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STUDIES ON THE ORIGIN OF THE MUCIN IN MUCINOSIS FOLLICULARIS

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Mucinosis follicularis (m.f.), described as an unclassified entity by Lehner and Szodoray (21) became widely known after the work of Pinkus (27). He named it alopecia mucinosa owing to the loss of hair, which is a characteristic feature of this condition. Braun-Falco (3) demonstrated by thorough histochemical studies that the substance in the hair follicles is a mixture of acid mucopolysaccharides, partly combined with proteins. He thought that the mucin owes its origin to the release of mucopolysaccharides from combination with proteins, and proposed accordingly the name Mucophanerosis intrafollicularis et seboglandularis. Credit is also due to Braun-Falco for distinguishing the symptomatic variety developing in various malignant lymphoreticular proliferations.

Since the changes affect exclusively the follicular organ, i.e., the root sheath and the sebaceous gland, the name mucinosis follicularis was proposed (17). This name seems all the more justified because the lesions often occur outside the areas of the scalp and, therefore, without alopecia.

The etiology and pathogenesis of m.f. remain completely obscure. Suggestions of viral etiology (21, 27) have yet to be confirmed, and so has incrimination of bacteria (2). Haber's surmise (14) that m.f. is a kind of eczema affecting follicles seems unconvincing because it is as a rule absent in eczematous lesions.

The presence of the mucinous substance in the follicles is undoubtedly a fact and can be demonstrated not infrequently even on gross examination by expressing it from the follicles (14, 17, 24, 32).

The nature of the lesions in the symptomatic form is the same as that in the idiopathic form (3, 5, 6, 7, 8, 9, 10, 11, 12, 14, 27, 29, 30, 31).

Histochemical studies demonstrated a mucinous substance in the degenerating follicular organ. This mucin is undoubtedly of epithelial origin (13, 18, 32), but the mechanism responsible for its formation is unknown. It is composed chiefly of acid mucopolysaccharides and to a lesser degree of neutral ones. Much, or even the better part of it is digested by hyaluronidase (18, 19, 26), but apart from the nonspecificity of this enzyme there may be also hyaluronsulphate, which is abundant especially in mucin of epithelial origin (3). In an excellent work Asada and Morita (1) call attention to the very compound composition of mucin in m.f. They believe that it still remains to be settled whether the mucin substance is here an acid mucopolysaccharide, a mucoprotein, or sulphomucin (after Tsurumi). The thorough histo-enzymatic research by Tappeiner *et al.* (30, 31) has yielded no conclusions on the composition of mucin in m.f.

Okun and Chorzelski (23) used special procedures which indicated that metachromasia of the dendritic cells found within epithelial structures is due to the presence of sulphated mucopolysaccharides.

We therefore believe that studies with the aid of ^{35}S-sulphate are justified in m.f., for even if sulphated mucopolysaccharides

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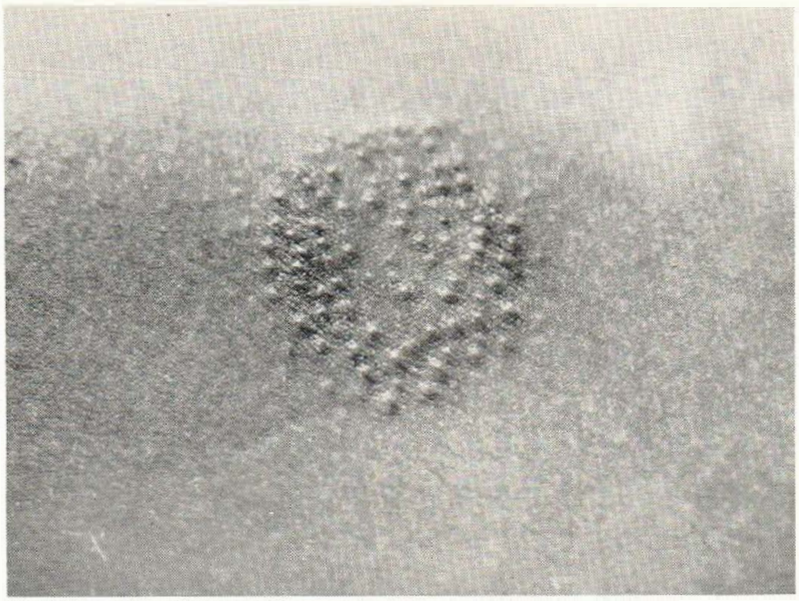

Fig. 1. Mucinosis follicularis—idiopathic form. Aggregated perifollicular papules on the chest.

