The dimensions of skin fragments dispersed into the air during activity

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SUMMARY

There was a 1000-fold difference between the highest and the lowest mean rate of dispersal of bacteria-carrying particles during exercise among a group of three male and three female subjects. Differences in the numbers of skin fragments dispersed and in the proportion of these carrying bacteria were almost equally responsible for this. Since there is little difference between individuals in the rate of skin replacement the differences in the rates of dispersal of skin fragments during exercise must reflect large variations in the amount of skin surface removed in other ways. The skin fragments dispersed had a wide size range extending below 5 μm for the minimum projected diameter (MPD). The median MPD was about 20 μm with 7–10% less than 10 μm. Many of the particles could therefore pass freely through tightly woven fabrics with pores up to 10–15 μm which might seem to be impenetrable to whole corneocytes, typically larger than 30 × 40 μm in the hydrated state.

INTRODUCTION

In an earlier paper (Lidwell, Mackintosh & Towers, 1978) we examined the capability of a series of fabrics worn as trousers to reduce dispersal of bacteria by a group of volunteers. One of the most striking facts shown by this and other similar studies (e.g. Noble *et al.* 1976) is the very wide variation in the rates of dispersal by different individuals. The bacteria dispersed in these experiments are carried on desquamated skin scales (Davies & Noble, 1962). The numbers of bacteria enumerated in dispersal experiments will therefore depend on the numbers of scales dispersed into the air, the proportion of these carrying viable bacteria and, if the sampling method separates the bacteria carried on a single scale, the numbers of separable bacteria on each scale.

There have been a number of studies of the degree of bacterial colonization of different skin areas of the body (e.g. Noble *et al.* 1976). Estimates have also been made of the 'median equivalent particle diameters'* of the skin fragments dispersed and of those carrying micro-organisms (Noble & Davies, 1965). The penetration of these through fabrics worn as protective garments is obviously dependent on their physical size but there do not appear to be any published data for their actual dimensions.

* The equivalent particle diameter is the diameter of a sphere of unity density which would have the same settling rate in still air as the particle in question.

Measurement of these dimensions is complicated in samples of environmental dust by the presence of textile fragments and old, possibly further fragmented, skin particles. The experimental arrangements we have used (Lidwell *et al.* 1978) with a HEPA filtered air supply have produced samples of skin scales dispersed from below the waist almost entirely free of such contamination. We have, therefore, carried out a series of measurements on these samples and compared the results with those obtained by examination of wet scrub samples and mini-vacuum cleaner samples taken from a variety of skin sites on the same individuals.

METHODS

Observations were made on six subjects, three male and three female, known from previous experiments to be high, medium and low dispersers for their sex. Each subject was examined by each method on two separate occasions.

Dispersal during exercise

The equipment and procedure was similar to that described earlier for 'control' dispersal without protective trousers (Lidwell *et al.* 1978). The subjects, stripped to underclothes, exercised for $2\frac{1}{2}$ min at 84 steps/min in the sampling box which enclosed the body below the waist.

Samples were taken during the exercise period and the following $1\frac{1}{2}$ min onto nutrient agar plates in a slit sampler at either 160 or 24 l/min. The plates were incubated overnight at 37 °C and the colonies counted. Portions of the plate surface were examined with a binocular microscope and the numbers of skin scales estimated as well as the proportion of these which had given rise to colonies.

Samples were also taken by a second slit sampler onto a stationary glass slide. Half of the airborne particles collected in this sampler were deposited on a thin layer of Vaseline and half on a block of agar. These samples were examined microscopically and photographed. They gave a second estimate of the numbers of skin fragments dispersed and were used to determine the size distribution of these both dry, as suspended in the air, and when rehydrated, as would be the case if they were stained.

Mini-vacuum cleaner samples

A mini-vacuum cleaner incorporating a slit sampler has been designed and used for sampling from surfaces, such as textiles and skin onto either a nutrient agar plate (which can be rotated) or onto any convenient stationary collecting surface (Fig. 1).

