metachromatic glycoprotein.

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MATERIALS AND METHODS

Psoriatic scales were obtained from the affected skin sites of untreated patients with psoriasis vulgaris. The scales were defatted by ether extraction in a Soxhlet for 24 h and dried at room temperature.

Protein N was determined by micro-Kjeldahl, hexoses were assayed with orcinol[5] (standard: galactose) and uronic acids with carbazol[6] (standard: glucuronic acid). Paper electrophoresis was carried out in a conventional horizontal apparatus, using a veronal buffer pH 8.6, $\mu = 0.05$, for 5 h at 2 mA per strip; agar electrophoresis according to WIEME[7] in the same buffer, $\mu = 0.04$, for 15 min at 20 mA per slide. Electrophoresis in agarose was performed on microscope slides by the same technique, the agarose being prepared from Difco Agar Noble by the method of HJERTEN[8]. Immuno-electrophoresis was carried out by the micromethod of SCHEIDEGGER[9], using diffusion against a polyvalent anti-human serum (rabbit) after electrophoresis in agar (Anti Human Serum, lot No. 484, Behringwerke AG, Marburg/Lahn).

Staining procedures:

Protein staining with Amidoblack 10B after fixation of the agar slides in alcohol–acetic acid–water (90 : 5 : 5); carbohydrates with periodate-Schiff modified after LISON[10]. The method of BARNETT AND SELIGMANN was adopted for use in the detection of SH-groups in proteins on agar slides, as described by ZWAAN[11]. Metachromatic stains: for paper strips toluidine blue or Alcian Blue according to HEREMANS[12] or thionin according to ROE[1]; for agarose slides either toluidine blue or a combination of this dye and Lissamine Green protein stain as described by VAN ARKEL *et al.*[13]. The chondroitin sulphate marker was a commercial preparation (N.B.C.).

EXPERIMENTAL

In earlier experiments ROE's buffer system was used, but eventually we adopted the procedure outlined in the following experiment.

Dried and defatted psoriatic scales (20 g) were homogenized in a Waring Blendor with 800 ml Tris–HCl buffer 0.1 *M*, pH 9.2; extraction was continued for 24 h at 4° with stirring. The insoluble material was centrifuged and re-extracted with 200 ml buffer for 3 h; the undissolved residue was discarded. The pooled extracts were dialyzed against running tap water and dried by lyophilization. A yellow, very hygroscopic material was obtained (fraction A).

Fraction A (4 g) was dissolved in water (320 ml) and the proteins in the solution were fractionated with $(NH_4)_2SO_4$ at 4°. A period of 24 h was allowed for maximum precipitation at any given salt concentration, except in the case of the 30–80% precipitate which was collected after 2 h in order to avoid coprecipitation of mucopolysaccharides.

Three fractions were obtained, precipitating between 0–30%, 30–80% and 80–100% salt saturation respectively. From the solution in water of the 0–30% and the 30–80% precipitates, prolonged dialysis against water produced insoluble material which was centrifuged and studied separately. The supernatant (650 ml, pH 4.9) remaining after complete salt saturation was treated with calcium acetate (final concentration 0.1 *M*) after exhaustive dialysis and the precipitate obtained by the addi-

