THE MECHANISM OF SELF-DISINFECTION OF THE HUMAN SKIN AND ITS APPENDAGES

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Introduction

In a previous investigation (Burtenshaw, 1938) into the disinfectant ability of the human skin I attempted to show correlation between the degree of acidity of the skin and of its power to kill homolytic streptococci. The present work throws more light on the mechanism of this self-disinfection of the skin.

First, I determined the effect of *p*H on the strains of haemolytic *Streptococcus* used. Then I tested the power of skin scrapings suspended in normal saline at various degrees of acidity to kill the *Streptococcus*. As a consequence of the work of Brann (1928) I compared the streptococcocidal power of ether and alcohol extracts with that of saline extracts of skin. I also compared the effect on the *Streptococcus* of extracts of hair, nails, and cerumen with that of extracts of skin, and investigated the influence of hair extracts on a number of common bacteria.

In order to compare the disinfectant properties of skin and hair with those of lysozyme, the substance found in various animal and vegetable tissues and fluids by Fleming (1922), I studied the diffusibility and the resistance to heating and to changes of *p*H of saline suspensions of skin scrapings and hair extracts, and tested the ability of egg albumen, a substance very rich in lysozyme, to kill streptococci.

I succeeded in identifying the chemical group to which the streptococcocidal constituents of hair fat belong, and, as a result, examined for streptococcocidal power several fatty acids present in human sweat and skin and in animal fats.

Some observations of Stevens (1936, 1937) suggested the use of reducing substances, such as cystein, in an attempt to inhibit the activity of ether extracts of hair, and of ultra-violet light to increase this activity. Similarly, the work of Hill & White (1933) and of Bayliss (1936) led me to study the inhibiting effect of blood on these extracts.

Technique

The bactericidal substance of skin was derived from the palms and forearms mainly of a single subject (J. M. L. B.), but also from those of four young males and of two corpses, by using the frame and cylinder previously described (Burtenshaw, 1938).

About 2 c.c. of normal (0·85 %) saline, ether or alcohol, were pipetted into the cylinder, and the submerged area of skin scraped with the end of a microscope slide until the fluid became opalescent from detached epithelium. The

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fluid was then removed with a Pasteur pipette, and the operation repeated until 6–8 c.c. of epithelium-containing fluid were obtained. Hair, cerumen and nails (derived from J. M. L. B.) were extracted for 24 hr. with saline, ether, or ether and methyl alcohol. Saline extracts or suspensions were directly used in the tests, but ether and alcohol extracts, after the addition of talc and an interval of a few hours with frequent shaking, were evaporated to dryness, and the residues resuspended in saline (test suspension) were used. Talc was used in the proportion of 200 mg. to 75 c.c. of subsequently added saline; its purpose was to adsorb the water-insoluble lipoids. Talc was added in the same proportion to saline extracts, and also to a litre of sterile normal saline used as a control suspension (control saline).

For most of the tests sterile test-tubes, $6 \times \frac{5}{8}$ in., were set up in a double-row 24-tube stand (test rack), and in a single-row 12-tube stand (dilution tube rack). A standard quantity, usually 2·5 c.c., of each of the test suspensions and of the control saline was pipetted into one or more tubes of the front row (suspension tubes) and into the corresponding tubes of the back row (*p*H tubes) of the double-row stand. About 5 drops of B.D.H. Universal Indicator were placed in each of the *p*H tubes, and the reaction of any one suspension tube adjusted by delivering into its corresponding *p*H tube the required number of drops of dilute HCl or NaOH, and then delivering the same number of drops into the suspension tube itself. Solutions of alkali and acid ranging between *N*/400 and *N*/10 were found to be suitable. At the end of an experiment the reaction of the suspension tubes was checked by adding indicator to them also. This method of varying the *p*H avoids the introduction of buffers or indicators into the test suspensions themselves. Into each suspension tube and *p*H tube was then pipetted 0·02–0·1 c.c. of a normal saline suspension of the test organism, at a density of 10–30 million streptococci per c.c. as measured by Burroughs and Wellcome opacity tubes (standard suspension). The test rack was kept in the incubator with frequent shakings for 45–150 min. Then 0·05–0·1 c.c. of the contents of each suspension tube was transferred to 5 c.c. of normal saline in the corresponding dilution tube, and 0·02–0·03 c.c. of saline from the dilution tube mixed in a Petri dish with 12 c.c. of agar and 0·5 c.c. of horse blood. The quantities transferred were varied according to the probable number of colonies which would appear in the plate. After incubation of the plates at 37° C. for 24 hr. the colonies were counted with the help of a Pake's disk over an illuminated counting box.

The strains of haemolytic *Streptococcus* were obtained from the following sources: (1) A from an acutely inflamed mastoid process; (2) B and C from scarlatinal throats; (3) S from the inflamed tonsils of a patient convalescent from diphtheria; (4) 3 from the fauces of a patient suffering from influenza; (5) H from an accidentally infected needle puncture on a blood-donor's arm. Their origin and their production of β-haemolysis and of a soluble haemolysin make it likely that they belonged to the group *Streptococcus pyogenes* (Topley & Wilson, 1936).

Experimental work

(1) *Effect of pH on haemolytic streptococci*

Avery & Cullen (1919) recorded that human strains of *Str. haemolyticus* growing in broth never produced a final acidity higher than *p*H 5·0; Dernby (1921) stated that the *Str. pneumoniae* grew in broth culture only within the *p*H range 8·3–7·0, and Eggerth (1926) found that exposure for 2 hr. in buffer solution at *p*H 5·5 killed *Str. pyogenes*.

As Marchionini (1928) observed that the *p*H of many parts of the skin surface varied between 3 and 5, and ascribed the disinfectant action of skin to its acidity, I carried out experiments to determine the effect of acidity on the strains of *Streptococcus* used in this investigation (Table 1).

It appears that in all these experiments there was progressive fall in the number of organisms recovered from normal saline as the *p*H decreased from 7·5 to 5·0; at a *p*H lower than 5·0 the fall in recovery rate was accelerated in nearly all the experiments.

(2) *Effect of extracts of skin and its appendages on haemolytic streptococci*

In a preliminary test 0·5 c.c. of a saline suspension of *Streptococcus* B, diluted to the standard density (10–30 million per c.c.), was mixed in a test-tube with about 5 c.c. of scrapings in normal saline from both palms of the subject A. F. In a second test-tube 0·5 c.c. of the streptococcal suspension was mixed with 5 c.c. of saline to serve as a control. After 3 min. and again after 35 min. had elapsed since the addition of organisms to the two tubes, 0·1 c.c. of the contents of each tube was embodied in a blood-agar plate. After 12 hr. incubation both control plates showed numerous colonies of the *Streptococcus*; of the plates containing suspension of palmar skin as well as organisms the 3 min. plate showed a few colonies, the 35 min. plate none at all.

Subsequent observations (Table 2), made according to the procedure already described under Technique on dead as well as on living skin, fully confirmed the result of the preliminary experiment.

Does this sterilizing power reside in the saline used to scrape off the skin particles, or in the skin particles themselves? In other words, is the disinfecting agent water-soluble? Exp. 29 showed that the skin scrapings were disinfectant even after washing, but that the suspending saline was inactive. This result was confirmed by the different technique of Exp. 30, where the skin scrapings kept their sterilizing power after preliminary washing of the hands with soap and water. Ether extracts of skin from the palms of five subjects were now compared for sterilizing power with the extracted skin particles resuspended in saline. The results of the experiments in Table 4 indicate that the disinfectant agent was ether-soluble, but that it may be absent in some subjects (Exp. 32).

Further tests were done comparing the disinfectant power of saline and ether extracts of palmar and forearm skin, of hair, of nails and of cerumen (Table 5). The saline extracts in this series of tests were almost completely inactive. This disagreement with the results of the experiments in Tables 2 and 3 is perhaps explicable by three supervening factors. First, all the experiments of Table 5, except Exp. 37, were carried out a year later than those of Tables 2 and 3 and in a different place. Secondly, different strains of *Streptococcus* were employed. Thirdly, the streptococcal suspensions used in the tests of Table 5 were about twice as dense as those used in the earlier tests. On the other hand, Table 5 shows that the ether extracts of hair, nails and cerumen were powerfully, that of palmar skin moderately, and that of forearm skin feebly lethal to the *Streptococcus*.

Finally, the results obtained with skin scrapings were confirmed by a different method, illustrated by the experiments of Table 6, whence it appears that preliminary scraping of a skin area with ether greatly reduced its ability to kill streptococci subsequently deposited on it. In four out of the six experiments preliminary scraping with normal saline increased the sterilizing power of a skin area; no explanation of this finding has been suggested. In Exps. 55 and 56 there has apparently been multiplication of organisms in the saline suspension over the test period; allowance has been made for this increase in calculating the figures for surviving organisms. The great disparity in Table 6 between the figures for O.R. from the skin and those for O.R. from 'Control' suggests that drying on the skin surface and the manipulations of recovery contributed to the lethal effect of the ether soluble substance.

(3) *Effect of ether extracts of hair, cerumen and nails on various organisms*

A few tests were done to determine the effect of ether extracts of skin appendages on strains of (1) *Str. viridans*; (2) *Str. pneumoniae*; (3) *Staphylococcus aureus*; (4) *Staph. epidermidis albus*; (5) *Corynebacterium diphtheriae*; (6) *Bacterium typhosum*; (7) *Bact. coli commune* (Table 7).

It appears from Exp. 57, and also from Exps. 46 and 47, that *Streptococcus viridans* was killed by ether extracts of hair, nails and cerumen. Ether extract of hair was bactericidal to *C. diphtheriae* (Exps. 63 and 64), and also to two later subcultures of a strain of *Staph. aureus* (Exps. 59 and 60), but not to an earlier one (Exp. 58). Hair extract almost sterilized the first of three successive subcultures of one strain of *Staph. epidermidis*, but the later two not at all, whilst a second strain was unaffected (Exps. 58–60). There was partial sterilization of an early subculture of a strain of *Str. pneumoniae*, but not of a later one (Exps. 61 and 62). Strains of *Bact. typhosum* and *Bact. coli* were uninfluenced (Exps. 61–64).

