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Contents

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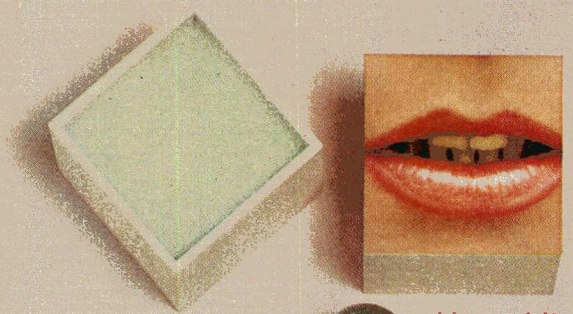

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SYNOPSES FOR CARD INDEXES

The following synopses can be cut out and mounted on 127 × 76 mm index cards for reference without mutilating the pages of the Journal.

The relationship between water-borne bacteria and shampoo spoilage: S. A. MALCOLM and R. C. S. WOODROFFE. *Journal of the Society of Cosmetic Chemists* **26** 277–288 (1975)

Synopsis—Bacteria capable of surviving and multiplying in shampoos appear to represent only a very small proportion of the total population carried by mains water. Examination of mains water (from a single source) showed these bacteria occurring with a frequency of approximately 5 in 100 l.

The ability of small numbers of bacteria to initiate heavy contamination in shampoos is unrelated to the volume of product inoculated. As few as 50 bacteria are capable of initiating contamination in 1 l of shampoo and this same small number may be capable of initiating contamination in much larger volumes, e.g. a factory batch. The implications of this observation on the methods of detection of contamination and the time after manufacture at which products should be examined for the presence of contaminants are discussed.

Hair breakage: the scanning electron microscope as a diagnostic tool: A. C. BROWN and J. A. SWIFT. *Journal of the Society of Cosmetic Chemists* **26** 289–297 (1975)

Synopsis—Physical techniques for studying the mechanical properties of human hair have been well established for many years and since the introduction of the scanning electron microscope (SEM) 8 years ago, there has been some attempt to correlate, retrospectively, the structural appearance of deformed fibres with data obtained during mechanical straining experiments. A logical development is to combine these two techniques so that both physical and structural data could be collected simultaneously, thereby enabling a more detailed and accurate assessment of the breakdown of structural components to be made.

The SEM, because of its great depth of focus, wide range of magnification and large area for specimen manipulation, has been adapted for conducting dynamic experiments *in situ*. In addition, the manner in which the visual information was processed made direct recording of the results onto videotape possible.

A number of simple specimen stages have been constructed for use in examining the structural deformation of hair fibres under various conditions of mechanical stress. The incorporation of d.c. micromotors into the SEM allowed accurate control of sample movement during experiments and miniature strain gauges were used so that continuous recordings of

The following papers have been accepted for publication in the Journal:

ORIGINAL SCIENTIFIC PAPERS

Skin impedance and moisturization
E. J. Clar, C. P. Her and C. G. Sturelle

Analytic procedures for the determination of chlorhexidine in oral products
E. Cropper, P. Platt and N. A. Puttnam

Estimation of the general incidence of specific lanolin allergy
E. W. Clark

Cough irritation by deodorant sprays
E. M. Staal, W. Bree and P. L. C. A. Rijnbeek

The role of essential fatty acids (EFA) in the regulatory processes of Malpighian cell membranes
M. Cambrai, Ph.D.

The examination of particulate inclusions in toothpaste, by freeze-fracture, replication and transmission electron microscopy
W. B. Davies, B.Sc., A.I.M., D.A.E., M.Sc. and A. C. Macdonald, B.Sc., Ph.D.

The promise and the product
J. B. Wilkinson, M.A., B.Sc., F.R.I.C.

the changes in strain could be made.

The nature of the structural breakdown in human hair during combing has been investigated, together with the changes taking place as a result of natural weathering.

An appraisal of human head hair as forensic evidence: J. PORTER and C. FOUWEATHER. *Journal of the Society of Cosmetic Chemists* **26** 299–313 (1975)

Synopsis—The evidential value of human head hair in forensic science is discussed. As well as basic techniques such as morphology and the identification of cosmetics, an experiment to put the measurement of hair colour on a less subjective basis, is described. Results show that with the techniques currently in use a definite method of relating a hair fibre to a particular individual is still not a reality, but, in cases of rare hair colour and the presence of cosmetic treatments, good discrimination can be achieved.

Microbiological quality control—a case history: G. C. BREACH. *Journal of the Society of Cosmetic Chemists* **26** 315–322 (1975)

Synopsis—A history of the introduction of microbiological quality control (MQC) into factories manufacturing non-sterile pharmaceuticals, toiletries and cosmetics throughout Europe and Africa is outlined. The development of a microbiology manual and appropriate standard operating procedures to up-grade good manufacturing practice (GMP) is explained. Problems with particular products and organisms are discussed, especially the presence of *Achromobacter* and *Pseudomonas* organisms in plant water in some sites. The inter-relationship of QC, R & D, and Production which enabled MQC to proceed smoothly is emphasized as is the application of one overall 'in house' standard.

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J. Soc. Cosmet. Chem. **26** 277–288 (1975)

The relationship between water-borne bacteria and shampoo spoilage

S. A. MALCOLM and R. C. S. WOODROFFE*†

Presented on 28th August 1974 in London at the IFSCC VIIIth International Congress on 'Cosmetics—Quality and Safety' organized by the Society of Costmetic Chemists of Great Britain

Synopsis—BACTERIA capable of surviving and multiplying in SHAMPOOS appear to represent only a very small proportion of the total population carried by mains WATER. Examinations of mains water (from a single source) showed these bacteria occurring with a frequency of approximately 5 in 100 l.

The ability of small numbers of bacteria to initiate heavy contamination in shampoos is unrelated to the volume of product inoculated. As few as 50 bacteria are capable of initiating contamination in 1 kg of shampoo and this same small number may be capable of initiating contamination in much larger volumes, e.g. a factory batch. The implications of this observation on the methods of detection of contamination and the time after manufacture at which products should be examined for the presence of contaminants are discussed.

INTRODUCTION

The microbiological quality of process water in the food industry has been of concern to manufacturers for many years. It is only comparatively recently, however, that the same concern has been shown in the manufacture of toiletries and cosmetics.

* Unilever Research, Isleworth Laboratory, Isleworth, Middlesex.
† R. C. S. Woodroffe died in December 1974.

Originally the reported contaminants of toiletries and cosmetics covered a wide spectrum of organisms (1). However, more recent reports show that the typical product contaminant is in fact drawn from a much narrower range of organisms (2–7). The discrepancy between earlier and more recent reports is due to the fact that earlier investigators failed to differentiate between multiplying contaminants and those which were merely chance residents or transients. The most frequently reported contaminants in recent years have belonged to such genera as *Pseudomonas*, *Klebsiella*, *Achromobacter* and *Alcaligenes*. These bacteria are common residents in water, both fresh (8–12) and distilled (13, 14) and it is now widely believed that the water used in the preparation of toiletry products is their likely source (11, 15, 16).

In the UK mains water as supplied generally contains low numbers of bacteria (usually less than 300 ml^{-1}) and if water were used in this condition it seems probable that it would contaminate only the more susceptible products since it is likely that contaminants capable of multiplication in products represent only a small proportion of water flora. However, in the production of toiletries it is not always possible to use water directly from the main and it is frequently held in a storage tank before use. The ability of water-borne bacteria to multiply on storage is well known (9, 16, 17) and total counts of greater than 10^7 ml^{-1} have been recorded (18). Indeed the readiness with which bacteria will multiply in stored water has led Chambers and Clark (11) to suggest that an absence of bacteria indicates that the water is toxic. Growth is probably at the expense of organic materials and salts present in the water (9) and perhaps materials dissolving in the water from the container (19). Growth may also be at the expense of dissolved volatile organic materials if these are used in the vicinity of the storage tank (20).

Multiplication of bacteria will occur in pipelines where velocities are low (21) and it has been reported that polythene pipelines are prone to supporting large growths of bacteria, sufficient even to block the pipes (9), although Burman (22) has claimed that polythene piping has little effect on bacterial numbers.

There is little question, however, that deionizing columns are sites at which bacterial numbers may increase (11, 23, 24). The bed concentrates Ca^{2+} and Mg^{2+} from the water and at the same time collects organic materials by a process of sieving (24). The bacteria present in the water are either entrapped within the matrix of the bed or adsorbed on to the resin surface and so act as the inoculum. The numbers of bacteria in the effluent

from the column vary with the frequency with which the column is used or cleaned and disinfected (23).

There is now considerable practical evidence indicating a positive correlation between high levels of bacterial contamination in water used in manufacture and the incidence of contaminated toiletry products. This is presumably because the greater the number of contaminants in the water the wider the range of resistances to unfavourable conditions and the greater the probability of bacteria being present which are capable of surviving and multiplying in the product. The range of resistance is likely to be widest where the population is made up from a range of different species of bacteria rather than large numbers of only a small number of species. It would also be theoretically possible to have very heavily contaminated water presenting no threat to product stability because of the low resistance of the bacteria or, in reverse, water containing small numbers of bacteria which, because they are well suited to growth in the product, will give rise to contamination.

In order to perform Challenge Tests on shampoos in the laboratory it is often found necessary to use very large inocula, usually of the order of 10^6–10^7 bacteria per gram of product. This is, however, much higher than would normally be found as an initial inoculum during manufacture in a well-ordered production unit and product contamination is known to occur even when the level of contamination in the plant is low and water of good bacteriological quality is used.

Materials and methods

Empicol ESB 3/S (sodium lauryl ether sulphate—27.3% active) a specially prepared preservative-free batch supplied by the Marchon Division of Albright and Wilson Ltd.

Lauryl Isopropanolamide—Marchon Division of Albright and Wilson Ltd.

Nutrient Agar CM3—Oxoid Ltd.

Tryptone Soya Agar CM131—Oxoid Ltd.

Peptone Bacteriological Neutralized L34—Oxoid Ltd.

Because of the wide variety of different ingredients used all experimental shampoos are atypical. It is, however, possible to produce a model system which is representative of a general shampoo formulation. For this work the following formulation was used.

	% w/w	
Empicol ESB 3/S (sodium lauryl ether sulphate) (27.3% active)	36.6	(equivalent to 10% active detergent)
Lauryl isopropanolamide	1.5	
Sodium chloride	0.5	
Sterile distilled water to	100.0	

After preparation the model shampoo was sterilized by filtering it through a 0.2 μm filter membrane.

Sodium lauryl ether sulphate (SLES) was chosen as the detergent because products containing it are known to be amongst those most susceptible to contamination, possibly due to its relatively low toxicity to Gram-negative bacteria. It is also widely used as a detergent in shampoos.

The bacterium selected as the experimental contaminant was a strain of *Enterobacter cloacae* isolated from a contaminated shampoo. We have found this organism to be particularly resistant to anionic detergents.

Three 16 oz nutrient agar slopes were each inoculated with 1 ml of a 24 h nutrient broth culture of *Enterobacter cloacae* and incubated at 28° overnight. The bacteria were washed from the slopes using distilled water and washed three times with water after centrifugation. The bacteria were finally re-suspended in distilled water and stored at 4° for 4 days before use.

Total viable counts

Total viable counts on bacterial suspensions and inoculated shampoos were performed in Tryptone Soya Agar using the pour-plate method with 0.1% peptone water as the diluent. Total viable counts on mains water were performed in Nutrient Agar using sterile mains-water as the diluent. In each case the plates were incubated at 28° for 7 days before counting.

Influence of inoculum size on the survival and multiplication of bacteria in a shampoo

Total viable counts were performed on the stored suspension of *E. cloacae*. A series of decimal dilutions of the suspension were then made in distilled water so as to give the following range of concentrations of bacteria: 10^9, 10^8, 10^7, 10^6, 10^5, 10^4, and 10^3 ml^{-1}.

0.2 ml of each suspension was added to duplicate 20 g amounts of sterile shampoo. The inoculated shampoo was incubated at 28° and total viable counts were performed at intervals up to 14 days.

Influence of shampoo volume on the survival and multiplication of bacteria

10 g, 100 g and 1000 g aliquots of the model shampoo were each inoculated with either 50 or 400 bacteria contained in 0.2 ml of suspension. After inoculation the shampoo was mixed thoroughly and then incubated at 28°. Total viable counts were performed at intervals up to 14 days.

Isolation from mains water of bacteria capable of multiplying in a model shampoo

To determine the minimum volume of water necessary to contaminate the model shampoo a series of 100 g quantities of the shampoo were inoculated with bacteria isolated from volumes of water from 1 ml to approximately 10 l. With the exception of the 1 ml inoculum the shampoos were not inoculated with water but with bacteria-proof membrane filters through which the water had been passed. Five replicates of each volume of water were inoculated into model shampoo.

The water for testing was collected from a tap fed directly from the mains. Before collection the tap was swabbed with alcohol which was then burned off. The tap was then opened fully and allowed to run for 3 min to ensure that the sample was composed of water directly from the mains and excluded water which had stagnated in the pipes.

