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Simulated human skin scales

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SUMMARY

Human skin scales which have been shed naturally bear a flora of micro-organisms which is unknown until tested. To replace these scales in a study of the micro-environment of both the human body and of models a method has been devised of making synthetic scales which behave both physically and aerodynamically in a similar way to the natural material. The synthetic materials carry no natural flora and it is possible to include in them test markers of several kinds to assist in identification after dispersion.

INTRODUCTION

At the boundary between animal bodies and the air surrounding them there exists a layer of convected air which moves continually upward (Lewis *et al.* 1969). It has been shown (Clark, Cox & Lewis, 1971) that small particles may be carried upwards in this layer. It has also been shown (Davies & Noble, 1962) that micro-organisms are carried on naturally shed skin squames, and it is possible that aerial microbial contamination is caused at least in part by the movement of infected squames at first in the boundary layer, and later in the environment after the mixing of the boundary layer.

As part of the study of the micro-environment of the human body a supply of particles was required which could be introduced into the boundary layer, and which would carry test markers of several kinds, both animate and inanimate. Although natural skin squames can be collected from laundries, such particles carry a microflora which is unknown until tested and identified. We therefore decided to prepare synthetic squames having the same physical characteristics as the natural material, but which were free of natural micro-organisms and were non-antigenic.

Several natural polymers were tried, and were finally discarded in favour of a mixture of ethyl cellulose and stearic acid; this was found to have the nearest physical properties to those of human squames judged by particle size distribution, shape, density, aerodynamic behaviour, electrical charge and moisture regain capacity.

MATERIALS AND METHODS

Preparation of synthetic particles

Particles were initially prepared from gelatin, agarose, dextran T70 and bovine serum albumin, by preparing 10% (w/v) aqueous solutions of the polymers and spreading them in a thin film on siliconed glass sheets. When dry, the film was scraped off the glass and the particle size reduced by roller-ball milling for 3–5 hr. Alternatively a vibrating-ball mill was used for 15 min. The fraction of powdered material which passed through a 300 mesh sieve (i.e. all particles $< 53\ \mu$m.) was collected. To assist in the study of the aerodynamic properties of the particles while in flight, gelatin particles were made fluorescent by the addition of fluorescein isothiocyanate and rhodamine isothiocyanate (FITC and RITC, BDH).

Subsequently particles were made using ethyl cellulose, grade N50 (Hercules Powder Co. Ltd.), and finally ethyl cellulose mixed with stearic acid was used in the proportions by weight 50:50 and 60:40. These were made up as 10% (w/v) solutions in chloroform and the addition of 0·05% dimethyl POPOP (Packard, Zurich) to the solution made fluorescent particles. Thin films were spread on glass, dried and scraped off, as before.

Particle-size reduction was initially carried out using a vibrating-ball mill (Podmore), in which the scraped particles were wet-ground for 10 min. intervals, wet seived through a 325-mesh sieve (particle size $< 44\ \mu$m.), the eluate centrifuged to concentrate, and dried in a hot-air oven at 40° C. This process was tedious and gave particles of wide size range. Size reduction was finally affected using another method.

When a pressure of 20 tons/in.^{2} is applied to ice at $-25°$ C. a change in crystal structure occurs. A press (X press Biox, Nacka, Sweden) using this principle was employed to shear particles suspended in ice at $-25°$ C. Particles obtained by this method had a narrower size distribution than particles prepared previously.

After size reduction a variety of drying methods were tried: rotary evaporation, freeze-drying and finally filtration of the thawed suspension through sintered glass filter porosity no. 3, followed by desiccation of particles retained on the filter.

Evaluation of particles

Size and shape

Particle-size distribution and shape were determined microscopically using a calibrated eyepiece. A few skin scales were obtained for comparison by lightly scraping the skin with a scalpel from arms and legs of several individuals. Measurements were also made by means of fluorescent incident differential interference contrast microscopy of the scales on the surface of human and 2-day-old rat skin.

Density measurements

Density of synthetic particles was determined by calculation and that of skin scales taken as the equivalent density of alcohol in which the scales remained suspended in dynamic equilibrium.

