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The transmission of bacteria and viruses on gummed paper

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The widespread practice of using the tongue to moisten envelope flaps, gummed labels and postage stamps not only is unattractive but could involve a danger to health. In order to assess the possible risk of infection associated with this practice, some simple studies were undertaken.

MATERIALS AND METHODS

Samples

A visit was paid to a local factory which produces envelopes and other gummed stationery. Samples of three representative adhesives were collected in sterile jars from the machines and from the stock barrels. Gum 'A' was a white plastic product ('433–0553', National Adhesives Ltd.); gums 'B' and 'C' were of the dextrin type ('Golden Grain R. 655', Alfred Adams Products; and 'Tragacine 2568', Tragacine Adhesives Ltd., respectively). Envelopes were taken from the output of several machines, and other samples were obtained from the stationery store of this Department.

Samples of commercial gum arabic as used in the manufacture of British postage stamps were obtained from a local chemical supplier. The solid gum was dissolved in Seitz-filtered tap water (30 g. in 100 ml.—'gum D').

Growth studies

Each gum was dispensed into eight 30 ml. bottles. Pairs of bottles were inoculated with approximately 100 cells, respectively, of *Staphylococcus aureus* (bacteriophage propagating strain '75'), *Escherichia coli*, *Salmonella paratyphi B* (both freshly isolated in the diagnostic laboratory of this Department) and *Klebsiella aerogenes* (isolated from the roller used to moisten gummed labels in the diagnostic laboratory). After thoroughly mixing the contents, samples were removed from each bottle for initial viable counts (by the method of Miles & Misra, 1938). One of each pair of bottles was incubated at 37° C. and one at room temperature (18–22° C.). Subsequent counts were made each day for one week, and thereafter at less frequent intervals.

Survival tests on bacteria and viruses

Bottles of the various gums were inoculated, in pairs, with approximately 10^8 cells of *Staph. aureus*, and *Salm. paratyphi B*, respectively. Other bottles were inoculated with echovirus Type 1 and adenovirus Type 3, respectively (10^7 tissue culture doses per bottle). Bottles were incubated at room temperature.

Streaks of these artificially infected gums were made on note paper (equivalent to the films produced on envelope flaps). They were stored in closed containers at room temperature.

Bacterial survival in gums was tested by inoculating a loopful on horse-blood-agar plates. Tests for survival in films and contamination of envelope flaps were made by rubbing the streaks with swabs moistened in nutrient broth. Gums containing virus were examined by inoculating 0·1 ml. of the gum solution into roller tube tissue cultures (HEp-2 cell line for the adenovirus, and primary monkey kidney cells for the echovirus). Dried films containing virus, and gummed flaps of envelopes, were cut away, and each strip was dropped into 2 ml. of Hanks's balanced saline. After the gum had completely dissolved, 0·2 ml. samples were transferred to tissue culture tubes. The Hanks's solution contained 500 units of penicillin and 500 μg. each of streptomycin and nystatin per ml., while the tissue culture media contained one-tenth of these concentrations.

Identification of organisms

Representative colonies of coagulase-positive staphylococci were subcultured and tested for susceptibility to bacteriophages of group III (including '75'). The other bacteria were subcultured on MacConkey's agar and tested biochemically, and by the slide agglutination test in the case of *Salm. paratyphi B*. If tissue cultures showed cytopathic effects, the presence of virus was confirmed by the neutralization technique.

RESULTS

Micro-organisms on envelopes and in gums

Neither pathogenic bacteria nor viruses were isolated from any of the 50 envelopes examined. Scanty growths of *Bacillus* spp. and saprophytic micrococci were obtained from the gummed flaps and from other parts of the envelopes. Similar cultures were obtained from the liquid gums, and one sample of gum arabic also yielded a moderate growth of *K. aerogenes*. This sample was not used in any further tests, but no attempt was made to sterilize any of the gums used.

Bacterial multiplication in gums

The test organisms did not grow in gums A, B or C, although within 2 weeks moulds and *Bacillus* spp. appeared as surface growths in B and C. The pH meter readings were 6·1, 3·8 and 7·6 in gums A, B and C, respectively. These values were unchanged after storage.

