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SOME OBSERVATIONS ON SURVIVAL OF PATHOGENIC BACTERIA ON COTTON-WOOL SWABS

DEVELOPMENT OF A NEW TYPE OF SWAB

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This inquiry was prompted by our doubts on the reliability of culturing swabs when examination had been delayed by sending the specimens through the post or by depositing them after laboratory hours. Our laboratory is often obliged to carry out such delayed examinations, but similar delays must be fairly common in hospital and public health bacteriology. Apart from a few isolated statements, little attention has been given to the question of survival of the common pathogenic organisms on cotton-wool swabs.

The earliest account describing the use of cotton-wool swabs in clinical pathology appears to have been given by Councilman (1893), who recommended their employment in the bacteriological diagnosis of diphtheria. About this time Continental and English workers were using a variety of bizarre methods. For example, in France, Roux and Yersin (1890) described the use of a platinum wire flattened at the end for sampling from diphtheritic membranes. Goldscheider (1893), in Germany, used a platinum scoop; Hamilton (1895), working in London, suggested a goat's-hair brush; Hardman (1895) preferred to collect the discharges on a white paper which could then be dried before a fire and sent to the laboratory by post. Preston (1896) and Hewlett and Nolan (1896) were among the first English bacteriologists to recommend the use of cotton-wool swabs for the isolation of diphtheria bacilli. By 1900 swabs were in common use (Kolle and Wassermann, 1903).

Although 50 years have now elapsed the bacteriologist can still give the clinician or the field investigator little information about the survival time of common pathogens on these simple appliances. It is generally agreed that the bacteriological examination should be done as soon as possible after swabbing; but this vague recommendation, however conservatively it might be interpreted, cannot always be met in practice. Van Reimsdijk (1924) studied the survival of diphtheria bacilli on swabs and found that by inserting the swab in a serum-agar gel contained in the holder tube the survival of these organisms was assured for 24 hours at room temperature. This method was cumbersome and has not been adopted. Brahdy *et al.* (1934) suggested dipping swabs in serum and incubating for four hours before culture and direct smear. Parish (1935) made a similar recommendation, but both these investigators were interested in developing a rapid method of diagnosis of diphtheria and not in extending the survival time of the organism on the swab.

Downie (1940) investigated various methods of prolonging survival of meningococci on nasopharyngeal swabs, and found that these organisms could be cultured 24 hours after swabbing, provided the swab was placed in contact with a blood-agar medium contained in the swab tube. Hynes (1942) stated that rectal swabs carrying intestinal pathogens should be cultured very soon after collection, or, if not, the swab should be kept moist by immersion in saline agar. Mitchell *et al.* (1946) described an elaborate method of prolonging survival of haemolytic streptococci, impossible for routine use, and Saint-Martin (1949) stated that gonococci would survive 4 to 18 hours on swabs if the latter were thrust into Hirschberg's medium immediately after swabbing. More recently, Aycock and Mueller (1950) recommended immersion of the swab in sterile horse blood for recovery of meningococci on delayed plating. Finally, none of the current textbooks in bacteriology or clinical pathology give any indication of the time limits for delayed culture of swabs.

It is evident from this survey of literature that this important problem requires further elucidation so that the results of delayed culture from swabs can be interpreted with some degree of certainty.

Laboratory Results

Two rather slight observations served as a starting-point of the present study. First it was noted that the survival time of *Str. pyogenes* on swabs from infected wounds was far greater than on throat swabs carrying the same organisms. The second observation was that 24-hour-old throat swabs mounted on wire were often discoloured by solution of the iron in the presence of saliva. The lines of investigation which followed soon showed that the former was a more fruitful approach to the problem of survival than the latter, which, however, could be easily overcome by discarding wire applicators for wood.

