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THE INFLUENCE OF SODIUM DODECYL SULPHATE UPON SKIN REACTIONS TO BACITRACIN

By

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On the basis of their histochemical findings, *MacKee, Sulzberger, Herrmann & Baer* (6), as early as 1945, concluded that when some anionactive surfactants are added to aqueous solutions of iron and bismuth salts, sulphonamides and dyes, the percutaneous penetration of these substances is increased. Subsequently, addition of surface active agents into patch test solutions in order to increase penetration has been recommended (5). *Shelley & Melton* (12), however, were unable to show that anionic and nonionic surfactants had an enhancing influence on the penetration of histamine from aqueous solutions. Similarly, according to *Blank* (1), sodium dodecyl sulphate (sodium lauryl sulphate) in 0.01 to 0.04 molar concentrations has no effect on the penetration of alcohols of low molecular weight. *Nilzén & Wikström* (8) found that guinea-pigs could be sensitized to potassium bichromate and nickel sulphate, provided that these substances were mixed with sodium dodecyl sulphate. However, while in similar studies some authors have reported positive results with nickel, others have not been able to sensitize the animals as appears from the reviews of *Magnusson* (7) and *Blank* (1). Therefore, *Magnusson* (7) studied the influence of different surfactants

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upon sensitization of guinea-pigs to some strong contact allergens. His results indicated that the surface active effect *per se* does not influence the contact sensitization, but, if a surfactant is employed at a toxic concentration, sensitization is more easily attained than when the allergen is used alone. This is in agreement with *Riska's* (11) observation that 3, 4-benzpyrene penetrates the epidermis of the rat in greater amounts from strong solutions of surfactants than from aqueous solutions alone.

However, the last word has not yet been written, and the influence of surface active agents upon penetration of water and other substances through the epidermal barrier and upon contact sensitization is still a subject of controversy. It is often forgotten that, as chemically active substances the surfactants affect the skin constituents and the allergens in many ways. For instance, *Blohm* (2) pointed out that the skin irritating and protein-denaturing effects of a homologous series of alkyl sulphates, including sodium dodecyl sulphate, are closely connected.

The starting point of the present study was that bacitracin as a polypeptide can be expected, like proteins, to react with the anionic surfactants. Indeed, in preliminary investigations with ordinary patch tests on patients sensitized to bacitracin the reactions to mixtures of bacitracin and sodium dodecyl sulphate (SDS) tended to be weaker than to bacitracin alone. This observation suggested that either the eczematogenic effect of bacitracin is, at least in part, destroyed by SDS, or SDS decreases the rate of penetration of bacitracin. Therefore the following investigations were conducted.

As a simple method of demonstrating the reaction between SDS and amino groups in bacitracin, Albustix (Ames) slips, manufactured for detecting protein in urine were used as the indicator when titrating a given bacitracin solution with SDS. It was found that the colour reaction for protein which was clearly positive with bacitracin alone became weaker with increasing SDS concentration, turning negative when the molar ratio of SDS to bacitracin exceeded approximately four to one