Table 1

s.g. H_2SO_4	% H_2SO_4	Relative humidity	Vapour pressure
	$CaCl_2$ desiccator	0	0
1·50	61·0	18·8	3·3
1·40	51·0	37·1	6·5
1·30	40·0	58·3	10·1
1·20	28·0	80·5	14·0
1·00	1·0	100·0	17·4

Where 1·83 g./ml. = 100% H_2SO_4.

Aerodynamic behaviour

Aerodynamic behaviour of the particles was compared using a vertical laminar air-flow channel (Clark, Cox & Lewis, 1970) with constant upward air velocity of 20 cm./sec. Particles injected into the upward moving layer of air from a capillary tube by tapping, either impinged on the side walls or on a filter at the head of the channel. Particles deposited at graduated increments up the front glass wall of the channel were counted and a particle-size distribution at three equidistant positions determined.

Electrophoresis and surface charge

Electrostatic charges on different particles were found to affect their aerodynamic behaviour. Measurement of electrostatic charges of particles in air proved to be difficult, hence intrinsic surface charge on particles was compared by particle electrophoresis in a conducting liquid (Brinton & Lauffer, 1959). Particle electrophoresis was carried out in a small glass electrophoresis chamber filled with a suspension of particles in barbiturate buffer (ionic strength = 0·01, pH 8·5). Movement of particles was observed microscopically and the time taken for particles to travel a fixed distance (using a calibrated eyepiece grid, 1 division = 180 μm.^{2}) was determined over a range of voltages (0–100 V.).

Moisture regain capacity

Determination of moisture change of ethyl cellulose/stearic acid (60:40) particles and human skin scales over a range of relative humidities at room temperature (21° C.) was carried out in equilibrated sealed humidity chambers, using differing sulphuric acid concentrations to give known relative humidities (Table 1). Moisture change was taken as the difference in weight of the particles before and after equilibration for 2 weeks in the sealed chambers.

RESULTS

The large numbers of particles counted for particle-size distribution gave a normal Gaussian distribution, with the mean sizes (length × width) shown in Table 2. The figures for scraped skin scales (Table 2) refer to the scales scraped from four individuals, the back of the hand being used in each case; the fifth value refers to scraped scales from legs used in the aerodynamic evaluation.

Table 3 shows specific gravity of natural and synthetic particles.

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Table 2. *Mean sizes (μm.) of natural skin scales and synthetic particles*

Human skin scales			Synthetic particles	
Scraped	*in situ* on skin	Rat scales: *in situ* on skin	Ethyl cellulose (vibrating ball mill)	Ethyl cellulose/ stearic acid (X-press)
34·0 × 22·6	23·0 × 17·1	38·1 × 25·0	20·5 × 14·0	32·4 × 32·6
35·1 × 22·8				
37·3 × 29·3				
28·6 × 19·2				
43·2 × 24·8				

Table 3. *Specific gravity of natural and synthetic particles*

Scraped skin scales	0·95
Gelatin	1·27
Ethyl cellulose	1·15
Ethyl cellulose/stearic acid	1·01

Table 4. *Distribution of natural and synthetic particles in laminar-air flow channel*

Particle	Weight used (mg.)	Position along channel from point of injection (Y)									
		28·6	35·0	41·2	47·7	54·0	60·3	66·6	72·9	78·0	83·1
Scraped skin	2·15	61	58	69	70	60	40	41	62	73	74
Gelatin	20·90	15	14	26	32	39	30	24	40	36	23
Ethyl cellulose	20·60	68	72	59	62	54	55	33	36	49	75
Ethyl cellulose/stearic acid, 60:40	4·10	55	61	41	43	55	53	46	64	40	45

Y = length of channel travelled (cm.).

Table 4 shows weights of particles injected into the laminar air-flow channel and the numbers of particles deposited on the front wall.

Agglomerates and particles > 40 μm. fell out of the channel as a low upward velocity was used. Fig. 1 shows the channel wall deposition of the particles mentioned in Table 4. Although low weights of skin scales and ethyl cellulose/stearic acid particles were used, similar counts to those of ethyl cellulose were obtained, indicating that the size ranges of the two former types of particle were narrower than that of ethyl cellulose.

