allergens in maltworkers with extrinsic allergic alveolitis than in the rest of the maltworker population, and (c) atopic maltworkers had a significantly lower rate of skin sensitivity to occupational allergens than non-atopic workers. The first observation is not surprising, since extrinsic allergic alveolitis is not due to a type 1 allergic reaction. The second is of interest, since it suggests that the type 3 allergic reaction responsible for the disease may be accompanied by a type 1 reaction to the same allergen. The third is difficult to explain except, perhaps, on the rather dubious hypothesis that skin sensitivity to common allergens in some way inhabits the development of skin sensitivity to occupational allergens. It would appear, however, that this phenomenon does not protect such patients from the type 3 antigen-antibody reaction believed to be responsible for extrinsic allergic alveolitis.

With increasing mechanisation of the malting process the prevalence of this disease is likely to diminish rapidly, and there may never be another opportunity to confirm or refute the hypothesis that in most cases extrinsic allergic alveolitis is a pulmonary allergic reaction to inhaled spores of *A clavatus*.

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Topical chemoprophylaxis with silver sulphadiazine and silver nitrate chlorhexidine creams: emergence of sulphonamide-resistant Gram-negative bacilli

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Summary

Controlled trials of 0·5% silver nitrate compresses (SN), 1% silver sulphadiazine cream (SSD), and a cream containing 0·5% silver nitrate and 0·2% chlorhexidine digluconate (SNC) showed that all were comparably effective in protecting burns from infection. SN compresses were much less active against miscellaneous Gram-negative bacilli than the other preparations, and the mean morning and evening temperature and respiration rates in the patients treated with SN compresses were higher than those of patients treated with SSD. Pseudomonas aeruginosa and Proteus spp, though rare in all groups, were less often found in the patients treated with SN compresses. Sulphonamide-resistant Gram-negative bacilli became predominant during the trial of SSD cream on extensive burns and the prophylactic effectiveness of that preparation was thus reduced in the later stages of the trial.

Introduction

Earlier controlled trials in this unit have shown the effectiveness of various forms of topical chemoprophylaxis against bacteria that commonly colonise burn wounds and occasionally cause invasive infection.[1-3] Apart from polymyxin, antibiotics have proved unsuitable, even when effective, for routine prophylaxis, mainly because their use encourages the emergence of resistant variants of organisms that are usually sensitive; it is obviously important to reserve such agents as gentamicin for therapeutic use in severe established sepsis.

With the introduction of silver nitrate (SN),[4] mafenide,[5] and silver sulphadiazine (SSD),[6] topical chemoprophylaxis has been more widely used and received further support from controlled trials.[7-9] We report here the results of two trials, one comparing the prophylactic effects of SSD and SN on severe burns, and one comparing the prophylactic effects of SSD and silver nitrate chlorhexidine (SNC) on both extensive and less severe burns.

Silver sulphadiazine compared with silver nitrate

METHODS

Patients with extensive burns were allocated on admission alternately to a control group in which treatment was with compresses of 0·5% SN—the best established method for treating extensive burns in our previous experience[7 8 10]—and to a group treated with 1% SSD cream (Flamazine), which has been shown[9] to be effective prophylaxis for less extensive burns. Patients were eligible for the trial if they had a mortality expectation of 0·1 or more (calculated from their age and area of burn on Bull's[11] table of expected mortality). Patients with burns usually treated by the exposure method—those of the face and some burns of the trunk—were excluded from the trial. Children under 3 years of age were excluded because of the special danger in them of electrolyte imbalance caused by treatment with hypotonic silver nitrate solution. Dressings were applied and serum electrolytes controlled as described.[7]

Patients treated with SSD cream had their burns dressed as soon as possible after admission, the cream being applied thickly on strips of sterile gauze, which were covered with cotton wool and held in

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place by crepe bandage. SSD dressings were changed daily or every other day until the burns were ready for grafting or had healed.

Assessment of prophylactic value—Bacterial colonisation of burns in the treatment and control groups was assessed by culture of moist swab samples taken from all burns on admission and at every change of dressings. Bacteriological examination was the same as that described,[7] but we used cetrimide-nalidixic acid agar[12] as the selective medium for *Pseudomonas aeruginosa*. Strains of *Staphylococcus aureus*, *Ps aeruginosa*, and other Gram-negative bacilli were tested for sensitivity to SN and to sulphadiazine as described elsewhere.[9 13 14] Clinical assessments included mean morning and evening temperature and respiration rates taken from the patients' temperature charts, and mortality of patients in the trial.