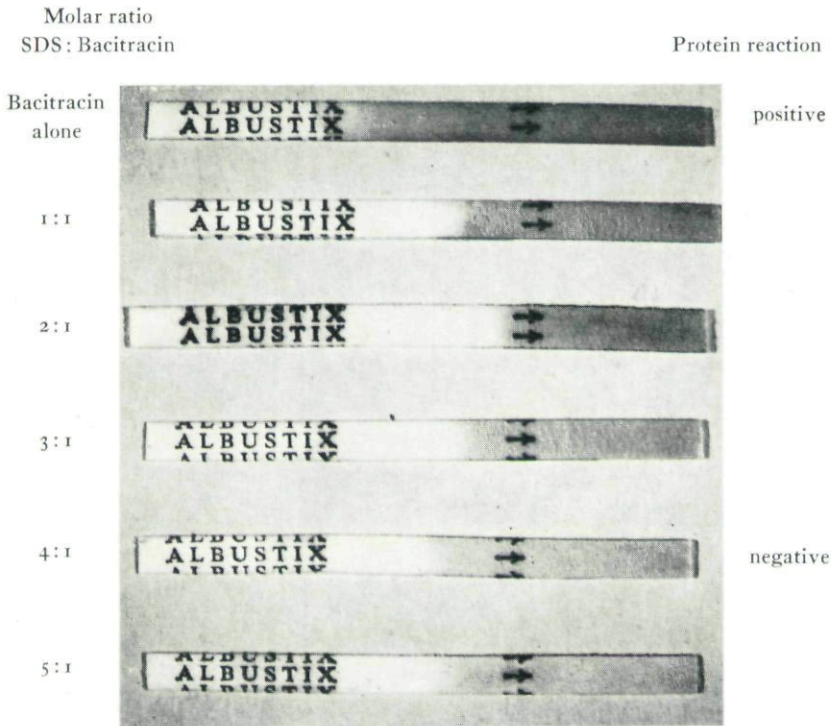


Fig. 1.

The influence of sodium dodecyl sulphate upon the protein reaction of bacitracin.

(Fig. 1). Thus, it appeared that one dodecyl sulphate ion blocks one of the four free amino groups of the bacitracin molecule and that in 4 : 1 mixture all these amino groups have been blocked. This mixture, however, and even a 5 : 1 mixture of SDS and bacitracin, gave an ultraviolet spectrum characteristic of the thiazoline ring, the group that has been held essential for the antibiotic potency of bacitracin (3) and, according to our earlier investigations, for its allergenic effect also (10).

As the supposed allergenic structure was preserved undamaged, it appeared that the suppression of epicutaneous reactions to bacitracin through the addition of SDS, suggested by

our preliminary investigations, is most probably due to a decreased rate of penetration. To confirm this, additional skin testing was performed. In intracutaneous tests 0.05 cc was injected, and for the epicutaneous testing Rokstad's chamber method, slightly modified (9) was used.

Intracutaneous tests with bacitracin solutions at concentrations from 0.01 to 0.1 per cent caused in control persons immediate reactions, wheals, up to 12 mm in diameter, surrounded by a red halo, as earlier shown by *Hjorth* (4). These reactions are due to liberation of histamine, like those caused by another polypeptide antibiotic, polymyxin, which has been thoroughly studied by *Zachariae* (14). The addition of SDS into the bacitracin solution had no significant effect on the test results.

TABLE 1
The Influence of Addition of Sodium Dodecyl Sulphate in ratio 5:1 upon Skin Reactions to Bacitracin.

Persons tested	Testing technique	Type of reaction	Influence of SDS
Control persons	Intra-cutaneous	Wheal due to histamine liberation	No effect
Patients sensitized to bacitracin	Intra-cutaneous Epicutaneous (chamber)	Delayed eczematous reaction	No effect Marked depression

With four patients sensitized to bacitracin, who were tested by the adhesive chamber method it could be confirmed that when SDS in a molar ratio of five to one was added to the bacitracin solution the strength of the reactions to bacitracin was markedly reduced and the threshold concentrations were correspondingly increased. (This was the case in spite of the fact that SDS alone at highest test concentrations tended to cause slight toxic reactions). In contrast, with intracutaneous

tests significant differences between the reactions to bacitracin alone and to its mixture with SDS were found neither in the strengths of the reactions nor in the threshold concentrations.

Thus neither the histamine-liberating effect of nor the hypersensitivity reactions to bacitracin were influenced by SDS in the intracutaneous tests, whereas in the epicutaneous reactions—evidently due to decreased rate of penetration—a marked depression was seen.

In order to study the mechanism of the decreased penetration, the stability of the bacitracin-dodecyl sulphate complex and its behaviour in an electric field was investigated using paper electrophoresis.

