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The fate of a toxigenic strain of *Staphylococcus aureus* in vacuum-packaged bacon

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SUMMARY

Pork was cured by (*a*) the Wiltshire method and (*b*) a hygienic sweet cure process. Representative samples of both bacons were inoculated at 'low' density (10^3 organisms/g.) and 'high' density (10^6 organisms/g.) with a toxin-producing strain of *Staphylococcus aureus*, 'High' and 'low' density samples of both bacons were each stored at 5° C. for 42 days and 15° C. for 21 days.

Results indicated that the test organism at high inoculum density grew slowly in both bacons at 5° C. The organism survived at 5° C. in both 'low density' bacons. At 15° C. the test organism grew; growth being more pronounced in the 'hygienic' than in Wiltshire bacon.

INTRODUCTION

Previous work has shown that the shelf life of packaged bacon is influenced by, among other factors, the bacterial load at the point of packaging and the temperature of subsequent storage (Dempster, 1972). To ensure a satisfactory shelf life every effort must therefore be made to reduce initial bacterial numbers to a minimum. However, reducing the initial bacteriological count may, inadvertently, introduce a further hazard, namely the growth of potentially pathogenic organisms, particularly coagulase-positive staphylococci (CPS). Ingram (1962) considered that pathogens are hindered from growing in vacuum-packed bacon by what he called the 'population pressure' of an already established microflora. Thus he showed that staphylococci introduced into anaerobic packs of normal bacon at a concentration of 10^3 per g. increased to 10^4 to 10^5 per g. when the indigenous flora was first drastically reduced by irradiation. He concluded: 'accordingly in so far as the method of packing weakens the spoilage flora more than the pathogen it seems possible that packaging might conceivably encourage the more frequent survival of the latter'. By improving hygiene in the manufacture of packaged bacon and thereby reducing the number of spoilage organisms, accidental contamination of the product with CPS might therefore result in their multiplication. Furthermore, Ingram (1960) considered that a warm vacuum pack of bacon might act as an unintentional selective medium for staphylococci since

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they can reduce nitrate, are proteolytic, facultatively anaerobic and have a temperature optimum for growth near 37° C.

Despite Ingram's (1960) suggestion, vacuum packaging *per se* has been shown by Christiansen & Foster (1965) to be inhibitory. They reported that *Staphylococcus aureus* increased about six generations on sliced ham at both 20° and 15° C. under vacuum, but went through 11–12 generations in the absence of vacuum and reached numbers which were 20-fold greater than the maximum populations which developed under the anaerobic conditions of a vacuum pack.

However, results of particular investigations have shown that staphylococci have grown at < 10° C. Thus Angelotti, Foter & Lewis (1961) found food-poisoning strains grew well in custard at temperatures from 5° to 45° C. Ginsberg (1945) studied the effects of immersion in commercial bacon brines on meat inoculated with various organisms including *Staph. aureus* and reported that this latter organism survived but did not multiply for 21 days at 8° C. Similarly Buttiaux & Moriamez (1958) observed that *Staph. aureus* survived in curing brines for 16 days at 6° C. although numbers decreased slowly over this period. Bardsley & Taylor (1960) reported a slight initial rise (up to 24 hr.) in staphylococcal count in Danish smoked bacon which was vacuum packed and stored at 5° C. They attributed this to growth during the initial stages of the experiment before the bacon was cooled to the holding temperature. However, they found that over 44 days staphylococci decreased only from about $10^{6\cdot8}$ to about $10^{5\cdot5}$ per g., that is, about 80·0 %. According to Bryan (1968), for a staphylococcal intoxication to occur, there must be (*a*) a reservoir of *Staphylococcus aureus*, (*b*) a mode of dissemination of the organisms, (*c*) contamination of a food that is capable of supporting the growth of the organism, (*d*) a suitable temperature for such time as to allow adequate multiplication and toxin production, and (*e*) consumption of sufficient amounts of toxin by a susceptible person.

In view of these comments it was thought desirable to study the fate of a toxin-producing strain of *Staphylococcus aureus* after its introduction to packs of sliced bacon. Although a considerable quantity of vacuum-packaged bacon is still produced from Wiltshire-cured middles, an increasing amount is made from pork cured in short-time (48 hr.) sugar brines. This product is usually produced and stored in the factory under refrigeration (5° C.) but may be exposed to higher temperatures during distribution. Commercially produced packaged bacon may be contaminated with variable numbers of staphylococci.