Volumes of water from 10 to 1000 ml were collected in sterile conical flasks and filtered through membranes of mean pore-size 0.22 μm supported in Millipore Sterifil filter holders. In order to estimate the number of organisms trapped on the membrane total viable counts were performed on the water before filtration.

It was not convenient to filter a 10 l. volume of water using the Sterifil filter holder so these volumes were filtered through a 0.22 μm membrane held in a stainless steel Carlson–Cox Model 1000 filter holder. The filter holder was attached to a length of sterilized flexible plastic hose the other end of which was fitted to the tap.

After the water had been passed through the membranes each membrane was transferred to 100 g of sterile shampoo in a 4 oz sterile glass bottle. To dislodge as many organisms as possible from the surface of the membrane the bottle was shaken vigorously in a Griffin flask shaker for 5 min before transferring to an incubator.

Total viable counts were performed immediately and at various intervals during incubation at 28°.

RESULTS

Influence of inoculum size on the survival and multiplication of bacteria in a shampoo

At all inocula levels the addition of bacteria to shampoos resulted initially in a reduction in bacterial numbers (*Fig. 1*). This reduction was between 80% and 90% after 4 h incubation for all but the largest and smallest inocula. In the case of the smallest inocula no bacteria could be detected at 4 h. Within 24 h, however, all shampoos, except that inoculated with the largest number of organisms, contained a greater number of organisms than had been initially introduced. After the initial reduction in numbers in this latter shampoo the bacterial population remained approximately constant for the remainder of the incubation period. After incubation for 7 days all the shampoos, irrespective of the original inoculum size, had approximately

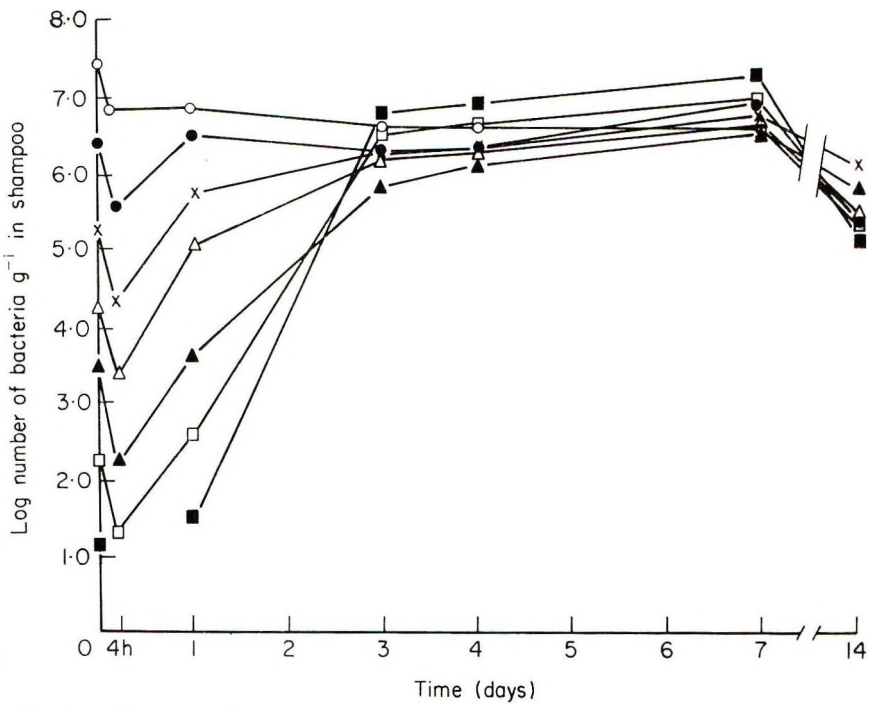

Figure 1. Influence of inoculum size on the survival and multiplication of *E. cloacae* in a model shampoo. Approximate initial inoculum: O, 10^7 g^{-1}. ●, 10^6 g^{-1}. X, 10^5 g^{-1}. Δ, 10^4 g^{-1}. ▲, 10^3 g^{-1}. □, 10^2 g^{-1}. ■, 10 g^{-1}.

the same bacterial populations. After 14 days the populations were still similar but lower, by a factor of approximately 10, than at 7 days.

Influence of shampoo volume on the survival of bacteria

As few as 50 organisms were necessary to contaminate 1000 g of shampoo so that after 4 days' incubation a population of 10^6 bacteria g^{-1} was obtained (*Fig. 2*).

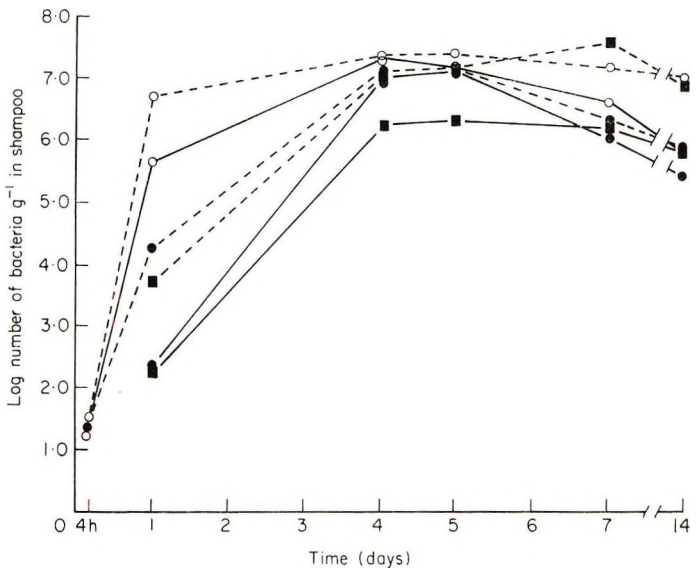

Figure 2. Influence of inoculum size and shampoo volume on the survival and multiplication of *E. cloacae* in a model shampoo. Initial inoculum: —○— = 50 bacteria in 10 g shampoo; - - -○- - - = 400 bacteria in 10 g shampoo; —●— = 50 bacteria in 100 g shampoo; - - -●- - - = 400 bacteria in 100 g shampoo; —■— = 50 bacteria in 1000 g shampoo; - - -■- - - = 400 bacteria in 1000 g shampoo.

As might be expected the maximum supportable population was achieved most rapidly in the system in which the initial number g^{-1} was highest but all systems were in the early stationary phase of the growth cycle within 4 days of inoculation. After 7 days the total number of bacteria in each system was proportional to the volume of shampoo inoculated (*Fig. 3*).

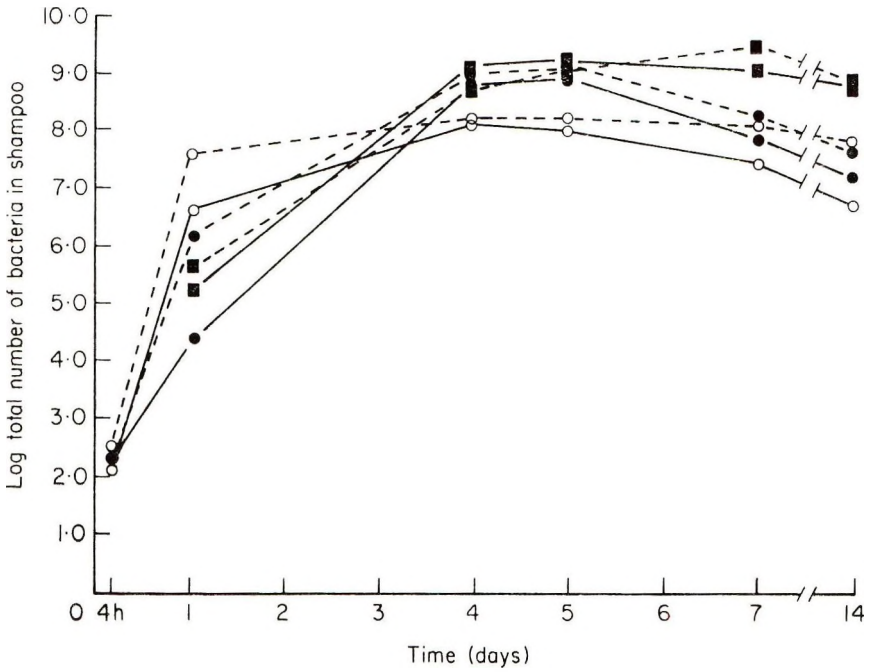

Figure 3. Influence of inoculum size and shampoo volume on the total population of *E. cloacae* in a model shampoo. Initial inoculum: —○— = 50 bacteria in 10 g shampoo; - - -○- - - = 400 bacteria in 10 g shampoo; —●— = 50 bacteria in 100 g shampoo; - - -●- - - = 400 bacteria in 100 g shampoo;—■— 50 bacteria in 1000 g shampoo; - - -■- - - = 400 bacteria in 1000 g shampoo.

Isolation from mains water of bacteria capable of multiplying in a model shampoo

Inoculation of shampoos with filters derived from filtration of 1000 ml of water or less did not result in contamination with bacteria capable of multiplying in the shampoo. Of five inoculations with filters derived from 10 l volumes two resulted in contamination of the shampoo (*Table I*).

Viable counts on the shampoos immediately after inoculation in every case failed to reveal the presence of bacteria. Despite the relatively low population which developed in the contaminated shampoos (2.7×10^5 and 3.6×10^3 g^{-1}) contamination was associated with obvious visual changes, i.e. the production of turbidity and strings of slime, after only 7 days' incubation.

Table I. Multiplication of bacteria isolated from mains-water in a model shampoo system

Volume of water filtered	Calculated no. of bacteria in inoculum	No. of shampoos† showing	
		Growth	No growth
*1 ml	1.55×10^2	0	5
10 ml	1.55×10^3	0	5
100 ml	1.55×10^4	0	5
1 l	1.55×10^5	0	5
10 l	1.34×10^6	2	3

* 1 ml volumes were not filtered but were added directly to the shampoo. Since the 10 l volumes were not all filtered on a single occasion the number of bacteria in the inoculum expressed in the table is an average value.

† Five replicates.

Both contaminated shampoos were contaminated with a single bacterial species. First stage identification using the Cowan and Steel procedure (25) indicated that both organisms belonged to the genus *Alcaligenes*. Biochemically and morphologically both contaminants appeared to be the same organism.

Discussion

The maximum volume of shampoo contaminated was 1000 g. However, it is clear that the organisms which survived and multiplied in this, because of their low numbers, did so as individuals and were in no way influenced by the presence of other organisms. In other words because of the enormous dilution factor involved it is unlikely that the initial growth and division of any single organism was assisted by the presence of metabolites or enzymes produced by other bacteria in the shampoo. Thus it is reasonable to postulate that since 50 bacteria are sufficient to contaminate 1000 g of shampoo the same number would be capable of contaminating a whole factory batch —as much as 2000 kg. It is apparent that the minimum number of bacteria required to contaminate a shampoo need not be expressed in terms of numbers per gram but in numbers per batch. However, the number per gram is important in terms of detection. In products in which bacterial multiplication is possible contamination will only become detectable when

the concentration of contaminants has reached a level to which the chosen method of analysis is sensitive. In the case of total viable counts using the pour plate technique this means that a product containing less than, say, 30 organisms g^{-1} would be unlikely to be found to be contaminated. This has important implications when selecting the time after manufacture at which a product should be sampled for contaminants. For example, the experiments reported here show that in the model shampoo system *E. cloacae* had a generation time (the time required for the bacterial population to double in size) of between 70 and 92 min. Now in a manufacturing unit where batches of 2000 kg of shampoo are made there would need to be a total of 6.0×10^7 bacteria present for contamination to be detectable in the bulk, i.e. 30 bacteria per gram. Contamination would not be detectable until at least 30 h after manufacture if the original inoculum was 50 bacteria and the generation time 90 min. Even with an initial inoculum of 5×10^5 bacteria, more than 10 h would elapse before detectable numbers of organisms would be present. Thus except in those cases where gross contamination of a product has occurred microbiological examination of either the bulk product or the product in the final container immediately after manufacture is unlikely to reveal the presence of bacteria. Thus products should be examined not only on the day of manufacture but also some days later; 7 days would be a convenient period. In view of our observation that in a model shampoo with a low inoculum bacteria become detectable within 24 h, an incubation period of 7 days might seem excessively long. However, in some products the phase of rapid multiplication, the logarithmic phase, may be preceded by a lag phase in which no multiplication occurs and also the generation time may, under less favourable conditions, be considerably longer than 90 min.

Since it may take a further 7 days for the results of microbiological examination to be known it may be thought necessary in the future to prevent distribution of finished products until 2 weeks after manufacture. However, this will depend upon experience, the ease or otherwise of recalling products once they have left the factory and the risk the manufacturer is prepared to take.

The ability of small numbers of bacteria to contaminate large volumes of shampoo helps to explain the occasional observation that contamination may be found in some packs from a batch but not others. If the original inoculum is very small or unevenly dispersed then only a certain proportion of all packs will contain bacteria capable of multiplying and giving rise to detectable numbers of contaminants. The length of the logarithmic phase, i.e. the time required for contaminants in a product to achieve their maximum

number, will increase with an increase in batch size. However, during this phase the longer a product is stored prior to packing the greater will be its bacterial population and the greater the proportion of packs containing contaminants. So products should be packed as soon as possible to reduce the frequency of pack contamination. If storage prior to packaging is unavoidable, viable counts should be performed to establish the advisability of packaging.