Gum arabic solution (D) had a pH of 4·6, but it nevertheless supported bacterial growth, as shown in Table 1. Following multiplication and death of the bacteria at 37° C., the pH fell to 4·0 in the case of *Staph. aureus*, and 3·8 in bottles containing the Gram-negative bacilli. The drop in pH was less marked in bottles kept at room temperature. To minimize the overgrowth of indigenous bacteria on subculture, it was found helpful to perform viable counts in parallel on plates which had been incubated anaerobically.

Table 1. *Growth of bacteria in gum arabic solution*

Organism	Temp. (° C.)	Log. of viable count per ml. after (days) 0	1	2	3	4	5	6	7	10	20	30
Staph. aureus	37	1·7	3·6	5·0	4·8	3·9	3·6	2·0	0·9	–	–	–
	20	1·9	2·1	2·7	5·6	6·1	7·0	7·2	5·6	4·0	1·3	–
Salm. paratyphi B	37	1·8	5·5	6·7	3·2	1·3	0·7	–	–	–	–	–
	20	1·7	2·3	2·3	4·2	6·2	4·0	2·3	2·4	2·0	1·6	1·4
Esch. coli	37	1·9	6·6	7·5	4·3	4·0	2·3	0·5	–	–	–	–
	20	1·9	2·6	3·2	4·8	4·6	6·3	7·6	6·2	5·8	4·6	1·8
K. aerogenes	37	2·0	5·6	7·8	7·0	7·2	5·8	3·6	3·0	1·6	–	–
	20	2·1	3·6	4·3	6·2	8·0	8·6	6·3	6·6	5·3	4·0	2·3

– = no growth.

Survival of bacteria and viruses

Dried films. As shown in Table 2, *Staph. aureus* was still viable after 6 months in dried films of C and D. *Salm. paratyphi B* survived for 4 months in C, and 3 months in D. The echovirus could not be recovered after a month, and the adenovirus not after 10 days.

Table 2. *Survival of bacteria and viruses in gums*

	Survival period (days) in: Dried films A	B	C	D	Liquid gums A	B	C	D
Staph. aureus	21	10	180+	180+	10	6	(21+)	30
Salm. paratyphi B	21	18	120	90	18	21	(21+)	48
Adenovirus 3	3	1	10	3	0*	0*	6	3
Echovirus 1	18	6	30	21	3	1	10	7

* Virus was isolated from samples examined after 30 min. but not after 1 day.

Liquid gums. Organisms usually remained viable for considerably shorter periods than in dried films (Table 2). Survival in C could not be investigated after 21 days because of overgrowth by moulds.

Adhesion of bacteria to gummed paper

The efficiency of gummed paper in the transfer of *Staph. aureus* from a solid surface was compared with that of self-adhesive cellulose tape (Sellotape). This kind of tape has been used for several years in hospital to study the bacterial contamination of surfaces (Selwyn, Maccabe & Gould, 1964).

By means of a glass rod, 0·5 ml. of an overnight broth culture of *Staph. aureus* diluted 1/10,000 in broth was spread over a sterile ceramic tile. After the broth had dried, the surface of the tile was sampled by pressing alternate strips of Sellotape and gummed paper on to it for 1 sec. (The gum arabic film on the paper had been slightly moistened with a damp swab.) The strips of tape and paper were then pressed on to blood-agar in Petri dishes for a few seconds. Fresh strips were

placed on the tile, and transferred to blood-agar in the same way. After incubation, the growth of *Staph. aureus* was almost confluent on all the plates that had been inoculated during the first series of transfers. Relatively low colony counts were obtained on all the plates inoculated during the second series—thus demonstrating the comparable efficiency of slightly moist gummed paper and Sellotape in the original transfer process.

