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In vitro release of nickel sulphate of varying particle size from paraffin bases

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Summary

The in vitro rate of release of nickel from suspensions of nickel sulphate in soft paraffin, as used in patch testing for contact dermatitis, showed a marked dependency on particle size in the range 12–320 μm (geometric mean size), release being fastest with the largest particles. Increasing the proportion of hydrophilic base by admixture of cetomacrogol 1000 with the soft paraffin increased nickel release. Determination of water uptake supplemented by conductance measurements suggested that in vitro release correlated with penetration of water into the base. The presence of nickel sulphate itself enhanced water penetration perhaps by percolation mechanism. Patch tests on patients, however, assessed by clinical scoring, and supplemented by infra-red thermography, laser Doppler flowmetry and skin reflectance measurements failed to show a clear correlation between in vivo response and in vitro release into a large volume of water. It is argued that in the presence of the restricted amounts of moisture available to the formulation, in vivo dissolution will occur preferentially from the smaller crystals.

Introduction

It is now accepted that the vehicle in which topical therapeutic agents are applied to the skin can control the bioavailability of the active ingredient. The release of active material from the vehicle is determined by its solubility in the vehicle and its partition coefficient between vehicle and skin. There is therefore no standard base in which all drugs can be applied to the skin for optimal effect; each vehicle must be optimised for a given agent. However, it is still the practice in patch testing for allergic contact dermatitis to use a single vehicle for a wide variety of topical allergens. This vehicle is usually yellow soft paraffin (paraff. molle flav., PMF) which is used regardless of the physicochemical properties of the allergen involved. The universal use of PMF has been criticised before by van Ketel (1979) and by Wahlberg (1980). Fischer and Maibach (1984) have also re-appraised petrolatum as a vehicle and found many preparations wanting in basic pharmaceutical specifications, e.g. in relation to homogeneity of the active ingredient, stability and particle size control, all essential for reproducibility of effect.

Crommelin and de Blaey (1980a and b) found the release rate of water-soluble drugs from vehicles in which they are insoluble, increased

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with increasing particle size, contrary to naive expectation and to experience with most other pharmaceutical systems. Izgu and Gungor (1981) have obtained similar results with paracetamol in Witepsol bases, and suggest that the smaller particles agglomerate thus reducing their surface area. A rank-order in vitro–in vivo correlation was obtained. The release of salicylic acid from triglyceride bases is increased by increasing the amounts of water incorporated into the base (Turakka and Tiokkanen, 1983).

We have recently shown (Mendelow et al., 1985; Mendelow and Baillie, 1985) that the vehicle can influence the outcome of patch testing with nickel sulphate, apparently by modifying the quantity of nickel released into the skin to elicit the allergic response. Nickel sulphate is insoluble in yellow soft paraffin and as a result the mechanism of release is likely to be the dissolution of crystals in the limited amounts of moisture present at the interface between the preparation and the skin surface. In this paper we examine the influence of the particle size of the nickel salt, as this parameter can vary between proprietary test preparations. Although we are principally concerned with particle size effects, we have also investigated the role of the vehicle in nickel release. The putative allergen cannot be considered in isolation; allergen and vehicle must be considered to be part of the one test system.

Materials and Methods

Nickel sulphate (Analar grade, $NiSO_4 \cdot 7H_2O$) was obtained from BDH Chemicals U.K. Yellow soft paraffin B.P. and Cetomacrogol Cream (Formulation A) were obtained from Evans Medical U.K.; Cetomacrogol 1000 B.P. was obtained from Duncan Flockhart, Edinburgh.

The materials were used as received, except for nickel sulphate which was dried at 50 °C overnight, and fractionated by seiving. The largest crystal size fraction had a geometric mean diameter of 320 μm, and the medium size fraction, 41 μm. The finest fraction was obtained using a fluid energy mill at a pressure of 80 lbs/sq. in. The resulting crystals had a geometric mean diameter of 12 μm. (Figs. 1a, b, c).

