Bacteriological Swabs

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British Medical Journal, 1969, 3, 450-451

Summary: *Streptococcus pyogenes* survives poorly on plain cotton-wool swabs, whereas serum-dipped swabs permit its survival but also allow overgrowth by other bacteria and are likely to contain virus inhibitors. The use of swabs dipped in a solution of 20% bovine albumin (fraction V) and sterilized by steam is therefore recommended, since these permit bacterial survival without growth and are unlikely to contain virus inhibitors.

Introduction

Plain cotton-wool swabs commonly used for collecting material for bacteriological examination were found by Rubbo and Benjamin (1951) to be inimical to the survival of bacteria, especially *Streptococcus pyogenes*, and swabs dipped in ox serum and sterilized by heat were advocated instead. Furthermore, these workers suggested that swabs coated with egg albumen were just as good as serum swabs. Serum swabs were also recommended by Cruickshank (1953), and Holmes and Lermitt (1955) drew attention to the imperfection of the usual methods of detecting streptococcal carriers.

In 1965 White reported the failure of bacteria to survive on commercially supplied cotton-wool swabs sterilized by gamma-rays; similar swabs sterilized by heat instead of irradiation were, in White's words, "as satisfactory as our own laboratory-made swabs." In correspondence following White's communication his observations were confirmed by Mair and McSwiggan (1965), but Anderson (1965) thought that variation in the pH of cotton-wool rather than irradiation might have made swabs inhibitory to bacteria. It was clear from these letters that some laboratories were still using plain swabs.

After this correspondence we tested our own swabs and concluded that steam-sterilized swabs made of non-absorbent cotton-wool were the best available. This opinion was held until a school streptococcal outbreak was investigated in 1968. We were dismayed to find that 10 out of 14 swabs taken from boys with clinical evidence of streptococcal tonsillitis failed to yield *Str. pyogenes* after overnight delay in transit. This incident led to the investigations described below.

A change from plain cotton-wool swabs to Rubbo and Benjamin's steam-sterilized serum swabs (S.S.S.) or to commercially available serum swabs sterilized by ethylene oxide (E.O.S.S.) was considered, but not carried out for two reasons. It was feared, firstly, that virus inhibitors which are known to be present in the sera of adult animals might make serum swabs unsuitable for virus isolation (this objection applied particularly to serum swabs sterilized by ethylene oxide, since virus inhibitors in steam-sterilized serum swabs would probably be denatured by heat); and, secondly, that serum swabs might act as miniature culture media on which the pathogenic bacteria might be outgrown by other organisms. We therefore tried various other types of swab as well.

Methods

Str. pyogenes (the same strain was used throughout) was grown overnight in Hartley's broth. The culture was centrifuged and the deposit washed three times and resuspended in quarter-strength Ringer's solution to give opacity equivalent to No. 3 Brown's tube. Tenfold dilutions of this suspension were prepared and surface-viable colony counts were done. On

the insides of sterile plastic Petri dishes drops of suspensions were laid out in rows, 0.02-ml. dropping pipettes being used. Four swabs of each kind were used at each dilution. Each of the swabs to be tested was used to take up a drop of suspension from the plastic Petri dish, thus simulating in a standardized fashion the act of swabbing a patient's throat. Swabs were held in their tubes at room temperature for various periods before being plated on horse-blood digest agar. The plates were incubated overnight at 37° C. and colonies of *Str. pyogenes* were counted.

Results

Illustrative results obtained with different kinds of swabs are summarized in the Table. Results similar to those recorded in the Table were obtained when albumin-coated swabs and plain absorbent wool swabs were tested with other unrelated strains of *Str. pyogenes*.

Before these experiments were finished it became necessary to search for streptococcal carriers in a school, and for this purpose swabs soaked in calf serum and sterilized in the autoclave were used. These swabs were plated less than four hours after collection. Swabs taken from two patients which yielded *Str. pyogenes* were replated 24 hours after collection and yielded *Staphylococcus aureus* but no *Str. pyogenes*. This observation confirmed one of the previously mentioned disadvantages of serum swabs and led to a comparison of the ability of heated calf serum and of heated 20% bovine albumin to support the growth of various kinds of bacteria. The fluids were poured into Petri dishes which were heated sufficiently to coagulate their contents. Overnight broth cultures of *Staph. aureus*, *Staph. aureus* (Oxford strain), *Staph. albus*, *Escherichia coli*, *Klebsiella* sp., *Str. pyogenes*, and *Str. pneumoniae* were prepared, and were diluted to opacity equivalent to No. 1 Brown's tube. A loop charged with each culture was drawn across a serum plate, a bovine albumin plate, and a control plate of blood agar. After overnight incubation at 37° C. none of the organisms had grown on the bovine albumin, but all except *Str. pyogenes* and *Str. pneumoniae* had grown on the coagulated serum.

Survival of *Str. pyogenes* on Different Kinds of Swabs

Type of Swab (All on Wooden Sticks)	No. of Colonies when Plated at Once	No. of Colonies when Plated after Intervals of	
		4 Hours	24 Hours
Absorbent wool	413	0	0
Non-absorbent wool	> 500	0	0
Charcoal-coated. Absorbent wool and stick boiled in buffer	254	95 (37%)	4 (1.5%)
Charcoal-coated. Untreated absorbent wool, buffered stick	270	52 (19%)	11 (4%)
Calcium alginate wool	206	27 (13%)	6 (2.9%)
E.O.S.S. (firm A)	343	357 (> 100%)	275 (80%)
E.O.S.S. (firm B)	128	98 (76%)	224 (> 100%)
S.S.S. (calf serum)	100	60 (60%)	35 (35%)
20% bovine albumin-coated, steam sterilized	296	275 (92%)	180 (61%)

For tabulation we selected dilutions which gave immediate counts in the range 100 to 500 colonies. Each figure is the mean of at least four counts.