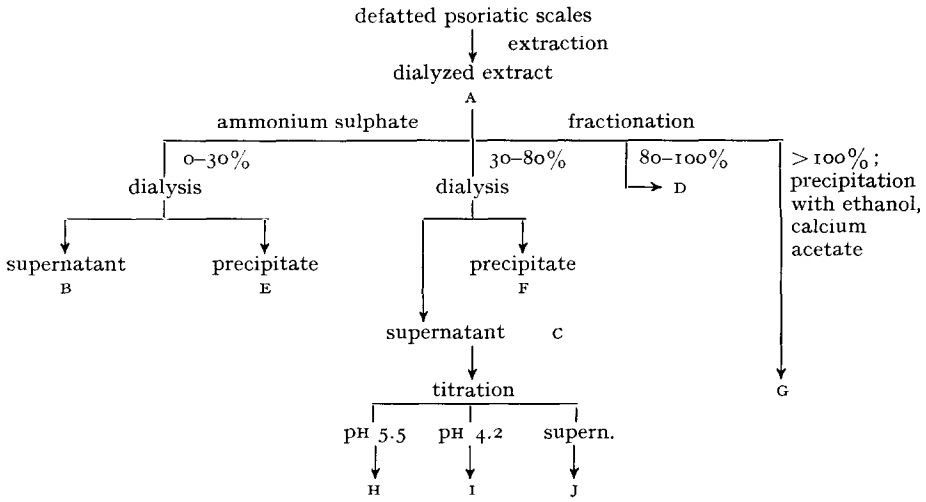

Fig. 1. Flow sheet illustrating the fractionation of soluble proteins in psoriatic scales.

tion of an equal volume of absolute ethanol was centrifuged, dissolved in 2 *N* HCl, and dialyzed.

All fractions were dried by lyophilization. The fractions obtained have been labelled according to the flow sheet in Fig. 1.

Fraction C, which should contain ROE's glycoprotein, was dissolved (400 mg in 100 ml of water; pH 8.9) and titrated with 0.01 *N* HCl. A relatively large amount of substance precipitating at pH 5.5 was centrifuged and kept apart; up to this point the uptake of H^+-ions was 0.05 mequiv. per 100 mg of proteins. Titration of the supernatant was continued to pH 4.2, which required an additional 0.12 mequiv. per 100 mg. The small quantity of precipitate appearing at this pH was centrifuged, dissolved in water at pH 8.0, and dialyzed.

RESULTS

The yields of some protein fractions, together with some data on their chemical composition, have been recorded in Table I.

TABLE I

ANALYSIS OF SOME SOLUBLE PROTEIN FRACTIONS FROM PSORIATIC SCALES

Fraction	% *in dried defatted scales*	% *Nitrogen*	% *Hexoses*	% *Uronic acid*
A	16–20*	9.4	1.5	0.7
B	1.5	13.4	2.0	0.5
C	4.0	12.5	1.9	0.6
D	0.04	**	2.4	2.5
E	1.4	13.3	2.0	0.4
F	0.4	12.3	2.2	0.7
G	0.03	**	8.5	6.4
I	0.12	**	**	1.3
J	3.0	10.3	1.5	0.5

* Not exact, because the material was very hygroscopic.
** Not determined.

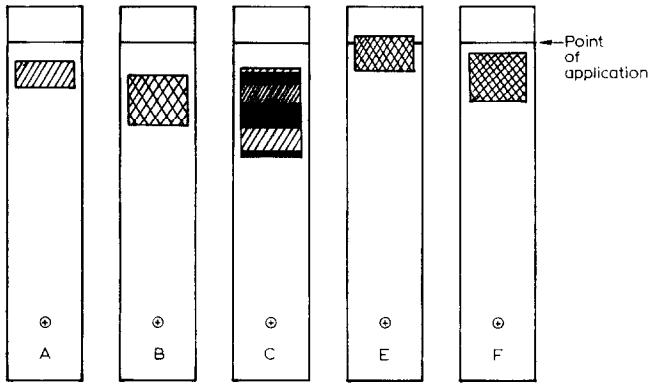

Fig. 2. Tracing of the patterns obtained by paper electrophoresis of some fractions. Stain: Amidoblack; conditions: see text.

The alkaline buffer extracts 16–20% of nondialyzable material from psoriatic scales; the comparative figure for normal human dandruff is 2.5–3%[14]. The protein content of the fractions is rather high, as indicated by the nitrogen values; it may be observed, moreover, that there is a gradual increase in the percentage of uronic acids parallel to the salt concentration used for precipitation of the fractions. In fact, fraction G contains chondroitin sulphate (see below) and it seems probable that a small amount of this mucopolysaccharide coprecipitates with protein in fraction D.