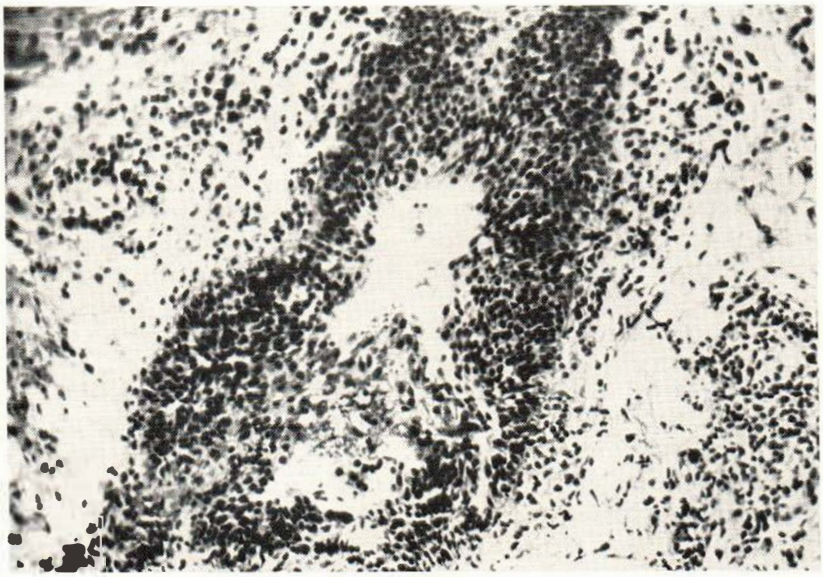

Fig. 2. Mucinosis follicularis—idiopathic form. Degeneration of hair follicles with cystic changes.

account only for a part of mucin, their increased synthesis should be demonstrable in this way. In an earlier work on normal skin (20) we have demonstrated that ^{35}S-sulphate was incorporated in considerable quantities into the intercelluar substance of the epidermis, with a certain reduction in the number of silver grains being evident after digestion with hyaluronidase, and so do also anagen follicles, where ^{35}S-sulphate incorporation is very intensive.

In our present study the purpose was to show whether the acid mucopolysaccharides are in m.f. actually synthesized in the follicles, or merely become demonstrable owing to their being released from combination with proteins (mucophanerosis) as supposed by Braun-Falco (3).

Material and Methods

Our material comprised five histologically confirmed cases of mucinosis follicularis; in four cases it was the idiopathic form (Figs. 1 and 2), and in one case, a man aged 48 years, it accompanied mycosis fungoides.

Use was made of the following stains and histochemical reactions:

1) PAS and Hale's reaction + digestion with diastase
2) alcian blue, pH 2.5
3) toluidine blue, pH 8.0
4) toluidine blue, pH 3.5

(2–4) + digestion with hyaluronidase

Autoradiography. Specimens approximately 2 mm thick were placed immediately in glucosol solution with an addition of 10 % calf serum and $Na_2{}^{35}SO_4$ (Amersham Radiochemical Centre) in amounts of 3 μC/ml, and incubated four hours at 37°C. The specimens were then fixed 24 hours in a 3 : 1 mixture of ethanol with 96 % glacial acetic acid, embedded in paraffin, and sectioned to a thickness of 4 microns. After hydration the sections were washed in running water and a solution of unlabelled Na_2SO_4 to remove the unincorporated precursor. The sections were coated with autoradiographic[1] emulsion after Pelc's method (25) and kept 10–15 days at 4°C. The developed and fixed autoradiograms were stained with haematoxylin-eosin or toluidine blue pH 3.5, covered with a 2 % solution of polyvinyl alcohol (Elvanol-Du Pont), and sealed in Canada balsam

In some cases, before coating the sections with autoradiographic emulsion, a 1 mg/ml hyaluronidase pH 8.0[2] was applied for 1 hour at 37°C.