RESULTS

Table I shows the growth yielded by swabs from patients in both groups while the burns were being treated with the topical antimicrobial applications. There was a higher incidence of no bacterial growth from burns treated with SSD cream than from those treated with SN compresses ($(\chi^2=7{\cdot}8$; $0{\cdot}01>P>0{\cdot}001$). This difference was due entirely to the greater prophylactic effect of SSD cream against miscellaneous Gram-negative (Coliform) bacilli ($\chi^2=156{\cdot}1$; $P<0{\cdot}001$). *Staph aureus* was, in fact, slightly more common in samplings from burns treated with SSD cream than in those from burns treated with SN compresses; *Proteus* spp and *Ps aeruginosa*, which were uncommon in both groups, were less common in the SN than in the SSD group. Heavy growth of miscellaneous Gram-negative bacilli was found more often in burns treated with SN compresses than in those treated with SSD cream; heavy growth of *Ps aeruginosa*, by contrast, did not appear in any of the 19 swabs from burns treated with SN compresses which yielded this organism, but it appeared in 13 of the 31 swabs which yielded *Ps aeruginosa* from burns treated with SSD cream. Significantly more patients showed *Staph aureus* in more than 20% of the swabs in the SSD series (13 out of 22) than in the SN series (6 out of 25) ($\chi^2=4{\cdot}6$; $0{\cdot}05>P>0{\cdot}02$); significantly more, however, showed coliform bacilli in more than 20% of the swabs in the SN series (22 out of 25) than in the SSD series (11 out of 22) ($\chi^2=6{\cdot}4$; $0{\cdot}02>P>0{\cdot}01$). The difference in numbers of patients in the two groups yielding different proportions of *Ps aeruginosa*, *Proteus* spp, and no bacterial growth was not significant.

The patients in both groups were comparable in age (SSD: mean 48·4 years, range 7-91; SN: mean 40·4 years, range 11-74), but the mean area of burn was slightly larger in the group treated with SN compresses (40·6%, range 16-87) than in the group treated with SSD cream (mean 28·2%, range 8-55). Mortality in the SN group was equal to the expected rate in the period since the introduction of silver nitrate in 1965 (10 out of 25), and in the SSD cream group it was lower (5 out of 22), but not significantly so, than the expected mortality[11] for that group (8 out of 22).

During the first week of treatment the mean (±SD) morning and evening temperatures of patients treated with SSD cream (37·27±0·042°C and 37·36±0·058°C respectively) were significantly lower than those of patients treated with SN compresses (37·38±0·035°C and 37·58±0·035°C) ($P<0{\cdot}05$ and $P<0{\cdot}001$ respectively). Mean morning and evening respiration rates of patients treated with SSD cream (23·04±0·245/min and 23·39±0·235/min respectively) were significantly lower than those of patients treated with SN compresses (24·00±0·357/min and 24·9±0·332/min) ($P<0{\cdot}05$ and $P<0{\cdot}001$ respectively). In the second week the mean evening temperature was significantly lower ($P<0{\cdot}01$) in patients treated with SSD then in those treated with SN compresses; the mean morning temperature and the respiration rates were not significantly different in the two groups.

Silver sulphadiazine compared with silver nitrate chlorhexidine

In earlier trials SSD cream[13] and SNC cream[10] were independently found to be more effective than 0·5% SN cream in protecting burns against bacterial infection. Our evidence that in some respects SSD cream may be at least as effective a prophylactic application as SN compresses prompted us to compare SSD and SNC creams as topical prophylactic applications in burns.

LESS EXTENSIVE BURNS

Methods—Patients with burns for whose mortality expectation was less than 0·1 were allocated, if otherwise eligible for the trial, alternately to groups for local prophylactic application of SSD cream and of a cream containing 0·5% silver nitrate and 0·2% chlorhexidine digluconate prepared with cetomacrogol 1000 emulsifying base (SNC cream).[10] Swabs were taken, inoculated on culture media, and examined as in the trial described above.

Results—Table II shows that the proportions of swabs yielding no bacterial growth and very scanty growth were virtually the same in the two treatment groups. The percentage of isolations and the density of growth of *Staph aureus*, *Ps aeruginosa*, *Proteus* spp, and coliform bacilli from patients in the two series were in the same range. The mean age (15·4 years, range 1-61) and mean area of burns (9·1%, range 1-25) in the SNC cream group were also comparable with the mean age (13·7 years, range 1-70) and mean area of burns (10·4%, range 1-29) of patients in the SSD cream group.