It was therefore concluded that the ability of S.S.S. swabs to maintain viability of pure cultures of *Str. pyogenes* (Rubbo and Benjamin, 1951), may not always be useful in practice,

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since other organisms may overgrow the streptococci if the swabs are not plated at once. Tubes containing uncoagulated sterile ox serum were inoculated with the same group of organisms to see if it was likely that E.O.S.S. might support growth. All the tubes gave visible growth except that containing *Str. pyogenes*, subculture of which showed that only slow multiplication had occurred.

Conclusions

In our opinion the use of plain cotton-wool swabs for the isolation of bacteria should no longer be considered as good practice. Charcoal-coated cotton-wool and alginate-wool swabs are good enough only when such swabs are plated within a few hours of collection. Serum-coated swabs preserve the viability of *Str. pyogenes* but permit overgrowth by other organisms. We therefore recommend that swabs soaked in 20% bovine albumin (fraction V) and sterilized by steam should be used. Bovine albumin is believed to be free from antiviral properties and is a constituent of virus transport and tissue culture maintenance media.

It was noted that serum swabs were absorbent, whereas albumin swabs were non-absorbent, but whether absorbency is an advantage or a disadvantage is not known.

We propose to continue to use charcoal-coated swabs with Stuart's (1956) transport medium for material suspected of containing *Neisseria gonorrhoeae*; gonorrhoea is not common enough in the Winchester area for a test of the ability of albumin swabs to preserve the gonococcus to be carried out. Though the use of charcoal swabs with Stuart's medium for all bacteriological swabs is an effective procedure it is too cumbersome for routine use.

We are grateful to Armour Pharmaceutical Co. Ltd. for supplying a solution of bovine albumin (fraction V) without azide, and to Stayne Laboratories Ltd. and Medical Wire and Equipment Co. (Bath) Ltd. for supplies of serum swabs sterilized by ethylene oxide.

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Preliminary Communications

Potential of Insulin Action in Normal Subjects by a Pituitary Polypeptide

British Medical Journal, 1969, 3, 451-452

Summary: A pituitary polypeptide, acceleratory polypeptide-growth hormone (ACG), has been found to increase the sensitivity of fasting normal people to intravenous insulin. It is suggested that diminished activity of this polypeptide—or increased activity of another polypeptide, inhibitory polypeptide-growth hormone (ING)—may have a role in the genesis of diabetes mellitus.

INTRODUCTION

The hypoglycaemic effect of a pituitary polypeptide (ACG)¹ produced by hydrolysis of growth hormone in diabetes mellitus has been reported (Armstrong *et al.*, 1969). It was suggested that the mechanism of this effect was the reversal of resistance to the endogenous insulin present in the patients studied.

This communication reports the influence of ACG on the degree and duration of hypoglycaemia induced in six normal volunteers by the intravenous injection of insulin.

PRESENT INVESTIGATION

Techniques.—The preparation of ACG and the blood glucose estimations (reported to the nearest 5 mg./100 ml.) were undertaken as described in the earlier report. The six volunteers, all of whom gave informed consent, ranged in age from 19 to 32 years. None was diabetic or had a family history of diabetes. All were of normal weight.

¹ ACG stands for acceleratory polypeptide-growth hormone. Experimentally it is capable of accelerating glucose uptake and fatty acid synthesis in various tissues. It may be derived by either non-specific or specific hydrolysis of purified growth hormones.

Experimental.—All volunteers were fasted for 18 hours previous to the study. This duration of fasting was chosen to induce the regulatory mechanisms which maintain blood glucose levels in the face of a pronounced depletion of liver glycogen. Among these mechanisms the anti-insulin effect of growth hormone might be expected to play a part, since fasting has been shown to increase growth hormone levels in plasma (Glick *et al.*, 1965). The blood glucose level was followed for 30 minutes to establish a baseline, and at zero time in the first experiment (series A, see Table) soluble insulin 0.025 unit/kg. body weight (1.4 to 1.6 units) was administered intravenously. Blood glucose was then measured at 10-minute intervals for 90 minutes. In a second experiment (series B, see Table), following an identical technique, insulin and ACG 1 mg. were administered simultaneously and blood glucose was followed for 90 minutes. The low dose of insulin was chosen, as it was thought that the standard dose of 0.1 unit/kg. + ACG might produce severe hypoglycaemia.

RESULTS

The Table shows the fall in blood glucose levels after insulin alone (A) and insulin with ACG (B) in each experiment, together with the mean changes. The Chart depicts the mean changes in blood glucose levels for the whole group.

Fall in Blood Glucose (mg./100 ml.) From Fasting Level

Volunteer:	1 (F. 24)		2 (F. 19)		3 (F. 21)		4 (M. 32)		5 (M. 20)		6 (F. 23)		Mean	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Time after Injection														
10 min.	5	10	5	0	0	10	0	15	5	0	10	15	4.2	8.3
20 min.	20	35	15	35	10	20	25	40	20	25	30	20	20.0	29.2
30 min.	25	45	30	40	20	35	25	45	5	30	30	40	22.5	39.1
40 min.	25	30	0	30	20	25	25	40	5	30	20	10	15.8	27.5
50 min.	10	20	0	5	0	10	15	0	20	15	0	0	4.1	13.3
60 min.	0	20	0	0	0	10	0	15	5	0	5	0	1.7	10.0
70 min.	0	10	0	0	0	5	0	10	0	15	0	0	0	8.3
80 min.	0	10	0	0	0	5	0	10	0	15	0	0	0	6.7
90 min.	0	0	0	0	0	5	0	5	0	0	0	0	0	1.7