Proprietary nickel sulphate test materials. These were obtained from Trolab-Hermal-Chemie, F.R.G. as a 5% preparation, and from Hollister-Steier, U.S.A. as a 2.5% preparation (Fig. 1d, e).

Preparation of nickel sulphate ointments. The 3 crystal fractions were used to prepare 3 ointments by trituration with PMF, taking care not to alter the particle size distribution of the nickel sulphate in the process. 1% nickel sulphate preparations were used for patch testing and 5% preparations for the in vitro dissolution experiments.

Another series of preparations were made, containing proportions of Cetomacrogol 1000 to allow investigation of the effect of the hydrophilicity of the vehicle on the nickel sulphate release profiles. Cetomacrogol 1000 concentrations above 60% were not used since a uniform dispersion of crystals could not be obtained on cooling the system. Where relevant, 1% nickel sulphate systems were used in the clinic and 5% systems for in vitro experiments.

Conductivity measurements. A small container of vehicle was placed on a 'Labjack' such that the vehicle could be raised up to parallel electrodes which then probed the vehicle to different depths. The electrodes, parallel pins 0.5 cm apart vanished to within a few millimeters of the tip, were connected, to a constant 15 V AC output and the current passing between them at different levels in the vehicle measured in milliamps.

Characterisation of products. The nickel sulphate ointments were examined microscopically. Angular, irregular crystals were observed especially in the formulations with the largest particle size of nickel sulphate. The crystals which had been micronized in the fluid energy mill were more regular in size. Proprietary formulations of nickel sulphate were also examined microscopically. Mixed vehicles of PMF and Cetomacrogol 1000 were not homogeneous. Domains of (presumably) Cetomacrogol 1000-rich composition demonstrate the inhomogeneous nature of the systems, which increases with increasing volume fraction of the surfactant.

Measurement of in vitro release from ointment bases. The release rates of nickel sulphate from