METHODS AND MATERIALS

To study the influence of various factors on the growth of *Staph. aureus* in bacon, an experiment was set up in which two types of bacon were each inoculated with large and small numbers of staphylococci: samples of each were then stored at either 5° C. for up to 42 days or 15° C. for up to 21 days.

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Staphylococcal culture

The culture no. 69/7015, phage type 85, had been isolated from ham and implicated as a source of a small (family) food-poisoning outbreak. It had been shown to produce enterotoxin A. After passage through 2–3 transfers in nutrient broth (NB) (each passage consisted of incubation for 24 hr. at 37° C.), and before inoculation into the bacon, the culture was serially diluted in quarter-strength Ringer's solution and plated on the tellurite–glycine–egg yolk medium (EGPTA) of Baird-Parker (1962). The bacterial population per ml. was calculated after incubation at 37° C. for 48 hr.

Bacon

A boned-out Wiltshire middle was cured and sliced in the factory and a second middle was cured according to the 'hygienic cure' procedure (Dempster, 1972). Before slicing, the second middle was dipped in a 10 gal. milk churn of boiling brine for 15 sec. with the object of reducing surface bacterial numbers. Both middles were sliced and sufficient slices then transported to the laboratory without delay and each divided into equal sub-lots. With aseptic care, individual slices were laid out on sheets of grease-proof paper and inoculated with a suspension of the test organism. One half of each subsample of bacon was seeded with a 'high density' inoculum and the second half with a 'low density' inoculum as follows: 0·5 ml. NB. culture was added as drops (0·1 ml. per drop) on to each slice using a graduated pipette. The drops were placed along the surface of the muscle. The number of staphylococci in the high-density inoculum was 168×10^6 per ml. and the number in the low-density inoculum was $43{\cdot}5 \times 10^3$ per ml.; this gave approximately 10^6 organisms/g. and 10^3 organisms/g. based on the weight of bacon in each pack. Two inoculated slices were then sandwiched and placed in a 'Metathene' (X320) pouch and vacuum-drawn. Sufficient material was treated to provide for the analysis of triplicate packs at each storage interval. Two sub-lots were stored at 5° C. for 6 weeks and sampled at weekly intervals. Two further sub-lots were stored at 15° C. for 3 weeks and sampled twice weekly. Uninoculated control slices of Wiltshire and 'hygienic-cured' bacon were similarly packaged and stored at the two temperatures.

Bacteriological analyses of bacon

At sampling the packs were opened aseptically and the entire contents macerated with scissers. Ten gram amounts from each pack were then transferred to 3×20 cm hard-glass Pyrex boiling tubes and 40 ml. of sterile water added. The mixture was blended for 1 min. on a 'Polytron' homogenizer and 0·1 ml. serial dilutions in Ringer–peptone solution added to poured plates of EGPTA and Oxoid Plate Count Agar (PCA) + 3 % of added salt. Plates were counted after 24 hr. at 37° C. (EGPTA) and 3 days at 25° C. (PCA).

Chemical analyses of bacon

The lean from the remaining macerate was minced and duplicate samples (5 g.) weighed into porcelain dishes. The samples were dried for 18 hr. at 100° C. and

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Table 1. *Total colony count, coagulase-positive staphylococcal counts, salt, nitrite and pH of uninoculated bacon*

Bacon	Total colonies* on PCA +3% NaCl	Coagulase-positive staphylococci* on EGPTA	NaCl (%, w/v)	Nitrite (μg./g.)	pH
Wiltshire	5·03	< 1·69	4·14	51	6·2
'Hygienic'	3·94	< 1·69	2·71	318	5·85

* Log_{10} colonies/g.

ashed for a further 18 hr. at 550° C. Chloride was determined on the ash by Volhard's method (Vogel, 1948). Nitrite was estimated by the method of Follet and Ratcliffe (1963) using 10 g. of minced lean. The pH values of the homogenates were measured with a portable meter (Radiometer, Copenhagen) standardized against a pH 6·50 phthalate buffer.

RESULTS

The bacterial population and chemical composition of samples of the two bacons before inoculation with the test organism are shown in Table 1.