The instrument was used in this study to collect samples from the skin by drawing the sampling nozzle to and fro across the selected skin area for a period of 10 sec. The areas selected were (*a*) the groin, at the fold between the trunk and the upper part of the thigh; (*b*) the front surface of the thigh; (*c*) the lateral surface of the calf. Samples were taken both onto nutrient agar plates, which were treated as described above for the dispersal experiments, and onto Vaseline coated slides. These last were examined directly under the microscope and after staining.
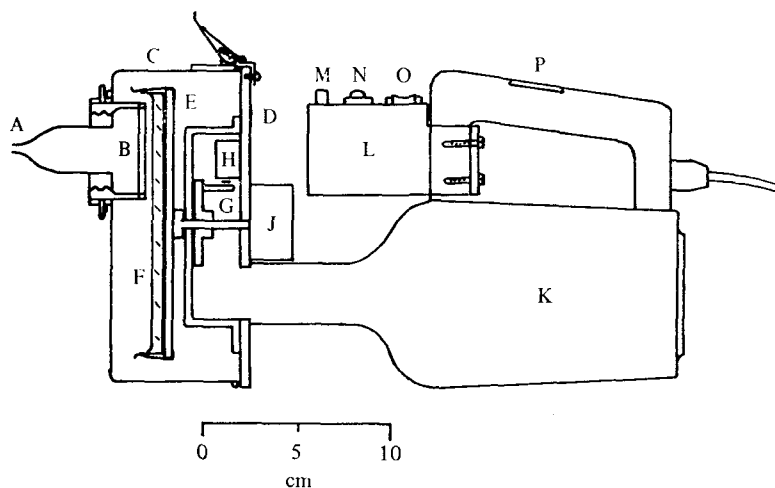
Fig. 1. Mini-vacuum cleaner sampler. A, Sampling nozzle, slit 30×2 mm. B, Impaction slit 50×0.5 mm, removable from head on bayonet pins and separable from sampling nozzle for cleaning. C, Head, held onto base by toggle clips. D, Base, carrying the rotating table E. F, Petri dish, 14 cm diam., held onto the table by clips. Glass slides or other sampling surfaces can be held in an unfilled Petri dish. G, Timing pin(s) operating micro-switch H; a single pin for full rotation samples, three pins for $\frac{1}{3}$ rotation samples. J, Synchronous motor, 2 rev./min. K, Hoover Dustette hand vacuum cleaner. L, Control module carrying: M, mode switch; N, neon indicator light; O, operative button; P, on/off switch. Weight, 3.25 kg. When the mode switch M is in position 1 the table motor, J, does not operate and sampling onto stationary surfaces is controlled by the on/off switch P. With the mode switch in position 2 the table rotates and the blower K operates when the on/off switch is closed, unless the micro-switch H is opened by a timing pin G. A sample is started with the micro-switch opened by a timing pin. On closing the on/off switch the neon light glows, pressing the operating button, O, starts the fan and the table motor. When the pin has cleared the micro-switch the neon light goes out and the button may be released. When a pin reaches the micro-switch both fan and table motor stop and the neon light comes on. The instrument is then ready for another timed sample after changing the sampling surface as required.

Scrub samples

Four areas on each subject, the groin, the thigh, the calf and the popliteal fossa were sampled by the method of Williamson & Kligman (1965) as modified by Stringer & Marples (1976). A sterile stainless steel cylinder, 26.5 mm internal diameter, was held against the skin. One ml of a 0.1% solution of Triton X-100 in 0.075 M phosphate buffer at pH 7.9 was added and the skin surface rubbed for 1 min with a sterile blunt-ended glass rod. The fluid was then removed by pipette and the procedure repeated without moving the cylinder. The two samples of liquid were pooled. Aerobic colony counts were made in serial dilutions and corneocyte counts made in a haemocytometer on the undiluted specimens after staining with crystal-violet.

Size determinations

Photomicrographs were taken, usually at 66× magnification, of all samples which were to be used for size estimations. For the measurements the negatives were projected on a screen to give a final 2000× magnification.