DISCUSSION

The quantitative studies of Heller (1941) defined the conditions necessary for the long-term survival of *Streptococcus pyogenes* and *Esch. coli* in the dried state. The most favourable suspending media were shown to be either hydrophilic colloids or crystalloids that are water-soluble and utilizable by the bacteria; moreover, the two types of substance together exert an additive protective effect. Applying these principles, Stamp (1947) reported the preservation of various bacteria for over 4 years in dried disks containing gelatine and ascorbic acid. Lyophilization was found to be unnecessary. Naylor & Smith (1946) preferred dextrin to gelatine as the colloidal component of the suspending medium; and more recently McCracken (1964) has re-explored Stamp's method of preserving bacteria without lyophilization.

The present work demonstrates that certain commercially important gums also provide a favourable environment for the preservation of bacteria and viruses under normal atmospheric conditions. The relatively short periods during which the adenovirus and the echovirus could be recovered might have been due to the insensitivity of the system used for viral isolation.

The ability of gum arabic (or 'gum acacia') to support the growth of the four strains of bacteria was perhaps surprising on account of both the acidity and the chemical nature of the material. Data on pH minima for the growth of bacteria are scanty, and are mainly derived from early work in which neither standardized media nor accurate methods of pH determination were used. Porter (1946*a*) and Thimann (1963) both cite various minima which range from 4·0 to 4·5 for the species of enterobacteria used in the present work. *Staph. aureus* is not included by these authors, though Dernby (1921) recorded a minimum of 5·6 for growth, and Wilson & Miles (1964) state that growth is possible down to a pH of 4·0. A consideration of the effect of pH on bacterial growth in gum arabic is further complicated by the intrinsic protection afforded by such substances (Porter, 1946*b*). Gum arabic consists mainly of salts of arabic acid which may hydrolyse spontaneously to give various sugars, but it is usually contaminated also with other plant products (Dispensatory, 1955). The growth of saprophytic organisms in the gum further increases its biochemical complexity. In the samples examined, the material evidently contained all the nutrients necessary for the growth of widely differing bacteria.

During the manufacture of gummed paper and related products, contamination of the liquid gums by pathogenic bacteria and viruses can readily occur. This could be of particular importance in the production of postage stamps, for if relatively small numbers of pathogenic bacteria are introduced into the gum arabic adhesive,

they may multiply and subsequently retain their viability in the dried gum for several months. The use by the stamp manufacturer of unchlorinated spring water for the preparation of gum solution raises additional possibilities of contamination. Furthermore, postage stamps are often handled very carelessly when issued over the counter, and yet the purchaser will usually lick them without hesitation. The present work shows how readily bacteria can adhere to the surface of gummed paper which has been slightly moistened; and the finger is a suitable source both of moisture and of bacterial contamination.

There is an interesting parallelism between the probable consequences of contamination as revealed in this study, and the hazards associated with the entry of small doses of intestinal pathogens into cans of meat. Despite the marked differences in the vehicle, the experiments of Meers & Goode (1965) on the growth of typhoid bacilli in tins of corned beef produced similar results to those of the present tests on paratyphoid bacilli in gum. The initial growth phase is comparable following the introduction of small numbers of bacilli, which may be waterborne. Thereafter, at room temperature, bacterial survival in the acid gum is not very greatly inferior to survival in a can of meat. Moreover, the dried and uniformly infected gum shows as little evidence of danger as did the tins of meat which Meers and Goode infected with typhoid bacilli. Finally, the direct route of infection of the individual is the same in each case. However, the person who licks stamps runs the risk of acquiring respiratory tract as well as alimentary tract infection.

As long as this insanitary habit persists, adhesives used in the production of gummed paper should contain non-toxic agents which are active against the common pathogenic micro-organisms.

SUMMARY

The possible risks of infection associated with the practice of licking envelopes, stamps and labels were investigated. Although pathogenic bacteria and viruses were not isolated from sample envelopes obtained from various sources, the gums used in manufacture were found to exert a protective effect against death from desiccation on the bacteria and viruses which had been introduced into them. *Staph. aureus* and *Salm. paratyphi B* remained viable for several months in dried films of two out of four gums tested. An echovirus could be recovered from similar films for up to 30 days, and an adenovirus for up to 10 days.

Bacterial multiplication occurred in the gum used for the manufacture of postage stamps. The comparison is drawn between the possible consequences of minimal contamination of this gum and of cans of corned beef in the light of recent studies.

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