Figs. 2–5 give a size distribution of the four different types of particles counted above at three positions along the channel wall, where Y equals 35·0, 54·0, 72·9 cm. respectively from the point of injection. In each case, particles were evenly distributed up the channel face, a complete size range being counted at each position.

The results of particle electrophoresis are shown in Fig. 6. All particles were negatively charged, all moving in the same direction, towards the anode.

Fig. 7 shows percentage moisture change of ethyl cellulose/stearic acid particles and skin scales with humidity, after 2 weeks equilibration.

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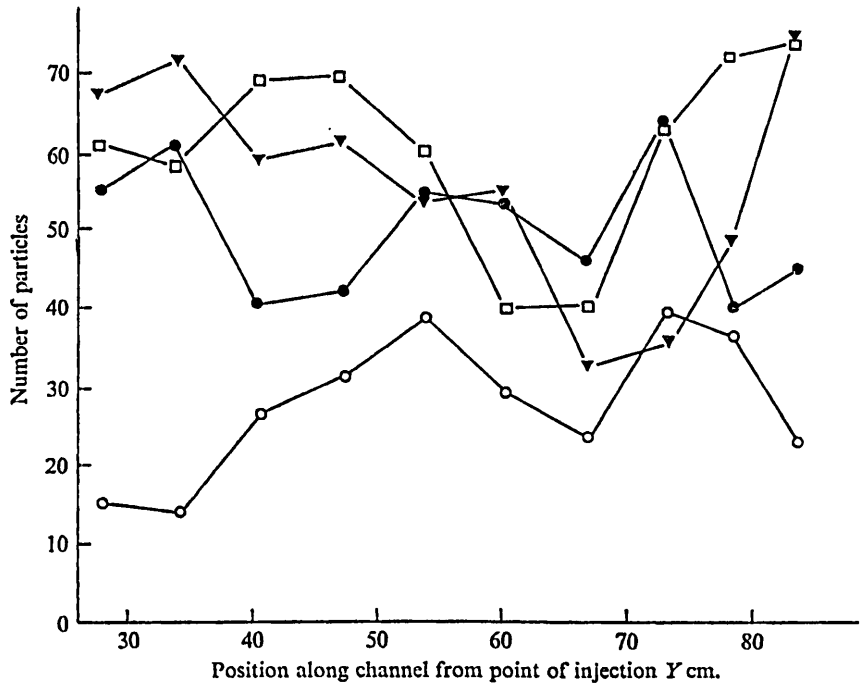

Fig. 1. Channel wall deposition of (○) gelatin, (▼) ethyl cellulose, (●) ethyl cellulose/stearic acid particles, and (□) skin scales.

DISCUSSION

Manufacture of the synthetic particles by ball milling was discontinued as the process was too lengthy and gave too wide a particle size distribution. The time taken to produce discrete particles in the range 15–50 μm. was greatly reduced using the X-press. Agglomerates readily broke down into discrete particles after desiccation.

Initially natural polymers were chosen to resemble keratinized skin squames chemically, however, their physical properties proved them to be unsuitable. Particles of bovine serum albumin and dextran were extremely friable and developed such high electrostatic charges as to make them unsuitable. A further defect was that owing to their antigenic nature they were likely to present a hazard to workers in subsequent air flow experiments. Gelatin, although the most suitable natural polymer since the size and shape of milled particles closely resembled those of skin scales, was too dense and its hygroscopic nature caused particles to become even more dense with a tendency to agglomerate, as shown by the unusual flight properties (compared with other particles) in the channel.