Two measurements were made using a square grid drawn on white cardboard with lines at 4 mm spacing, i.e. equivalent to 2 μm on the original object. The minimum projected diameter (MPD) was the minimum distance between two parallel straight lines between which the image of the particle could be fitted. The longer projected diameter (LPD) was the minimum distance between two parallel lines at right angles to the above which would contain the image of the particle. These two dimensions define the narrowest rectangle into which the image of the particle can be fitted and correspond to the breadth, B, and length, L, as defined by Heywood (1963). No particles with dimensions less than 5 μm were measured owing to difficulties of identification. Such particles were not numerous. An approximate estimate of the area of the particle was also made by considering the proportion of this rectangle occupied by the particle. Particles were classed as filling about 90, 75 or 50% of the enclosing rectangle. Photographs of stained corneocytes from the scrub samples and of the dry particles collected by the mini-vacuum cleaner are shown in Plate 1. Plate 2 shows, on the same scale, the dry dispersed skin fragments collected from the air of the sampling box, together with a photograph of talc particles for comparison.

RESULTS

Table 1 shows the mean numbers of colonies of aerobic bacteria recovered from the six subjects in the chamber experiments, together with the mean number of skin fragments > 5 μm. The proportion of these carrying viable bacteria is compared with the proportion among the skin fragments collected by the mini-vacuum cleaner from the different skin sites. The mean numbers of corneocytes collected in the scrub samples are also shown.

It is clear that there are only small differences between the numbers of corneocytes removed from the individual subjects. There was also no significant difference between the numbers collected from the four sites examined. Bacterial counts on the scrub samples showed substantial differences between individuals and sites. The highest dispersers showed the highest counts and the numbers recovered from the groin were greater than those from the other sites. However, the variability of the observations was extremely high. This is in part due to the fact that the method separates the bacteria carried on individual skin scales so that the results are greatly influenced by the random distribution of micro-colonies on the skin. The bacterial counts reached, on some occasions, over 100 times the number of corneocytes in the sample.

The number of bacteria-carrying particles dispersed varied more than 1000-fold between subjects. The proportion of skin fragments carrying bacteria varied by about 25-fold and there is a 40-fold variation, in the same sense, in the numbers

Table 1. *Skin particles liberated and the proportions carrying bacteria*

Subject	Dispersal during 2½ min exercise			Collected by mini-vacuum sampler, proportion (%) from:			Scrub samples, no. of corneocytes/cm² × 10⁵
	Skin fragments	Colonies	Proportion (%)	Groin	Thigh	Calf	
Males							
OML	53 150	28 700	54	36	16	9	1·14 (1·23)
CAM	13 530	6 300	47	26	12	3·9	1·05 (1·62)
GM	11 356	2 840	25	8·1	5·2	7·2	1·35 (3·31)
Females							
AGT	7 548	343	4·5	2·6	2·0	0·7	0·93 (1·41)
JFR	2 920	385	13	6·3	1·7	0·9	0·69 (2·04)
GH	1 225	27	2·2	4·8	1·4	0·9	0·64 (2·63)
Max./min.	43	—	25	—	—	—	—
Median for all subjects	—	1 063	—	—	—	—	0·93 (1·31)

The first three columns of figures are derived from dispersal experiments in the chamber as described in the text. The values are geometric means from duplicate experiments combining, where appropriate, the results from the several methods of estimation. The values for the mini-vacuum cleaner samples are arithmetic means obtained from three samples, on different occasions, from each site. Arithmetic means were used since there were some zero values. The corneocyte counts, except for AGT, four samples only, are the geometric means of eight samples, three each from the groin and thigh and two from the calf. The figures in parentheses give the geometric standard deviations of the distributions, approximately log-normal, i.e. the ratio of the 84th percentile to the median or mean.

Table 2. *Size distribution of skin cells and fragments*

Subject	Dispersed fragments (dry)		Corneocytes (stained)	
	MPD	LPD	MPD	LPD
Males				
OML	24 (1·58)	41 (1·56)	34 (1·29)	46 (1·17)
CAM	18 (1·80)	31 (1·48)	32 (1·18)	42 (1·17)
GM	25 (1·48)	39 (1·41)	ND	ND
Females				
AGT	28 (1·61)	42 (1·48)	ND	ND
JFR	20 (1·60)	31 (1·61)	ND	ND
GH	15 (1·67)	24 (1·67)	33 (1·27)	44 (1·14)
All subjects	21 (1·62)	33 (1·58)	33 (1·21)	44 (1·14)

This table shows the geometric mean values in μm, with the geometric standard deviation of the distribution, approximately log-normal, i.e. the ratio of the 84th percentile to the median or mean, given in parentheses. MPD, the minimum projected diameter, is the shortest distance between two parallel straight lines between which the particle can be fitted. LPD, the longest projected diameter, is the shortest distance between two parallel lines at right angles to the first pair. These two measurements define the narrowest possible rectangle within which the whole particle can be contained.