Storage at 5° C.

The survival of the test organism at 5° C. in both the Wiltshire and hygienically cured vacuum-packaged bacon stored for 6 weeks is presented in Fig. 1. Numbers increased marginally in the 'high density' Wiltshire bacon (Fig. 1*a*) up to 14 days ($10^{6 \cdot 265}$ to $10^{6 \cdot 44}$) and in the hygienic sweet-cured bacon (Fig. 1*c*) up to 7 days ($10^{6 \cdot 18}$ to $10^{6 \cdot 46}$). Thereafter numbers decreased. However, even after 6 weeks, 94·7% and 87·8% of the initial staphylococcal numbers were still viable in the Wiltshire and 'hygienic' bacons respectively. The total viable count (inoculated samples) increased progressively, reaching maximum numbers in 35 days (Wiltshire) and 42 days (hygienic cure). Despite the initial rise in growth the numbers of staphylococci did not increase in either bacon.

In both 'low density' bacons (Figs. 1*b*, 1*d*) staphylococci decreased throughout storage; only 65·0% and 62·0% of the original inoculum was still viable in the Wiltshire and 'hygienic' bacons respectively at the end of storage. The total viable count increased to reach maximum numbers in 35 days in both types of bacon. No coagulase-positive staphylococci were isolated from uninoculated control packs stored at 5 °C.

Storage at 15° C.

The fate of *Staph. aureus* on bacon stored for 3 weeks at 15° C. is shown in Fig. 2. Numbers increased in the 'high density' Wiltshire (Fig. 2*a*) up to 11 days and in the 'high density' 'hygienic' bacon (Fig. 2*c*) up to 17 days. Staphylococci did not die out; the final count was about equal to the initial count on the Wiltshire and exceeded the initial count (112·0%) on the 'hygienic' bacon. The total viable count reach maximum numbers in 7 days on the Wiltshire and in 17 days on the

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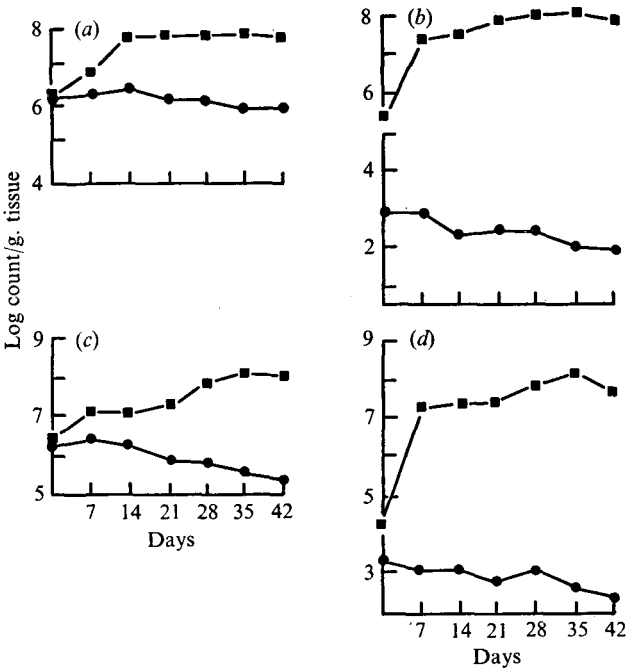

Fig. 1. Changes occurring in Wiltshire (*a*, *b*) and hygienic (*c*, *d*) sweet-cured vacuum packaged bacon at 5° C., inoculated with high (*a*, *c*) and low (*b*, *d*) levels of *Staphylococcus aureus*. Squares, total viable count on PCA + 3 % NaCl; circles, Staphylococcus count on ETGPA.