Inert non-antigenic materials were then used, but pure ethyl cellulose particles made by milling had a wide size distribution and were too dense compared to skin scales. Addition of the free fatty acid, stearic acid, to ethyl cellulose reduced its density to 1·01, and the waxy nature of the mixed particles simulated the physical characteristics of the lipid-keratin composition of skin squames. Particles made with equal proportions of ethyl cellulose and stearic acid were unsuitable as they

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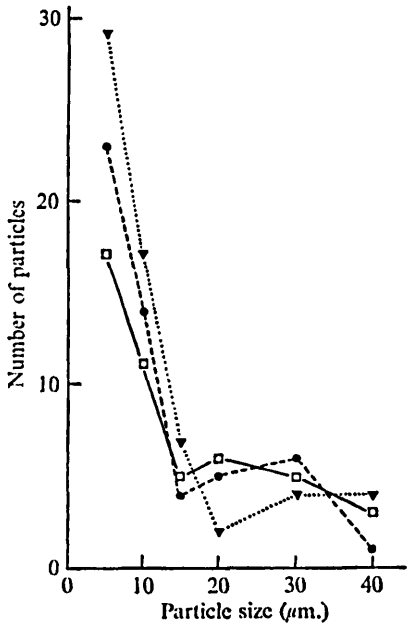

Fig. 2. Ethyl cellulose particles.

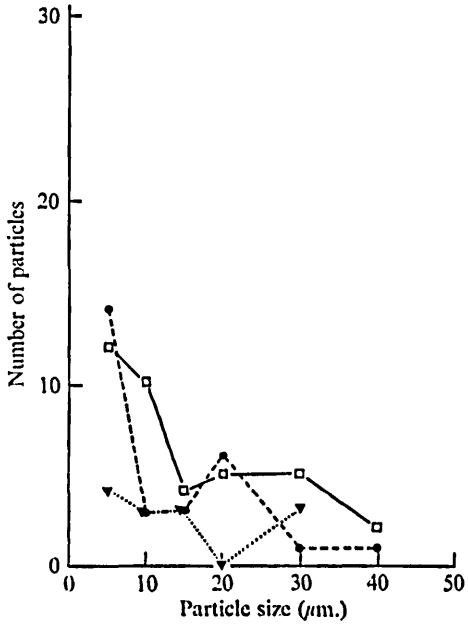

Fig. 3. Gelatin particles.

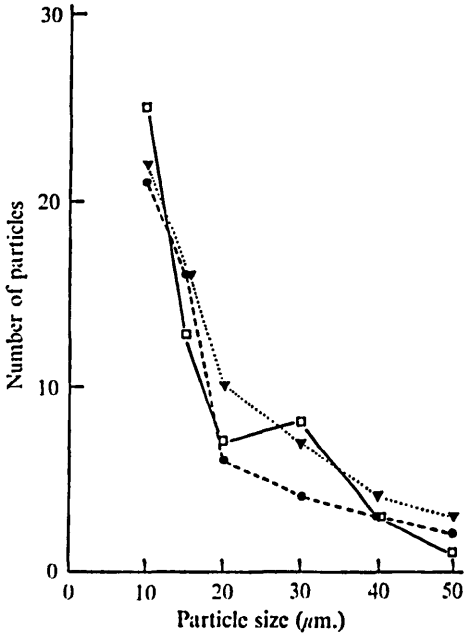

Fig. 4. Ethyl cellulose/stearic acid particles.

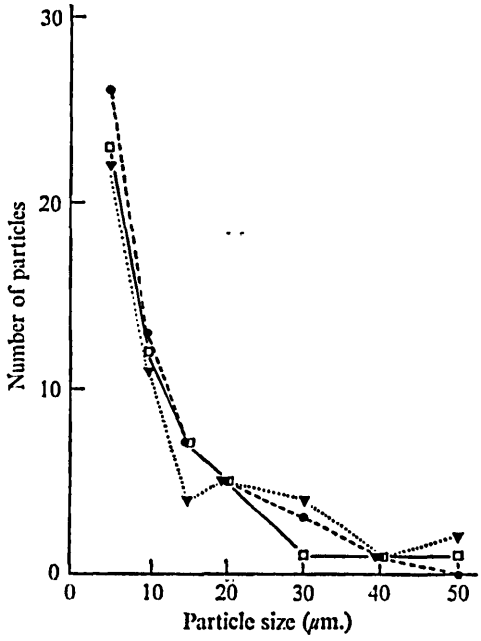

Fig. 5. Skin scales.