Table 3. *Variation in estimated size of skin particles collected or examined by different methods*

Source of sample	Collected on Vaseline (dry)		Collected on agar or stained (wet)	
	MPD	LPD	MPD	LPD
Dispersed fragments	18 (1·80)	31 (1·48)	25 (1·52)	42 (1·48)
Mini-vacuum sampler				
Groin	29 (1·69)	44 (1·59)	34 (1·47)	49 (1·59)
Thigh	34 (1·50)	50 (1·50)	39 (1·44)	52 (1·54)
Calf	35 (1·37)	50 (1·38)	35 (1·37)	49 (1·37)
Scrub samples (corneocytes)				
Groin	—	—	32 (1·16)	40 (1·15)
Thigh	—	—	31 (1·19)	40 (1·15)
Calf	—	—	33 (1·12)	44 (1·14)

The table shows the geometric mean values in μm, with the geometric standard deviation of the distribution, approximately log-normal, given in parentheses. MPD and LPD are the minimum projected diameter and the longer projected diameter as defined in Table 2.

of skin fragments dispersed. Examination of the proportion of fragments carrying bacteria from the various sites examined by the mini-vacuum cleaning procedure suggest that the groin may be the major source for the airborne material.

Table 2 shows the size distribution of the airborne skin fragments collected on Vaseline-coated slides in the chamber experiments together with that of the corneocytes recovered in the scrub samples. The variation in mean size of the fragments between subjects is barely significant, the total range amounting to no more than $\pm 33\%$. The ratio of the longer to the minimum projected diameter was about 1·4. The variation in the dimensions of the corneocytes from the scrub samples was insignificant.

In Table 3 the dimension of the fragments collected and examined in different ways from one individual are compared. The samples collected by the mini-vacuum cleaner contain substantially larger particles than those found in the air as a result of exercise. The mini-vacuum cleaner samples contained appreciable numbers of groups of cells or cell fragments and the median size was between 50 and 100% greater than the airborne material. This latter showed more than a 30% increase in the minimum projected diameter when collected onto a wet substrate, either due to swelling on rehydration or to flattening of the curled fragments. In contrast, the mini-vacuum cleaner samples showed little size change when wetted. The corneocytes in the scrub samples were obtained in the wetted condition and were appreciably larger than the airborne fragments although smaller than the mini-vacuum cleaner collected material.

DISCUSSION

The skin surface is formed by a tesselation of irregular polygonal flakes, mostly hexagonal, derived from flattened cells (Plate 1). The mean projected dimensions of these are about 33×44 μm (MPD $\times$ LPD), Table 2, and they are some 3–5 μm thick. Since the whole cell, the corneocyte, occupies about 75% of the rectangle its area is about 10^{-3} mm². These dimensions are very similar to those given by Plewig & Marples (1970). The average body surface is about 1.75 m² so that a complete layer of skin corresponds to about 2×10^9 cells or about 4×10^8 for the skin of the legs which comprise nearly 20% of the total skin area.

A complete layer is replaced on average in less than 24 h (Jansen, Hojyo-Tomoto & Kligman, 1974) so that more than 4×10^8 cells are shed in that time from the skin of the legs alone. This corresponds to an average dispersal exceeding 7×10^5 every $2\frac{1}{2}$ min. Our experiments recovered about 24% of the airborne fragments dispersed (Lidwell *et al.* 1978) so that the figures in Table 1 must be multiplied by 4.2 to give the total numbers of scales dispersed during the $2\frac{1}{2}$ min of exercise. For the experiments listed in Table 1 this varies from 5×10^3 to 2.2×10^5, a figure which includes fragments as well as a small proportion of whole cells. Our shedding rates are, therefore, considerably less than the 24 h average even though the rate of shedding during exercise is much greater than during less active periods. Friction with clothing and bed-clothes as well as bathing must remove very large numbers of the surface cells and the above calculation suggest that the principle mechanism of loss is probably to be found among these.