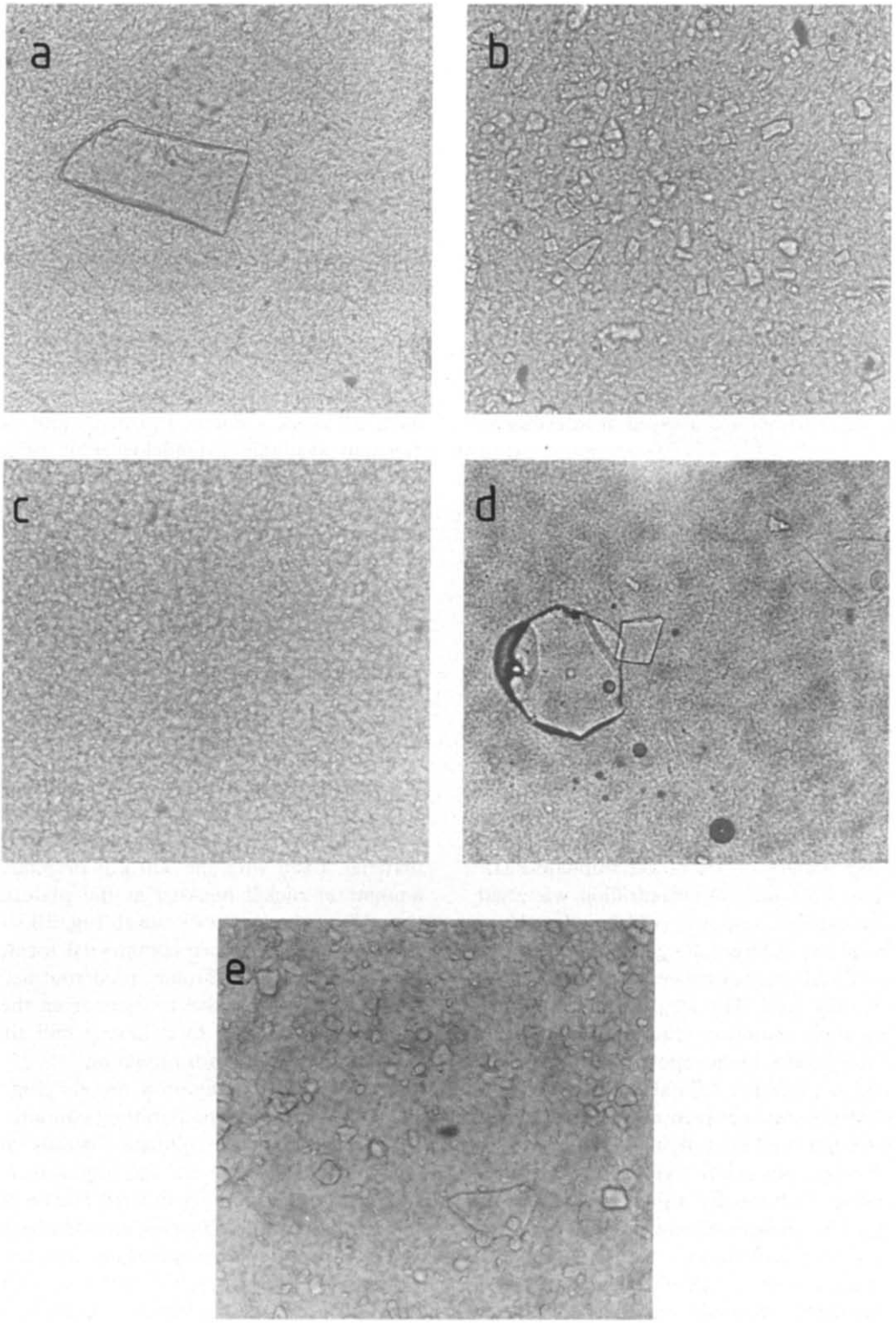

Fig. 1. Photomicrographs of the 3 nickel sulphate preparations: (a) mean size 320 μm; (b) mean size 41 μm; and (c) mean size 12 μm. (d) and (e): photomicrographs of the two commercial nickel preparations: (d) Hollister-Steier; and (e) Trolab.

the various semi-solid preparations were determined in vitro. The ointments were placed in dialysis sacs (Visking Cuprophane Tubing, Medicell) 4 cm long × 0.5 cm diameter, the ends sealed with Mediclips, and placed in water (200 ml) at 30 °C. At intervals 5 ml samples of dialysate were taken and analysed for nickel content at 232 nm using a Pye SP9 Atomic Absorption Spectrophotometer. Each 5 ml sample was replaced with an equal volume of water to maintain constant volume.

In an alternative system, the preparation was filled into a Teflon ring so that only one surface of diameter 35 mm was exposed. The ring was placed in 200 ml water which was assayed at intervals as before.

To determine water uptake by the vehicle, water (2 ml) was placed on the surface of a known weight of ointment in a small plastic beaker which was allowed to stand for 120 min, the water poured off and the sample reweighed.

In vivo testing of formulations. In vivo testing was carried out on nickel-sensitive patients at the Contact Dermatitis Investigation Unit, Belvidere Hospital. The trial was divided into two parts. The nickel sulphate-PMF preparations were studied in 28 patients. After the routine initial patch test patients who had shown a positive allergic response to the 5% standard nickel test were invited to participate in the trial. All were then challenged with the test battery of 1% nickel sulphate-PMF formulations. This nickel concentration was used to minimise extreme response (which reduced patient discomfort). Lower allergen concentrations should permit differences between preparations to be more readily seen. The formulations were applied using Finn chambers (Epitest, Finland) attached to the patient by Scanpore tape. Chambers were placed at twice the normal distance apart to minimise interference between adjacent reactions. Patches were removed after 48 h and the responses assessed by the physician (AF). Some patients were assessed additionally using laser Doppler flowmetry, skin surface reflectance and, or infra-red thermography techniques.