Particle-size distribution at channel positions $Y = 35{\cdot}0$ cm. (▼); $Y = 54{\cdot}0$ cm. (●); $Y = 72{\cdot}9$ cm. (□).

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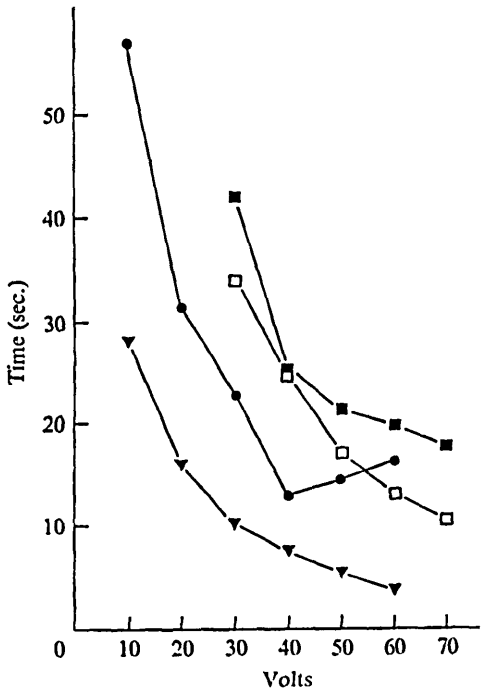

Fig. 6

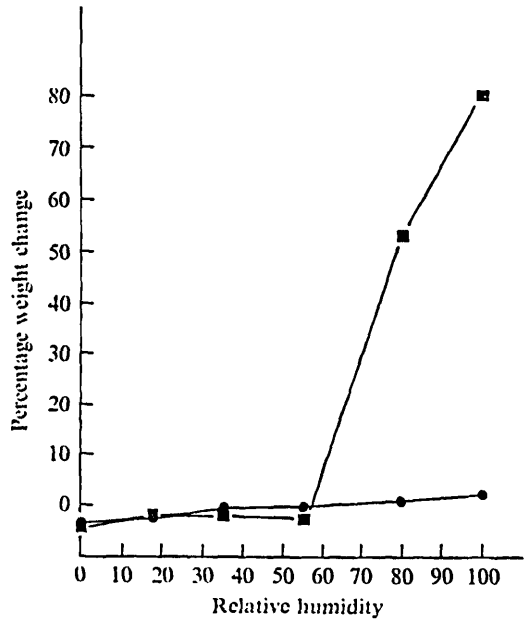

Fig. 7

Fig. 6. Particle electrophoresis of ethyl cellulose (▼), ethyl cellulose/stearic acid (●), scraped-skin scales (□) and naturally shed skin scales (■).

Fig. 7. Moisture regain of ethyl cellulose/stearic acid (●) and skin scales (■).

proved to be too friable, with a tendency to agglomerate readily owing to their extreme waxiness. However, with a lower proportion of stearic acid (ethyl cellulose:stearic acid, 60:40) particles remained coherent and discrete.

Particle electrophoresis showed that the intrinsic charge of ethyl cellulose/stearic acid (60:40) particles was closest to that of skin; however, the difference between all three types of particles was not significant.

From the above data, ethyl cellulose/stearic acid (60:40) particles were those most closely resembling normal skin scales in their physical properties (see Plate 1, Figs. 1, 2).

In the moisture regain experiments comparison of moisture uptake over the range RH (0–60) showed good correlation between ethyl cellulose/stearic acid particles and skin scales. However, above RH 60, there was a difference between the two kinds of particle, moisture uptake by skin scales far exceeding that of the synthetic particles. Increasing relative humidity causes physical uptake of moisture by the scraped skin scales, and although the effect of increasing RH on skin *in vivo* has been attributed to an increase in insensible perspiration (Mole, 1948) uptake of moisture from the atmosphere may also occur, forming a dynamic equilibrium between diffusion of water vapour across the skin. Ambient room humidity is normally in the range of 40–60% RH; and it is of interest to speculate that physiological degree of comfort may be attributed to the sharp transition of moisture regain of the keratinized layer of skin.