In short, the bactericidal effect of hair extract on *Str. haemolyticus* and *Str. viridans*, and on *C. diphtheriae* was pronounced and constant, on *Str. pneumoniae*, *Staph. aureus* and *Staph. epidermidis* inconstant, and on *Bact. typhosum* and *Bact. coli* absent.

(4) *Experiments bearing on the relationship of the sterilizing substance* (s.s.) *in skin and hair to lysozyme*

The significance of the following experiments will be discussed later.

(i) *Diffusibility of* s.s.

Enough of a saline suspension of *Streptococcus* 3 was incorporated in nutrient agar plates and agar plates containing various concentrations of horse blood to produce on incubation a just countable number of colonies. Holes about 5 mm. in diameter were punched out of the set agar and filled with saline suspensions of actively streptococcocidal ether extract of hair, and also with control substances, such as butter, vaseline, paraffin wax and olive oil. There was no sign of inhibition of growth in the colonies immediately round any of the implanted substances. The inference from this observation is that s.s. cannot diffuse in a watery medium.

(ii) *The effect of heating and cooling* s.s.

From Exps. 65–67 it appears that heating saline suspensions of skin above 75° C. reduces, and may abolish, their sterilizing power, if heating is done at an acid reaction as in Exp. 66, but the skin suspensions used in Exps. 66 and 67 were but feebly bactericidal without heating. In Exps. 68 and 69, where an active ether extract of hair was used, heating at 100° C. did not in the least reduce its sterilizing power. Saline suspensions containing s.s. were often left for weeks in the refrigerator, sometimes even frozen, without losing any of their streptococcocidal ability.

(iii) *The effect of egg albumen* (*lysozyme*) *solutions on haemolytic streptococci*

Exps. 70–73 show that saline solutions of egg albumen, both fresh and dried, in concentrations presumably very rich in lysozyme (Fleming, 1929), had no sterilizing effect on three strains of a haemolytic *Streptococcus* easily killed by s.s.

(5) *Identification of the sterilizing substance*

The fats in a sample of hair were split up by the following procedure:

(i) 1·055 g. hair of J. M. L. B. were extracted with 95 % alcohol at 65° C. under a reflux condenser. The filtered extract contained *fatty acids, hydroxy acids, fats, soaps, alcohols, sterols, sterol esters.*

(ii) The extract was treated with 1 % alcoholic solution of digitonin and the precipitate filtered off. The filtrate contained *fatty acids, hydroxy acids, fats, soaps, alcohols, sterol esters, excess digitonin* (α). The residue contained *sterol digitonide* (β).

(iii) The filtrate α was evaporated over a water-bath at 65° C. to volatilize most of the alcohol. After the addition of water α was extracted with ether. The ether extract from α contained *fatty acids, hydroxy acids, fats, higher alcohols, sterol esters* (γ). The residue of α contained *soaps, lower alcohols, excess digitonin* (δ).

(iv) The ether extract γ was shaken with dilute potash, which extracted *fatty acids, hydroxy acids* (ϵ) as soaps. The ether residue of γ contained *fats, higher alcohols, sterol esters* (η).

(v) The extract ϵ was acidified and extracted with ether, which removed the *fatty and hydroxy acids* (θ).

(vi) The residue δ was acidified and extracted with ether, which removed any *fatty acids or hydroxy acids* (ι) present as soaps in the original alcoholic extract.

Each of the following fractions, η, θ, ι, and A+E, an alcohol and ether extract of another 1·055 g. hair serving as a positive control, were mixed with 140 mg. talc in an evaporating dish and dried at 38° C. Each residue was then suspended in 53 c.c. of normal saline. Difficulty was experienced in recovering the sterols from digitonide (β), so a suspension of 45 mg. cholesterol (Analar) and 180 mg. talc in 75 c.c. saline was substituted (Chol.). The quantities of talc and saline were based on those found suitable in previous experiments with the total fats of hair. The weight of the residue of θ, fatty and hydroxy acids, was 30 mg. The saline suspensions, η, θ, ι, A+E, and Chol. were then tested for sterilizing ability by the method employed in previous experiments.

Exps. 75 and 76 demonstrate that the only fraction having streptococcocidal power was θ, which contained fatty and hydroxy acids. The slight activity of η at *p*H 5·0 in Exp. 75 was belied by the corresponding test in Exp. 76.

As it seemed possible that the unsaturated acids of θ might be the active moiety, bromination and iodization of θ were carried out as follows:

(1) *Bromination.* A fresh quantity of θ was prepared by removing the fatty acids from an ether and acetone extract of hair with weak potash, and 7·5 mg. in 50 c.c. ether divided into two equal portions, to one of which 4 c.c. of bromine were added. This brominated portion was kept at 37° C. with frequent shaking for 2 hr., and the excess bromine then removed with $Na_2S_2O_3$. To serve as a control 25 c.c. of ether were similarly treated. The three portions, untreated θ, brominated θ and bromine control (B.C.) were each mixed with 75 mg. talc, evaporated and suspended in 32 c.c. saline. This procedure afforded no proof either of any unsaturated acids in θ or of the bromination of these acids.

(2) *Iodization.* The iodine number of 40·5 mg. θ in ether was estimated by Wij's method, and found to be 49. After iodization θ weighed 59·5 mg.; thus 19 mg. iodine had been taken up. This procedure proved the presence of unsaturated acids in θ, and determined, by a volumetric and a confirmatory gravimetric estimation, the quantity of combined iodine. Then 22 mg. untreated θ in ether, 22 mg. iodized θ in ether, and an iodine control (I.C.), similar to the bromine control above, were each mixed with 120 mg. talc, evaporated, and suspended in 50 c.c. saline. The remaining fats (*RL*), amounting to 33 mg., in the hair, which yielded θ for the foregoing saturation experiments, were suspended with 120 mg. talc in 50 c.c. saline, and compared for sterilizing power with saturated and unsaturated θ.

From Exps. 77 and 78 it appears that bromination and iodization both diminish the sterilizing activity of θ, but only slightly. It may be argued that the bromo- and iodo-acids are almost as streptococcocidal as the unsaturated acids, and that the activity of these latter would be more effectively reduced by saturation with hydrogen. Work described later (§ 6) on the sterilizing power of known fatty acids helps to remove this ambiguity.

An unexpected finding was the sterilizing power of *RL*. Since this could be caused by the liberation of further acids through hydrolysis of the extremely dilute esters, an ether extract of 250 c.c. of saline suspension of *RL* was shaken with weak alkali and the fatty acids from any removed soaps liberated by acidification and extraction with ether. Fatty acids amounting to 34 mg. were recovered and were suspended with 341 mg. talc in 142 c.c. saline (*RL* θ). The remaining fats in the ether extract of *RL* were divided into two portions: (1) amounting to 14·3 mg., which was evaporated with 41 mg. talc and suspended in 17 c.c. saline (*RL*2); (2) amounting to 42·9 mg., which was split by saponification at 80° C. with 10 % KOH into (*a*) fatty acids (19·6 mg.) and (*b*) residual fats (42·0 mg.). Fraction (*a*) was mixed with 197 mg. talc and suspended in 82 c.c. saline (*RL*2 θ); fraction (*b*) was mixed with 98·0 mg. talc and suspended in 50 c.c. saline (*RL*3).

It can be inferred from Exps. 79 and 80 that *RL* became active owing to release by hydrolysis of free acids. In Exp. 80 *RL*2 was becoming active, presumably through further hydrolysis. This presumption was verified by separation with weak potash into fatty acids (*RL*2 θ^2) and residual fats (*RL*3^2). Exp. 81 illustrates the relative activities of these fractions. *RL*3, the unsaponifiable residue of *RL*2, remained inactive.

(6) *The sterilizing power of certain organic acids*

A number of fatty and other acids occurring in the skin and in the secretions of skin glands, such as the sweat and milk, were tested for streptococcocidal activity. The organic acids present and their amounts in sweat and butter, as given by Peck, Rosenfeld, Leifer & Bierman (1939), and Davies (1939) respectively, are the following:

(*a*) Composition of sweat (Acids mainly free)

	mg. %
Acetic	9·6
Propionic	6·2
Caproic—caprylic	9·6–37·7
Lactic	100·0
Citric	10·0
Ascorbic	4·0
Sodium chloride	700·0
Urea	Trace
Uric acid	Trace
Water	99,020·0

(*b*) Acids in butter (Obtained from glycerides)

	%
Butyric	3·4
Caproic	1·8
Caprylic	0·9
Capric	1·9
Lauric	3·1
Myristic	9·7
Palmitic	27·6
Stearic	12·2
Oleic	34·3
Linoleic	4·4
Unidentified	0·7

The acids used in the tests of Table 10 all figure in one or both of the above analyses. The fatty acids up to caprylic are inert, whilst the acids from capric

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upwards and their soaps are actively streptococcocidal, as also are the total butter acids. The hydroxy acids, lactic and citric, and the hexose derivative, ascorbic acid, are inactive. Incidentally, the ratio of the activity of the saturated stearic to that of its unsaturated homologue, oleic acid, is comparable with the ratio of the activity of the iodized acids to that of the untreated acids in Exp. 78. This finding disposes of the objection raised in § 5 to saturating with halogens, which probably reduce the activity of unsaturated acids in the same way as hydrogen, by abolishing an unsatisfied linkage.

(7) *Effects of cystein, blood, aeration and* U.V.L. *on the sterilizing action of* S.S. *and of fatty acids*

Cystein, added as the water-soluble hydrochloride to extracts of skin, hair, etc., and to certain fatty acids, inhibited their bactericidal power. It only exerted this inhibition on the acid side of *p*H 7·0; therefore, after the addition of the strongly acid hydrochloride, the extract-containing saline was left for some time before any further adjustment of the reaction and addition of test organisms. Presumably the diminishing activity of cystein towards *p*H 7·0 was due to its increasing precipitation as the water-insoluble base.