Since there are only insignificant differences between individuals in the rates of skin replacement (Jansen *et al.* 1974), which is confirmed by the similar numbers of corneocytes recovered from our subjects by the scrub sampling method (Table 1), it would seem that the very large differences we have observed in the rates of shedding during exercise, a range of over 40:1, must arise from large differences in the proportions of desquamating skin removed at other times, e.g. by bathing, etc. This could be related to differences in the mechanical stresses needed to remove or break up the individual cells in the outer layer.

Variations in the level of skin colonization combine with differences in the

rates of shedding to give an even greater variation, over 1000:1, in the numbers of bacteria-carrying particles dispersed into the air during exercise. It is interesting, and suggests the operation of some common factor, that the subjects with the highest degree of skin colonization and the highest proportion of dispersed skin fragments carrying bacteria also dispersed the largest number of skin fragments. As a result of this the 40-fold difference in the numbers of skin scales dispersed combines with the 25-fold difference in the proportion of fragments carrying bacteria to give the observed 1000-fold difference in the rates of dispersal of bacteria-carrying particles. The more active dispersers seem in these respects to approach the characteristics of patients with skin disease who both disperse very large numbers of skin fragments and often exhibit a very high degree of skin colonization (Noble & Davies, 1965). It is, perhaps, worth noting that the most vigorous disperser in our series was also a persistent nasal carrier of *Staphylococcus aureus* and that the nasal strain could be recovered from various sites on his skin.

Variations in the size of the skin fragments dispersed during exercise (Table 2) were not correlated with the rates of dispersal. It is apparent from the numerical values, and from Plate 2, that the dispersed material contains many fragmented corneocytes, including pieces down to 5 μm or less. The dispersed fragments have a wide size range. The geometric standard deviation is about 1·6 so that, with a mean value for the minimum projected diameter of 21 μm for fragments larger than 5 μm, one quarter have an MPD less than 15 μm and between 7 and 10% an MPD below 10 μm. If the measured sizes of the particles over 5 μm are treated as a truncated log-normal distribution the estimated fraction with an MPD less the 5 μm in the parent distribution does not exceed 1%. The median MPD and the proportions with an MPD less than 15 and 10 μm are not significantly affected.