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EXPLANATION OF PLATE

Fig. 1. Particles of ethyl cellulose/stearic acid.

Fig. 2. Human skin scales. Scale: 1 division = 10 μm.

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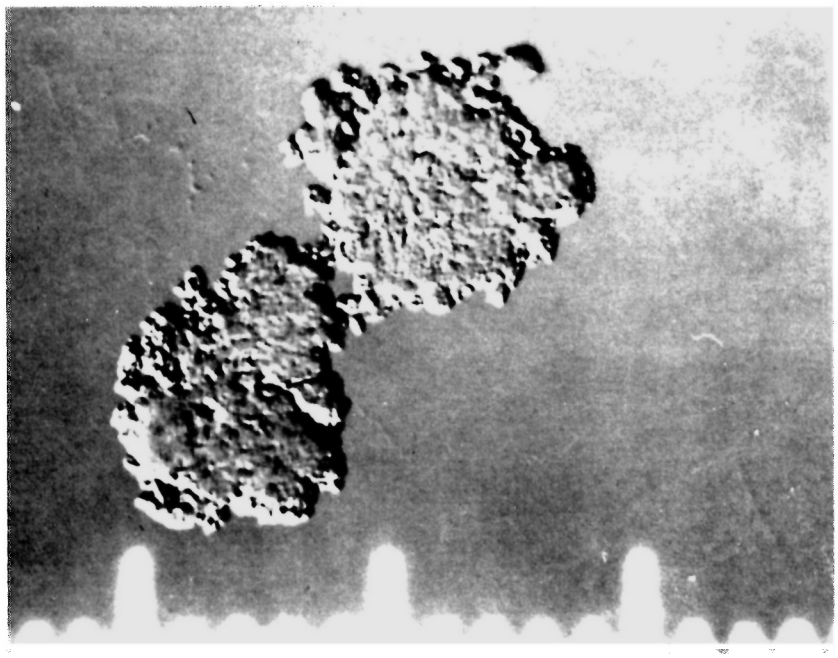

Fig. 1

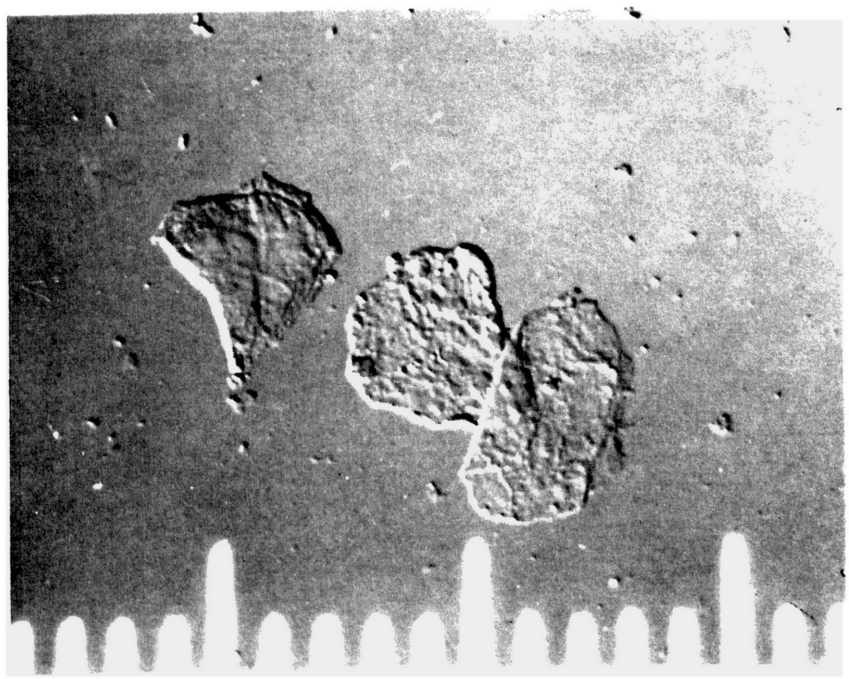

Fig. 2

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