Table 11 gives examples of the inhibiting action of cystein, and also of blood, on S.S. and a number of fatty acids. The inhibition of S.S. by cystein was often marked but seldom complete; the only fatty acid showing considerable inhibition was stearic, though lauric was slightly and inconstantly inhibited. Blood was more effective; it inhibited θ and all the fatty acids more or less. Exps. 93, 94, 100 and 101 illustrate the effect of *p*H on the inhibition exercised by cystein on stearic acid. Ultra-violet light failed to enhance the activity of ether extract of hair (Exp. 102); but, if this activity had been reduced by cystein, it was restored by drying in air with or without accompanying ultra-violet radiation (Exp. 103). Ether extract of cerumen, normally in the dark and poorly aerated, was rendered more active by drying in air, though this increased activity was partly annulled by accompanying ultra-violet radiation (Exp. 104).

DISCUSSION OF RESULTS

It emerges from the foregoing work that there are present in the skin, its appendages and its secretions, lipoids[1] extremely lethal to *Str. pyogenes* and *viridans* and to certain other organisms. Fatty acids are the active constituents of hair fat, and it may be assumed that these substances endow the other skin fats with disinfectant power. That these acids have long chains is supported by their low volatility, high ether-water repartition ratio, and the proved streptococcocidal power of commonly occurring long chain acids and soaps, contrasted with the lack of this power among the short chain acids and soaps.

[1] I use 'lipoid' generically to include fats, their derivatives (e.g. lecithin), and their components (e.g. fatty acids, cholesterol).

The demonstration in Table 10 of the susceptibility of the *Streptococcus* to oleic, stearic, lauric, and capric acids and their soaps, and of its resistance to the lower fatty acids and soaps, harmonizes with the findings of many workers, notably Lamar (1911), Nichols (1920), Walker (1924), Eggerth (1926), Bayliss (1936), and of Belin & Ripert (1937).

Reichenbach (1908), Walker (1924) and Bayliss (1936) showed that members of the group *Bacterium* are almost unaffected by the unsaturated soaps, but are susceptible to 1 % concentrations of the saturated soaps. Walker & Bayliss found *Staph. aureus* very resistant to all soaps, but Hettche (1934) records destruction of this coccus by a number of unsaturated acids. Walker (1925) noted the extreme sensitiveness of *C. diphtheriae* to oleates and other unsaturated soaps.

The results of the workers mentioned on the action of soaps on members of the group *Bacterium*, on *Staph. aureus*, and on *C. diphtheriae* conform with my observations of the effect of hair extracts on these organisms (Table 7).

Certain fungi possess quite different susceptibilities to various fatty and hydroxy acids. Thus, Peck *et al.* (1939) found that 0·009 % caproic, 0·03 % propionic and caprylic, 0·2 % lactic, and 0·3 % citric and ascorbic acids sufficed to kill *Trichophyton gypseum*. Longer chain fatty acids were not tested.

What, then, are the acids and acid-yielding lipoids present in the skin, its appendages and secretions?

Sweat contains, besides lactic acid at a concentration of about 100 mg. % (McSwiney, 1934; Whitehouse, 1935), and traces of citric (Leake, 1923) and ascorbic acids (Wright & MacLenathen, 1939), small amounts of the fatty acids up to caprylic, perhaps derived from decomposed sebum (Schwenkerbecher, 1929).

There has been much controversy about the secretion of lipoids by the ekkrine sweat glands. Unna (1894, 1898, 1928) found droplets staining with osmic acid in the alveolar cells of sweat glands, and concluded that they were oleic acid. He also argued that, as fat was detectable in the sweat of the palm and sole and there are no sebaceous glands in these regions, the fat must be secreted in the sweat. It was pointed out, however, by Rothman (1929) that droplets staining with osmic acid are not necessarily oleic acid, that such droplets are never seen in the duct lumen of sweat glands, and that the presence of fat in the absence of sebaceous glands on the palms and soles does not prove that fat is excreted in sweat, since fat is contained in the horny layer of the skin. On the other hand, the apocrine sweat glands of the axilla and perinaeum undoubtedly secrete fat (Schiefferdecker, 1922).

Sebum, according to Linser (1904), is composed of both long chain and short chain fatty acids and their esters, of about 1 % cholesterol, and of 40–50 % unsaponifiable remnant, called by Röhmann (1905) 'Dermocerin' and found by Ameseder (1907) to consist largely of eikosyl alcohol ($C_{20}H_{41}OH$). Sebum also contains some soaps (Ziemssen, 1883). The ceruminous glands, though homologous with the sweat glands (Quain, 1912; Testut, 1922), produce a secretion

resembling sebum (Linser, 1904; Unna & Golodetz, 1909; Schwenkerbecher, 1929).

Compared with the secretion of the skin glands the fat of the epidermis and nails is rich in cholesterol and its esters, which amount in these structures to 16 % of the lipoids (Unna & Golodetz, 1909). From the appearances after staining with osmic acid Unna (1928) inferred that oleic acid and its esters are 'die eigene Fett der Hornschicht'. By staining methods Koga (1934) demonstrated the presence of fatty acids and their soaps and esters in the horny skin, whilst Partridge (1938) concluded that the epidermis contains numerous highly unsaturated hydrocarbons. Eckstein & Wile (1926) found that about 2·5 % of epidermal fat is phospholipoid. Much of the fat normally present on and in the superficial layers of the skin is derived from sebum and perhaps from sweat (Schwenkerbecher, 1929; Cerutti, 1934).

Mark reported that 4 % of hair is lipoid, of which only 3 % is embodied in the hair; the rest is absorbed sebum (Cerutti, 1934). According to Eckstein (1926), of the 4·5 % total fat in rat's hair, 11·9 % consists of cholesterol and 0·86 % of lecithin.

In brief, fat from the palmar skin and nails, where there are no sebaceous glands, contains oleic and other unsaturated acids with their esters and soaps, and traces of the short chain fatty acids; whereas fat from skin supplied with sebaceous glands, from hair, and from cerumen contains numerous long and short chain fatty acids with their esters and soaps derived from, or characteristic of, sebum.

A partial analysis of an ether extract of human hair used in the present investigation yielded the following figures:

Total fat in hair	5·86 %
Unsaponifiable fraction	36·1 % of total fat
Water-insoluble fatty acids	63·9 % of total fat
Total acid number	201·9
Iodine value of fatty acids	49·0
Cholesterol	4·6 % of total fat
Phospholipoid	1·5 % of total fat

These figures, compared with those given by Unna & Golodetz (1909) for various skin fats, suggest that the fat in ether extract of hair is a mixture of sebum and sweat lipoids together with a small contribution of epidermal cholesterol. I have discovered in the literature no estimate of the phospholipoid content of the skin secretions; on the assumption that they contain no phospholipoids, the amount found by me, using the method of Hawk (1938), in hair fat is presumably derived, like part of the cholesterol, from the scalp. The iodine value is in approximate agreement with Linser's for sebum fat, 36–44 (Schwenkerbecher, 1929); it indicates that more than half the acids are unsaturated, if oleic (iodine value = 90) is the chief unsaturated acid present.

The facts given justify the conclusion that the various suspensions and extracts of skin, hair, cerumen, etc. used in the present work were bactericidal owing to their content of oleic and other long chain fatty acids and of their soaps. Other constituents, to wit, the esters of these acids, sterols, higher alcohols (Table 9), and acids such as lactic, citric, and ascorbic, and their sodium salts (Table 10) were inactive. Such substances as lecithin, and less strongly cholesterol, were probably even inhibitory to fatty acids and soaps (Eggerth, 1927), but phospholipoids are very labile (Kooyman, 1932), readily yielding highly bactericidal fatty acids.

In nearly all the experiments of the present work the sterilizing substance of skin, hair, etc., the acids of hair fat, and the individual fatty acids, with the exception of stearic, were far more bactericidal at an acid than at a more alkaline reaction. This increase of sterilizing activity with diminishing *p*H is not entirely ascribable to the toxicity of H ion; thus the effect of acidity and of s.s. (skin scrapings, extracts of hair and skin, fatty acids, etc.), acting together is greater than the sum of the effects of both factors acting separately, since the figures for O.R. in most of the above tests reveal the following relationship:

$$(1)\ \frac{\text{O.R. of s.s. tube } p\text{H } 7{\cdot}5\text{–}7{\cdot}0}{\text{O.R. of s.s. tube } p\text{H } 5{\cdot}5\text{–}4{\cdot}0} > (2)\ \frac{\text{O.R. control tube } p\text{H } 7{\cdot}5\text{–}7{\cdot}0}{\text{O.R. control tube } p\text{H } 5{\cdot}5\text{–}4{\cdot}0},$$

where the denominator of (1) is lower than would be expected from simple summation of the effects of acidity and s.s. The fact that the disinfectant power of skin increases with rise of acidity has led Marchionini (1928) and myself (1938) to surmise that H ion alone is the sterilizing agent on the skin surface, whereas the experiments above described suggest that increase of H ion acts not only directly, but also by releasing fatty acids from their soaps.

Eggerth (1926), who proved that the fatty acids are nearly always more lethal than their soaps to *Str. pyogenes*, *Staph. aureus*, *C. diphtheriae*, *Vibrio cholerae*, and *Bact. typhosum*, advanced four reasons for the greater effectiveness of the acids: (1) The acid reaction may sensitize the bacterium to the soap or fatty acid, (2) Decrease of *p*H in soap solutions lowers the surface tension, which leads to greater concentration of the soap or acid at the water-bacterium interface, (3) Coulter (1924) discovered that, if the acidity of a suspension of erythrocytes is raised, the inside of the cells does not become as acid as the outside fluid. If the same is true of bacteria, it is likely that rising acidity in a soap solution would drive the increasingly insoluble soap into the more alkaline bacterial protoplasm, (4) The fatty acid molecule, being less dissociated than the soap molecule, may penetrate more easily into the bacterium (Osterhout, 1925).