EXTENSIVE BURNS

The results of the comparison of these creams in prophylaxis of smaller burns encouraged us to compare the same creams in the prophylaxis of extensively burnt patients.

Methods—The conduct of this trial, which was started on completion of the comparison of SN compresses with SSD cream, was similar to the conduct of that trial, but children under three years of age were eligible, as silver nitrate compresses were not being used.

Results—The preliminary results of this trial, which was stopped because of the emergence of sulphonamide resistance, are shown in table III. Although the numbers of patients initially admitted to the two groups in the trial were the same, more of those treated with SNC cream were withdrawn because their burns were tangentially excised and grafted within the first week. More swabs from patients treated with SNC cream (though not significantly more) than from

TABLE I—*1% Silver sulphadiazine cream compared with 0·5% silver nitrate compresses on extensive burns*

	% Of swabs that yielded:							No of		
	No growth*	Very scanty growth†	No or very scanty growth	*Staph aureus*	*Ps aeruginosa*	*Proteus* spp	Coliform bacilli	Swabs	Burns	Patients
SSD cream	24·0	15·0	39·0	26·9	4·9	4·3	29·8	655	120	22
SN compresses	18·0	11·2	29·2	15·3	2·2	1·5	62·2	867	143	25

*No bacterial growth on solid or in liquid medium.
†No growth on solid medium, but growth in liquid medium.

TABLE II—*Silver nitrate chlorhexidine cream compared with silver sulphadiazine cream on less extensive burns*

	% Of swabs that yielded:							No of	
	No growth	Very scanty growth	No or very scanty growth	*Staph aureus*	*Ps aeruginosa*	*Proteus* spp	Coliform bacilli	Swabs	Patients
SNC cream	36·2	24·8	61·0	21·9	3·1	4·8	10·3	483	52
SSD cream	34·7	25·0	59·7	21·8	1·2	2·2	8·8	510	62

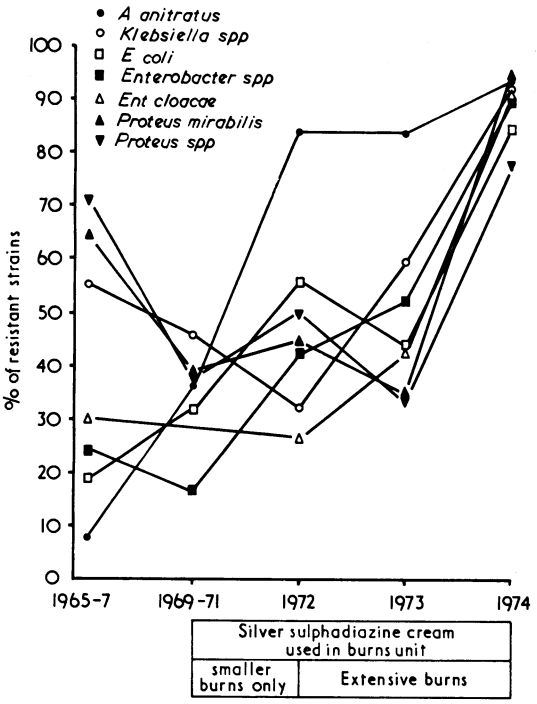
TABLE III—*Silver nitrate chlorhexidine cream compared with silver sulphadiazine cream on extensive burns (preliminary report June 1975)*

	% Of swabs that yielded:									No of		
	No growth	Very scanty growth	No or very scanty growth	*Staph aureus*	*Ps aeruginosa*	*Proteus* spp	Coliform bacilli	*Klebsiella* spp	*A anitratus*	Swabs	Burns	Patients
SNC cream	20·1	20·1	40·2	29·9	7·6	16·0	13·9	11·1	9·0	144	39	8
SSD cream	19·7	14·9	34·6	25·5	4·0	6·6	32·4	10·1	25·0	376	74	17

those treated with SSD showed no bacterial growth or only scanty growth. *Staph aureus*, *Ps aeruginosa*, *Proteus* spp, and *Klebsiella* spp were isolated from more swabs in the SNC series, but *Acinetobacter anitratus* and miscellaneous coliform bacilli were found in significantly more samplings from burns in the SSD series (*A anitratus*: $\chi^2=15{\cdot}3$, $P<0{\cdot}001$; coliform bacilli: $\chi^2=17{\cdot}1$, $P<0{\cdot}001$).