Clinical assessments. These were made according to standard methods, grading responses according to the scale: +1 = erythema, +2 = erythema, palpable, +3 = vesicles, oedematous and weeping, +4 = irritant, and E = equivocal. The diameter of the spread of reaction was measured in 18 patients and most reactions were photographed.

Laser Doppler flowmetry, skin reflectance and infra-red thermography. The techniques used are discussed in detail elsewhere (Mendelow et al., 1986). Laser Doppler measurements were made with a Periflux KB instrument and variations in the skin blood flow at each site were compared with normal untreated sites. Skin reflectance measurements were made with a Haemelometer (designed and built by the Medical Physics Department of Leeds General Infirmary and not commercially available) (Mendelow et al., 1986). Infra-red thermography was carried out using an AGEMA Thermovision 782 System under standardised conditions. Typical infra-red thermograms of patch test sites obtained with this system are shown in Mendelow and Baillie (1985).

Results and Discussion

In vitro

The influence of nickel sulphate particle size on its in vitro release from PMF preparations is shown in Fig. 2A. Most rapid and extensive release is observed from the largest particle size material. Even with the 320 μm preparation, the amount of nickel released at the plateau is less than 1% of the available nickel. Fig. 2B shows the nickel release from two commercial formulations, Hollister-Steier and Trolab, used routinely in the clinic. Release of nickel is faster from the former preparation, in spite of it having half the nickel content of the Trolab formulation.

Microscopic examination reveals (Fig. 1) that the Hollister-Steier preparation contains significantly larger nickel sulphate crystals and thus gives further support for the importance of particle size in determining in vitro release from this type of preparation (Fischer and Maibach, 1984). The two proprietary preparations are not directly comparable due to the different viscosities of the paraffin used as the vehicle. A lower viscosity might allow greater spread of the preparation on

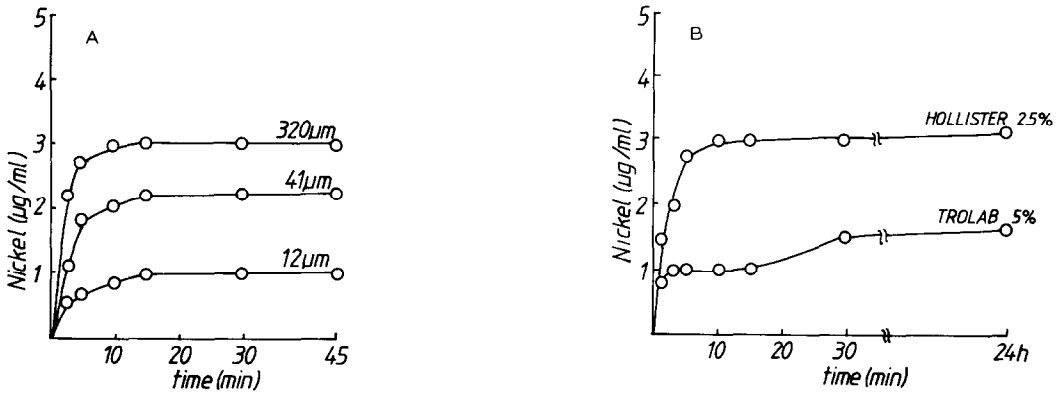

Fig. 2. A: rate of solution of nickel from a 5% nickel sulphate preparation in the PMF vehicle into 200 ml water, showing the effect of the mean particle size of nickel sulphate. B: the rate of solution of two commercial formulations of nickel sulphate in paraffin vehicles, into 200 ml water. Note that the Hollister-Steier preparation contains 2.5% nickel sulphate, while the Trolab preparation has 5%.

the skin from the test chamber and might allow more rapid sedimentation of the nickel sulphate crystals (density = 1.948 $g \cdot cm^{-3}$) and greater contact with the skin.