That a number of organic acids are more lethal than their salts to certain fungi was shown by Peck *et al.* (1939); on the other hand, Lamar (1911) asserted that soaps are more effective than their acids in killing the pneumococcus.

What is the mechanism of the lethal action of the fatty acids? It has long

been known that the disinfectant power of many hydrocarbons and their ability to reduce surface tension increase with the length of their carbon chain. This has been demonstrated for the alcohols by Wirgin (1904), Traube (1919), Cowles (1938), and Kokko (1939), for the acids by Lamar (1911) and Reid (1932), and for the soaps by Berczeller (1917), Walker (1924), and Stock & Francis (1940).

Yet lowering of surface tension is not alone responsible for the lethal action of these substances; their water solubility and molecular constitution and the kind of organism acted upon also play an important part. As a rule unsaturated are more active than saturated acids of comparable length of chain against *Str. pneumoniae* and *pyogenes* and *C. diphtheriae*; and often this increased activity runs parallel with greater ability to lower surface tension than that of comparable saturated acids. Lamar (1911) pointed out that the long chain unsaturated acids, oleic ($C_{18}H_{34}O_2$), linoleic ($C_{18}H_{32}O_2$) and linolenic ($C_{18}H_{30}O_2$), and their soaps with iodine values of 90·07, 181·42, and 243·2 respectively fall in that order of increasing toxicity to the pneumococcus, and that the soaps being more soluble are more toxic than the acids. Of all the acids and soaps tested potassium linolenate showed the greatest toxicity correlative with its high iodine value, its long chain, and its great water solubility. Crotonic acid, however, ($C_4H_6O_2$), though it has an iodine value of 295·28, has a short chain and is but slightly toxic; also chaulmoogric acid with an iodine value of 90·3, an isomer of linoleic, is partly of ring structure and is less toxic than oleic. Walker (1924, 1925, 1926) found that *Str. pneumoniae* and *pyogenes*, *C. diphtheriae*, *Neisseria meningitidis* and *gonorrhoeae* are extremely susceptible to the oleates, linoleates, and linolenates, but the saturated laurates, perhaps because of their power of reducing surface tension, are as effective as the linoleates. Bayliss (1936) observed that 1·0 % sodium stearate, 0·1 % palmitate, 0·01 % myristate, 0·04 % laurate, 0·004 % oleate, and 0·005 % linoleate kill *Str. pneumoniae* in 15 min., but 0·03 % α-elaeostearate and 0·4 % β-elaeostearate, isomers of linolenate, are needed to kill it in the same time. Eggerth (1929.*a*, *b*) found that α-hydroxyl or α-bromine increases the effectiveness of the saturated acids, but that α-hydroxyl decreases the effectiveness of the unsaturated acids, a finding confirmed by Bayliss (1936). Stock & Francis (1940), working with the influenza virus, noted that it is inactivated most strongly by the unsaturated acids with 18 carbon atoms in their chain, oleic, linoleic, and linolenic. They emphasized that intensity of disinfection does not always vary with degree of unsaturation. Thus, chaulmoogric, undecylenic, pyromucic, and β-elaeostearic are almost inactive, whilst lauric and less markedly myristic acids are active. Ability to lower surface tension is very commonly, but not invariably, correlated with virocidal power. For example, undecylenic, ricinolic, chaulmoogric and palmitic acids powerfully depress surface tension, but have little effect on the virus.

In contrast with the streptococci, with certain members of the genus

Neisseria, with *C. diphtheriae*, and with the influenzal virus, intestinal bacilli of the genus *Bacterium* are far more susceptible to the saturated than to the unsaturated acids and soaps (Reichenbach, 1908; Walker, 1924, 1926; Bayliss, 1936), though they withstand all these substances far better than the group sensitive to unsaturated acids. According to Reichenbach 1 % sodium palmitate acting for 50 min. kills *Bact. coli*, whilst only 10 % oleate suffices to kill in the same period. Walker obtained similar results with the typhoid and dysentery bacilli.

Staph. aureus is very resistant to most of the long chain acids and soaps. Bayliss (1936) found 1 % laurate, abietate, and undecylenate slowly effective, and Hettche (1934) recorded partial sterilization with 0·1 % oleate and complete sterilization with 0·1 % linoleate and linolenate in 60 min., but none of the soaps used by Walker (1924) affected the *Staphylococcus*.

Many workers, including Wren (1927), Harris, Bunker & Milas (1932), Sears & Black (1934), Stevens (1935, 1936), have reported the formation in vegetable and mineral oils on exposure to oxygen and ultra-violet light of volatile peroxides disinfectant to a number of organisms, especially the haemolytic streptococcus. Also Stevens (1937) discovered that the lipoids of guinea-pig skin absorb oxygen in the dark, but more rapidly on ultra-violet light irradiation, and that their lethal action on streptococci is proportional to the oxygen absorbed. Stevens supported the view that this sterilization is due to peroxides by showing that it is annulled by cystein. Stock & Francis (1940) inquired whether the ability of unsaturated fatty acids to form peroxides (Holm, Greenbank & Dreysher, 1927) is related to their sterilizing power. They observed that 1 % H_2O_2 kills the influenza virus, whilst 0·1 % is inadequate, a finding which agrees with mine (Exp. 74, Table 8) on subjecting the haemolytic *Streptococcus* to H_2O_2. They failed to detect peroxides in their fatty acid solutions, as I failed to detect them, using the benzidine reaction and the Kerr-Kreis test (Bolton, 1928), either in fatty acid solutions or in suspensions of hair fat. Moreover, boiling of these suspensions did not impair their activity, which therefore could hardly have been due to volatile peroxides (Table 8). In any case the concentration of peroxide lethal to virus and streptococcus could not arise from the amounts of fatty acid sufficient to kill these organisms (23–65 mg. %).

I have confirmed the inhibition by cystein of the streptococcocidal power of fats on ether extracts of skin, hair, etc. and on one or two fatty acids, and in a few experiments I have noted that exposure to air with or without ultra-violet light irradiation increases or restores the disinfectant properties of cerumen or cystein-inhibited hair fat (Table 11); but, as these substances could not be shown to form peroxides and as the activity of the saturated stearic acid was more strongly suppressed than that of any other acid tested, I cannot conclude that cystein inhibits solely by reducing peroxides. Again, I have shown that blood, an oxidizing agent, is more efficient than cystein in diminishing the streptococcocidal power of fatty acids (Table 11), whilst Bayliss (1936) has

noted the diminution by blood, and Noguchi (1907), Lamar (1911), and Walker (1924) the diminution by serum of the disinfectant property of soaps.

According to du Nouy (1922) the surface tension of water solutions of serum, egg albumen, gelatin, etc., lowered by the addition of oleates, spontaneously returns to normal, a recovery which he attributes to the adsorption of the oleate upon the substrate molecules. Possibly cystein, as well as blood or serum, inhibits the bactericidal power of lipoids by interference with their lowering of surface tension, though cystein may sometimes act by reducing peroxides.

From reviews such as those of Rideal (1923, 1930) and Harris & Bunker (1931) the action of long chain alcohols, fatty acids, and soaps on bacteria in a watery medium may be pictured as follows: The molecules of these substances owing to their water-insoluble carbon chains collect at the water-bacterium interface, where their OH, NH_2 and COOH groups protrude into the water and their fat-soluble carbon chain is adsorbed to the partly lipoid envelope of the bacterial cell. By intercalating themselves between the surface molecules they effect a marked difference between the surface tension of the outside and inside of the cell envelope, which undergoes 'peptization', i.e. disruption. Short of thus destroying the cell active groups of the lipoid, such as OH and COOH, combine with active groups of the cell surface and impede its chemical exchanges, whilst peroxides may form around unsaturated linkages in the lipoid or around such groups as —CHO and =CO and disorganize the protein metabolism.

It is curious that, as appears from Table 11, no other long chain molecules in skin fat besides the fatty acids, for example, esters and alcohols, evince streptococcocidal power. The following facts may account for this inactivity. The active groups of the component parts of the esters are largely neutralized by internal combination, and the alcohols of skin fat are, according to Ameseder (1907), mainly of the type of eikosyl alcohol ($C_{20}H_{41}OH$), which has a very long chain and but a single hydroxyl group. Therefore both esters and alcohols must be nearly insoluble in water and chemically almost inert. It is significant that, according to Eggerth (1926) in a homologous series of saturated soaps, the sterilizing power for various organisms reaches its peak in the member containing 12 or 14 carbon atoms and falls away rapidly in the higher members. A similar peak may well exist in an alcoholic series.

Is there any relation between the skin fatty acids and lysozyme, the substance discovered by Fleming in animal and plant tissues and found by him to be lytic and lethal to many bacteria? From the work of Fleming (1922, 1929, 1932) and of Wolff (1927*a*) the properties of lysozyme may be summarized as follows: It occurs in nearly all the body fluids except sweat, urine, and cerebro-spinal fluid, but is most concentrated in tears. Of the tissues cartilage yields the most potent extract in normal saline, skin a weak extract, brain the weakest, though Wolff has shown that lysozyme is abundant, but almost inseparably fixed, in brain lipoids. Hen's egg white is the richest source

known, being twice as lytic as human tears. The test organism most used by Fleming owing to its extreme sensitivity to lysozyme is a Gram-positive coccus from nasal secretion, called by him *Micrococcus lysodeikticus*, but other organisms are in varying degrees sensitive. Wolff (1927*b*), testing some common pathogens, found that these could be arranged in the following groups of descending sensitivity to lysozyme: (1) *N. gonorrhoeae*; (2) *N. meningitidis*, *Str. haemolyticus*, *Staph. aureus*, *Mycobacterium tuberculosis*; (3) *Bact. typhosum*. A group comprising *Bact. coli*, *C. diphtheriae*, *Str. pneumoniae* and *viridans* was quite insensitive. Lysozyme is inhibited, but not destroyed, by minute additions of alkali or acid. Its strength is reduced to a quarter by heating at 75° C. for 30 min., and destroyed by boiling for the same time. It is soluble in water and normal saline, but is precipitated by alcohol, ether or acetone.