The inability of total viable counting techniques to detect less than about 30 bacteria per gram of product might be considered to be an argument for their replacement by other more sensitive techniques, e.g. inoculation of a liquid nutrient medium with a sample of product or filtration methods. However, unless the complete absence of contaminants or the absence of specific types of organisms is required these methods will be of doubtful value since a manufacturer will not reject a batch of product, until it has been shown that the contaminants present are capable of multiplying to an unacceptable level. Growth in liquid media has other disadvantages: chance contamination may give rise to false positive results, no information concerning the numbers of bacteria in the product is provided, bacteria capable of growth in the product may be outgrown by those of no significance from a product contamination viewpoint and a standard of sterility is imposed on the product.

Experimental inoculation of shampoos with bacteria derived from mains-water indicates that the bacteria capable of growth represent only a small proportion of the total population carried by water (our experience is of course with water from a single source). Using Probability Tables (26) by extrapolation, our results suggest that the number of bacteria capable of multiplying in our model shampoo system is approximately 5 in 100 litres (less than 0·00005% of the total number of bacteria present). However, a 2000 kg batch of our model shampoo would contain 1228 l of water or 60 bacteria capable of surviving and multiplying in this product. This is, according to our results, a sufficient number to contaminate it.

Had we used more inhibitory detergent systems (e.g. monoethanolamine lauryl sulphate) or had we used test bacteria less well adapted to growth in sodium lauryl ether sulphate, it is probable that we would have found that more than 50 bacteria were necessary to contaminate our model shampoo. Similarly the frequency of occurrence in water of bacteria capable of survival and multiplication in a product will be least when that product provides an inhospitable environment for bacteria. But when selecting water treatment devices or designing plant cleaning and disinfection procedures it is

necessary to consider extreme conditions, i.e. the product most likely to support bacterial growth. Our results indicate that for certain products a very small number of bacteria only is necessary to contaminate a large batch of product.

(*Received: 20th March 1974*)

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Hair breakage: the scanning electron microscope as a diagnostic tool

A. C. BROWN and J. A. SWIFT*

Presented on 28th August 1974 in London, at the IFSCC VIIIth International Congress on 'Cosmetics—Quality and Safety' organized by the Society of Cosmetic Chemists of Great Britain

Synopsis—Physical techniques for studying the mechanical properties of HUMAN HAIR have been well established for many years and since the introduction of the SCANNING ELECTRON MICROSCOPE (SEM) 8 years ago, there has been some attempt to correlate, retrospectively, the structural appearance of deformed fibres with data obtained during mechanical straining experiments. A logical development is to combine these two techniques so that both physical and structural data could be collected simultaneously, thereby enabling a more detailed and accurate assessment of the breakdown of structural components to be made.

The SEM, because of its great depth of focus, wide range of magnification and large area for specimen manipulation, has been adapted for conducting dynamic experiments *in situ*. In addition, the manner in which the visual information was processed made direct recording of the results onto VIDEOTAPE possible.

A number of simple mechanical specimen stages have been constructed for use in examining the structural deformation of hair fibres under various conditions of mechanical stress. The incorporation of d.c. micromotors into the SEM allowed accurate control of sample movement during experiments and miniature strain gauges were used so that continuous recordings of the changes in strain could be made.

The nature of the structural breakdown in human hair during COMBING has been investigated, together with the changes taking place as a result of natural weathering.

INTRODUCTION

As the fashion for long hair has grown more popular in recent years so interest in hair weathering has increased. An opinion poll conducted

* Unilever Research, Isleworth Laboratory, Unilever Ltd, 455 London Road, Isleworth, Middlesex.

recently (1) showed that 36% of all women between the ages of 16 and 24 had long hair and of those interviewed, 71% would have grown their hair longer had it not been for the problems involved. It is significant that two of the most widespread problems associated with long hair are the tendency for tangles to form during combing and the presence of split ends.

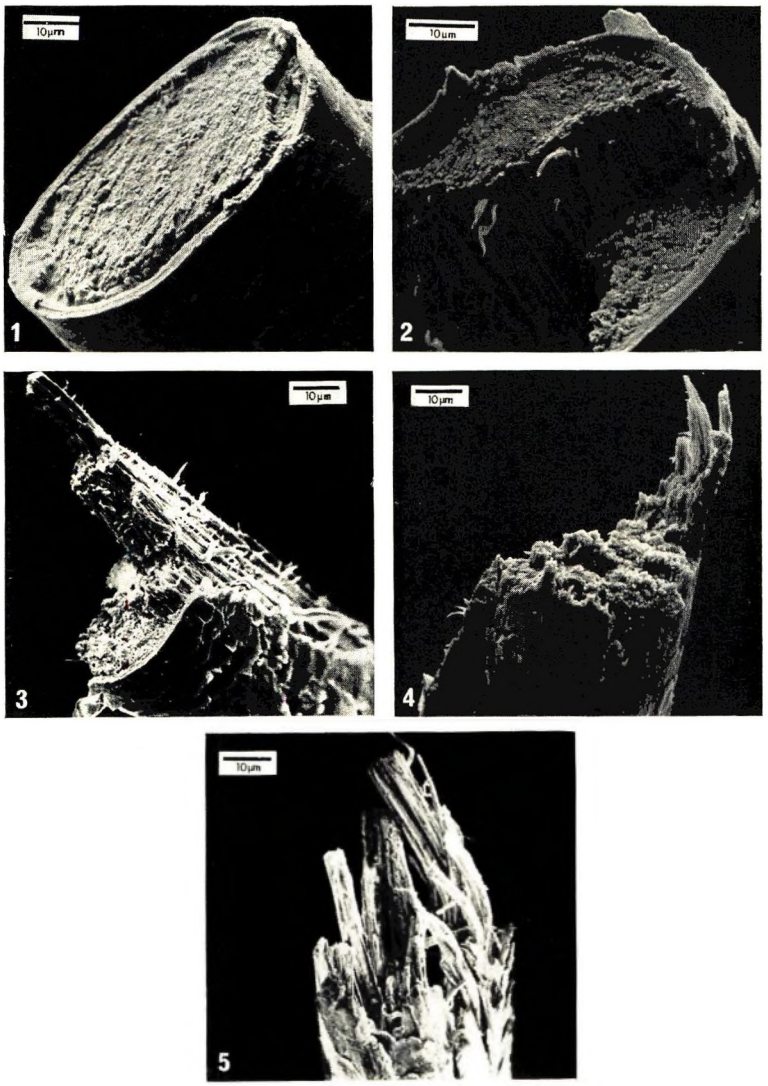
Human head hair has a growth cycle of between 3 and 6 years and reaches a final length before natural shedding, and providing it is not cut, of 50–80 cm. During this growth period the exposed fibres undergo progressive change which is generally referred to as weathering. The predominant cause of this deleterious change is thought to be sunlight and this results in a variation in the chemical and physical properties from the root to the tip of the fibres (2–4). In addition, cosmetic treatment and handling during brushing and combing increase the effects of environmental exposure. It is well known that in extreme cases of over-treatment with certain cosmetic agents or over-exposure to sunlight, premature fracture of the hair may occur resulting in a condition referred to by dermatologists as trichorrhexis nodosa (5–7). This is commonly known as 'paint brush hair' because of the longitudinal fibrillation or separation of the hair cortex which gives each hair the appearance of the bristles of a paint brush. It is interesting to note that these structural characteristics are similar to those encountered in the split tips of moderately weathered hairs.

The present paper is concerned with a study of the fracture of human hair and an investigation of those processes which lead to longitudinal splitting of weathered hair. Because of its moderately high resolution, its wide range of magnifications and high depth of focus, the scanning electron microscope (SEM) has been used exclusively in this work. Novel techniques have been devised for stressing fibres in the microscope in such a way that the fracture process can be followed continuously at high magnification and the results recorded on video tape.

Although the video tape results will be shown at the actual Symposium it is only possible in the present published paper to show a representative selection of static photographs.

An examination of hairs fractured from root to tip

The hair of six young women was chosen for use in our experiments. The hair was in excess of 50 cm length and varied in the extent of weathering as assessed by loss of surface cuticle and the presence of split ends (8). Hair



Figures 1–5. Examples of the type of fracture observed from the root end (*Fig. 1*) to the tip end of severely weathered hair (*Fig. 5*).

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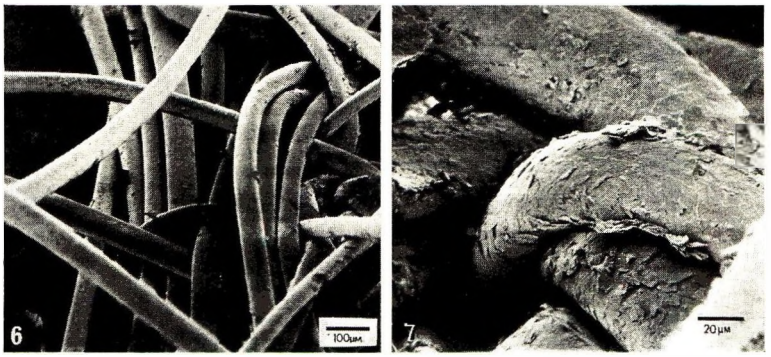

Figure 6. Appearance of a tangle at low magnification.

Figure 7. At higher magnification (× 120) the disruption of cuticle is seen clearly.

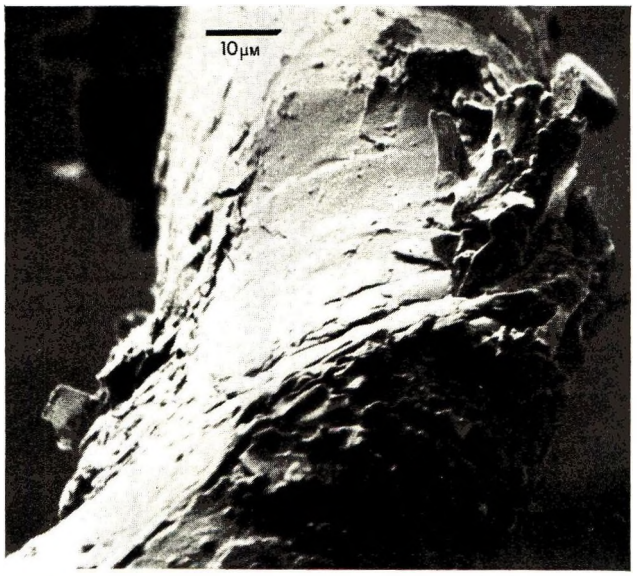

Figure 8. A groove worn in the cuticle of a hair fibre by the abrasive action of a second fibre.

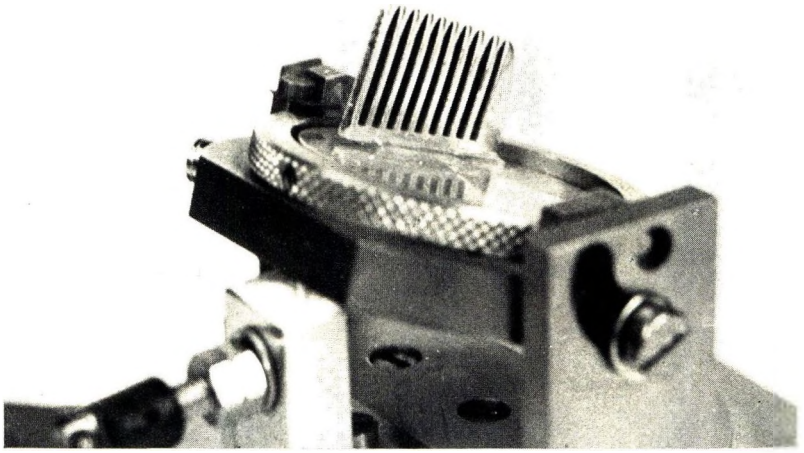

Figure 9. The specimen stage use for combing small switches of hair.

Figure 10. A micrograph at low magnification showing a tangle forming in the comb.

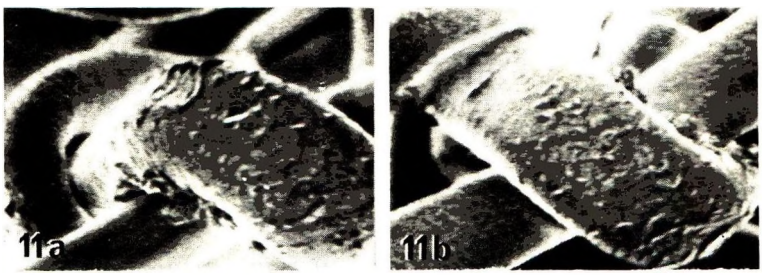

Figure 11. Two stills, photographed from the TV screen during a dynamic experiment, showing the formation of loops and lifting of cuticle during combing.