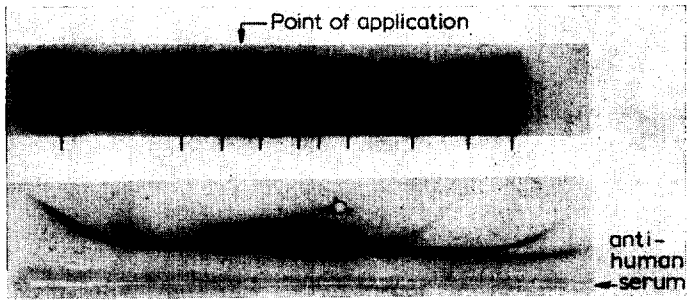

Fig. 3. Agar and immunoelectrophoresis of psoriatic protein fraction C; conditions: see text. The positive electrode is on the left. Stain: Amidoblack.

Paper electrophoresis revealed that there is a considerable overlap in the precipitation of protein constituents, as indicated schematically in Fig. 2. However, fraction C actually did show three protein bands on the strips, in confirmation of ROE's results; precipitation of C between 30–60% salt saturation did not change this pattern, nor did we – in separate experiments – notice any influence of the kind of buffer used for extraction. The three proteins detected in a similar fraction from normal human dandruff migrate at approximately the same rate[14].

Agar electrophoresis reveals that in fraction C we are actually dealing with an extremely complex mixture of proteins (Fig. 3). Moreover, it was found by immunoelectrophoresis that, among the ten different proteins in C, there are at least seven deriving from the serum (Fig. 3). The presence of serum proteins is by no means restricted to fraction C; it has been reported in a separate communication that A, B, E

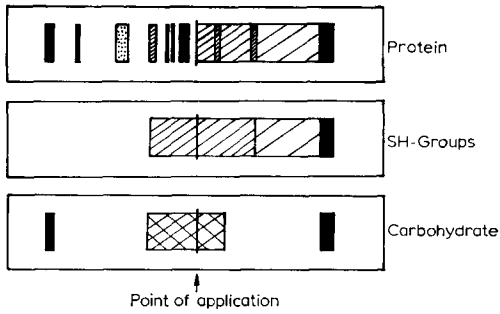

Fig. 4. Tracing of the patterns obtained by differential staining techniques after electrophoresis of fraction C in agar.

and F also contain fair amounts of various serum constituents[4]. This contamination with serum, of course, seriously invalidates the use of differential staining for sulphydryl groups or carbohydrate for the localization of ROE's glycoprotein. Consequently, at this stage, positive reactions with specific stains were not restricted to a single protein (Fig. 4). None of the C– proteins, moreover, exhibited metachromasia with toluidine blue or thionin, either on paper or in agar gel.

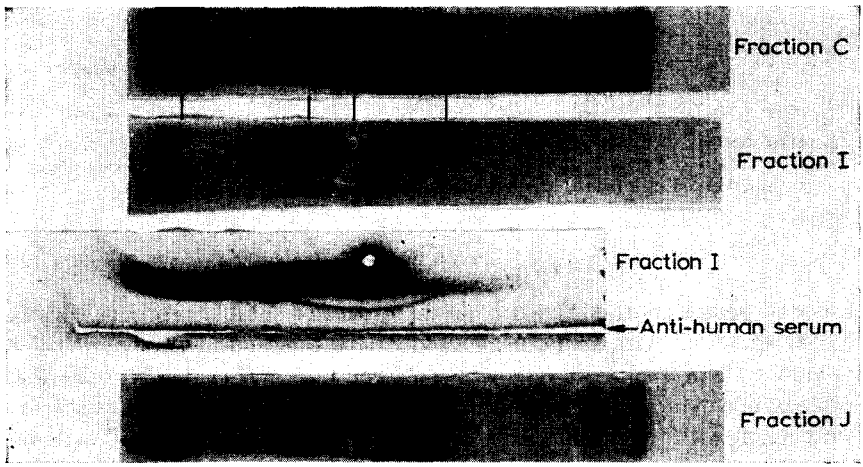

Fig. 5. Agar- and immunoelectrophoresis of psoriatic protein fractions. Top to bottom: Fraction C, fraction I, fraction I (immuno) and fraction J. Stain: Amidoblack. Positive electrode on the left.