The long chain fatty acids, on the other hand, abound in and are easily recovered from brain tissue and skin, and it is improbable that there is more than a trace in such substances as tears and cartilage. They are extremely lethal to *Str. viridans* and *pneumoniae*, as well as to *Str. haemolyticus*, and also to *C. diphtheriae*, but are variable in their effect on *Staph. aureus*. Moreover, a 1/20 saline solution of egg albumen is harmless to a strain of *Streptococcus* killed by very small amounts of hair fatty acids (Table 8*b*). The fatty acids are as a rule more active in acid than in neutral or alkaline solution, where they are mostly converted to soaps. They are stable at 100° C. and over, and are easily dissolved by the fat solvents, but are nearly insoluble in water. The soaps, however, though soluble in alcohol, are insoluble in ether and form true or colloidal solutions in water. Streptococcocidal hair fats, which the foregoing experiments prove to be active through their fatty acid content, diffuse hardly at all through agar media. Some of these properties are illustrated in § 4 and Table 8.

From these almost diametrically opposed qualities it seems unlikely that lysozyme and the higher fatty acids are nearly related. Indeed, recent research, such as that of Roberts (1937), of Abraham & Robinson (1937), and of Epstein & Chain (1940), indicates that lysozyme is a protein of low molecular weight with the properties of a polysaccharolytic enzyme. The acids of hair and skin fat have more in common with the alcohol-soluble, heat-stable bactericidin described by Conradi (1902), and are probably the sterilizing constituent of the ether-soluble substance extracted by Brann (1928) from hair and skin.

It is questionable whether Fleming in his investigation was always dealing with the same lysin. Tears and egg-albumen were the sources of lysozyme chiefly used in determining its properties, and it was assumed that the same substance was responsible for the lytic activity of skin, hair and nails. In the light of the present work the activity of these epidermal structures may more probably be ascribed to fatty acids and soaps.

The generalization may be hazarded that two groups of unspecific bactericidal substances co-exist in the animal body. One, to which lysozyme belongs, operates in the neutral environment of the internal organs and in gland

secretions, such as mucus and tears. The other, represented by the fatty acids and soaps, operates not only internally, but also on the skin, where the reaction, normally acid in most areas, and the absence of inhibiting proteins intensify their activity.

Finally, this opinion of Noguchi (1907) is worth quoting: 'Die im Blute und in der Lymphe enthaltenen Seifen- und Fettsaüremengen bilden die Grundlage für die Meinung, dass ein gewisser Teil der Schutzkraft des Organismus diesen Seifensubstanzen zuzuschreiben ist.'

SUMMARY AND CONCLUSIONS

1. Four β-haemolytic strains of *Streptococcus*, suspended in normal saline, showed progressive increase in mortality, as the *p*H was lowered from 7·5 to 5·0; below *p*H 5·0 the mortality was greatly accentuated.

2. Ether and alcohol extracts of human skin and its appendages were powerfully, saline extracts more weakly and inconstantly, lethal to the haemolytic *Streptococcus* and to certain other organisms.

3. The long chain fatty acids and soaps are the chief, if not the only, bactericidal constituents of skin and its appendages.

4. Experiments are described illustrating the streptococcocidal effect of various acids occurring in animal fats.

5. Cystein and blood inhibit the streptococcocidal activity of skin fats and certain fatty acids. Ultra-violet light may increase this activity, or abolish the inhibition exercised by cystein.

6. The mechanism of the sterilizing power of fatty acids and soaps, the influence exerted by cystein, blood, and ultra-violet light on the sterilizing power of skin fats and fatty acids, and the relationship of skin fatty acids to lysozyme are discussed.

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Table 1. *The influence of hydrogen ion on strains of the haemolytic* Streptococcus

No.	Date	*p*H ...	7·5	7·0	6·5	6·0	5·5	5·0	4·5	4·0	E.T. min.	Organism
1	10. i. 39	O.R.	—	700	700	628	652	391	—	271	30	*Strept.* A
2	11. i. 39	,,	—	688	670	597	550	470	—	140	90	,,
3	23. ii. 39	,,	4036	—	3528	—	3200	—	2092	—	45	,,
4	10. iii. 39	,,	2576	—	—	2240	—	420	—	54	112	,,
5	7. ii. 39	,,	—	480	460	455	320	240	—	200	170	*Strept.* B
6	28. ii. 39	,,	—	1320	—	1180	—	872	380	—	145	,,
7	7. iii. 39	,,	3320	—	—	2976	—	150	—	72	107	,,
8	20. iii. 39	,,	3296	—	—	2912	2544	—	246	—	135	,,
9	16. vii. 40	,,	1250	—	840	—	952	—	95	—	50	*Strept.* S
10	22. ix. 40	,,	1352	—	—	1280	—	1224	—	4	45	,,
11	28. x. 40	,,	640	530	540	492	—	—	20	7	50	,,
12	22. xii. 40	,,	1544	1256	1136	—	—	832	—	1	50	,,
13	28. x. 40	,,	1100	1004	—	—	—	556	—	65	50	*Strept.* 3
14	14. xii. 40	,,	6056	—	—	4176	—	3952	2080	—	50	,,
15	22. xii. 40	,,	3640	—	—	4400	3272	—	1792	—	50	,,
16	1. i. 41	,,	—	—	—	2848	1680	608	—	320	55	,,

Notes. The strains of *Streptococcus*, prepared from 24 hr. cultures on blood agar, were suspended in normal saline, adjusted to the required *p*H, at a concentration of about 10–30 millions per c.c. The suspensions were all fine, except that used in Exp. 5, which was very coarse. Twenty-two experiments were done in all. The organisms incorporated in a plate constituted a 1/1250th part of the organisms in the corresponding suspension tube. The various numbers of organisms recovered at a given *p*H are mainly due to differences of initial concentration, of exposure time, and of strain of organism.

O.R. = organisms recovered, i.e. colonies counted in plate.

E.T. = exposure time, i.e. the interval between addition of organisms to saline and incorporation of an aliquot part in blood agar.

The numbers in the *p*H columns show the colonies counted in blood agar plates.

Table 2. *The effect of skin scrapings from (a) living and (b) dead subjects on the haemolytic* Streptococcus

No.	Date		(*a*) S.C.		(*a*) L.S.		E.T. min.	Comment
17	10. ii. 39	*p*H	7·5	4·0	7·0	4·0		*Strept.* A used in somewhat granular suspension. Scrapings initially at *p*H 4·0
		O.R.	960	464	383	8	175	
18	13. ii. 39	*p*H	7·0	4·5	7·0	4·0		*Strept.* B used in smooth suspension
		O.R.	1804	1554	1690	684	17	
19	14. ii. 39	*p*H	7·0	4·5	7·0	4·0		*Strept.* B used in smooth suspension. Scrapings initially at *p*H 5·0
		O.R.	1700	1135	824	28	90	
20	19. ii. 39	*p*H	6·5	4·0	6·5	3·8		*Strept.* B used in smooth suspension
		O.R.	210	155	120	21	67	
21	21. ii. 39	*p*H	7·0	4·0	7·0	4·0		*Strept.* B used in very granular suspension
		O.R.	140	70	95	40	50	
22	23. ii. 39	*p*H	7·5	4·0	7·5	4·0		*Strept.* A used in smooth suspension
		O.R.	4036	2092	2092	784	45	
23	28. ii. 39	*p*H	7·0	4·5	7·0	4·5		*Strept.* B used in smooth suspension
		O.R.	1224	160	880	6	145	
24	2. iii. 39	*p*H	7·5	4·5	7·5	4·5		*Strept.* B used in smooth suspension. Scrapings initially at *p*H 6·0. *Hands washed before scraping*
		O.R.	880	576	600	34	80	
25	7. iii. 39	*p*H	7·5	5·0	7·5	5·0		*Strept.* B used in smooth suspension. Scrapings initially at *p*H 4·5
		O.R.	3340	1500	1544	58	107	
26	10. iii. 39	*p*H	7·5	5·0	7·5	5·0		*Strept.* A used in smooth suspension. Scrapings initially at *p*H 5·0
		O.R.	1962	950	712	10	112	

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Table 2 (*continued*)

(*b*)

	Date		S.C.			L.S.			D.S.			E.T. min.	Comment
27	20. iii. 39	*p*H	7·5	5·5	4·5	7·5	5·5	4·5	7·5	5·5	4·5		*Strept.* B
		O.R.	3112	2320	900	1950	57	21	1864	720	130	135	
28	15. iv. 39	*p*H	7·0	—	4·0	7·0	5·5	4·5	7·0	5·5	4·5		*Strept.* A
		O.R.	2856	—	1300	486	26	14	2816	252	9	135	

Notes. Live skin was obtained from the palms of J. M. L. B.; dead skin in Exp. 27 from the palms of a woman, 45 years old, dead about 12 hr.; in Exp. 28 from the palms of an old man, dead about 6 hr.

S.C. = saline control. L.S. = skin from live subject. D.S. = skin from dead subject.

Table 3. *The insolubility of the skin sterilizing substance in water*

			S.C.		Sup.S.		Scr. + S.		E.T. min.
29	5. v. 39	*p*H	7·0	4·5	7·0	4·5	7·0	4·5	
		O.R.	2504	2432	4512	2800	380	56	130

Notes. Scrapings from palms of J. M. L. B. were centrifuged free of supernatant, washed twice with normal saline, and finally added to fresh saline equal in volume to the original supernatant. To the original supernatant was added talc in an amount corresponding to that in the control saline. *Strept.* A used in smooth suspension.

Sup.S. = original supernatant saline.
Scr. + S. = washed scrapings added to fresh saline.