SDS was added to 10 per cent aqueous bacitracin solution in a series of molar ratios from one to one to five to one. Each of these five samples and bacitracin solution alone were subjected to paper electrophoresis (6V/cm, 6 hours in a Shannon apparatus) using three different buffer solutions: acetate buffers pH 5 and pH 7 and borate phosphate buffer pH 8.4. The dried paper strips were sprayed with ninhydrin 0.3 per cent in ethanol.

At pH 5 in the bacitracin solution two fractions, bacitracin A and F were seen, both moving towards the cathode. This movement was depressed by the addition of SDS.

At the ratio of 2:1 the slower moving component, bacitracin F, remained on the starting line, and with increasing concentrations of SDS all the material moved towards the anode (Fig. 2). At pH 7 the bacitracin fractions moved towards the anode at lower concentrations of SDS already, and at pH 8.4 even without addition of it.

Thus it seems reasonable to assume that at the acid pH of the skin surface the negative charge of the complex formed tends to prevent its absorption into the living layers of the epidermis where the pH is higher than on the skin surface (3). Another factor decreasing the penetration is the larger molecular size of the bacitracin-dodecyl sulphate complex.

In conclusion, we would like to emphasize that although

Molar ratio
SDS : Bacitracin

Bacitracin
alone

+

2 : 1

+

4 : 1

+

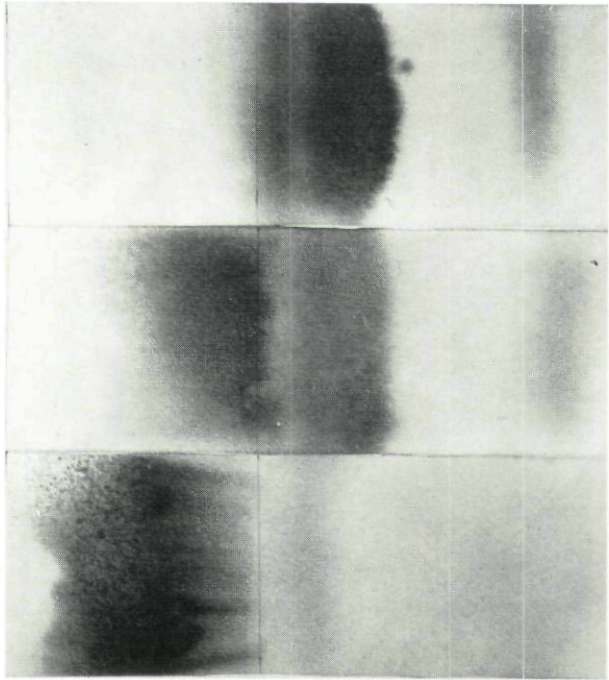


Fig. 2.

The influence of sodium dodecyl sulphate upon the behaviour of bacitracin in paper electrophoresis (pH 5).

SDS has been used by many authors to enhance sensitization or to increase the strength of patch test reactions to various allergens, it may also have the contrary effect, for instance, suppression of patch reactions to bacitracin. This phenomenon was shown to be due to decreased penetration of the bacitracin-dodecyl sulphate complex which has a negative electric charge on the acid surface.

In addition, the results presented are in good conformity with our earlier belief that the thiazoline ring of bacitracin A is essential for its eczematogenic effect (10). This structure is not impaired by addition of SDS, whereas in bacitracin F, which is an eczematogenically ineffective degradation pro-

duct of bacitracin A, it has been converted into the thiazole ring.

SUMMARY

The authors have shown that although sodium dodecyl sulphate has been used by many workers to enhance sensitization or to increase the strength of patch test reactions to various allergens, it may also have the contrary effect—for instance suppression of patch test reactions to bacitracin, a polypeptide, the amino groups of which are bound by sodium dodecyl sulphate. With the aid of both epicutaneous and intracutaneous tests, as well as paper electrophoresis, this phenomenon was shown to be due to decreased penetration of the bacitracin-dodecyl sulphate complex, which has a negative electrical charge on the acid skin surface.

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