The experimental technique adopted involved inoculating a predetermined site on the swab with a tap-water suspension of the organism recovered from the centrifuged deposit of a 24-hour broth culture. The turbidity of the suspension was made equivalent to a thousand-fold dilution of No. 3 Burroughs Wellcome opacity tube. All swabs were mounted on wooden applicators and sterilized by autoclaving. Some were moistened before testing by immersion in sterile tap-water. Each swab was plated on an appropriate medium and then discarded, and the degree of growth was expressed according to the following key:

+ + + +	signifies	more than 200 colonies
+ + +	,,	50–200 colonies
+ +	,,	20–50 colonies
+	,,	5–20 colonies
±	,,	1–5 colonies
−	,,	no growth

The results of a typical experiment are recorded in Table I, which shows that two of the most important factors determining the survival of a given species were the nature of the cotton-wool and the degree of moisture present. For example, it will be noted that the survival patterns of *Str. pyogenes* differed according to the wool used. With No. 1 sample of plain absorbent wool the organisms rapidly succumbed on both moist and dry swabs, whereas with No. 4 sample of plain non-absorbent wool viability was retained for at least two days. Samples 2 and 3 of absorbent and non-absorbent plain wools respectively were different again in that the presence of moisture accelerated death. In contrast, the survival of *Sh. flexneri* (Types 1, 2, 3, and 6 were tested) was unaffected by the different types of plain wool so long as the swab remained moist.

TABLE I.—*Survival of Str. Pyogenes and Sh. Flexneri on Plain and Serum Swabs (Temperature of Storage, 17° C. to 22° C. Moistening Fluid, Sterile Tap-water)*

Organism	Type of Wool	Type and Condition of Swab	Degree of Growth on Plating at: 0 hr.	8 hr.	24 hr.	48 hr.
Str. pyogenes	No. 1 absorbent	Plain, dry	+ + + +	−	−	−
		,, moist		−	−	−
		Serum, dry		+ + + +	+ + +	+ + +
		,, moist		+ + +	+ + +	+ + +
,,	No. 2 absorbent	Plain, dry	+ + + +	+ + +	+ + +	+ +
		,, moist		+	−	−
		Serum, dry		+ + +	+ + +	+ + +
		,, moist		+ + +	+ + +	+ + +
,,	No. 3 non-absorbent	Plain, dry	+ + + +	+ + +	+ + +	−
		,, moist		+ + +	−	−
		Serum, dry		+ + +	+ + +	+ + +
		,, moist		+ + +	+ + +	+ +
,,	No. 4 non-absorbent	Plain, dry	+ + + +	+ + +	+ + +	+ + +
		,, moist		+ + +	+ + +	+ +
		Serum, dry		+ + +	+ + +	+ + +
		,, moist		+ + +	+ + +	+ + +
Sh. flexneri	Nos. 1, 2, and 4	Plain, dry	+ + + +	−	−	−
		,, moist		+ +	+	−
		Serum, dry		+ +	+	−
		,, moist		+ + +	+ + +	+ +

It was apparent in these and other experiments that the main characteristic of the survival of *Str. pyogenes* was its unpredictability on different samples of plain cotton-wool. However, the survival time of serum-treated swabs was constant and prolonged and uninfluenced by the nature of the wool or the presence of moisture. Although we have recorded growth in these experiments up to 48 hours, in many other experiments of a similar type it has been found that *Str. pyogenes* remained viable on serum swabs for at least 10 days. As a result of investigations of this type a serum-treated swab was developed which has been used as a routine in these laboratories for over four years.

Preparation of Serum Swabs.—Pledgets of cotton-wool (absorbent or non-absorbent) are rolled on wooden applicators (Peerless brand) and then dipped into ox serum for 10 to 30 seconds. The swabs are spread loosely on a sheet of blotting-paper and placed in a 37° C. incubator until dry (usually 30 minutes). The swab is then finished in the usual way by mounting in a 6 by ½ in. (15 by 1.25 cm.) test-tube and sterilized by autoclaving for 20 minutes at 15 lb. pressure.

