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CLINICAL ARTICLES

IMMUNOLOGIC PATTERNS OF SOLUBLE PROTEINS OF CUTANEOUS SCALES IN SQUAMOUS DERMATOSES.

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Increased amounts of soluble proteins in psoriatic scales have been reported by Flesch and Esoda (1957, 1958, 1964), Fisher (1965) and Singh et al (1969). Whether this quantitative variation has also a concomittant change in the quality of proteins has evoked a wide interest and many studies have been conducted in this direction in recent times (Roe, 1958; Forsey et al, 1965; Fisher, 1965 and Scott, 1965). The present paper reports the results of a study undertaken to observe immunologic patterns of soluble proteins obtained from extracts of scales of psoriasis, exfoliative dermatitis, callus and ichthyosis.

MATERIAL AND METHODS

For this, an antiserum was prepared against psoriatic scale extract proteins and then tested by immunoelectrophoresis against extracts made from scales of individual patients of psoriasis, exfoliative dermatitis, callus and ichthyosis.

PREPARATION OF ANTI-PSORIATIC-SCALE-EXTRACT SERUM:

Scales obtained by gentle scraping of untreated lesions of 5 proven cases of psoriasis were pooled and homogenized in 0.05 M Borate buffer, pH 9.4 (Roe, 1958). This was placed at 6°C overnight and the supernatent collected by centrifugation at 1500 rpm for 15 minutes. It was then dialyzed against double distilled water in cold and concentrated in a lyophilizer to give 10 mg of protein per ml, as estimated by the Biuret method (Kingsley, 1939).

Three Haffkine strain rabbits were immunized by injecting each one of them with 0.75 ml of psoriatic scale extract along with an equal volume of complete

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Freund's adjuvant at 3 sites, 2 subcutaneously and 1 intraperitoneally. These injections were repeated every fortnight to a total of five. Three weeks after the last injection each rabbit was given 2.5 mg psoriatic scale protein in 0.15 M sodium chloride at the same sites and the animals were bled 7 days later. The presence of antibodies in the immunized serum was confirmed by immunoprecipitation in 2% saline agar (Ouchterlony, 1962). This antiserum was labelled anti-psoriatic-scale-extract-rabbit serum (APSK). Half of the antiserum was mixed with lyophilized human serum and kept overnight at 6°C. The precipitate formed was removed by centrifugation and the supernatent was further repeatedly treated, till no antibodies against normal human serum were demonstrable by immunoprecipitation. This was designated as absorbed anti-psoriatic-serum (APSK-ABS). These absorbed and unabsorbed anti-psoriatic-sera were tested against extracts made from scales of 20 cases of psoriasis, 5 cases of exfoliative dermatitis, 5 cases of ichthyosis, 4 cases of planter callosities and 1 case of congenital palmoplanter hyperkeratosis.

Immunoelectrophoresis was carried out in 2% Veronal agar (2% agar in 0.05 M veronal/HCl buffer, pH 8.2) on 75x25 mm glass slides employing 4 mA current per slide for 70 minutes. One of the wells contained scale extract while the other one contained normal human serum. After electrophoresis, the central trough was filled with the antiserum, APSK in 2 slides and APSK-ABS, in the other 2. These were then kept at 37°C overnight and later at 6°C for 1 week. The precipitation formed were photographed.

OBSERVATIONS

On immunoelectrophoresis using unabsorbed antipsoriatic serum, scale extracts of all the cases of psoriasis showed 4 immunoprecipitation arcs. In relation to serum proteins they were placed in the albumin, beta globulin, gamma globulin and post gamma globulin regions respectively (Fig. 1). In one case an additional band in the alpha globulin region was also present. After absorption with human serum all the samples of psoriatic scale extracts showed the presence of 3 proteins, in the albumin, beta globulin and post gamma globulin regions respectively (Fig. 2). Density of all these precipitation lines was found to be less when compared to the results obtained with unabsorbed serum.