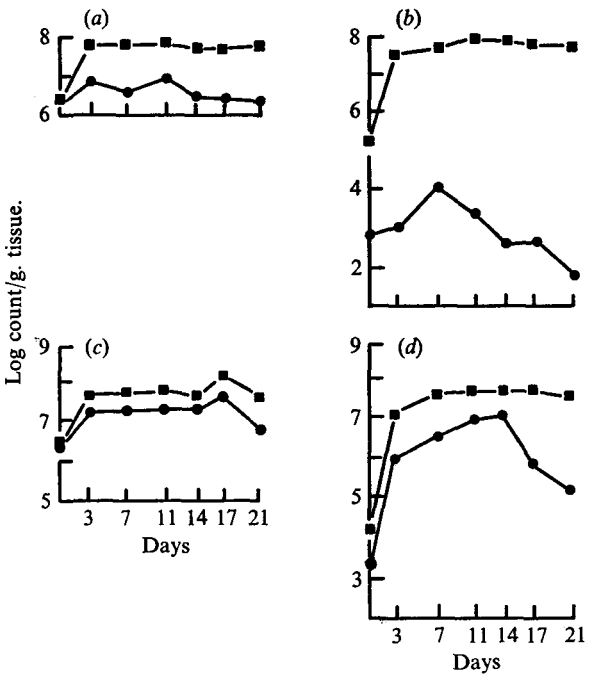

Fig. 2. Changes occurring in Wiltshire (*a*, *b*) and hygienic (*c*, *d*) sweet-cured vacuum-packaged bacon at 15° C., inoculated with high (*a*, *c*) and low (*b*, *d*) levels of *Staphylococcus aureus*. Squares, total viable count on PCA + 3 % NaCl; circles, 'Staphylococcus' count on ETGPA.

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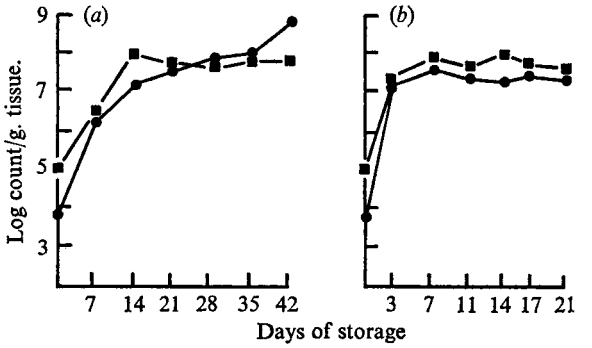

Fig. 3. Changes in total count (PCA + 3% NaCl) in vacuum-packaged bacon (uninoculated controls) stored at (*a*) 5° C. and (*b*) 15° C. Squares, Wiltshire; circles, hygienic, cured bacon.

Table 2. *Composite pH values of vacuum-packaged bacon held at 5° C.*

Days of storage	Wiltshire Bacon inoculated with: *c.* 10^6/g	*c.* 10^3/g	Control	'Hygienic' Bacon inoculated with: *c.* 10^6/g	*c.* 10^3/g	Control
0	6·20	6·20	6·20	5·85	5·85	5·85
7	5·68	5·63	5·60	6·01	5·90	5·80
14	6·05	5·87	5·90	6·23	6·13	5·90
21	6·01	6·00	5·50	6·06	6·00	5·40
28	5·51	5·55	5·50	5·65	5·68	5·50
35	5·56	5·58	5·40	5·65	5·73	5·55
42	5·71	5·71	5·50	5·85	5·85	5·70

Table 3. *Composite pH values of vacuum-packaged bacon held at 15° C.*

Days of storage	Wiltshire bacon inoculated with: *c.* 10^6/g	*c.* 10^3/g	Control	'Hygienic' Bacon inoculated with: *c.* 10^6/g	*c.* 10^3/g	Control
0	6·20	6·20	6·20	5·85	5·85	5·85
3	5·86	5·76	5·60	5·68	5·93	5·70
7	5·45	5·80	5·45	5·51	5·56	5·65
11	5·63	5·63	5·30	5·50	5·66	5·30
14	5·78	5·91	5·85	5·95	5·80	5·75
17	5·56	5·63	5·30	5·72	5·70	5·60
21	5·65	5·53	5·50	5·45	5·60	5·20

'hygienic' bacon. In the 'low density' bacons (Figs. 2*b*, *d*) staphylococci increased to maximum numbers in 7 days on the Wiltshire and in 14 days on the 'hygienic' bacon. Thereafter numbers decreased to less than the initial level on the Wiltshire (< 58·0%) but in excess of the initial inoculum on the 'hygienic' bacon. Total viable numbers increased considerably during the first 7 days on both bacons to maxima in 11 days on the Wiltshire and 17 days on the 'hygienic' bacon. Thereafter numbers decreased slightly. Again no coagulase-positive staphylococci were isolated from control packs stored at 15° C.