The finished product is a compact honey-coloured pledget, 3–5 mm. in diameter, in which the fibres are firmly bound to one another and to the applicator. It is therefore impossible to dislodge the wool when taking a specimen. The surface is smooth and does not unduly abrade the medium on plating. No special precautions are taken for storage purposes.

The above results (Table I), which have been repeatedly confirmed, foreshadowed the usefulness of serum swabs for a wider range of pathogenic organisms. In order to test this point plain and serum swabs were inoculated with suspensions diluted as indicated above and plated on appropriate media after 0, 8, 24, and 48 hours' storage at room temperature. Swabs plated immediately (0 hours) invariably produced a heavy growth—that is, more than 200 colonies. The results, listed in Table II, show the longest storage time up to 48 hours, at which a positive culture was obtained. They represent a summary of repeated experiments carried out at different room temperature and relative humidities.

TABLE II.—*Survival of Gram-positive and Gram-negative Organisms on Plain and Serum Swabs (Temperature of Storage, 16° C. to 22° C.; Moistening Fluid, Sterile Tap Water)*

Organism	Survival after Hours of Storage on: Plain Dry	Plain Moist	Serum Dry	Serum Moist
Gram-positive organisms:				
Str. pneumoniae, Type III	+ at 8	+ at 8	+ at 48	+ + at 24
Cl. welchii	+, 24	+, 8	+ +, 24	+ +, 24
C. diphtheriae gravis	+, 48	+, 8	+ + +, 48	+ + +, 48
Str. pyogenes	+, 48	+, 8	+ + +, 48	+ + +, 48
Str. viridans	+, 48	+ +, 24	+ + +, 48	+ + +, 48
Staph. aureus	+ +, 48	+ +, 24	+ + +, 48	+ + +, 48
Gram-negative organisms:				
N. meningitidis and *N. gonorrhoeae*	− at 8	− at 8	− at 8	− at 8
V. cholerae	−, 8	−, 8	−, 8	+, 24
H. pertussis	−, 8	−, 8	+ +, 24	+ +, 24
Sh. sonnei	−, 8	−, 8	+, 8	+ +, 48
Sh. flexneri and *Salm. typhi*	−, 8	+, 24	+, 24	+ +, 48
Pr. vulgaris	−, 8	+ + +, 24	+ + +, 24	+ + +, 48
Klebs. pneumoniae and *E. coli*	−, 8	+ + +, 48	+ +, 48	+ + +, 48

The salient features brought out in this Table are: (1) With the exception of *Neisseria* serum swabs prolonged survival and yielded heavier growth (when compared with plain swabs) of all organisms tested (16 species). (2) Moisture tended to accelerate death rate of Gram-positive types on plain swabs but had no such effect with serum swabs. (3) In contrast, the survival of Gram-negative bacilli was favoured by moisture, and

this was even more pronounced on the serum swabs. (4) Of the Gram-positive cocci *Staph. aureus* survived best, then the streptococci, whilst the pneumococci tended to die off more rapidly. (5) Of the Gram-negative enteric bacilli *V. cholerae* was the most sensitive, while *Sh. sonnei, Sh. flexneri,* and *Salm. typhi* were intermediate in their survival times between *Vibrio* and the more resistant coliform types of the genus *Bacterium.*

Some interesting side-issues arose from these experiments which would appear to be worth recording. For example, the rapid death of Gram-negative enteric pathogens on plain dry swabs has not been sufficiently stressed in the literature or textbooks, and a clear-cut demonstration of this phenomenon is indicated. *Sh. flexneri* was used as being typical of this group of organisms, and serum and plain swabs were moistened with tap-water and examined, after storage at room temperature, under three sets of conditions. In the first series the moisture was retained by storing in sealed tubes; in the second, slow evaporation was allowed to proceed by plugging the swab tube, containing 1 to 2 ml. of water, with cotton-wool; and in the third rapid drying was achieved by exposing the swab direct to air. The rate of evaporation was determined by frequent weighing of eight swabs (four plain and four serum) in each series and viability determined by plating at 1, 2, 8, 24, and 48 hours. The results are shown graphically in the Chart. It will be seen that the death rate closely followed the rate of drying. For example, in curve 3 and in curve 1 (after unsealing) air-drying precipitated a sudden dying out of the organisms. While serum tended to lessen this lethal effect it must be emphasized that a high moisture content is the most important single factor in favouring survival of Gram-negative bacilli.