Evidence obtained thus far indicated that fraction H, precipitated from C at pH 5.5, probably represents part of ROE's epidermin, which is incompletely removed by ammonium sulphate precipitation at a degree of saturation of 30%. Fraction C contains only a very small percentage of substance precipitable at pH 4.2 (about 3%); we have been unable to increase this yield by substituting dialysis against a 0.1 *M* sodium acetate buffer, pH 4.2, for titration, nor by increasing the ionic strength of the solution with neutral salts. Fraction I therefore represents the optimum yield of metachromatic glycoprotein supposed to be present in psoriatic scales. The substance proved to be still inhomogenous (Fig. 5) and immunoelectrophoretic analysis revealed it to contain a fair amount of serum albumin and, presumably, and α-globulin (Fig. 5).

Attempts to stain the substance, or the individual components, in fraction I metachromatically failed throughout. Fig. 6 shows the result of metachromatic staining with toluidine blue after electrophoresis in agarose, as well as of a combination of this procedure with protein detection according to VAN ARKEL *et al.*[13]. Finally, in the same figure, the evident, positive metachromatic reaction of fraction G has been reproduced, from which it may be deduced that G, but not I, contains chondroitin sulphate as the main mucopolysaccharide. Neither serum proteins, nor any other polypeptides were detected in fraction G.

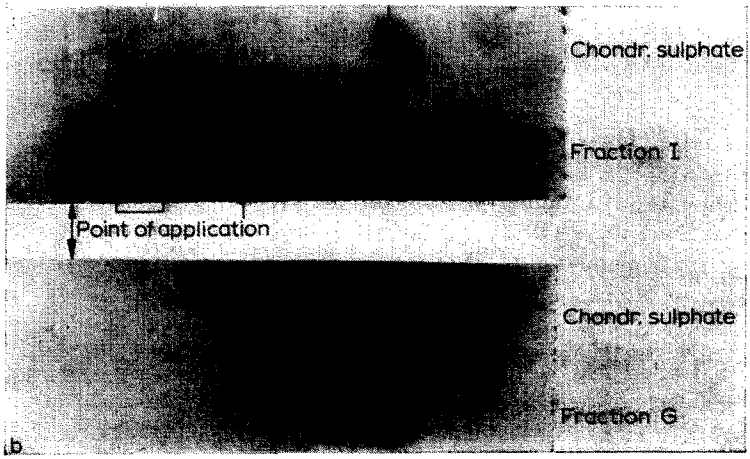

Fig. 6. Electrophoresis in agarose gel. (a) chondroitin sulphate (marker) and fraction I, stained with Lissamine Green and toluidine blue combination. (b) fraction G and the chondroitin sulphate marker, stained with toluidine blue. Positive electrode on the right.

DISCUSSION

Psoriatic scales contain appreciable quantities of soluble proteins, but in view of the contamination with serum proteins the detection and identification of specific factors among them becomes a tedious task. Even more so because statements about a possible abnormality of the remaining proteins seem to gain full force only after due comparison with the normal case. That, in this respect, we are still rather a long way off from definite conclusions may be illustrated by the results presented here.

The experiments, in our opinion, show that obviously there is no specific metachromatic glycoprotein in alkaline extracts of psoriatic scales. This conclusion is based on the discovery that the material precipitated at pH 4.2 (fraction I) consists mainly of serum albumin (which indeed has its IEP at pH 4.2–4.5); this protein, of course, gives positive SH and carbohydrate stains. ROE's analytical results and the metachromasia can only be explained reasonably by assuming that, in the course of her isolation procedure, chondroitin sulphate coprecipitated with the albumin and did not separate from it in paper electrophoresis. As we have shown, the extract certainly does contain chondroitin sulphate, though in its free form (fraction G).

On these grounds, we believe it justifiable to reject the presence of a metachromatic glycoprotein in psoriatic scales and to doubt any theory which may have been based upon its existence.

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