Silver grains over individual follicles were counted.

Results

Toluidine blue of pH 3.5 and pH 8.0 demonstrated in all cases a metachromatically staining substance; it also stained with alcian blue at pH 2.5 and in Hale's reaction. A slight reduction of metachromasia after digestion with hyaluronidase was noticeable. The PAS reaction was occasionally weakly positive, and diastase—negative. In autoradiograms the number of silver grains was over changed follicles very small, much like over follicles during telogen; it was virtually the same as in the background (Fig. 3). No reduction in the number of silver grains after ingestion with hyaluronidase was observed.

Over uninvolved follicles at anagen on the other hand, it was very large (Fig. 4), surpassing considerably that over epidermis cells.

No differences in the incorporation of ^{35}S in hair follicles could be detected between the idiopathic and the symptomatic forms of mucinosis follicularis.

Discussion

Incorporation of inorganic sulphate by the cells of the epidermis supplies evidence of the synthesis of acid mucopolysaccharides (4, 20). In experiments *in vitro* (incubation in glucosol with ^{35}S-sulphate for 4 hours) we have demonstrated incorporation of ^{35}S-sulphate in the living epidermis, but have never seen silver grains over the horny layer or keratogenic zone. Although it is possible that inorganic sulphate is incorporated into sulphur-containing amino acids in experiments made *in vivo* (15), there was no indication of this process in our four-hour experiment *in vitro*. The labelled material over the squamous layer was only slightly digested by hyaluronidase.

In work still unpublished we observed intensive incorporation of ^{35}S-sulphate in anagen hair follicles. Major amount of ^{35}S incorporated into hair follicles confirms the synthesis of acid mucopolysaccharides in them (22).

Our investigations do not indicate synthesis of acid mucopolysaccharides in hair follicles undergoing destruction in m.f. Even if sulphated mucopolysaccharides (ir-

[1] Kodak AR-10.
[2] Koch-Light.

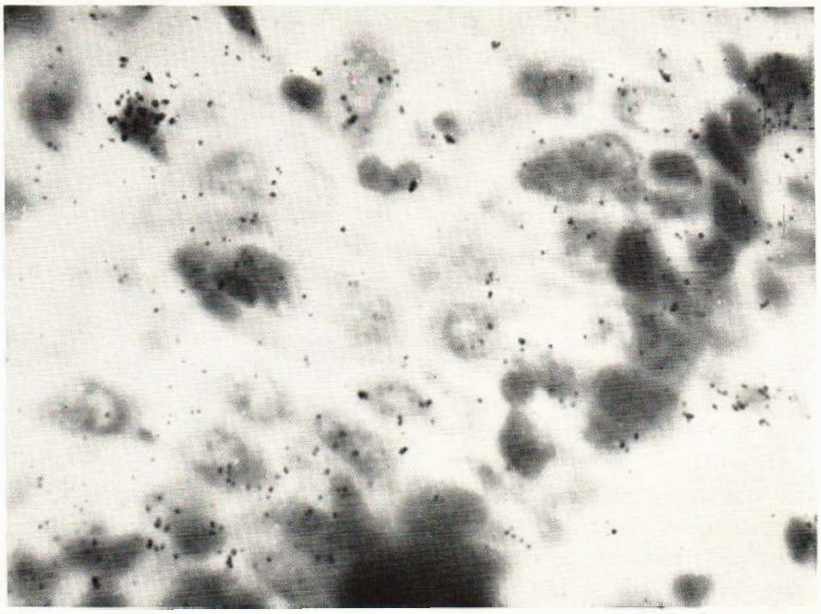

Fig. 3. Mucinosis follicularis—idiopathic form. Slight incorporation of ^{35}S into a hair follicle undergoing considerable mucinous degeneration.