It is of interest to note that the behaviour of *Str. pyogenes* under the same conditions was quite different, in that rapid drying of the swab definitely favoured survival whilst slow drying associated with a high moisture content was unfavourable.

It is appropriate at this stage to refer to numerous survival measurements carried out with *Str. pyogenes* and *C. diphtheriae* using different moistening fluids. It was found that on certain batches of cotton-wool the rate of death was almost as rapid in the presence of saliva as with saline or tap-water and that this result could be effectively countered by serum treatment. Hence, from the clinical point of view, no reliance should be placed on the delayed culture—for example, 24 hours—of plain cotton-wool throat swabs, which are almost invariably moistened with salivary secretions (see Tables III and IV below).

Although it has been stated in several textbooks (e.g., Wadsworth, 1947; Mackie and McCartney, 1948) that absorbent wool should be used in preference to non-absorbent, our observations lend no support to this conclusion. In fact we are of the opinion that if a choice must be made the non-absorbent type of wool is preferable (see Table I).

In regard to the type of applicator, we have almost exclusively used the wooden applicator in preference to wire. The latter is unsuitable for serum swabs, as the serum discolours the wool in the presence of wire. With plain swabs no difference in survival time of *Str. pyogenes* was detected when wire and wooden applicators were compared, although Buchbinder *et al.* (1947) preferred the latter for sampling of food utensils.

In view of the work of Dunklin and Puck (1948) on the influence of relative humidity on the death rate of