Increasing the hydrophilic nature of the vehicle by addition of Cetomacrogol 1000 increases the rate of release of nickel. Fig. 3 shows the effect of Cetomacrogol 1000 concentration on the release of nickel from preparations containing the 320 µm nickel sulphate particles. Similar increased nickel release was observed from the other formulations with smaller particle sizes. The Cetomacrogol 1000 concentration-dependent increased nickel release showed a rank order correlation with water uptake (Fig. 4).

The greater weight gain of ointments containing 5% w/v nickel sulphate suggests that percolation effects, which would enhance water penetration, might be in operation, and might also occur in the Cetomacrogol-PMF vehicle. The initial rates of nickel release from the pure PMF vehicle (Fig. 2A) are so rapid that it seems that release must be by direct dissolution of exposed surface crystals. With homogeneously dispersed particles to which solvent has free access, the smaller particles, having the greater surface area should dissolve more rapidly. If, however, water penetration into the

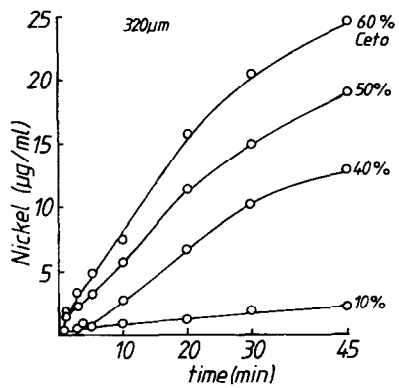

Fig. 3. Release of nickel from 5% nickel sulphate (320 µm) preparations in PMF-containing percentages of Cetomacrogol 1000 increasing from 10% to 60%.

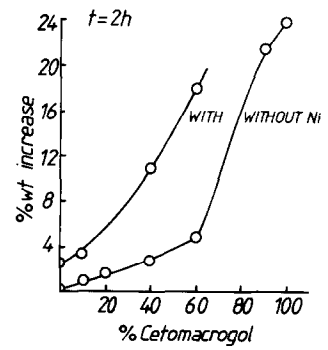

Fig. 4. Water uptake into the vehicles leading to increased weight of PMF bases with and without 5% nickel sulphate as a function of Cetomacrogol 1000 concentration.

vehicle is limited release begins to be determined by the exposed rather than the theoretical surface area. As an example, the dissolution into 200 ml water of one cubic 320 μm particle would produce levels of nickel of 0.065 $\mu g \cdot ml^{-1}$; the maximum level actually achieved of about 3 $\mu g \cdot ml^{-1}$ (Fig. 2A) suggests that a relatively small number – in this example 46 – of the large crystals have dissolved, that is some 5% of the available particles in the ointment mass used in the dissolution experiment. That is, water penetrates 5% of the 6 mm mass, or to a depth of some 300 μm. This is approximately the dimension of the crystals involved and suggests that only the nickel sulphate crystals exposed at the vehicle surface are dissolving.

Applying this mechanism to the preparation containing the smallest crystals, water penetration to a depth of the crystal dimension represents the equivalent of 0.2% of the depth of the ointment mass. However, it can be seen from Fig. 2A that with the smallest crystals the maximim nickel release is 1 $\mu g \cdot ml^{-1}$, which is over 1.6% of the available nickel in the mass. This is in accord with water penetrating to a depth much greater than the crystal dimension. Water movement through the PMF base itself will be negligible and percolation of the water through the channels provided by the dissolution of water-soluble crystals is a more probable mechanism.

To test the percolation hypothesis, addition of Cetomacrogal 1000 to the PMF as a means of introducing hydrophilic domains, led, as expected, to an increase in nickel release rate (Fig. 3). If small quantities of water are added to such a mixed vehicle, the ability to allow passage of an electric current proves a simple indication of interconnecting aqueous domains which are a prerequisite for the percolation mechanism. At a fixed amount of incorporated water (10% w/v), PMF–Cetomacrogol 1000 mixtures do not conduct until the percentage of Cetomacrogol reaches circa 15% w/w (or 0.126 volume fraction) (Fig. 5). Connecting pathways are unlikely to exist in systems with > 85% PMF. The threshold volume fraction for percolation to occur in a continuous model in which the "allowed" volume (i.e. the volumes equivalent to nickel or Cetomacrogol 1000