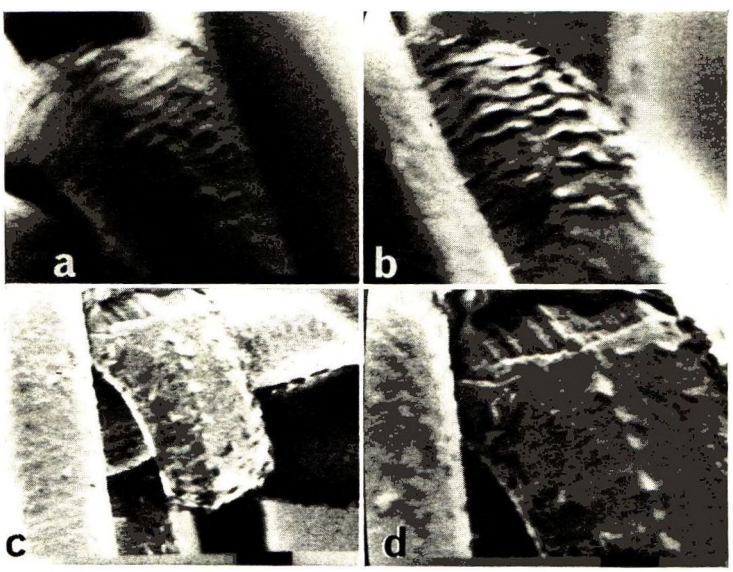

Figure 12. A sequence of stills, photographed from the TV screen, showing the final breakdown of a tightened loop.

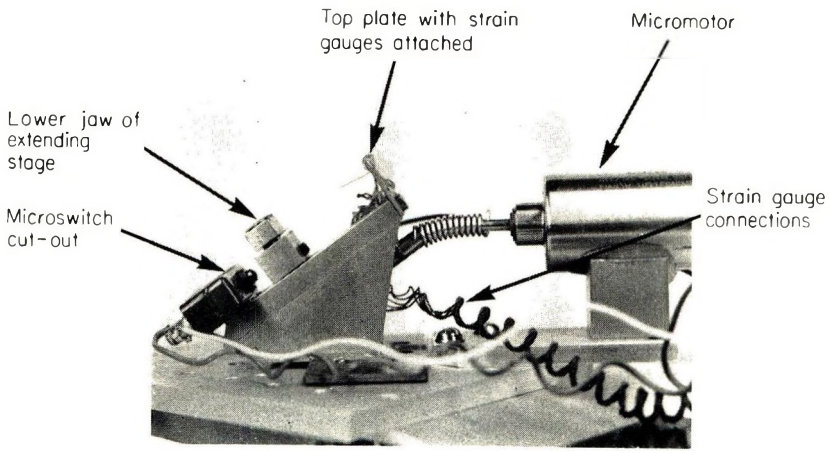

Figure 13. The specimen stage used for extending hair loops.

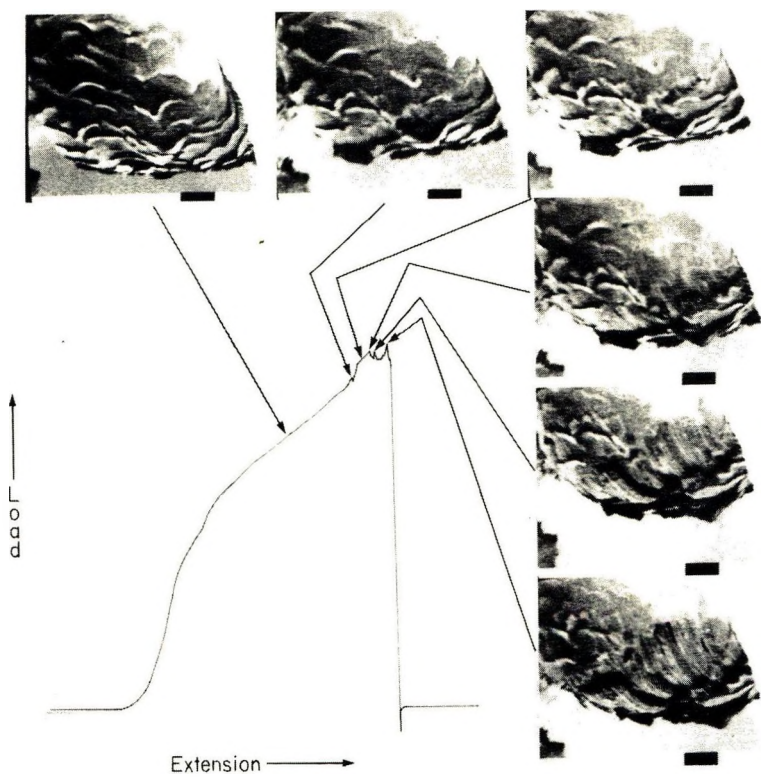

Figure 15. Load/extension curve obtained during a 'static-loop' breaking experiment. The sequence of still micrographs was photographed from the TV screen during the experiment. Irregularities in the curve just before fracture relate to the breakdown of structural elements in the hair fibre.

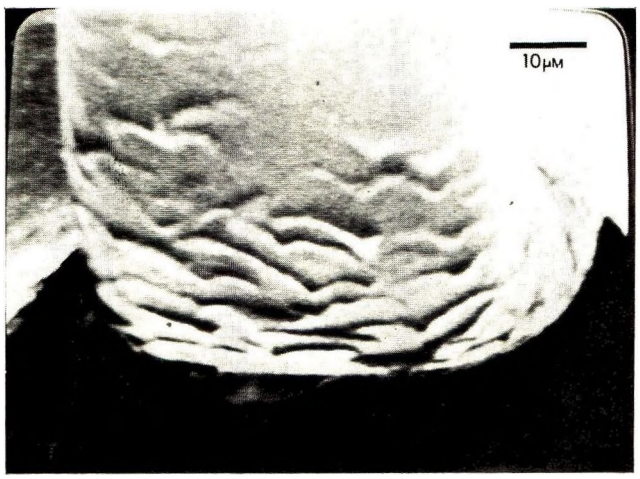

Figure 16. Static loop break, root end of fibre. A single shot from the TV screen showing cuticle cell lifting just before fracture.

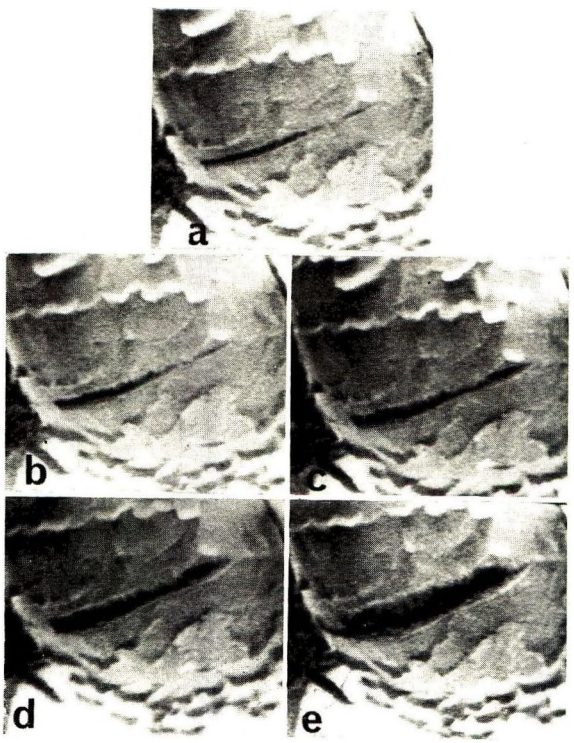

Figure 17. A sequence taken just after the point in *Fig. 16* showing the initiation of fracture.

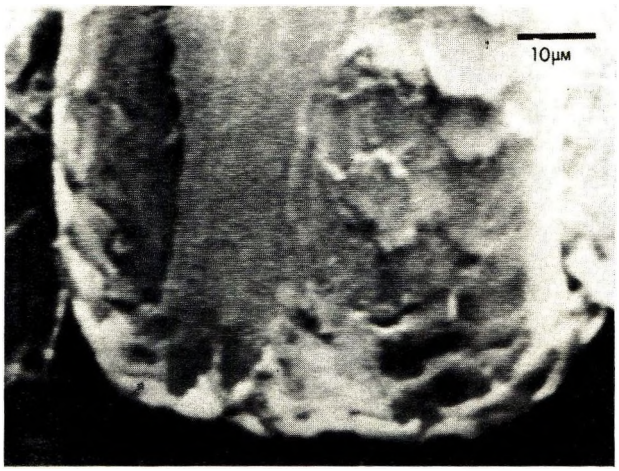

Figure 18. Static loop-break tip end of fibre. A single shot from the TV screen showing the initiation of fracture.

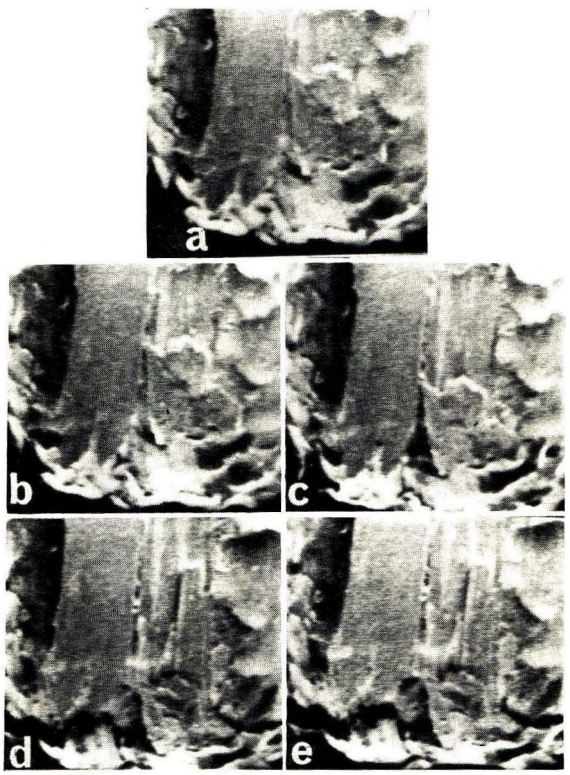

Figure 19. A sequence showing the separation of cuticle and cortical elements during a loop-break at the tip end of a weathered fibre.

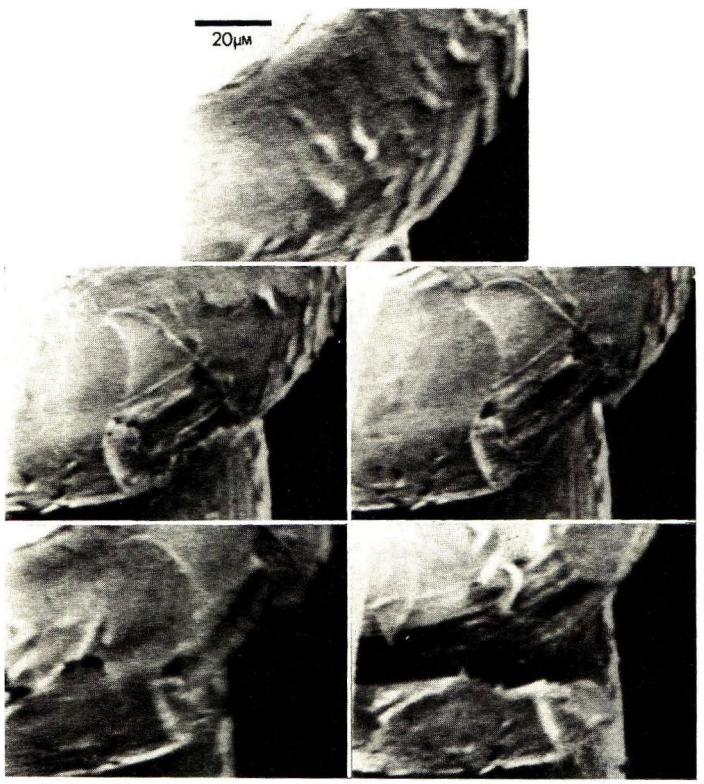

Figure 20. A sequence photographed during a 'running-loop' break at the root end of a hair fibre. Note how initial fracture of the cuticle is circumferential and the cortex begins to fracture transversely. As the loop 'runs', the fracture continues longitudinally.

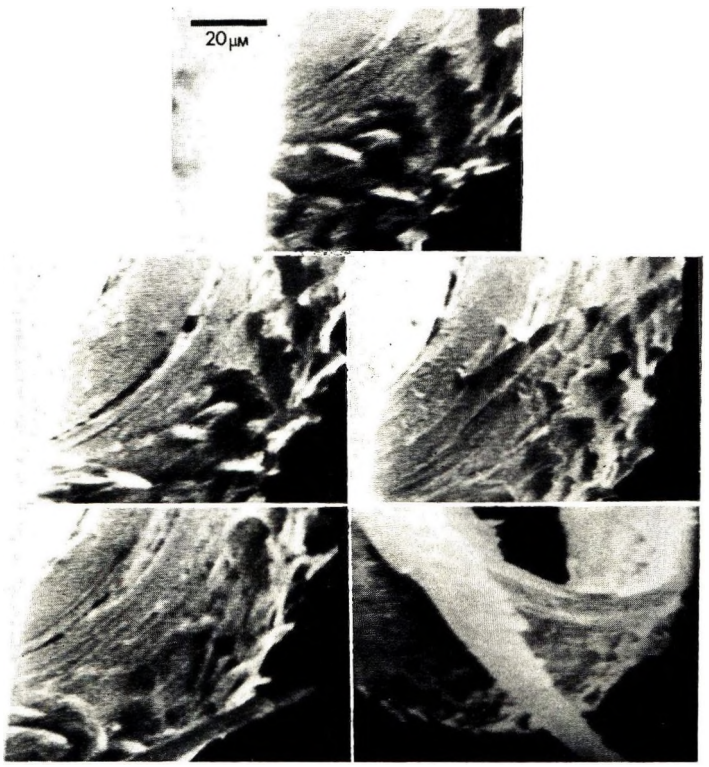

Figure 21. A sequence showing a 'running-loop' break at the tip end of a fibre. Longitudinal splits develop quite early in the process leading to a separation of the cellular structure.

samples were collected by retrieving those which fell out naturally during brushing and combing and only those which had an intact root were selected. Starting at the root end a 40 mm long segment was clamped between the cross heads of an *Instron* tensile tester and, at ambient room temperature and relative humidity, extended at various rates of extension until fracture occurred. Fractures were obtained in this manner from root to tip of the various fibres and all were mounted for examination in the SEM.