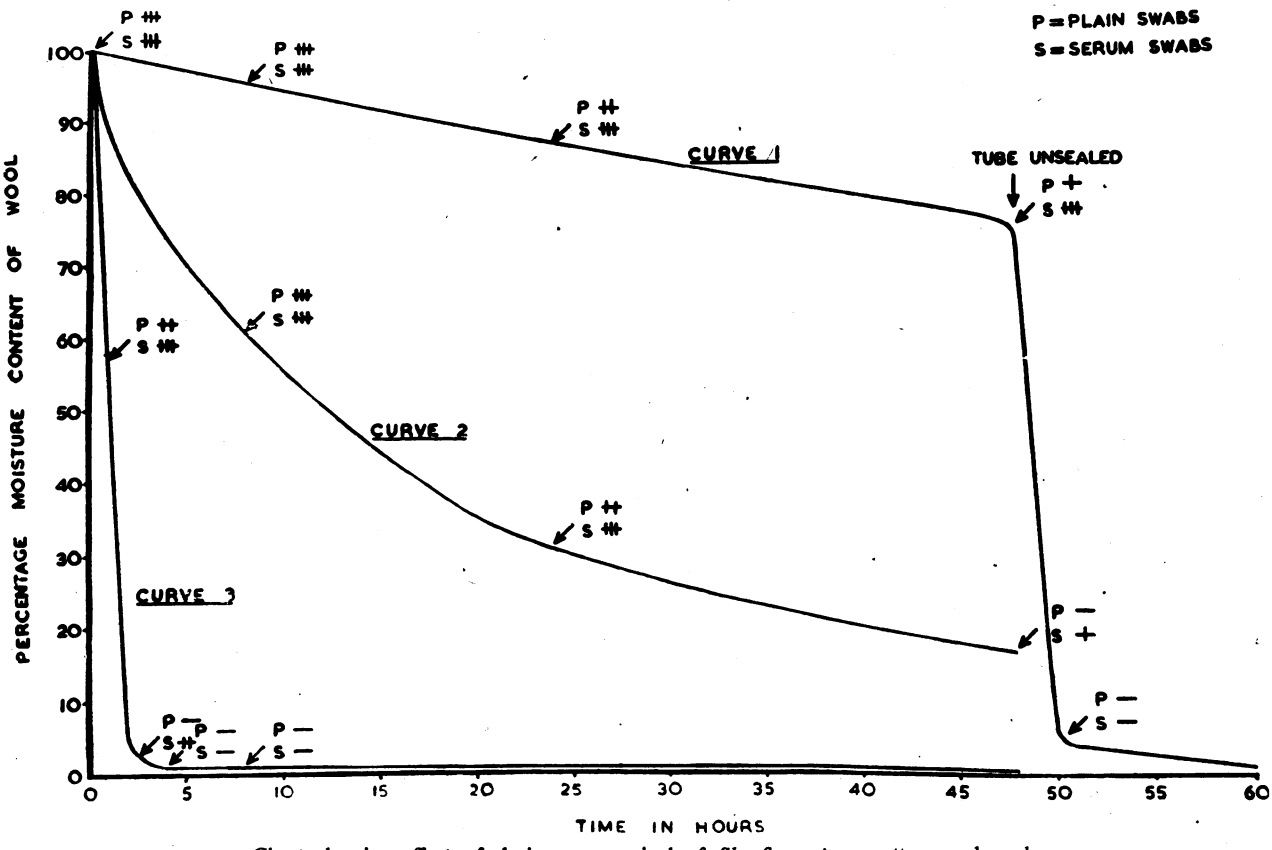

Chart showing effect of drying on survival of *Sh. flexneri* on cotton-wool swabs.

Gram-positive cocci in droplet spray, this factor was investigated in connexion with survival on swabs. Inoculated dry swabs were held between 18° C. and 23° C. in sealed containers over the solid and saturated solutions of various inorganic salts, anhydrous $CaCl_2$, and water to obtain the following levels of relative humidity: 0% R.H., $CaCl_2$ anhyd.; 35%, $CaCl_2.6H_2O$; 42%, $Zn(NO_3)_2.6H_2O$; 56%, $Ca(NO_3)_2.4H_2O$; 66%, $NaNo_2$; 90%, $ZnSO_4.7H_2O$; 100%, H_2O. Many tests were made using *Str. pyogenes* and *Sh. flexneri* as test organisms, but results were too variable to record in a tabular form. In general, it appeared that a high humidity (100%) favoured the survival of *Sh. flexneri*, and a low humidity (0%) favoured that of *Str. pyogenes*. Over the intermediate ranges the results were equivocal and we concluded that relative humidity, although it obviously plays a part in determining the rate of drying, was not a major factor in determining death or survival on plain swabs.

The influence of temperature variations was investigated over the ranges normally encountered in temperate climates—for example, 15° C. to 24° C.—and was found to be unimportant, except indirectly by affecting the rate of drying of moistened swabs. In spite of the fact that the preservation of *Shigella* requires the presence of moisture, refrigeration (4° C.) as a means of maintaining this is contraindicated. However, when survival of *Str. pyogenes* was compared at 4° C. and room temperature it was found, in contrast to *Sh. flexneri*, to be slightly prolonged at the lower temperature.

Other factors which have been studied may be briefly summarized as follows:

1. Anaerobiosis did not prolong survival. Hence it seems improbable that death on swabs is due to oxidation of cell constituents by atmospheric oxygen.

2. Ether and water extraction of absorbent and non-absorbent wools failed to alter survival when compared with untreated samples. Accordingly, serum protection does not operate by neutralization of toxic lipoid or water-soluble substances present in cotton-wool. This contention was further supported by noting that serum treatment improved the survival of *Str. pyogenes* on cellulose fibres other than cotton-wool—for example, filter-paper pledgets.

3. Colloids, other than serum, were tested, and it was found that treatment with egg albumen or ether-extracted serum was equally as effective as serum itself, but that 2% agar, 7% gelatin, 10% peptone, and 20% serum were ineffective.