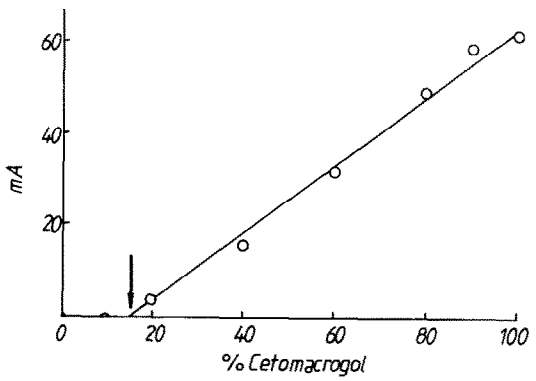

Fig. 5. Conductance of PMF–Cetomacrogol 1000 mixtures exposed to water as a function of Cetomacrogol 1000 concentration. A threshold concentration of Cetomacrogol of below 0.2 volume fraction is indicated.

in this paper) is 0.29 or 0.25 depending on the details of the model (Shanks and Kirkpatrick, 1971; Kirkpatrick, 1973). In the systems under consideration here, the volume fraction of water itself is 0.10 making the threshold effectively 0.20–0.21.

Conductivity measurements also provided a means of determining water penetration into Cetomacrogol/PMF vehicles on exposure of the vehicle surface to the solvent. Admixture of 40% or more Cetomacrogol to PMF gave measurable conductivities down to a depth of about 4 mm and for a 100% Cetomacrogol vehicle, apparent penetration was down to more than 10 mm. Even after 24 h exposure, there was no water penetration, as determined by conductivity, into PMF vehicles with Cetomacrogol concentrations of 20% or less. Fig. 6 shows the proximity of the hydrophilic conducting domains in PMF–Cetomacrogol 1000 mixtures. When either soluble crystalline material or Cetomacrogol 1000 forms the channel for percolation, there must be regions where the barrier of PMF is sufficiently thin or irregular to allow transport of water and conduction of ions.

Pathways will be stepped and determined by the major axis of the particular crystal or domain and by the volume fractions of the additive and to an extent by the size distribution of the additive. Critical PMF lamellae between the conducting domains will be more likely to conduct as the temperature of the media is increased.

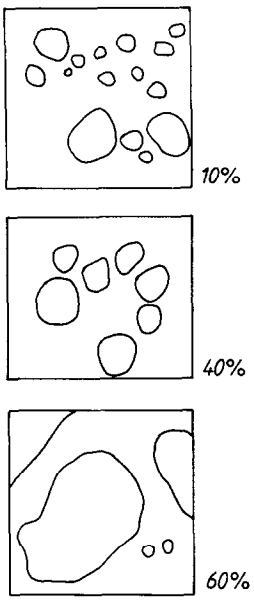

Fig. 6. Representation (from photomicrographs) of the discrete Cetomacrogol-rich hydrophilic domains in PMF systems containing 10%, 40% and 60% Cetomacrogol 1000.

In vivo responses

In vivo patch tests might prove positive in spite of any lack of optimisation because extremely small amounts of nickel are required to elicit a biological response (Allenby and Goodwin, 1983). We now examine whether the release rate characteristics of the systems have any relevance to the clinic.

Table 1 shows the patch test results for skin reactions in 17 patients to the 3 formulations containing 3 particle sizes of allergen. In all cases it was impossible to differentiate clinically between the responses induced by the 3 formulations. All patients were known to be nickel-sensitive and therefore overwhelmingly positive reactions were anticipated. The mean diameters of the areas of response indicate a rank order opposite to that expected from the in vivo data, the formulations containing the smallest nickel crystals producing the greatest spread of inflammatory reaction, the mean diameters being 11.8 mm (320 μm), 13.8 mm (41 μm) and 15.2 mm (12 μm).