Five main types of fracture were encountered and these are illustrated in *Figs 1–5*. Type 1 (*Fig. 1*) was found at the root end of all the hairs but was also observed towards the tips of the less weathered hair samples. It consists of a clean transverse fracture and appears almost as if cut with a knife. Also, the cuticle has split circumferentially about the transverse fracture through the cortex. Type 2 (*Fig. 2*) was also found near the root ends of the hairs. It consists mainly of a transverse fracture with the cortex stepped and with some disturbance of the cuticle behind the point of fracture, either in the form of a longitudinal split back from the main fracture or a narrow circumferential split some distance from the point of primary fracture. In type 2 (*Fig. 3*) part of the primary fracture is transverse but the remainder tails off with segments of cortex pulled out. Damage to the cuticle behind the point of fracture is more severe, usually in the form of lifting of the cuticle as well as more longitudinal and circumferential splitting than in type 2. Types 4 and 5 (*Figs 4* and *5* respectively) are typical of fractures occurring close to the tip of extensively weathered hair. In type 4, although the hair may still retain its cuticle, there is no clean transverse fracture of the type encountered in types 1–3. Instead the fracture is ragged with the cortex separating into fibrillar elements (presumably individual cortical cells and macrofibrils) and there are nearly always one or more longitudinal splits back along the fibre from the point of primary fracture. In type 3 there is extensive fibrillation of the cortex resembling that encountered in trichorrhexis nodosa. There is a general progression in the type of fracture 1–5 proceeding from root to tip of the various hairs, with 2, 3, 4 and 5 being reached more quickly for badly weathered hairs than for hairs which have not been so extensively weathered. In the case of hairs which have not received appreciable weathering, types 4 and 5 are not encountered even at the tips of the hairs.

The classification of fracture types has served well as a guide to the extent of degradation of the hair and correlates satisfactorily with other methods we have used for assessing the extent of hair weathering. By determining the type of fracture at a given distance from the scalp and scoring

1–5, some measure of quantification amongst the hair of the six women could be obtained. Although this is a useful method for assessing certain aspects of hair weathering it is evident from our examination of natural fractures that the circumstances leading to the fracture of hair on the head is more complex than simple tensile fracture of single hairs. It is already well known that one of the most damaging forms of grooming is the combing out of hair tangles and particularly those produced in backcombing (teasing). This aspect was therefore considered in more detail using the SEM.

Examination of tangled hair (static studies in the SEM)

In this experiment a lock of hair was lightly backcombed (teased) and then partially combed out to the point where the usual tangling occurred. The tangled lock was mounted in the SEM. At low magnification a mass of fibres looped round each other was seen (*Fig. 6*). At high magnification it was possible to see where the hair cuticle of individual fibres had lifted and had been stripped off (*Fig. 7*). Where hairs passed over one another or had become twisted round each other, grooves had been formed in the cuticle surface (*Fig. 8*) and in some cases loose cuticle debris was observed. In such a complex situation it was difficult to assess the causes of these effects without watching the whole process as it was taking place. At this stage therefore we turned to an examination of the comb-out operation actually taking place within the SEM, the whole experiment being recorded on video tape.

Dynamic combing experiments in the SEM

Before giving details of these combing experiments it is necessary to elaborate on the conditions that must be satisfied for the successful use of the SEM. Hair fibres are electrically non-conducting and tend to accumulate an unstable electrostatic charge under the influence of the electron beam in the SEM which leads to instability of the video image. Usually this problem is overcome by providing the specimen with a thin surface film of metal evaporated under vacuum. Naturally such a metal film is undesirable in our present studies. Some authors have used antistatic coatings for eliminating electrostatic charging (9–11) but these are also undesirable in that they affect

the surface properties of the hairs. An alternative procedure is to examine the hairs at low electron accelerating voltages (8, 12). Although image resolution tends to be somewhat poorer at these low accelerating voltages, with experience and by using minor modifications of the SEM we are now able to obtain very acceptable results (13). In addition we have established that at these low voltages, the electron beam causes imperceptible damage to the uncoated hair surface (13).

The combing experiments were set up with the minimum of modification to the microscope specimen stage. A small section of an aluminium comb was fixed to a suitable brass base (*Fig. 9*) and a small lock of 30–40 hairs, which had been teased and then partially combed-out, was laid over the comb. Continuation of the comb-out operation was effected by pulling the 'root ends' through the tines of the comb on to a shaft which was rotated from the outside of the microscope. A Cambridge Stereoscan 600 SEM was used in this work. The microscope gave a TV video output compatible with standard closed circuit TV equipment and was also linked to a Shibaden $\frac{1}{2}''$ video tape recorder so that the results of the work could be played back for further detailed analysis. In addition, by stopping the comb-out operation at various points, still micrographs were obtained.

One striking feature of the present dynamic studies of hair comb-out was the wealth of information contained in even a short run. In particular there were so many processes occurring at the same time that one had to be very selective in choosing an appropriate field of view or specific feature to study at high magnification.

At low magnification the hairs moved from all directions to line up and pass between the tines of the comb (*Fig. 10*). During this process, the hairs became twisted together to form tight bends and loops. As loops were pulled over stationary hairs, cuticle cells were stretched and lifted on the outside of the bend (*Fig. 11*). These exposed scale margins readily broke off as they came into contact with other fibres. While 'running loops' lost cuticle in this manner the stationary fibres with which they were in contact became distorted at the point of contact and became grooved by the continual abrasion. Deformation and stripping of cuticle also occurred as fibres were pulled through stationary loops of other hairs and this became an important factor as the loops tightened. Eventually the tangle tightened to the extent that a few individual hairs began to break and this occurred predominantly at a loop (*Fig. 12*). This proved such a common occurrence that we decided to investigate loop fracture in more detail using a simple system of straining a hair over a metal wire of comparable diameter.

Examination of dynamic 'loop breaks' in the SEM

The investigation of loop breaks was carried out using a small extending specimen stage (*Fig. 13*). In one set of jaws the two ends of a short length of 100-μm diameter Constantan wire was clamped. Looped through this wire and attached at both ends to the other jaw was the hair under investigation. One jaw was fixed and the other was driven on a screw thread by means of a d.c. micromotor controlled from a power supply outside the microscope. The speed of separation could be varied from 1 mm s^{-1} to 10 μm s^{-1}, the lower speed being necessarily slow so that details of the fracture propagation could be recorded at magnifications up to × 10,000. The fixed jaw of the stage carried four small semiconductor strain gauges arranged to measure the minute bending forces of the jaw support as load was applied to the loop under test. Output from the strain gauges was fed through an amplifier to a chart recorder so that at constant speed of extension a load/extension curve was obtained at the same time as the structural information was recorded on video tape (*Fig. 14*). In this way we have been able to relate small irregularities in the load/extension curve to corresponding changes taking place in the

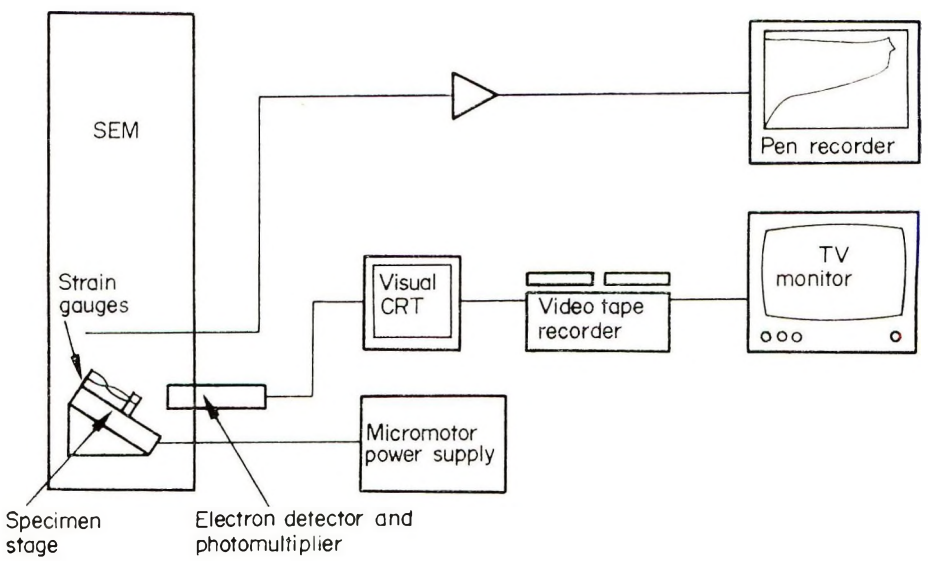

Figure 14. Block diagram of the equipment used in the 'loop-break' experiments.

structure. This is illustrated by the example in *Fig. 15* where the chart trace shows irregularities near the yield point which relate directly to structural failures in the cuticle and cortex of the fibre as the fracture developed.

In examining loop breaks, hair was obtained from the same women as for our earlier fracture experiments. Two types of loop breakings were studied. The first type, which we will refer to as the 'static loop', was produced when both ends of the hair were pulled so that no slipping of the hair on the wire occurred. The second type was a 'running loop' where one end of the hair was fixed to the specimen stage and the other end held firmly in the extending stage and pulled. In this case the hair moves slowly over the wire as the hair elongates under load.

Several interesting effects were observed. For static loops, the type of fracturing process and the final structure of the fibre end at break, for hairs from the various women and for various positions along each hair, were in good accord with the results described in Section 1 of this paper (i.e. *Figs 1–5*). At the root end, stretching of the cuticle about the loop was observed, usually accompanied at moderate loads by cuticle cell lifting (*Fig. 16*). Fracture usually began in this region as a split through the cell layers of the cuticle along a circumferential segment (*Fig. 17*). When this split had propagated about halfway round the fibre with the exposure of the underlying cortical surface, the rest of the fracture was catastrophic, the cortex fracturing transversely or in low transverse steps. At the tips of weathered hairs where little or no cuticle remained, the fracture process was initiated by the separation of surface fibrils from the cortex (*Fig. 18*). As the load increased further, separation of fibrillar elements from the cortex occurred (*Fig. 19*). At this stage some gross longitudinal splitting of the fibre occurred with more and more cortical fibrils rupturing until the two parts of the fibre separated abruptly.

Running loops always resulted in longitudinal splitting of the hair irrespective of whether root or tip segments were examined. At the root end the fracture began transversely through the cuticle but split longitudinally once this crack had reached the middle of the fibre (*Fig. 20*). At the tip the fracture started in much the same way as for the static loop but longitudinal splitting was accentuated (*Fig. 21*).

Conclusions

It is clear from the experiments that we have performed that tangling of hair during combing seriously damages the hair surface. In particular it appears that the types of fracture that result from this treatment yield longitudinal splitting. This splitting process occurs far more readily at the tips of hairs than at their roots and we must therefore conclude that the weathering the hair has received has facilitated the ease of splitting. On the other hand it is possible that combing and mechanical atrophy of the hair are essential requirements for splitting and that in the absence of mechanical perturbation splitting will not normally occur. In addition to reaching these conclusions we believe that we have demonstrated the value of the scanning electron microscope as a diagnostic tool.

Acknowledgment

We are indebted to Miss Paulene McCarthy for her technical skills in the use of the scanning electron microscope.

(*Received: 29th April 1974*)

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An appraisal of human head hair as forensic evidence

J. PORTER* and C. FOUWEATHER†

Synopsis—The evidential value of HUMAN HEAD HAIR in FORENSIC science is discussed. As well as basic techniques such as MORPHOLOGY and the identification of COSMETICS, an experiment to put the measurement of hair COLOUR on a less subjective basis, is described. Results show that with the techniques currently in use a definite method of relating a hair fibre to a particular individual is still not a reality, but, in cases of rare hair colour and the presence of cosmetic treatments, good discrimination can be achieved.

INTRODUCTION

At the present time the evidential value of hair in the forensic context is limited because no reliable means of characterization has yet been developed which allows the complete individualization of human hair (Simpson (1); Polson (2); Peterson, Haines and Webster (3)). This state of affairs is unsatisfactory as human hairs are often recovered from crimes of violence and many other serious crimes.