Clinical Results

The foregoing experiments established the superiority of serum swabs over plain, and it now remained to apply these findings to clinical material. To this end a number of haemolytic streptococcal carriers were examined. The swabs were stored at room temperature in the dark for 24 and 48 hours, then plated on to gentian-violet horse-blood–agar medium and incubated for 48 hours. When serum swabs were included in a series these were always taken last. To obtain the results recorded in Table IV not more than four swabs were taken from any particular subject at a session, although two or three sets of swabbings were made on the same day. In all, approximately 650 swabs were examined. The results are shown in Tables III and IV.

To discriminate between plain and serum swabs (Table III) an analysis of the main factors (type of carrier, type of swab, and time of plating) has been considered. Combining the interactions of these factors, the experimental error was estimated. The results

TABLE III.—*Survival of Haemolytic Streptococci on Plain and Serum Throat Swabs (Temperature of Storage, 17° C. to 19° C.)*

Type of Streptococcal Carrier	No. of Subjects	Type of Swab	No. Positive After Storage for: 0 hr.	24 hr.	48 hr.
Light*	15	Plain	15	5	3
		Serum	15	15	9
Moderate† ..	16	Plain	16	10	5
		Serum	16	16	16
Heavy‡ ..	28	Plain	28	28	21
		Serum	28	28	27

* Fewer than 20 haemolytic streptococcal colonies on plating at zero time.
† 20–50 colonies. ‡ Greater than 50 colonies.

indicated a significant superiority (at the 1% point) for serum swabs, particularly when swabs from light and moderate carriers are plated after 24 hours' storage.

During the course of this study an opportunity presented to investigate an outbreak of scarlet fever in a district 170 miles from Melbourne. Both plain and serum swabs from 44 individuals were examined 24 hours after swabbing, with the following results. With serum swabs Group A haemolytic streptococci were isolated from 39%, whereas only 23% of the plain swabs were positive. This incident illustrates the advantage to be gained by using serum swabs in the investigation of Group A haemolytic streptococcal infections.

Finally, we thought it necessary to show that our results for untreated wools were not due to a peculiarity of the wools available in Australia. In Table IV

TABLE IV.—*Survival of Haemolytic Streptococci on Swabs Prepared from Cotton-wools of Different Origin (Temperature of Storage, 17° C. to 19° C.)*

Origin of Wool	No. of Subjects	No. Positive After Storage for: 0 hr.	24 hr.	48 hr.
Edinburgh ..	18	18	10	5
Atlanta	18	18	7	4
Chicago	14	14	9	5
Liverpool ..	14	14	7	4
London	14	14	10	7
Melbourne ..	18	18	13	10
San Francisco ..	14	14	6	5

we record findings obtained by swabbing the same carriers with seven different wools procured in Australia, England, and the United States. Again the great variability of survival on plain wools is demonstrated, and the findings also show that the Melbourne brand of wool compared most favourably with any of the overseas types. The disappearance of oral flora was also assessed in this experiment, with results similar to those recorded for the haemolytic streptococci.

Discussion

It has already been pointed out that, apart from the well-known behaviour of meningococci and gonococci, a great deal of uncertainty exists concerning how long plating of swabs may be delayed before a significant change in the bacterial flora occurs. As a result of our experience we think it unwise to define any time limits when these refer to survival on plain cotton-wool swabs. This is subject to great variability even when standard inocula of pure cultures are used under controlled conditions. Apart from the number of cells on the original swab, these variations are largely due to the wool used and to the amount of moisture present. Neither of these factors can be adequately controlled in practice. To select a particular brand of cotton-wool, whether it be absorbent or non-absorbent, is no solution, as we

have found that different batches of the same brand of wool may yield significantly different survival patterns for most Gram-positive organisms. However, if we substitute serum swabs for the usual plain type then the first factor is automatically removed and the second becomes less critical. As a result, estimates of survival time on serum swabs may be accepted with greater confidence than those applying to untreated swabs.