TABLE I

In vivo response to nickel of 3 particle sizes in PMF using clinical assessment and diameter of skin reactions.

Patient	320 μm		41 μm		12 μm	
	Score	Diam. (mm)	Score	Diam. (mm)	Score	Diam. (mm)
1	+2	16	+2	20	+2	22
2	+3	14	+3	18	+3	20
3	+1	10	+1	8	+1	8
4	+2	8	+2	8	+2	6
5	+1	5	+1	10	+1	14
6	+3	10	+3	10	+3	14
7	+2	9	+2	17	+2	13
8	+2	8	+1	8	+2	10
9	+3	10	+3	12	+3	14
10	+3	8	+3	8	+3	8
11	+2	8	+2	10	+2	14
12	+3	12	+3	16	+3	18
13	+3	15	+3	17	+3	18
14	+2	10	+2	12	+2	14
15	+3	13	+3	20	+3	22
16	+3	13	+3	20	+3	22
17	+3	18	+3	20	+3	22
x		11.8		13.8		15.2
S.E.		0.8		1.2		1.3

It is clear from Tables 1 and 2 that clinical scoring could not distinguish between the preparations applied to individual patients, although there is a statistically significant difference between the mean diameters of the inflammatory response. These results are confirmed by the data in Table 2, obtained by Haemelometer along with clinical scoring of another group of patients in which the smallest crystals gave the greatest erythematous response. Two patients were assessed by laser Doppler flowmetry; readings which are the frequency of the rhythmical variations in human skin blood flow per minute increased from around 5% on normal skin to between 40% and 65% as seen in Fig. 7. The technique can distinguish between responses apparently identical to the eye; the results in one patient indicated that the smallest crystal formulation produced the largest blood flow increase, although in the second this trend was not seen.

In the first patient the response to the 12 μm formulation was significantly greater than to the other two systems. Infra-red thermograms of applied patches tend to confirm these data in individual patients.

We must conclude that the in vitro dissolution tests for nickel release that we have carried out are inappropriate for predicting in vivo performance in the patch test. This does not detract from the evidence that the formulation of the test patch is important. Further work must be done to find a valid in vitro test which will be able to compare formulations.

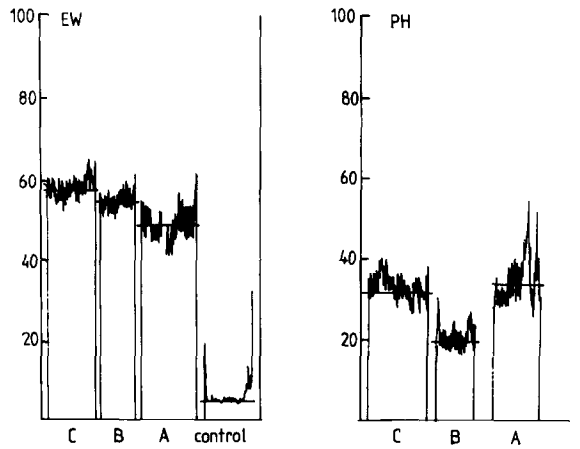

Fig. 7. Laser Doppler flowmetry results of two patients in which preparations A, B and C containing different nickel sulphate particle sizes have been applied. A = 350 μm; B = 42 μm; C = 12 μm.

TABLE 2

Haemelometer measurements of responses to 1% nickel sulphate in PMF

Patient number	Haemelometer reading [a]		
	320 μm	41 μm	12 μm
1	1.46	1.23	1.61
2	0.86	1.17	1.42
3	1.25	1.39	1.76
4	1.14	1.14	1.69
5	1.85	1.60	1.51
x	1.31	1.31	1.60
S.E.	0.15	0.0076	0.055

[a] Correct response which is calculated from: [post-test Hb reading/pre-test Hb reading] × [pre-control Hb reading/post control Hb reading].

Acknowledgements

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