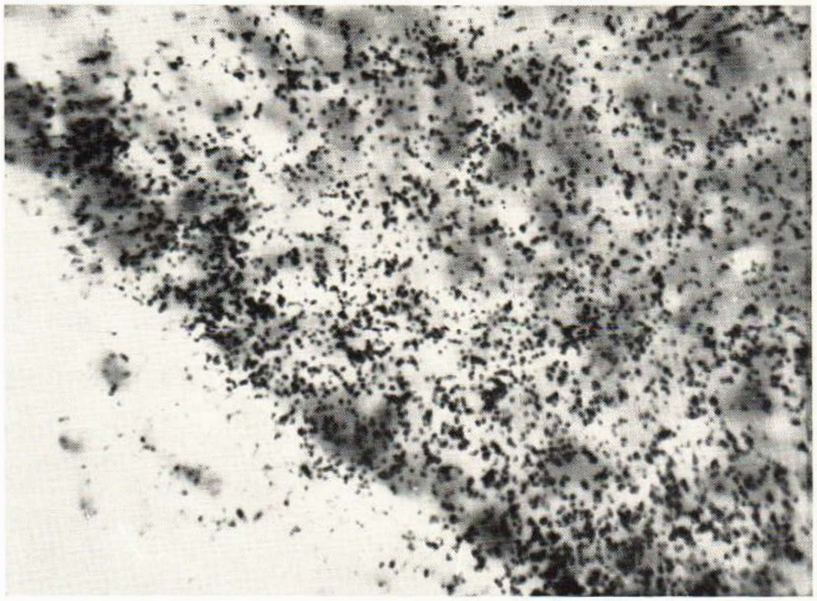

Fig. 4. Normal skin. Hair follicle during anagen, strongly labelled.

respective of that whether it is chondroitin sulphate or hyaluronosulphate which is digested by hyaluronidase, or both) account only for a part of mucin, their increased synthesis should be demonstrable in our experiments. In m.f. there is no synthesis of sulphated acid mucopolysaccharides even though the process involves anagen follicles, where ^{35}S-sulphate incorporation is usually very intensive, and which contain a substantially increased quantity of acid mucopolysaccharides, in-

cluding a proportion of sulphated ones. These considerations appear to support indirectly Braun-Falco's (3) hypothesis that in this disease the complexes of the mucopolysaccharides with proteins are broken up, which enables the freed former compounds to be detected by histochemical methods.

The break-up may be due to some undetermined proteolytic enzymes present in the specific and the nonspecific infiltrations in respectively the symptomatic and the idiopathic forms.

The similarity between the two forms of m.f.—the idiopathic and the symptomatic —as regards the mechanism responsible for the accumulation of mucin in follicles argues against their complete separation, as do also the observations of Pinkus (28) and Degos *et al.* (10).

Conclusions

1. In mucinosis follicularis, autoradiography in vitro with ^{35}S by 4 hours exposition time fails to indicate increased *in situ* synthesis of the sulphated acid mucopolysaccharides accumulating in considerable quantities in degenerating hair follicles.
2. In uninvolved hair follicles during anagen, unlike at telogen, it is possible to demonstrate substantial synthesis of the acid mucopolysaccharides.
3. Autoradiographic studies indicate that the mucin in mucinosis follicularis does not owe its origin to increased rate of actual synthesis of sulphated acid mucopolysaccharides. This seems to support the hypothesis of their liberation from the complexes with proteins.
4. Differences in the mechanism responsible for the accumulation of acid mucopolysaccharides could not be shown by autoradiography between the idiopathic and the symptomatic forms of mucinosis follicularis.

SUMMARY

Autoradiographic studies using ^{35}S-sulphate (experiments *in vitro*, incubation time 4 hours) were made in five typical cases of mucinosis follicularis (m.f.)—four of the idiopathic form, and one symptomatic form in mycosis fungoides. In these conditions ^{35}S-sulphate is incorporated into the living layers of the epidermis, and no increase in the number of silver grains is observed over keratin or keratogenic zone. The incorporation of ^{35}S-sulphate shows that epithelial cells produce the acid mucopolysaccharides of the intercellular substance of the epidermis, as do also anagen follicles. In both—idiopathic and symptomatic —forms of m.f. no increased synthesis of sulphated acid mucopolysaccharides was established, which in contrast was very intensive in uninvolved hair follicles at anagen.

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