The purpose of this review is to assess the present state of the available information, to discuss the results of recent work at the Home Office Central Research Establishment and finally to suggest possible lines of investigation.

* Home Office Central Research Establishment, Aldermaston, Reading, Berks, RG7 4PN, England.

† Home Office Forensic Science Laboratory, Shakespeare Street, Nottingham, NG1 4FR, England.

Hair is an appendage of the skin corresponding anatomically with the epidermis. It consists of a shaft, a root and bulbous extremity embedded in the hair follicle. Hair and wool, in their natural unstretched state, belong to a group of proteins called the α-keratins. Keratins occur as the principal constituents of the horny layer of the epidermis and of related appendages such as horns, hooves, scales, hair and feathers, that are derived from the skin. Keratin fibres are very complex both at the histological level and at the chemical level owing to the multiplicity of protein molecules which are effectively cross-linked to give an integral structure. A microscopic examination of a hair cross-section reveals the outer cuticular fibrous portion, an inner darker portion, the cortex, and in the centre of the cortex a central canal called the medulla. The outer cuticular layer takes the form of fine scales which cover the surface of the hair and act as a protective layer. The colour of the hair fibre is due to pigment granules (melanin), found principally in the cortex, and to the presence of air bubbles in the medulla.

The basic techniques that have found application are as follows:

Morphology of hair

Diameter

In an early paper Wynkoop (4) concludes that hair shaft diameter bears little or no relationship to the age of an individual. Trotter (5) and Trotter and Dawson (6) came to similar conclusions in a later study. A more detailed examination (Kind (7)) of human head hair diameters revealed that there is little correlation between hair shaft diameters and a given individual. The variation of head hair diameters over an individual head was very similar to the variation of hair diameters over the population (*Fig. 1*). The diameter of hair is known to vary over the length of an individual fibre but recent work (Fouweather (8)) has shown that the variation of hair diameters along an individual fibre is not a useful parameter for hair characterization.

Medullary index

Owen (9) has demonstrated that the medullary index or fraction (the ratio of width of medulla to the width of the hair) is of little or no use in the characterization of human head hairs. However, the medullary index is known to be a means of discriminating between some animal species (e.g. cat from dog) (Kind (10)).

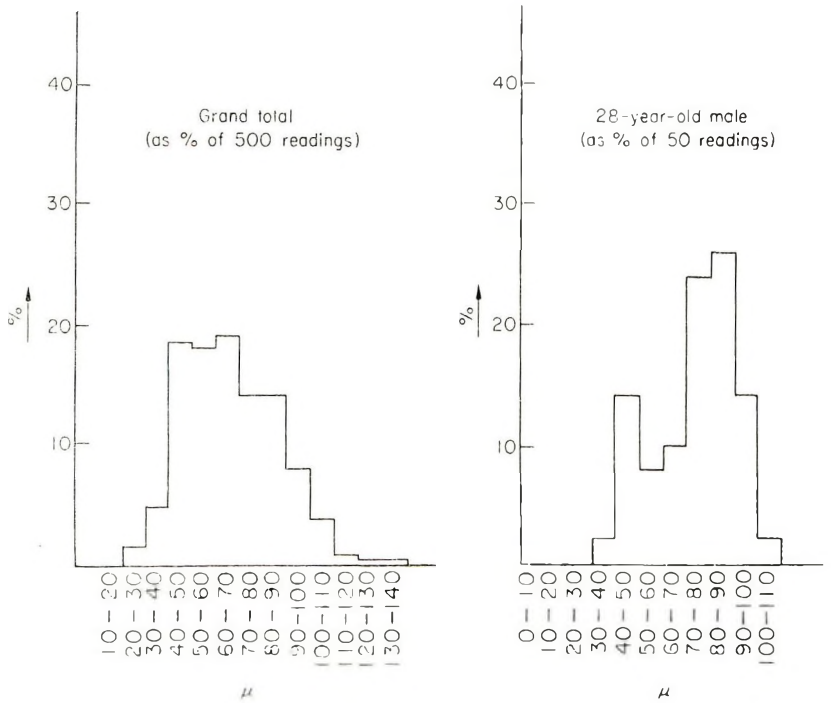

Figure 1.

Colour

Many attempts have been made to put the measurement of hair colour on a more objective basis. Trotter (11) has presented a summary of many such systems. Garn (12) has attempted to measure hair colours by reference to standard *Munsell* Colour Chips (13), but this system has not found general acceptance. More recent work in this laboratory has demonstrated that *Munsell* Colour Chips are totally unsuitable for the measurement of the colours of single fibres, and of only limited use in the measurement of the colours of hair 'tufts'. A system based on dyed nylon tufts is described in detail in 'The description of human hair colour' section below. A micro-spectrophotometer can be used for the measurement of hair colour but it is only suitable for measurements on hair tufts (1 g or greater) (Unilever (14)). Any data obtained for human hair colours will be of only limited use as the variation of colour over a single human head is considerable.

Scale counts and scale patterns

A considerable amount of work (9 and Gamble and Kirk (15)) has been

done in this area, and the results suggest that scale patterns are sufficiently characteristic to allow broad classes or species to be distinguished, i.e. a human hair can be readily distinguished from a cat hair, but not a dog hair from a cat hair.

An Atlas (16) of hair cross-sections, longitudinal views and scale patterns is available as a reference work. However, scale counts (number of scales in unit length) do not provide a means of distinguishing human hairs as the variation across a single human head is similar to that across the population, a similar distribution to that in *Fig. 1* is obtained.

Amino-acid analysis of hair

The amino-acid composition of human hair does not show any significant variation from person to person (Bogaty (17)) and is therefore unlikely to provide a means of hair characterization. The amino-acid analyses of wool, human hair, animal hairs and finger-nails are all known to be similar.

Pyrolysis gas chromatography

This has been investigated by two workers (De Forest (18) and Fouweather (19)) but does not provide an effective means of hair discrimination. The pyrograms of all the individuals studied were essentially similar and any small differences between individuals were within the experimental error. A comparison of two pyrograms from two individuals is shown (*Fig. 2*).

Physical parameters of hair

A study (Dabbs (20) and Greenwell, Wittmer and Kirk (21)) has been made of the refractive index (RI) of human head hairs and, whilst the RI varies from person to person, the variation of RI across a single head reduces the discrimination to 1 in 2/3, e.g. in a typical case studied an individual displayed hairs of RI from 1.546 to 1.550 compared with the variation over the population from 1.542 to 1.555. However, in view of the limited data available for hair characterization, it does at least provide a method of dividing the hair population in two.

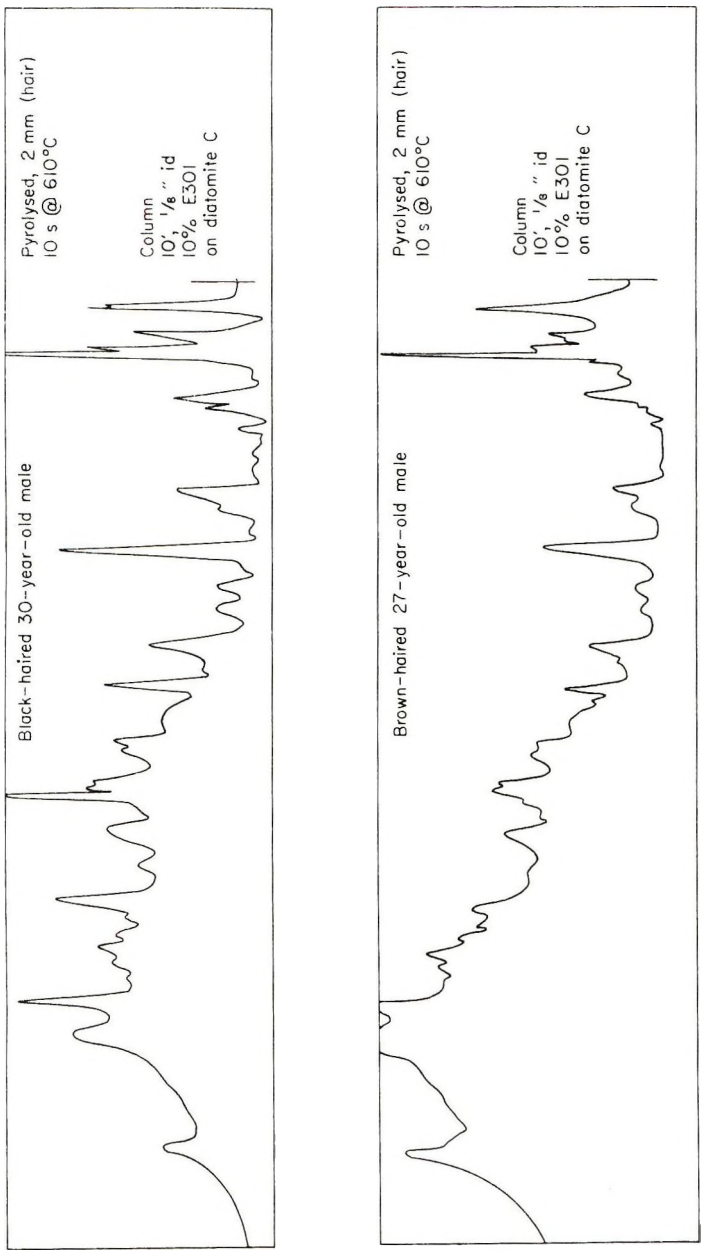
Black-haired 30-year-old male
Pyrolysed, 2 mm (hair)
10 s @ 610°C
Column
10', 1/8" id
10% E301
on diatomite C
Brown-haired 27-year-old male
Pyrolysed, 2 mm (hair)
10 s @ 610°C
Column
10', 1/8" id
10% E301
on diatomite C

Figure 2.

The tensile and elastic properties of hair have been studied using sophisticated equipment in constant temperature and constant humidity environment. However, the results indicate that these measurements are unlikely to provide a means of hair characterization. The equipment used is based upon similar equipment used to study the tensile and elastic properties of plastics and other elastomers.

Fatty acids in hair sebum

The free fatty acids in hair sebum were analysed by GLC after conversion to their methyl esters using diazomethane. The variation between individuals was little greater than the daily variation of an individual and was not pursued further. A similar result was obtained when the glycerides in hair sebum were hydrolysed and the fatty acids so produced methylated. A typical chromatogram is shown (*Fig. 3*). At least 50 fibres are required to produce a satisfactory analysis.

Trace element analysis

This is an extensive field and will not be discussed in this paper in any detail save to point out that it is one of the more promising techniques for hair characterization. However, problem areas still exist with regard to the interpretation of the results because of the correlation between elements, the variation of the elemental composition over the head, along the length of the hair fibre and also across the hair diameter.

Low temperature luminescence of hair

At 77°K a tryptophan-like fluorescence and phosphorescence emission can be observed (King (22)) using 10×1 cm lengths of hair (*Fig. 4*). By measuring the relative intensities of the phosphorescence and fluorescence emissions, it is possible to distinguish between numerous animal species, e.g. the species cat, cow and vole can be distinguished from one another. However, there is a large coefficient of variation within individual species and cat and dog hairs are normally indistinguishable using this technique. Similar problems arise with human head hairs. The species discrimination achieved

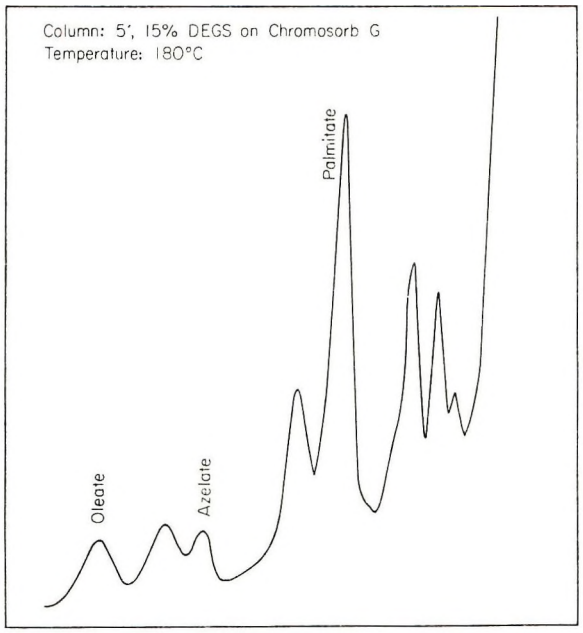

Figure 3. Chromatogram of methyl esters of fatty acids from hair (50).