			S.C.		Scrapings (1)		Scrapings (2)		E.T. min.
30	29. iv. 39	*p*H	7·0	4·5	7·0	4·5	7·0	4·5	
		O.R.	2500	548	988	73	1500	60	55

Notes. Scrapings (1) were obtained from two areas, one on each palm, of the unwashed hands of J. M. L. B. The hands were then thoroughly washed and rinsed, and scrapings (2) were obtained from two fresh areas, one on each palm. *Strept.* B used in smooth suspension.

Table 4. *The solubility in ether of the skin sterilizing substance*

No.	Date		S.C.		Scrapings		Extract		E.T. min.	Comment
31	5. vi. 39	*p*H	7·0	4·5	7·0	4·5	7·0	4·5		Two areas on left palm of J. M. L. B. scraped. *Strept.* A used. Scrapings originally at *p*H 5
		O.R.	2480	1216	2160	1960	91	5	67	
32	6. vi. 39	*p*H	7·0	4·5	7·5	4·5	7·0	4·5		One area on each palm of R. S. scraped *Strept.* A used. *Extract and scrapings inactive*
		O.R.	2350	1160	2304	1464	2200	1360	70	
33	7. vi. 39	*p*H	7·5	5·0	7·0	4·5	7·0	4·5		Two areas on left palm of J. M. L. B. scraped. *Strept.* A used. Scrapings originally at *p*H 4·5
		O.R.	1912	1080	1544	410	143	1	75	
34	8. vi. 39	*p*H	7·0	4·5	7·5	5·0	7·0	4·5		One area on each palm of S. L. scraped. *Strept.* A used. Scrapings originally at *p*H 6·0
		O.R.	2050	980	2610	940	160	2	80	
35	30. vii. 39	*p*H	7·0	5·0	7·0	4·5	7·0	5·0		One area on each palm of A. N. H. scraped. *Strept.* C used
		O.R.	3940	1860	4768	3030	6	2	77	
36	31. vii. 39	*p*H	7·0	5·5	7·0	5·5	7·0	5·5		One area on each palm of P. J. scraped. *Strept.* C used
		O.R.	3560	2040	4835	4520	33	10	72	

Notes. The skin was scraped under ether, and after 24 hr. extraction of the scrapings the ether was decanted and evaporated in contact with talc. The residue of fats and talc was suspended in saline (extract). The ether-extracted scrapings were washed with ether, dried, and suspended in saline (scrapings).

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Table 5. *The comparative effects of saline and ether extracts of (a) skin and hair, (b) nails, and (c) cerumen*

(a)

No.	Date		S.C.		E.P.		S.P.		E.F.		S.F.		E.H.		S.H.		E.T. min.	Comment
37	12. viii. 39	*p*H	7·0	5·5	—	—	—	—	7·0	4·5	7·0	4·5	7·0	4·5	7·0	4·5		*Strept.* C used
		O.R.	2088	1344	—	—	—	—	110	89	2640	1724	4	3	2392	1072	75	
38	24. vi. 40	*p*H	8·0	5·0	7·5	5·0	7·0	4·5	—	—	—	—	7·0	5·0	—	—		Mixture of *Strept.* 3
		O.R.	2144	1240	450	60	3230	360	—	—	—	—	2·4	0·5	—	—	50	and S used
39	19. vii. 40	*p*H	7·5	4·5	7·5	5·0	7·0	5·5	—	—	—	—	7·0	5·0	—	—		*Strept.* 3 used
		O.R.	2348	1544	1424	225	3800	2572	—	—	—	—	35·7	7·7	—	—	45	
40	29. vii. 40	*p*H	8·0	5·0	—	—	—	—	8·0	5·0	7·0	5·0	6·5	4·5	7·0	5·0		*Strept.* 3 used
		O.R.	550	286	—	—	—	—	392	220	450	410	6·0	0·2	980	522	60	
41	18. viii. 40	*p*H	7·5	5·0	8·0	5·0	8·0	8·0	—	—	—	—	7·5	5·0	—	—		*Strept.* S used. Palms
		O.R.	2200	1184	2140	43	1600	1420	—	—	—	—	83·5	15·4	—	—	45	extremely sweaty
42	20. viii. 40	*p*H	7·5	5·5	8·0	5·0	8·0	5·5	—	—	—	—	7·5	4·5	8·0	5·0		*Strept.* 3 used
		O.R.	1152	938	626	16	980	720	—	—	—	—	27·2	5·8	1290	340	50	
43	23. x. 40	*p*H	7·5	4·5	7·5	5·0	7·0	5·3	7·5	5·0	7·5	5·0	—	—	—	—		Dense susp. *Strept.* 3
		O.R.	12000	8300	9900	3300	9610	9040	11300	9400	12500	9020	—	—	—	—	45	used. E.P. *p*H 5 colonies small
44	15. xi. 40	*p*H	8·0	5·0	8·0	4·5	8·0	5·0	7·0	5·0	7·5	5·0	7·5	4·5	8·0	5·0		*Strept.* S used in very
		O.R.	200	96	64	1	112	56	162	6	118	95	3·2	0·6	420	380	50	dilute suspension

Notes. The skin was scraped off into ether (E.) or into saline (S.) from the palms (P.) or forearms (F.). Two equal portions of hair of J. M. L. B. unwashed for about 21 days were extracted, the one in ether (E.H.), the other in saline (S.H.) for 48 hr. Extracts were made up as described under Technique. The recording of a fraction in the O.R. column indicates that a quantity of organismal suspension above the standard for the experiment was incorporated into the plate from the Dilution tube, and the figure for O.R. had to be correspondingly reduced.

(b)

No.	Date		S.C.		E.N.		S.N.		E.T. min.	Comment
45	1. viii. 40	*p*H	7·5	5·0	7·5	5·0	7·0	5·5		*Strept.* 3 used in granular suspension
		O.R.	418	75	91	10	482	345	60	
46	4. viii. 40	*p*H	7·5	4·5	8·0	5·0	8·0	5·7		A very coarse suspension of *Strept.* S (O.R. 1), mixed with a strain of *Strept. viridans*
		O.R. 1	240	50	57	0	216	102		(O.R. 2), was used
		O.R. 2	250	65	2	1	580	550	55	

Notes. Two equal portions of mixed finger- and toe-nail parings of J M. L. B. were extracted, the one with ether (E.N.), the other with normal saline (S.N.) for 48 hr. The extracts were made up as in Technique.

(c)

No.	Date		S.C.		E.Cer.		S.Cer.		E.T. min.	Comment
47	5. viii. 40	*p*H	7·7	5·0	7·0	5·0	7·0	5·5		A smooth suspension of *Strept.* 3 (O.R. 1), mixed with a strain of *Strept. viridans* (O.R. 2),
		O.R. 1	21	12	0	0	340	382		was used
		O.R. 2	503	448	96	12	904	932	50	
48	9. viii. 40	*p*H	—	5·5	—	5·5	—	5·7		*Strept.* 3 used
		O.R.	—	602	—	174	—	896	45	
49	14. viii. 40	*p*H	—	5·0	—	5·0	—	5·0		*Strept.* S used
		O.R.	—	710	—	406	—	800	55	
50	22. viii. 40	*p*H	—	5·0	—	5·5	—	5·5		*Strept.* 3, considerably contaminated with a *Staphylococcus*, was used
		O.R.	—	210	—	26	—	728	45	

Notes. Cerumen from the ears of J. M. L. B. was extracted for 24 hr. with normal saline (S.Cer.), then for 24 hr. with ether and alcohol (E.Cer.). The extracts were made up as in Technique.

Table 6. *The comparative streptococcocidal power of untreated, of saline-washed, and of ether-washed areas of palmar skin*

No.	Date		Unwashed	Ether	Saline	Control	Position on palm
51	22. vi. 40	O.R.	5,800 (3)	34,560 (1)	9,040 (2)	192,000	*E*
		E.T.	13 min.	12 min.	14 min.		*U* *S*
52	19. x. 40	O.R.	11,105 (3)	30,400 (2)	27,940 (1)	12,000,000	*E*
		E.T.	17 min.	17 min.	14 min.		*U* *S*
53	25. x. 40	O.R.	22,800 (1)*	48,360 (3)	4,800 (2)	4,096,000	*U*
		E.T.	12 min.	14 min.	12 min.		*E* *S*
54	1. xi. 40	O.R.	8,904 (1)	12,000 (3)	216 (2)	760,000	*E*
		E.T.	12 min.	14 min.	12 min.		*U* *S*
55	26. xi. 40	O.R.	34,560 (3)	633,600 (1)	22,560 (2)	(*a*) 5,696,000	*E*
		E.T.	11 min.	12 min.	10 min.	(*b*) 6,080,000	*U* *S*
56	2. i. 41	O.R.	216,000 (2)	660,000 (3)	146,400 (1)	(*a*) 3,360,000	*U*
		E.T.	16 min.	17 min.	15 min.	(*b*) 5,152,000	*S* *E*

Notes. With the cylinder and frame (Burtenshaw, 1938) an area (*S*) on the left palm was scraped three times under 2 c.c. saline, a second area (*E*) was scraped three times under 2 c.c. ether, and a third area (*U*) was left untreated. On to each of these areas in turn was pipetted and spread 0·15 c.c. of standard streptococcal suspension. After a definite time (E.T.) the organisms on each area were scraped off by means of the frame and cylinder into three portions of 2 c.c. saline. 0·1 c.c. of each 6 c.c. scrapings in saline was incorporated in a blood-agar plate. As a control 0·15 c.c. of the standard suspension was added to 4 c.c. saline, 0·1 c.c. of this dilution was added to 10 c.c. saline, and 0·1 c.c. of this second dilution was incorporated in a blood-agar plate. This was done at the end of every experiment, and also at the beginning of Exps. 55 and 56 (controls (*a*) and (*b*)). In the 'Position on palm' column the letters indicate the relative positions on an observer's left palm of the tested areas. The figures in brackets indicate the order of testing the areas. J. M. L. B. was the subject, and *Strept.* 3 was used throughout.