Topical antimicrobial agents and emergence of resistance

The figure shows the percentage of strains of various species of Enterobacteriaceae (one strain, when present, per patient per week, or, after 1972, per fortnight) isolated from 1965 to 1974 that were resistant to sulphadiazine in a ditch-plate test with 100 mg sulphadiazine/l in the ditch; the medium used was lysed blood agar. All the species, including some that were commonly sensitive to sulphonamides before the trials of SSD in extensive burns, became predominantly resistant during these trials. In the most recent period (1974) all species showed a large preponderance of resistant strains.



Percentage of strains of Gram-negative bacilli isolated in burns unit from 1965 to 1974 (one isolation, when present, per patient per week or, after 1972, per fortnight) that were resistant to sulphadiazine.

The minimum inhibitory concentration of a selection of resistant strains tested by a plate dilution method was greater than 1000 mg/l. Sulphadiazine-resistance was found in more strains from patients treated with SSD cream (41 out of 59, 70%) than from patients treated with SN compresses (23 out of 52, 44%) ($\chi^2=6{\cdot}26$; $P<0{\cdot}02$).

The emergence of sulphonamide-resistant strains affected the prophylactic value of SSD. Forty-six out of 335 (14%) swabs from burns treated with SSD cream and 244 out of 363 (67%) swabs from burns treated with SN compresses yielded miscellaneous coliform bacilli during 1971-2, while during 1973-4 miscellaneous coliform bacilli were isolated from 149 out of 320 (47%) swabs from burns treated with SSD cream and from 295 out of 503 (59%) swabs from burns treated with SN compresses. Similar differences were not found in respect of *Ps aeruginosa* and *Proteus* spp. The numbers of swabs yielding no bacterial growth in 1971-2 were 107 out of 335 (32%) from burns treated with SSD cream and 72 out of 364 (20%) from burns treated with SN compresses; in 1973-4 no bacterial growth was found in only 50 out of 320 (16%) swabs from burns treated with SSD cream, but only a slightly smaller proportion (83 out of 503; 17%) of swabs from burns treated with SN compresses yielded no bacterial growth in the later than in the earlier period.

Tube dilution tests with chlorhexidine digluconate on a series of strains of *Staph aureus*, *A anitratus*, *Klebsiella* spp, *Proteus* spp, and *Escherichia coli* isolated from patients treated with either SNC cream or SSD cream showed no appreciable difference in the range of MICs for the strains of bacteria isolated in the two treatment groups; all appeared sensitive, the MIC for *Staph aureus* being 0·25-2 mg/l, and for Gram-negative bacilli 0·5-32 mg/l.

The proportion of "silver nitrate-resistant" strains of coliform bacilli[7]—that is, strains that would grow in nutrient agar made up with 0·5% silver nitrate—was 11 out of 44 (25%); the proportion of such strains isolated from burns in this unit was 4 out of 33 (12%) in 1965-6 and 27 out of 112 (24%) in 1969.[13]

Discussion

These and earlier trials in the unit have shown that SSD cream is a highly effective topical prophylactic agent, but its value during treatment of extensive burns has been reduced by the emergence of sulphonamide-resistant strains of various Gram-negative bacilli. The recent predominance of these strains in the unit made it necessary to suspend the use of SSD cream and also to place the sulphonamides and co-trimoxazole on the unit's reserve list of antimicrobial drugs[15] until the proportion of sulphonamide-resistant Gram-negative bacilli has fallen substantially. Fortunately, comparative trials showed that SNC cream had a propylactic value comparable to that of SSD; indeed, our trial on extensive burns—unfinished because of the emergence of sulphonamide resistance—showed SNC cream to be, probably because of this emergence of sulphonamide-resistant strains, more effective than SSD cream against some of the Enterobacteriaceae. Neither SNC nor SSD cream were as effective as SN compresses against *Ps aeruginosa*, and previous trials suggest that mafenide would also be more active against that organism.[9] Nevertheless, clinical results in these trials did not suggest that SN compresses were more effective in preventing invasive infection and death than SNC or SSD cream.