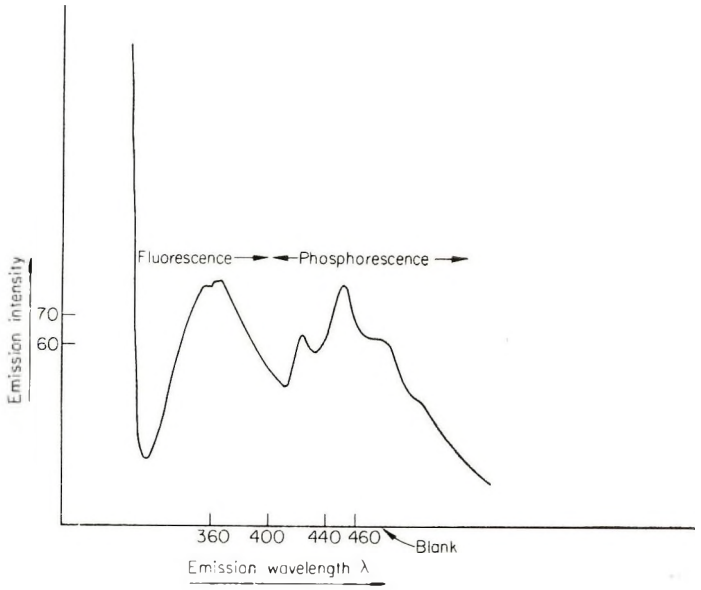

Figure 4.

with this technique can be more readily obtained by observation of scale patterns.

Serology and sexing

Whilst the identification of group substances in blood (Culliford (23)) has now become a routine technique in forensic laboratories no such advances have been made with hair. Japanese workers have claimed (Yada, Mori and Okane (24); Yada, Ishimoto and Okane (25)) to be able to group single human hairs but experiments in British laboratories have been unable to confirm these claims. Work in this laboratory has not yielded a successful technique, mainly due to the false reactions with A and O anti-sera. It is still not clear where the group substances are located.

Much work has been carried out on the sexing of human hairs (Phillips (26); Brinkman and Jobst (27)). Staining techniques were used to identify Y-chromosomes and Barr bodies and the results were very encouraging.

The description of human hair colour

Introduction

It is a common observation that the appearance of human hair (a description which includes colour, texture and many other subjective factors) is a particularly characteristic feature of an individual. The colour of human hair is one of its most distinctive features but its measurement needs to be put on a less subjective basis. The purpose of this experiment was to develop a hair chart suitable for the study of the variation of head hair colours across the population. Samples of hair from 100 men and 100 women were collected.

Experimental

Hair from each individual was gathered into a small tuft of approximately 50 hairs and mounted upon a small piece of neutral grey card. The colour of each tuft was compared with a *Munsell* hair colour chart (13) which contained matt painted chips arranged according to their hue, value and chroma. However, the results of this experiment demonstrated the inability of observers to match human hair (a highly textured material) to the matt surface chips used in the *Munsell* system. It is therefore essential that the colour of human hair is compared with coloured material of a similar surface texture. This is because the specular reflections from the

surface of the hair constitute the large part of the colour sensation. The other major limitation in hair colour comparisons is that human hair displays a very limited range of hues (581–606 nm), i.e. human hair differs mainly in the lightness or darkness of the colour, not in the wavelength of the reflected light. The 200 hair tufts were therefore compared with commercially available dyed nylon tufts (Wella (28)). Only 29 colour tufts were used to describe the colour of the hair samples, but on extending the colour comparisons to single fibres a hair colour chart containing 29 colours merely led to ambiguity in assigning the hair colour. This is because the colour of single fibres from a given individual varies along the length of the fibre and across the head of that individual. Successive experiments indicated that nine hair colours (from black to white) provided sufficient colours to describe the population with minimum ambiguity in assigning the particular colour. It is neither necessary nor desirable to use more than nine colours to describe hair because of the range of hair colours displayed by a given individual. Some of the individuals examined displayed a range of colours from group 3–6 and 2–5 (*Table I*). Hence it is important to have sufficient controls to establish the range of hair colour displayed by a given individual. A single fibre was chosen from the hair of each of 200 individuals at random and compared with the hair chart containing nine colours. A hair was described as grey/white if the original tufts prepared contained too many white hairs to preclude colour comparisons. The results of this survey are shown in *Table I.*

Table I. Distribution of hair colour in population

	Black	Very dark brown	Dark brown	Mid-brown		Blonde			White /grey
Colour group	1	2	3	4	5	6	7	8	9
Female % population	0	28	20	9	14	7	6	4	12
Male % population	0	43	16	12	9	1	0	0	19

Table I shows that a large proportion of the colours of the male and female population lie in groups 2, 3, 4, 5, 9. The remaining groups 1, 6, 7, 8 are sufficiently rare to provide useful discrimination. It can also be seen that no black hairs or genuine red hairs were found in this survey. So-called blonde hair (groups 6, 7, 8) were sufficiently rare in both male and female

populations to provide meaningful information. It can be seen that 17% of the hair of the female population can be described as blonde, whilst for the male population only 1% of male hairs were blonde. In group 6, six out of seven women had blonde hair as a result of chemical bleaching whereas, not surprisingly, none of the male population had bleached hair. One of the six females in group 7 had bleached hair, whilst one in four females in group 8 had bleached hair. Hence it will be obvious that a blonde, bleached hair is more likely to be female than male. A blonde female unbleached hair represents only 1% of the female population, and therefore it may be concluded that hair lighter than group 5, i.e. 6, 7, 8 can provide useful information.

Identification of hair cosmetic treatments

Bleaching

The use of commercially available bleaches to lighten hair brings about the bleaching of the hair pigment (melanin), together with structural damage to the α-keratin (hair protein). The cuticular layer containing the α-keratin is gradually abraded away due to the movement of individual hairs across one another and by brushing and combing. The extent of the damage to single fibres can be estimated by observation of the uptake of dye from an aqueous solution of methylene blue. The amino-acid composition of bleached hair is not greatly different from unbleached hair, with the exception that, in the former, a high proportion of the cysteine and cystine is converted into the corresponding sulphonic and cysteic acid. It is the presence of the anionic sulphonic acid groups which is responsible for the uptake of methylene blue (a cationic dye). It is apparent from the present work that the degree of hair damage is closely related to the extent of hair bleaching. For a given bleached fibre the uptake of methylene blue is greater at the tip of the fibre and least near the root. It has been established that if 1 cm lengths of hair are obtained from the same head but from different hairs and from the same distance from the root, then methylene blue uptake is similar in each case. The methylene blue test is therefore a useful comparative test for assessing hair damage caused by chemical bleaching. No other cosmetic treatments have been found to interfere with the methylene blue reaction. The occurrence of bleached hair for the different colour groups is shown in *Table II*.

Technique. A 1 cm portion of hair is fixed to a cavity slide using perspex cement or cellulose acetate, and several drops of aqueous methylene blue solution (0.5%) added to completely cover the hair. After 4 min the hair is

Table II. Occurrence of bleached hairs

Colour group (female)	1	2	3	4	5	6	7	8	9
% population (100)	0	28	20	9	14	7	6	4	12
No. hairs bleached	0	0	0	0	0	6	1	1	0

rinsed with water and observed. Extensive hair damage is indicated by intense blue/violet colouration. Unbleached hairs do not absorb any dye under these conditions.

Hair lacquer residues

A technique has been developed (Crockett (29)) for the examination of lacquer residues by infrared spectroscopy. This technique enables the type of resin used to be identified and requires only 2–3 cm of hair. However, before this technique can be used some data on the use of lacquer in the population is required. Polyvinyl pyrollidone (the major constituent of many hair lacquers) is, however, the basic material for a wide range of hair cosmetic preparations other than lacquers (e.g. sets, conditioners, etc).

Hair dyes

Hair dyes can be broadly classified into three types:

Temporary rinses. These are essentially coloured pigments which are dissolved in water and absorbed on the surface of the hair. They contain a wide range of compounds including azo-dyes, triphenylmethane dyes and anthraquinones.

Semi-permanent dyes. These are frequently nitroaromatic amines which are strongly absorbed on the surface of the hair and give a good depth of shade.

Permanent or oxidation dyes. Permanent dyes are mostly based on *p*-phenaminediamine. In the dyeing process the amine penetrates the hair shaft and enters the cortex, where it is oxidized with hydrogen peroxide to yield a complex polymeric product.

The development of a thin-layer chromatographic system to identify these types of dyes on single fibres has been studied (Logan (30)) but found to be impractical due to great similarity between the polymerized product of different manufacturers. Where sufficient fibres are available (i.e. > 50 cm) the dyes may be extracted and identified by TLC. However, a considerable

amount of information on the type of dye can be obtained from microscopic examination of the fibre. Dyed hairs frequently show a more uniform distribution of colour than un-dyed hairs, i.e. there are very few pigment granules visible. In natural hair there may be a gradual decrease in pigment from base to tip, but on para-dyed hair or bleached hair the decrease is normally abrupt. Hence the identification of dyed hair does not normally present any real problem in forensic examinations. Of 200 hairs examined only 1 individual displayed dyed hair (group 6, female). If the hair displays a root then an estimate of the time since dyeing can be made, as hair is known to grow at a rate of approximately 1 cm/month. Attempts to classify hair dyes from single fibres by TLC have been studied but were shown to be impractical because of the small quantities of dye used and the difficulties of extracting the dyes without modifying them chemically. Hair rinses contain dyes in solution which deposit a relatively uneven film of the dye on the surface of the hair fibre. (Some of these rinses are based on methylene blue and methyl violet.) Observation of an individual's hair has shown that many of these rinses persist for up to 6 days but at this time the dye is very uneven and difficult to detect. Even after 1 day a significant proportion of the hair dye has been removed by brushing and combing and is completely removed by washing.

Identification of medicated shampoos. One of the major limitations of the examination of cosmetic material in or on hair is the ease with which these materials can be removed by combing, brushing and washing. Phenols are known to be strongly absorbed (Breuer (31)) at polar sites in the hair keratin and are not readily removed. It was for this reason that attention was focused on formulations which contain phenols. An examination of the formulations of many medicated shampoos indicates that many of them are based upon a wide range of phenolic compounds or quaternary ammonium salts. It was not found possible, using known analytical reactions, to detect quaternary ammonium salts on hair fibres. However, examination of the common methods for identifying phenols produced a method based upon the formation of brightly coloured indophenols. The use of nitrous acid in concentrated sulphuric acid produced a reagent which partially destroyed the cuticle of the hair and allowed the reagent to penetrate the hair fibre. When a hair which had been washed using a medicated shampoo based on phenols was treated with nitrous acid an intense brown colouration was produced in the body of the fibre.

Hairs darker than group 6 were prebleached for 30 min using a commercial hair bleach. The advantage of commercial bleach over ammoniacal

peroxide is that the former contains a thixotropic agent which prevents the bleach running off the hair. The colour reaction with nitrous acid can be readily observed after the hair has been bleached.

One individual, whose hair gave a positive reaction, washed his hair on three successive occasions with a non-medicated shampoo based on lauryl sulphate. A positive nitrous acid test was obtained after each washing. If an individual continues to use a medicated shampoo then the phenol concentration in the hair shaft will rise, and one washing in a non-medicated shampoo does not remove all the absorbed phenols. In the survey 37% of the total population gave a positive reaction.

Technique. A single human head hair (0.5 cm) was attached to a cavity slide with perspex cement or cellulose acetate. An ice-cold solution of nitrous acid was prepared from 0.14 g of sodium nitrite in conc. H_2SO_4 (2 ml). Two drops of the above solution were added to the hair fibre and the colour reaction observed for up to 10 min. The crime and control samples can be examined in the same cavity. Dark hairs were prebleached for 30 min prior to the above procedure using a proprietary bleach.

Conclusions

The following questions frequently arise when hair is used as evidence and they remain appropriate to this discussion.

(1) Is the hair human or animal?
(2) From what part of the body did it originate?
(3) Is it from a male or female?
(4) What is the significance of special features the hair might display, e.g. lacquer residues, cosmetics, blood group substances, dyes, colours, etc.?
(5) Are the crime and control hairs similar using the available criteria?

Microscopy of a hair fibre will distinguish human hair from animal hair on the basis of its scale pattern and appearance. Dyed hairs can normally be distinguished from undyed hairs by microscopy but chromatography of hair dye obtained from a short fibre is not a practical technique.

The occurrence of lacquer and other cosmetic materials can be established but their significance must be limited as they are so readily removed by washing etc, and do not represent a method of hair individualization. Scale counts, medullary fraction measurement and the occurrence or absence of a medulla are of little or no use in the characterization of human head hairs.

Whilst the colour of human hair remains one of its most characteristic features, its use for a particular individual is limited by the variation of the colour of single fibres over an individual head. Hair colours are obviously significant for the rarer colours such as black, blonde, red-brown. Normally little difficulty is experienced in distinguishing human hairs from different parts of the body, e.g. moustache hairs are frequently triangular in section, whereas pubic hairs are short, curly with worn pointed tips. Trace elemental analysis may in the future provide a satisfactory means of characterizing a single fibre, but at the present time it still has problems.

The cosmetic treatments of bleaching and dyeing can be readily identified and these techniques can provide a useful addition to the limited techniques available at the present. The active ingredients of medicated shampoos can be identified using a simple colour reaction. A definite method of relating a hair fibre to a particular individual is still not a reality, but in favourable cases of rare colours, the presence of cosmetic treatments such as bleaching or dyeing, good discrimination can be achieved.