* This test was done on the corresponding area of the right palm.

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Table 7. *The effect of ether extract of hair on various organisms*

No.	Date		S.C.		H.E. 1		H.E. 2		E.T. min.	Comment
57	19. vi. 40	*p*H	8·0	4·0	7·0	5·0	8·0	5·0		A strain of *Strept. viridans*
		O.R.	836	148	35	9	180	32	55	from throat swab was used

No.	Date		*Staph. aureus*				*Staph. epiderm* (1)				*Staph. epiderm* (2)				E.T. min.	Comment
			S.C.		H.E. 3		S.C.		H.E. 3		S.C.		H.E. 3			
58	24. ix. 40	*p*H	7·5	5·0	7·0	5·0	7·5	4·3	7·0	5·0	—		—			The strain of *Staph. aureus*
		O.R.	4096	3970	4140	3950	6128	2584	7	4	—		—		50	was obtained from a boil
59	29. ix. 40	*p*H	—	4·5	—	5·9	7·5	5·5	7·0	5·0	—		—			The two strains of *Staph.*
		O.R.	—	1132	—	5	7520	7360	6575	7680	—		—		55	*epidermidis albus* were iso-
60	2. x. 40	*p*H	7·5	4·0	7·0	4·5	7·5	4·5	7·5	4·3	5·0		5·0			lated from the right palm
		O.R.	1340	1280	10	7	3785	2800	3380	2200	5632		7040		50	and fingers respectively of J. M. L. B.

No.	Date		*Str. pneumoniae*				*Str. haemolyticus*				*Bact. typhosum*				E.T. min.	Comment
			S.C.		H.E. 3		S.C.		H.E. 3		S.C.		H.E. 3			
61	12. x. 40	*p*H	7·5	4·0	7·0	4·5	7·5	4·5	7·5	5·0	7·5	5·0	7·0	5·0		The strain of pneumococcus
		O.R.	9600	8830	1635	1350	4800	110	4·6	0·7	4800	5280	5840	5504	50	(type II) was grown from
62	14. x. 40	*p*H	7·5	5·0	7·0	5·0	7·0	5·2	7·0	5·2	7·0	4·7	7·5	5·0		the sputum of a pneumonic
		O.R.	3220	2950	3140	2720	2100	2020	141·3	2·4	1540	1530	1645	1570	50	patient. The strain of *Bact. typhosum* came from a typhoid stool. The haemolytic streptococcus was *Strept.* 3

No.	Date		*C. diphtheriae*				*Bact. coli*				E.T. min.	Comment
			S.C.		H.E. 3		S.C.		H.E. 3			
63	26. ix. 40	*p*H	7·5	5·0	7·0	5·8	7·5	4·0	6·0	4·5		The strain of *C. diphtheriae*
		O.R.	20	15	0	0	4480	4680	4100	4350	50	was obtained from the
64	28. ix. 40	*p*H	7·0	5·0	7·0	5·0	—	4·3	—	5·0		fauces of a diphtheritic
		O.R.	3250	2784	928	70	—	4590	—	6745	60	patient. The strain of *Bact. coli* was obtained from a normal stool

Notes. H.E. 1, 2, 3 were saline suspensions of three different ether extracts of the hair of J. M. L. B. The method as for *Strept.* tests.

Table 8

(*a*) *The effect of heating the S.S. of skin and hair*

No.	Date		H*p*H	S.C.	20°	60°	75°	100°	H.T. min.	E.T. min.	Comment
65	April 1939	*p*H		5·0	5·0	5·0	5·0	5·0			Scrapings from the palms of J. M. L. B. were used as the source of S.S. The figures of Exp. 65 represent the averaged results of eight tests, those of Exp. 66 the averaged results of five tests. *Strept.* A and C were used
		O.R.	6·0	1074	29	22	31	130	42	60	
66	May–Aug. 1939	*p*H		5·0	5·0	5·0	5·0	5·0			
		O.R.	7·0	1033	438	558	211	504			
			4·5	463	463	646	316	1204	45	65	
67	22. iv. 39	*p*H		5·0	5·0	5·0	4·5	5·0			Scrapings from the palms of the corpse of a man yielded the S.S. *Strept.* A was used
		O.R.	6·5	1904	1132	336	508	1364	60	65	
68	17. vii. 40	*p*H		4·5	5·2	5·2	5·2	4·5			Another extract of hair from J. M. L. B. yielded the S.S. in Exps. 68 and 69. *Strept.* 3 was used
		O.R.	5·0	1400	23·5	13·0	5·3	7·5	35	45	
69	22. vii. 40	*p*H		4·0	4·8	5·5	4·8	5·0			
		O.R.	5·0	105	0·3	1·5	0·4	0·3	50	35	

Notes. A pair of test-tubes, each containing 2·5 c.c. of the saline suspension of S.S. was placed in each of three water baths, at 60, 75, and 100° C. respectively, for a definite time (H.T.) and at a definite *p*H (H*p*H). To one of each pair of tubes was added indicator, and the reaction was adjusted and maintained as described in Technique. After heating each pair formed the Suspension Tube and *p*H Tube in the test. The tubes for 'S.C.' and '20° C.' were left on the bench for the time that the others were left in the water baths. The further procedure was that given in Technique.

(*b*) *The effect of egg-albumen (lysozyme) solutions on the haemolytic* Streptococcus

No.	Date		S.C.		S.S. control			Albumen (1/500)			E.T. min.
70	11. v. 39	*p*H	7·0	5·5	8·0	6·0	4·5	7·0	6·0	4·5	
		O.R.	1020	770	1040	480	16	1580	1268	905	63
71	27. v. 39	*p*H	8·0	5·0	—	—	—	8·0	7·0	4·5	
		O.R.	1720	1465	—	—	—	2040	1940	1680	77

No.	Date		S.C.	Albumen concentrations 1/20	1/200	1/2000	1/20000	E.T. min.
72	14. iii. 41	*p*H	7·0	8·0	7·5	6·5	—	
		O.R.	1776	1840	1984	1816	—	50
73	15. iii. 41	*p*H	6·5	8·0	7·0	6·5	6·5	
		O.R.	2328	2500	2374	2400	2720	60

Notes. B.D.H. dried egg albumen dissolved in normal saline at a concentration of 1/500 was used in Exps. 70 and 71. Fresh egg albumen was used in Exps. 72 and 73. The S.S. control in Exp. 70 was done with palm scrapings of J. M. L. B. *Strept.* A was the test organism in Exps. 70 and 71, *Strept.* S in Exp. 72, and *Strept.* 3 in Exp. 73.

(*c*) *The effect of hydrogen peroxide solutions on the haemolytic* Streptococcus

No.	Date		S.C.	Concentrations of H_2O_2 solutions 1·0 %	0·1 %	0·01 %	0·001 %	0·0001 %	E.T. min.
74	24. vii. 40	*p*H	4·8	5·5	5·5	5·5	5·5	5·5	
		O.R.	1060	0	380	732	996	1020	35

Notes. H_2O_2 dilutions were prepared from hydrogen peroxide solution (B.D.H.) containing 10 vol. %. *Strept.* 3 was the test organism.

Table 9. *The streptococcocidal activity of components of hair fat*

No.	Date		S.C.		A.+E.		η		θ		ι		Chol.	E.T. min.
75	2. ix. 40	*p*H	7·5	4·8	7·5	5·5	7·5	5·0	7·5	5·0	7·5	4·8	5·5	
		O.R.	3314	2532	13	0	2760	816	15	1	2524	2380	3640	55
76	4. ix. 40	*p*H	7·0	4·5	7·0	5·0	7·0	5·0	7·5	5·0	7·5	4·8	5·5	
		O.R.	2232	600	98	2·2	2720	2440	0	0	3816	2860	1620	45

No.	Date		S.C.	θ	θ brom.	B.C.	E.T. min.
77	16. x. 40	*p*H	6·0	6·0	5·8	5·8	
		O.R.	4500	1·4	3·0	4200	45

No.	Date		S.C.		θ		θ iod.		I.C.	RL	E.T. min.
78	10. xi. 40	*p*H	7·5	4·3	6·5	4·3	6·5	4·3	7·5	7·0	
		O.R.	2672	1232	1·0	0·3	8·5	3·0	2480	42	50

No.	Date		S.C.	θ	RL	RL θ		RL2	RL2 θ	RL3	E.T. min.
79	27. i. 41	*p*H	6·0	5·8	5·5	7·0	5·5	7·0	5·8	6·0	
		O.R.	1520	0·6	10	1·5	2·0	1840	2·2	1150	65
80	30. i. 41	*p*H	7·5	5·5	5·8	7·5	5·8	5·5	5·5	5·2	
		O.R.	3512	0	0	0·2	0·5	30	1·0	2160	60

No.	Date		S.C.	RL θ	RL2 θ^2	RL3[2]	E.T. min.
81	12. ii. 41	*p*H	6·0	5·8	5·8	6·8	
		O.R.	2900	0·2	16·0	2720	58

Notes. The significance of the symbols heading the columns is explained in the text (§ 5). *Strept.* 3 was the test organism in Exps. 75, 77, 78, 79, and 81; *Strept.* S in Exps. 76 and 80.