Although the smaller fragments are less likely to carry bacteria than the larger ones, these figures are consonant with a significant degree of penetration of bacteria-carrying skin particles through tightly woven fabrics whose largest pores have diagonals of no more than 15–20 μm (Lidwell & Mackintosh, 1978). Pores as small as this would seem to be totally impenetrable to whole corneocytes with a median MPD in excess of 30 μm. However, the dimensions quoted for the corneocytes are derived from wet-stained specimens. The dried skin fragments swell or uncurl or both when collected onto a wet surface (Table 3). The increase in apparent size is sufficient to bring the median MPD up to 75% of that for the whole corneocyte. If a similar change was applicable to the whole corneocyte then its mean MPD in the dried state might be no more than 1·3 times that of the dry skin fragments. The corneocytes have, however, a relatively narrow size range, with a geometric standard deviation around 1·2, so that fewer than 1/100 if dried in this way would have an MPD as small as 18 μm (assuming that the estimated standard deviations are applicable to the tails of the distribution). Penetration through the tightly woven fabrics would therefore seem to be dependent on fragmentation of the dried corneocytes as these become detached from the skin surface.
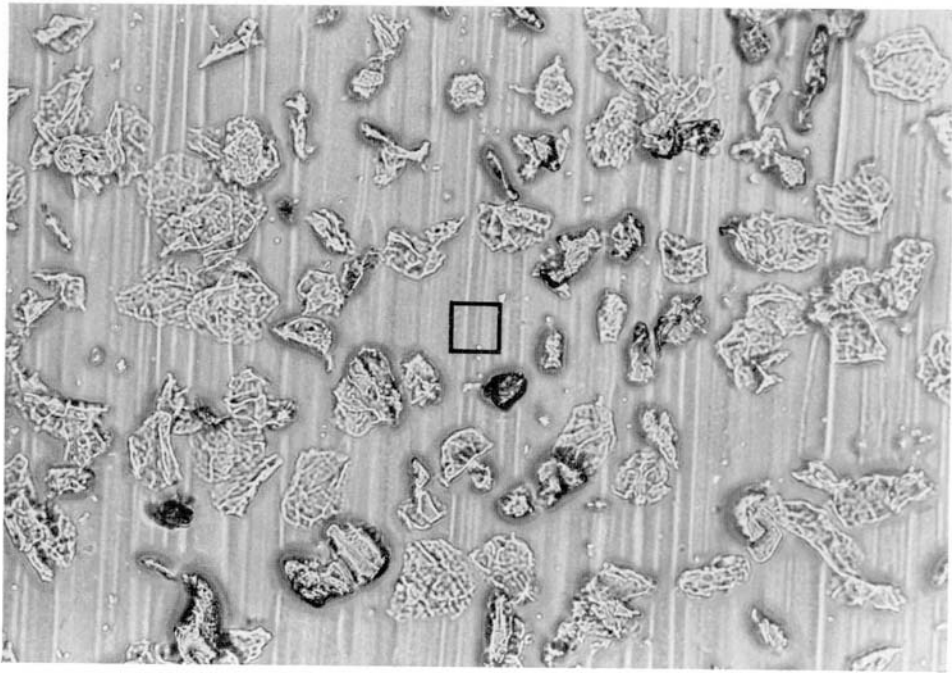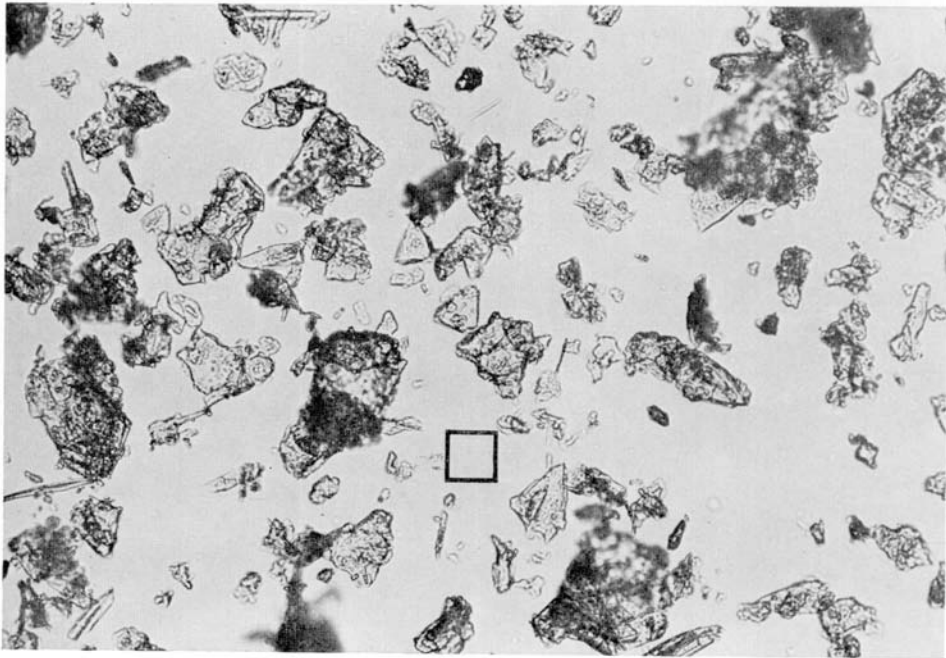
(a)

(b)

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(*a*)