Assuming a moderately heavy contamination of a serum swab (this is an important variable impossible to control clinically), we suggest that an examination may be expected to yield a valid result when cultured within the following time limits: (1) Organisms viable for less than eight hours: meningococci and gonococci. Downie's method or Aycock and Mueller's method is recommended for the former and Saint-Martin's for the latter. (2) Organisms viable for at least eight hours: *H. pertussis, V. cholerae* (moist swab is essential), *C. diphtheriae.* (3) Organisms viable for at least 24 hours: haemolytic streptococci, *Str. viridans, Cl. welchii,* pneumococci, *Shigella,* and *Salmonella* (moist swabs are essential for the last two genera). (4) Organisms viable for at least 48 hours: *Staph. aureus, Klebsiella, Proteus, Pseudomonas,* and *Escherichia* (moist swabs are essential for Gram-negative species).

The above estimates are based on laboratory experiments, but since the clinical findings for haemolytic streptococci closely paralleled the laboratory tests we consider that the same relationship will hold for most other organisms. However, in the case of *C. diphtheriae,* although good survival has been obtained in laboratory experiments (Table II), we do not recommend delaying plating longer than eight hours. In some clinical cases which we have examined the organisms failed to survive 24 hours on serum swabs.

Although autoclaved albumin-dipped swabs have not been studied so exhaustively the results were similar to those achieved with serum.

We have attempted to discover how serum prolongs survival, but without success. For instance, it could not be shown that serum inactivated a toxic lipoid or water-soluble agent in the wool or that it acted as a substrate for growth. The effect of serum protein is probably a physico-chemical one acting on the bacterial cells and prolonging their survival irrespective of the materials on which they are lodged.

It is felt that these findings may have a broader significance than the somewhat restricted object of this investigation. Two facts are worthy of consideration from an epidemiological point of view. The first is the rapidity with which enteric pathogens die off when dried in air, and the second is that organisms leaving their hosts in serous exudates will live longer than those discharged in secretions of low protein content. These two points may throw some light on factors determining the survival of pathogenic organisms outside their natural hosts.

Summary

A study has been made of the survival patterns of a large number of organisms on plain swabs under standard laboratory conditions. From this study the following conclusions have been drawn: (*a*) Survival time of Gram-positive organisms is variable, depending on the batch of cotton-wool used and the presence of moisture, the latter tending to accelerate rate of death. (*b*) Survival time of Gram-negative enteric pathogens is uninfluenced by the type of wool but depends on the degree of moisture on the swab. On moist swabs survival is greatly prolonged.

A new type of swab was developed, termed a serum swab, on which survival times of all organisms tested, except the *Neisseria,* were increased.

Other factors such as temperature of storage, relative humidity, and anaerobiosis were investigated and found to be relatively unimportant.

The mechanism of serum protection is unknown.

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INVESTIGATION INTO THE EFFECTS OF AUREOMYCIN AND CHLORAMPHENICOL IN HERPES ZOSTER

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Recent advances in antibiotic therapy have been sufficiently great to produce an effective remedy against most bacterial infections, and it is therefore natural that the effects of antibiotics on virus infections should be put to the test. Particular success accompanied such an investigation in the case of "aureomycin" and virus pneumonia. Finland *et al.* (1949) have described the effects of aureomycin on the course of 24 cases of herpes zoster; they conclude that this drug increases the rate of healing of the skin lesions, causes a rapid subsidence of herpetic pain, and seems to be followed only rarely by post-herpetic neuralgia. No controls were used in their series, and the total dosage for each case varied from 7.4 to 54 g. From this evidence they conclude that an effective dose might be 4 g. daily for two to four days followed by 2 g. daily for a further three to five days, and that particular benefit results if the treatment is begun early. They claim that in most of the patients there was no evidence of secondary infection, that no new lesions developed after the first day or so of treatment, and that pain was relieved after the first day.