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Microbiological quality control—a case history

G. D. BREACH*

Presented on 28th August 1974 in London at the IFSCC VIIIth International Congress on 'Cosmetics—Quality and Safety' organized by the Society of Cosmetic Chemists of Great Britain

Synopsis—A history of the introduction of MICROBIOLOGICAL QUALITY CONTROL (MQC) into factories manufacturing non-sterile pharmaceuticals, TOILETRIES and COSMETICS throughout Europe and Africa is outlined. The development of a microbiology manual and appropriate standard operating procedures to up-grade GOOD MANUFACTURING PRACTICE (GMP) is explained. Problems with particular products and organisms are discussed, especially the presence of *Achromobacter* and *Pseudomonas* organisms in plant water in some sites. The inter-relationship of QC, R & D, and Production which enabled MQC to proceed smoothly is emphasized as is the application of one overall 'in house' standard.

The introduction of microbiological quality control

We viewed microbiological control as a much needed extension of a quality assurance programme and the techniques, methods and standards which were developed were designed to control and improve hygiene standards in the plant. Where official government standards have been introduced these have been viewed as additive, rather than as a replacement to the 'in house' standards. For example, a government regulation which requires the absence of a particular species of bacteria from a product, utilizes a specific test designed for that organism and therefore it is felt that

* Vick International, 250 Bath Road, Slough, Bucks.

the product's ability to pass such a test does not measure the overall hygienic production of the product or indeed the actual level of bacterial contamination present.

It was also felt to be necessary to incorporate into the stability programme regular microbiological analysis, so that knowledge could be gained of the microbiological life-span of products and their preservative systems and sufficient data acquired for meaningful co-operation with government bodies, dealing in practicalities rather than airy-fairy theories.

All the QC managers were involved from the outset of the programme so that due economy in materials and time could be obtained. At the outset it was felt that it should be possible to introduce MQC to non-microbiologically qualified staff if methods and the results obtained by those methods did not contain elements of judgment and opinion. By this it was meant that the staff and premises available at that time were sufficient to initiate and maintain a programme of microbiological testing without:

(a) employing microbiologists in each plant;
(b) installing a separate microbiological laboratory in each plant.

To this end a manual was prepared by qualified microbiologists in R & D which outlined methods for use in QC laboratories to control the final product and establish accept/reject limits. Over the course of the last 4 years the programme has grown to adulthood and each plant now has at least one person involved in final product analysis, 'in process' control, and monitoring general factory hygiene standards. These people in the majority of cases are QC chemists who took over the task because of personal interest, the minority are fundamentally more microbiologically orientated who were taken on in the course of natural turnover of staff in the QC laboratory. It is generally felt that the introduction was a challenge well met rather than a traumatic experience.

As the author has said, elsewhere (1), 'Each manufacturer must assess and impose suitable standards for his product lines. Regrettably, standards will vary in an absolute sense throughout industry since the present structure of the cosmetic and toiletry industry interweaves with the manufacture of sterile and non-sterile pharmaceuticals and foodstuffs.' Throughout the plants regardless of product category the 'in house' standard imposed was, from the beginning, 100 organisms per g or per ml of product due to the fact that non-sterile pharmaceuticals and toiletries were and are manufactured alongside each other.

In the beginning all washing and disinfecting procedures were reviewed

and in sensitive product areas were checked by R & D personnel. Only then was final testing of finished packed products introduced, product by product, with priority given to products known to be at risk in the factory (aqueous 'cold mix' products) and if contaminated those of direct risk to the consumer, for example, baby toiletries and oral non-sterile pharmaceuticals.

The final check was by means of a total viable count per ml or gram of product, with the count carried out in such a way as to ensure the viability of as wide a range of organisms as possible and in the arithmetical treatment of numbers an inbuilt bias for counting high.

In the early days of the programme no attempt was made by QC personnel to identify organisms, their role was purely that of quantification.

If high counts were met, R & D personnel would visit the plant and after investigation pinpoint the source of contamination. In this way everyone, QC, Production and R & D learned from errors and mistakes. A plant without problems teaches nothing but merely adds to a sense of false security. The author has previously outlined the basic techniques for total viable count (1), but several points bear mention yet again.

The aim of a viable count is to provide a series of conditions under which each viable microbial unit in a material will give rise to a discrete visible colony which can then be counted. It is easier in microbiology to determine sterility (under defined conditions) than it is to provide accurate assessment of counts between sterility and 100 organisms per g or ml. This is due to the fact that very few products could be plated directly without dilution and such dilution rapidly affected the reliability of the count. For example, with accept/reject limits at 100 a dilution of 1 in 10 will require a decision on counts of less than 10. This is an order of magnitude lower than the density usually accepted as optimal.

It was fortunate that right at the beginning two temperatures of incubation (24°C and 37°C) were used rather than one as in the US Pharmacopoeia (2) (32°C). Bacteria such as *Achromobacter* which do not grow above 30°C have been a major problem over the years. Likewise, it was ensured that organisms with different growth requirements were catered for by using two non-specific growth media, nutrient agar for bacteria and Sabouraud dextrose agar for fungi. With various products at one time or another, spread plates, pour plates, membrane filtration and most probable number methods have been used for viable count.

Where a product produced in many centres has differing standards imposed by various governments, as a matter of good quality assurance the highest standard required has been imposed as the minimum company

standard for that particular product. This has ensured greater flexibility in supplying different countries from one centre.

As a result of 4 years' experience there is good confidence that because of the MQC programme the products leaving the plants are pure and wholesome.

Organisms which have caused problems

A review of the records places the culprits in the following order of importance.

1. *Achromobacter*
2. *Pseudomonas.*
3. *Bacillus.*

It is surprising perhaps that the Enterobacteriaceae and Micrococcaceae do not figure in our list but the survival value of pathogens in plants such as *Escherichia coli* and *Staphylococcus aureus* is essentially nil and they have never been a cause of control breakdown. Without doubt in places as far apart as Liverpool, Madrid and Johannesburg the *Achromobacter* have been the major problem. Both *Achromobacter liquefaciens* and *A. guttatus* have been isolated from mains water, deionized water, filling lines and finished product. They have caused a major rethink in the provision of deionized water to points in the plant and the use of filtered and boiled water for rinsing procedures.

Pseudomonas aeruginosa and another *Pseudomonas* spp. have been responsible on two occasions for the phenomenon of adaption.

On these two occasions the organisms were metabolizing a detergent present in the two products and were totally resistant to the paraben preservation in one case and benzalkonium chloride in the other. One plant had to be closed for sterilization and in the other the filling equipment was scrapped and replaced. This latter example serves to illustrate the problems that can be met in the factory environment when *Pseudomonas aeruginosa* is present.

Early in 1972 an overseas plant reported contamination in an aqueous topical non-sterile pharmaceutical. Filled stock was contaminated with an organism growing at both 24°C and 37°C on Nutrient Agar. This organism was present between 10^5 and 10^7 ml^{-1} and produced an intense green pigment. It was rapidly established that bulk product and packaging material were both free from contamination and it was, therefore, the filling equipment

which was the most likely source of contamination. An immediate monitoring of the filling equipment, raw materials and water, personnel, packaging and bulk product was undertaken. Contamination was only found 'downstream' of a rotation pump feeding bulk into the stationary line. Flushing through the system with sterile water and counting at the filling nozzles showed that approximately 10^3 organisms ml^{-1} were being delivered.

The entire filling equipment was dismantled and replaced with stainless steel parts throughout and the old pump was scrapped. A daily disinfection procedure was drawn up and process workers instructed in its use. With this new equipment and standard operating procedure the plant has been free of all contamination for over 2 years. The analysis of plant water showed that contamination was minimal and did not contain *Ps aeruginosa* but there were *Ps* spp. present.

The *Ps aeruginosa* isolated produced intense green pigmentation on nutrient agar, the pigmented colonies showed fluorescence and the plates smelled of methylamine. Oxydase was positive, it grew on and in Centrimide agar and broth and was typed as Pyocine 36 B.

When inoculated from contaminated product it could utilize Triton WR1339 as its sole source of carbon. Triton WR1339 is the non-ionic detergent present in the topical product.

Prompt response to the initial finding, the quarantining of stock and immediate clean up procedures prevented a major production disaster.

Final rinsing procedures after disinfection but before filling are now carried out with boiled deionized water or deionized water filtered through a final bacterial filter at the point of use. The use of mains water or non-sterilized deionized plant water does allow inoculation of equipment with various waterborne bacteria. The level of inoculation is extremely low but where there has been a breakdown in routine production the frequency of contamination problems rises considerably. For example a cosmetic lotion had two bulk batches pooled to adjust viscosity and on filling was shown to be contaminated with *Achromobacter liquefaciens* which was present in the plant water. The *Achromobacter* has been found somewhat more resistant to the usual parben preservatives so now this organism is included routinely in challenge tests carried out by R & D. *Achromobacter* is defined as Gram negative motile or non-motile rods which do not form pigment and whose optimum growth temperature lies at 30°C or below. An exact name has only been put to two species but the vast majority of isolates are of these two species, *A. liquefaciens* and *A. guttatus*. *A. liquefaciens* liquifies gelatine, *A. guttatus* does not, and neither produce nitrites from nitrates.

The *Bacillus* group of organisms have been infrequent contaminants of products and when the occurrence has been investigated no reasonable explanation has been made of that occurrence. These organisms are only remarkable for the low level of contamination found but because of the contamination being in the spore phase this low level of contamination remains in the product due to the preservatives present having no sporocidal activity but merely preventing exsporulation and growth. All species isolated so far have been aerobic in spite of considerable expenditure of time in attempting the isolation of Clostridia from talcs and similar materials.

Other than *Ps aeruginosa* classical pathogens such as *E. coli*, *Salmonella* spp. or *Staphylococcus aureus* have not been isolated.

When required by regulation to certify products as being free from these organisms but in the absence of approved test methods the following test structures were utilized.

E. coli

An aliquot is taken from the product sample and is used to inoculate a primary enrichment broth (Digest or Glucose) which is incubated at 37°C for 2 days. This broth is then subcultured onto a selective media containing bile salt, lactose and indicator (MacConkey Agar) at 37°C for 24 h.

Any Gram negative lactose fermenting colonies are inoculated into an equivalent broth (MacConkey Broth) and incubated at 44°C for 24 h. The production of both acid and gas at this temperature is considered to be presumptive of *E. coli*.

Ps aeruginosa

An aliquot is taken from the product sample and is used to inoculate a primary selective/inhibitory broth (Digest with 0.02% Cetrimide) which is incubated at 37°C for 2 days.

This broth is subcultured onto an equivalent agar (Nutrient with 0.03% Cetrimide) at 37°C for 24 h. Any Gram negative colonies growing on this plate which smell of trimethylamine and display greenish pigment and/or fluorescence under a UV lamp are considered to be *Ps aeruginosa*.

Salmonella spp.

An aliquot is taken from the product sample and is used to inoculate a primary enrichment broth (Digest or Glucose) which is incubated at 37°C for 2 days. This broth is subcultured into a selective/inhibitory broth (Selenite or Tetrathionate) at 37°C for 24 h. This broth is then subcultured

onto an inhibitory/differential agar (Wilson and Blair bismuth sulphite agar) at 37°C for 48 h.

Any Gram negative colonies growing on this plate which are black or green, have a metallic sheen or a diffuse black sulphide deposit around the colony are presumed to be *Salmonella* spp. The most certain and rapid confirmatory test is to subculture suspect colonies onto Nutrient agar and test the colonies after 24 h growth with appropriate polyvalent antisera.

Staphylococcus aureus

An aliquot is taken from the product sample and is used to inoculate a primary enrichment broth (Digest or Glucose) which is incubated at 37°C for 2 days. This broth is subcultured onto an inhibitory/differential agar (Tellurite-egg or Vogel–Johnson) at 37°C for 48 h.

Any Gram positive colonies growing on this plate (Vogel–Johnson) which are small, black surrounded by yellow zones are presumed to be *Staph. aureus*. For confirmation, subculture suspect colonies into tubes of mammalian plasma (0.5 ml) and incubate at 37°C. Examine at 3 and 24 h. Coagulation constitutes a positive test.

Lessons learned from the microbiological quality control programme

It has been recognized that only sterile products are expected to be free from detectable organisms and it is, therefore, important to recognize that non-sterile products can and do have organisms present in any product. It is, therefore, necessary to draw up techniques of viable counting with full laboratory instructions to determine such contamination and to set limits on that contamination.

The microbiological quality of any raw material that is used in the manufacture of products should be known and determined both in the development laboratory and in the factory. It is important to draw up realistic specifications for raw materials and great assistance may be had from most suppliers in doing this.

It is obvious that during the manufacture of a non-sterile product, the process should be so designed, so that the microbial content does not increase.

The function of a preservative is to prevent proliferation of an accidental contamination, not to free production from rigid hygiene standards and

because of the importance of good preservation consideration is now being given to the introduction of QC tests to determine the components of the preservative system into the final product specifications.

Packaging components have been found to play an important role in the microbiological integrity of a product and in the past insufficient attention was shown to the microbiological aspect when packaging was chosen. It is necessary to stress that not only could the components of the packaging introduce undesirable organisms into formulations but also they can interact chemically and physically with formulation ingredients (including preservatives) and thus change the susceptibility of the final formulation to bacterial insult. The package is part of the product and must be present for proper formulation evaluations.

Finally, without doubt, the provision of pure wholesome plant water is the number one priority of a microbiological control programme. The majority of incidents can be traced to water as the culprit.

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