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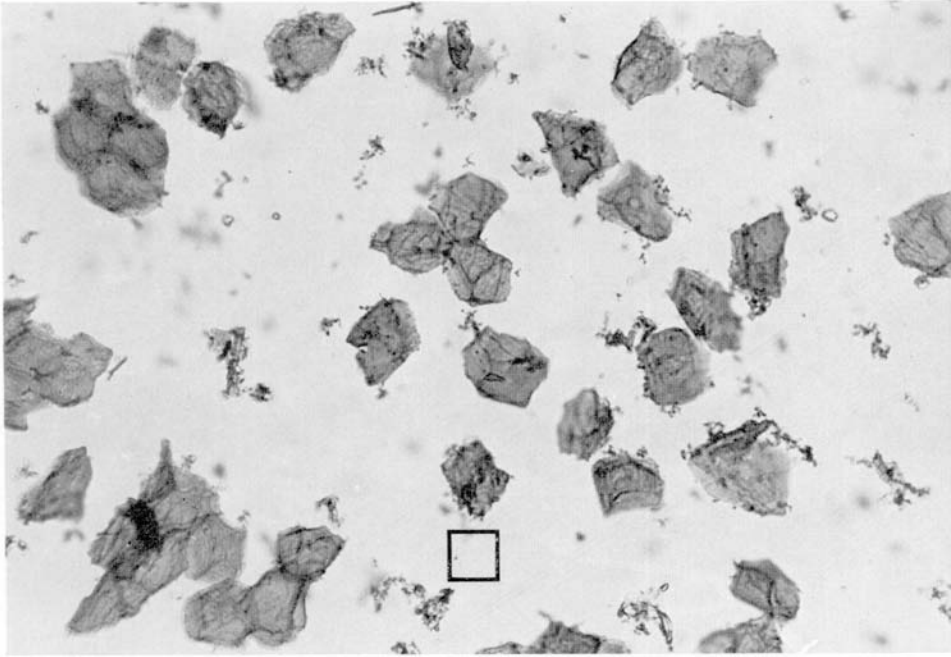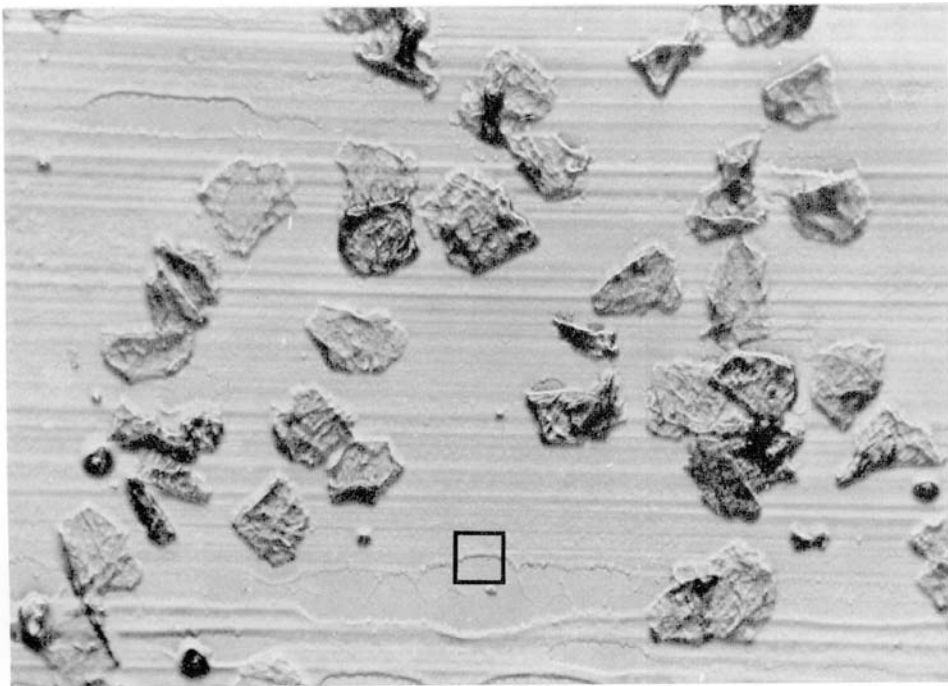
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EXPLANATION OF PLATES

PLATE 1

(a) Corneocytes removed from the skin by the scrub sampling method, stained with crystal violet. Groups of two or more cells still attached together, as in the original tessellation, can be seen. Measurements were made on the individual cells. (b) Skin fragments collected onto a Vaseline coated slide, photographed unstained and dry. Single cells, groups and fragments of cells can be seen. Measurements were made of all these as forming one distribution. The sides of the squares correspond to 25 μm.

PLATE 2

(a) Airborne skin fragments collected onto a Vaseline coated slide, photographed unstained and dry. There are many small fragments. (b) Talc powder Johnson & Johnson 00000. The similarity in size distribution and shape to the airborne skin fragments is obvious. The sides of the squares correspond to 25 μm.