Exfoliative dermatitis scales also showed 4 precipitation arcs in the albumin, beta globulin, gamma globulin and post gamma globulin regions respectively (Fig. 3) with unabsorbed antiserum. However, with the absorbed antiserum only 2 precipitation arcs in the beta globulin and post gamma globulin regions respectively were observed (Fig. 4).

Callus extracts showed 4 precipitation arcs in the albumin, alpha globulin, beta globulin and post gamma globulin regions respectively with unabsorbed antiserum and only a single precipitation line in the post gamma globulin region with absorbed antiserum (Fig. 5).

Ichthyosis failed to give any precipitation band on immunoelectrophoresis.

DISCUSSION

The present study shows the presence of serum proteins in all types of extracts in as much as after absorption of the antipsoriatic serum with serum proteins some of the precipitation lines disappeared altogether, while others became fainter. Among the different types of diseases studied, psoriatic scales seemed to contain the maximum amounts of serum proteins, followed by exfoliative dermatitis and callus.

Extracts of psoriatic scales showed 3 proteins distinct from serum proteins which resembled normal epidermal proteins in their electrophoretic mobilities (Pasricha and Kandhari). These findings however, differ from those of Fisher (1965), who showed psoriatic scale proteins to be placed in the post albumin, alpha–2 globulin (2 arcs) and post gamma globulin regions. Only in one sample, a single arc in alpha globulin region was also seen. Further still, the arcs in the post albumin and beta globulin regions did not join each other as reported by Fisher (1965).

In scales of exfoliative dermatitis, Roe (1958) had reported 2 bands while Singh et al (1969) reported 3 bands by paper electrophoresis. On immunoelectrophoresis, the present study showed only 2 bands in the beta and post gamma globulin regions respectively. Forsey et al (1965) also showed only 2 bands in the scales of exfoliative dermatitis.

Callus extracts showed only one band in the post gamma globulin region, while Roe (1958) and Scott (1965) showed 2 bands and Singh et al (1969) showed 3 or even 4 bands by paper electrophoresis. The extra number of bands could be attributed to the presence of serum proteins which cannot be differentiated by paper electrophoresis. Fisher (1965) showed 2 proteins by immunoelectrophoresis using an antipsoriatic serum and 4 components when anticallus serum was used.

Thus with the discrepancies observed in the findings of different workers which may be partly dependent upon differences in the methodology employed, more work is required to be done for getting further data to establish the cause of variations. However, for an assessment of the results of present study it must be taken into account that the proteins in different types of scales were detected by an antiserum raised against psoriatic scale proteins only. Thus a possibility still remains that scales of exfoliative dermatitis, callus and ichthyosis may actually contain more proteins than brought out by this study.

SUMMARY

Soluble proteins in scales of psoriasis, exfoliative dermatitis, callus and ichthyosis were studied by immunoelectrophoresis using an antiserum raised against pooled extract of psoriatic scales. Presence of serum proteins was detected in all types of scales; psoriatic scales containing the maximum amount.

Psoriatic scales contained 3 proteins, one each in the albumin, beta and post gamma globulin regions respectively; scales of exfoliative dermatitis contained 2 proteins, one each in the beta and post gamma globulin regions, while callus showed only 1 protein located in the post gamma globulin region. Protein content of scales of ichthyosis was too low to produce any precipitation lines.

LEGENDS TO FIGURES

Fig. 1. Immunoelectrophoretic patterns of psoriatic scale extract (PSK) and normal human serum (NHS) against antipsoriatic-scale-extract serum (APSK).

Fig. 2. Immunoelectrophoretic pattern of psoriatic scale extract (PSK) against absorbed antipsoriatic-scale-extract serum (APSK-ABS), showing one arc each in the albumin, beta globulin and post gamma globulin regions respectively.

Fig. 3. Immunoelectrophoretic patterns of exfoliative dermatitis scales (ED) and normal human serum (NHS) against APSK.

Fig. 4. Immunoelectrophoretic pattern of exfoliative dermatitis scales (ED) against APSK-ABS, showing one arc each in the beta globulin and post gamma globulin regions respectively.

Fig. 5. Immunoelectrophoretic pattern of callus extract (HKK) against APSK-ABS, showing one arc in the post gamma globulin region.

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