There is still a case for using SNC compresses on burns of the genitalia and perineum: no other method seems to give such good protection in this difficult area, where *Ps aeruginosa* colonisation so often begins. Like SN compresses and unlike the relatively insoluble SSD, SNC cream rapidly turns brown or black on standing. We are now studying a cream containing chlorhexidine with silver phosphate, which is as poorly soluble and as stable as SSD.[16]

We wish to thank our clinical colleagues and the nurses for their co-operation in these trials and Mrs Clare Wong for secretarial help.

ADDENDUM—In the six months after suspending the use of sulphonamides and silver sulphadiazine in this unit sulphadia-

zine sensitivity in most of the Gram-negative bacilli has reverted to its earlier levels.

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Epidermal architecture, growth, and metabolism in acromegaly

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Summary

Epidermal architecture, replication, and anabolic activity were studied in six patients with acromegaly. Patients with acromegaly had significantly larger viable epidermal cells than controls. The rates of incorporation of tritiated thymidine, proline, and histidine into skin slices in vitro was also significantly increased in acromegalic patients. The mean autoradiographic labelling indices after intracutaneous injection of tritiated thymidine were 8·4% in the acromegalic patients and 5·1% in the control group. None of the changes observed could be closely correlated with levels of serum growth hormone.

Introduction

Many of the physical features of acromegaly are the result of skeletal overgrowth and generalised thickening and enlargement of soft tissue structures. The skin seems to share in this general hypertrophic process—for example, the dermis becomes thickened.[1] There is also increased sebaceous gland activity.[2] The aim of our study was to determine whether epidermal structure, growth, and metabolism are altered in acromegaly and to determine whether there is a correlation between any changes observed and levels of serum growth hormone.

Patients and methods

Six women aged 31-67 years (mean age 56 years) were studied and compared with a control group of 25 normal adults (17 women and 8 men aged 18-77 (mean 51 years)). Five patients were untreated, and one had undergone surgical hypophysectomy two years earlier. Acromegaly was diagnosed in each patient by clinical and radiological assessment and measurement of serum growth hormone. All the patients studied were euthyroid at the time of the study.

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EPIDERMAL ARCHITECTURE

Skin biopsy specimens taken from the deltoid area were fixed in 10% formalin, dehydrated, embedded in paraffin, sectioned, and stained with haematoxylin and eosin. The mean thickness of the epidermis was determined by counting the numbers of cells along an axis perpendicular to the skin surface at 30 random points and expressed as mean epidermal cell thickness (MET).

The rete pattern of the epidermis was measured by calculating the ratio of the length of the basal layer to the length of the granular cell layer (B:G ratio) using a microscope drawing tube-projection technique.

The size of epidermal cells was expressed as the mean epidermal cell diameter (MECS). This was calculated by dividing the actual thickness of the viable epidermis (in μm) by the MET. Epidermal thickness was measured using a calibrated eye-piece graticule.

EPIDERMAL GROWTH

The replicative activity of the epidermis was studied in two ways.

In-vivo method—Biopsy specimens were taken from the deltoid area one hour after intracutaneous injection of 10 μCi of tritiated thymidine (specific activity 18 Ci/mmol) in 0·1 ml normal saline. Autoradiographs were prepared using a dipping method.[3] The number of basal and suprabasal cells that became labelled in DNA synthesis was expressed as a percentage of the total number of basal cells (labelling index (LI)).

In-vitro method—The rate of incorporation of tritiated thymidine into skin sheets was estimated (see below). Results were expressed as corrected counts per minute (cpm) mm^2 tissue^{-1} hour incubation^{-1} (thymidine units).

ANABOLIC ACTIVITY

Measurements were made of the rates of incorporation of tritiated proline (specific activity 18 Ci/mmol) and tritiated histidine (specific activity 44 Ci/mmol) into pieces of skin 0·4 mm thick and about 50 mm^2 in area that had been removed from the lateral aspect of the thigh with a Castroviejo keratotome using a method simliar to that of Marks *et al.*[4] The skin portions were incubated epidermis uppermost in Eagle's Minimal Essential Medium for four hours at 37° C in an atmosphere of 95% air and 5% CO_2. The radioactive precursors were added to the medium in a concentration of 1 mCi/l medium. Tritiated thymidine (specific activity 2 Ci/mmol) was used to assess replicative activity. After incubation the skin was washed, homogenised, and extracted with perchloric acid. The residue was solubilised with a highly basic biological solvent (Soluene, Packard) and the contained radioactivity was measured in a scintillation counter. The results were expressed as corrected cpm mm^2 tissue^{-1} hour incubation^{-1} (proline or histidine units).