Table 10. *The streptococcocidal power of a number of fatty and hydroxy acids*

No.	Date		S.C.	θ	Propionic		Butyric		Caproic		Caprylic		Capric		Lauric		Oleic	Stearic
82	6. iv. 41	*p*H	5·5	6·0	7·8	5·5	7·5	6·0	7·5	5·3	7·5	5·3	7·0	5·0	7·0	5·2	—	—
		O.R.	4112	20·0	4040	4170	3544	4620	4312	4320	4910	4520	1900	150	6	48	—	—
83	9. iv. 41	*p*H	5·0	6·0	7·5	5·0	7·5	4·8	7·5	5·0	8·0	5·0	5·8	4·3	7·0	4·5	5·5	5·5
		O.R.	900	8·0	2800	2064	2832	1600	3050	1720	2160	1760	312	4	0·5	4·0	0·5	14

No.	Date		S.C.		Caprylic		Capric		Lauric		Oleic		Stearic		B.A.	Lactic		Citric	Ascorbic
84	29. iv. 41	*p*H	8·0	5·3	7·5	5·0	7·5	4·5	7·0	5·3	7·5	5·5	7·5	5·0	5·5	7·5	5·5	5·0	5·5
		O.R.	6330	3440	4800	3240	1600	42	2·0	2·5	10·5	6·0	33	56	70	5800	4200	3900	4350

Notes. To 100 c.c. normal saline and 270 mg. talc enough of each acid (except of lactic) to form a 0·0028 *M* solution was added, volumetrically in the case of the fatty acids, of which lauric and stearic had to be melted, gravimetrically in the case of ascorbic and citric acids. This molar concentration was chosen because it gives a range of mass concentrations (20·6–79·5 mg. %) comparable to that of θ (23–44 mg. %) and of the total butter acids (64·9 mg. %). Lactic acid was used at a strength of 1/50 (2000 mg. %). The rest of the technique was that of previous tests. The test organisms were: *Strept.* 3 in Exp. 82, *Strept.* S in Exp. 83. *Strept.* H in Exp. 84. B.A. = butter acids.

Table 11

(a) The inhibition by cystein and blood of the streptococcocidal power of hair, cerumen, nails, skin, and hair θ of J.M.L.B.

No.	Date		S.C.		E.H.		E.H. Cyst.		E.T.	Organism used
85	12. vi. 40	*p*H	7·5	5·0	7·0	4·5	7·0	4·5		*Strept.* 3
		O.R.	662	330	3	0	320	20	60 min.	
86	19. vi. 40	*p*H	8·0	4·5	7·0	5·0	7·5	4·5		*Strept. viridans*
		O.R.	836	148	35	9	1251	446	50 min.	
			S.C.		E. Cer.		E. Cer. Cyst.			
87	5. viii. 40	*p*H	7·7	5·0	7·0	5·0	8·5	7·5		*Strept. viridans*
		O.R.	21	12	0	0	42	11	45 min.	
88	6. viii. 40	*p*H	8·0	5·0	7·0	5·0	8·0	7·0		*Strept. viridans*
		O.R.	500	450	96	12	260	420	50 min.	
			S.C.		E.N.		E.N. Cyst.			
89	4. viii. 40	*p*H	7·5	4·5	8·0	5·0	7·0	4·5		*Strept.* S
		O.R.	240	10	57	0	120	9	55 min.	
90	5. viii. 40	*p*H	7·5	4·5	8·0	5·0	7·0	5·0		*Strept. viridans*
		O.R.	250	20	2	1	264	18	60 min.	
			S.C.		E. Palm.		E. Palm. Cyst.			
91	20. viii. 40	*p*H	7·5	5·0	8·0	5·0	8·0			*Strept.* 3
		O.R.	1152	650	626	16	1300		45 min.	
92	15. xi. 40	*p*H	8·0	5·0	7·5	5·0	7·5			*Strept.* S
		O.R.	200	96	54	3	171		50 min.	
			S.C.		θ		θ Cyst.			
93	28. ii. 41	*p*H	6·0		5·5		5·5			*Strept.* S
		O.R.	950		0		(1) 50 (2) 312		60 min.	
94	14. iii. 41	*p*H	7·5		6·0		5·5			*Strept.* 3
		O.R.	2328		56·6		(1) 1560 (2) 2320		60 min.	

No.	Date		S.C.		θ		θ Cyst.		Blood	E.T.	Organism used
95	10. iv. 41	*p*H	7·5	4·5	6·5	4·5	8·0	4·5	7·0 (approx.)		*Strept.* 3
		O.R.	2672	1232	1·0	0·3	2368	50	2235	50 min.	
96	2. v. 41	*p*H	5·0		5·0		4·5		7·0 (approx.)		*Strept* S
		O.R.	450		23·3		180		425	55 min.	

(b) The inhibition by cystein of the streptococcocidal power of butter fatty acids

No.	Date		S.C.	B.A.	B.A. Cyst.	E.T.	Organism used
97	3. xi. 40	*p*H	6·0	6·0	5·5		*Strept.* S
		O.R.	520	40·6	700	50 min.	

Table 11 (*continued*)

(c) *The inhibition by cystein and blood of the streptococcocidal power of various fatty acids*

No.	Date		S.C.		Capric		+Cyst.	+Bl.	Lauric		+Cyst.	+Bl.	Oleic		+Cyst.	+Bl.	Stearic		+Cyst.	+Bl.	Organism used and E.T.
98	29. iv. 41	*p*H	8·0	5·0	7·5	4·5	5·0	—	7·0	5·0	5·0	—	7·5	5·5	6·0	—	7·5	5·0	5·5	—	*Strept.* H, 75 min.
		O.R.	6330	3440	1600	42	142	—	2·0	2·5	13	—	10·5	6·0	17	—	33	56	960	—	
99	5. vi. 41	*p*H	7·5	4·5	7·5	4·5	5·0	—	8·0	5·5	6·0	—	7·5	5·5	5·5	6·5	7·5	5·5	6·0	—	*Strept.* H, 50 min.
		O.R.	3280	2600	980	0	2	—	287	0	0	—	1·1	0·1	4	34	21·0	50·4	2264	—	
100	10. vi. 41	*p*H	8·0	4·5	6·5	4·5	5·0	6·5	7·0	5·0	6·0	6·5	—	—	—	—	6·0		6·0	6·5	*Strept.* H, 65 min.
		O.R.	1060	960	188	0	0	40	1·5	0	0	120	—	—	—	—	40·0	(1)	700	530	
																		(2)	130		
																		(3)	58		
																		(4)	41		
101	19. vi. 41	*p*H	8·0	5·0	7·5	5·0	5·0	6·5	7·5	5·0	6·0	6·5	—	—	—	—	5·5		6·0	7·0	*Strept.* 3, 75 min.
		O.R.	2424	1350	1040	35	1	1560	0·7	0·1	8	1450	—	—	—	—	114·0	(1)	924	4376	
																		(2)	2352		
																		(3)	1880		
																		(4)	128		

Notes. For testing the effect of cystein to 5 c.c. of the saline suspension of ether extract or fatty acid was added 15 mg. cystein HCl (B.D.H.), i.e. 0·3 %. The reaction of the suspension was either left unaltered (about *p*H 2·0) or alkalinized to the *p*H range 4·0–7·0 by adding drops of 1 *N*–1/50 *N* soda with a drop of B.D.H. Universal Indicator.

After the cystein had acted for 1–2 hr. 2·5 c.c. of the suspension was placed in each of two test-tubes. To one of these (*p*H Tube) was added 5 drops of Universal Indicator, the reaction noted, and, if necessary, altered (by adding dilute alkali), together with that of its fellow (Suspension Tube), to the *p*H chosen for exposure of the organisms.

For testing the effect of blood 1 drop of horse blood was added to 2·5 c.c. of suspension, i.e. in a concentration of about 1 %. The remaining procedure was as in previous experiments. In Exp. 93 cystein acted at (1) *p*H 6·0, (2) *p*H 4·0; in Exp. 94 at (1) *p*H 5·5, (2) *p*H 4·5; in Exp. 100 at (1) *p*H 2·0, (2) *p*H 5·0, (3) *p*H 6·0, (4) *p*H 7·5; in Exp. 101 at (1) *p*H 2·0, (2) *p*H 4·0, (3) *p*H 5·5, (4) *p*H 7·5.

E. Cer. Cyst. = saline suspension of ether cerumen extract treated with cystein.

Lauric + Bl. = saline suspension (or solution) of lauric acid with addition of blood, etc.

(d) *The effect of* U.V.L. *on S.S. and of* U.V.L. *and exposure to air on cystein-inhibited S.S.*

No.	Date		S.C.		E.H.		E.H. (7.5)		E.H. (15)		E.H. (33)		E.H. (62)		E.T.	Organism used
102	vii. 40	*p*H	7·5	4·5	7·5	5·0	7·5	5·0	7·5	5·0	7·5	5·0	7·5	5·0		*Strept.* 3
		O.R.	1546	834	48·6	7·2	77·1	5·0	82·4	4·7	93·7	8·7	93·4	8·1	45 min.	

No.	Date		S.C.	E.H.	E.H. Cyst.	E.H. Cyst. (aer.)	E.H. Cyst. (30)	E.T.	Organism used
103	viii. 40	*p*H	5·5	5·5	6·0	5·5	5·5		*Strept.* 3
		O.R.	850	24	975	16	1	45 min.	

No.	Date		S.C.	E. Cer.	E. Cer. Cyst.	E. Cer. (aer.)	E. Cer. (30)	E.T.	Organism used
104	viii. 40	*p*H	5·0	5·5	5·5	5·5	5·5		*Strept.* S
		O.R.	210	26	110	9	20	45 min.	

Notes. Exp. 102 (average of four tests). To ascertain the effect of U.V.L. on S.S. the fatty residue of an ether extract of hair of J.M.L.B. was exposed in an evaporating dish to an unscreened mercury-vapour lamp for varying periods (indicated as minutes by the figures in brackets). The residue was then suspended with talc in saline.

Exp. 103 (one of three tests). To ascertain the effect of aeration and U.V.L. on cystein-inhibited S.S. two portions of a cystein-treated saline suspension of hair ether extract were alkalinized, dried at 40° C. on a water-bath, and left in the dark for 3 days. One residue was resuspended in distilled water (aer.); the other was exposed to U.V.L. for 30 min., then resuspended in distilled water (30).

Exp. 104 (one of two tests). To ascertain the effect of aeration and U.V.L. on S.S. from cerumen two portions of a saline suspension of ether extract were dried on a water-bath at 40° C. One residue was resuspended in distilled water; the other was exposed to U.V.L. for 30 min., then resuspended in distilled water.

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