The total bacterial count in uninoculated samples of both bacons held at 5° and

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15° C. is presented in Fig. 3. Total numbers increased to maxima in 35 days at 5° C. and 14 days at 15° C. (Wiltshire) and in 42 days at 5° C. and 7 days at 15° C. ('hygienic' bacon).

The pH (mean of triplicate readings) of the bacon homogenates is shown in Tables 2 and 3. The pH decreased significantly from 6·20 to values between 5·71 and 5·53 in the Wiltshire bacon at both 5° and 15° C. and inoculated with high and low densities of staphylococci. There was little change, however, in the pH of the 'hygienic' bacon at either temperature or at either inoculum density.

DISCUSSION

In the experiments reported here, the effect of concentration of curing ingredients as well as the method of cure, storage temperature and the numbers of staphylococci artificially introduced into vacuum packaged bacon were studied. A coagulase-positive staphylococcus grew slowly in packaged bacon at 5° C. but only when the bacon was inoculated with high numbers. The fact that the numbers decreased over the experimental periods in both types of bacon after inoculation with small numbers suggests that the inoculum density *per se* exercises a significant effect on the ability of staphylococci to grow at low temperatures irrespective of the numbers or nature of the competing microflora. Thus in bacon inoculated with a 'high density' of staphylococci, 94·0 % and 87·0 % of the original numbers were still viable after 6 weeks at 5° C. in the Wiltshire and 'hygienic' bacon respectively. In the 'low density' bacons at 5° C. only 65·0 % (Wiltshire) and 62·0 % ('hygienic' bacon) of the initial inocula were still viable. This result agrees with the findings of Peterson, Black & Gunderson (1962), who observed a definite respressive effect on the growth of staphylococci when a mixture of saprophytic and psychrophilic bacteria was present. This effect was more pronounced as the staphylococcal portion of the population decreased. Obviously this is an example of Ingram's (1962) population-pressure hypothesis. Similar observations were made by Di Giacinto & Frazier (1966), who determined the inhibitory effect of coliform bacteria and *Proteus* spp. on the growth of staphylococci and showed that the time necessary for 2×10^4 staphylococci to reach 5×10^6 per ml. varied with the species of inhibiting ('effector') organism, the original ratio of effector organism to staphylococci, and the incubation temperature.

In the experiments described here, the test organism multiplied in both types of bacon held for 3 weeks at 15° C. Only in the 'low density' Wiltshire samples was the final count less than the original (< 58·0 %); in the 'high density' Wiltshire bacon 100·0 % of the initial inoculum was still viable. In both the 'high' and 'low' density 'hygienic-cured' bacon, the final staphylococcal count was 112·0 % and 115·0 % of the initial count respectively. Thus inhibition of staphylococci by other micro-organisms is affected by temperature; being less as the temperature rises. This has already been established, especially when bacon is stored at temperatures exceeding about 25° C. (Eddy & Ingram, 1962; Ingram, 1960). It was suggested by Eddy & Ingram (1962) that the principle obstacle to the multiplication of staphylococci is the preponderance of the normal microflora and they

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posed the question, 'why does this flora inhibit staphylococci?'. Sufficient evidence is available to show that certain genera, species and groups of bacteria exercise an inhibitory effect on staphylococci. Thus Graves & Frazier (1963) showed that *Lactobacillus* spp., *Leuconostoc.* spp, and *Streptococcus* spp. were inhibitory. Presumably the inhibition in this case was due to a lowering of pH. Acid pH is inimical to the growth of staphylococci as was shown by Bardsley & Taylor (1960). In bacon treated with lactic acid (pH 5·42) staphylococci failed to grow in 15 days while in control samples (pH 5·76), the test organism multiplied significantly. In fact these workers suggested that pork of low pH ($< 6{\cdot}0$) should be chosen for vacuum-packaged bacon as presenting conditions least favourable to growth of staphylococci. The results of the present experiment suggest that in no instance was the material acid enough to be solely responsible for staphylococci failing to grow.

Graves & Frazier (1963) also showed that certain other organisms, e.g. *Bacillus cereus*, *Proteus vulgaris* and *Escherichia coli*, produced an inhibitory substance specific for *Staphylococcus aureus*. Faecal streptococci and meat lactobacilli were shown to be inhibitory by Oberhofer & Frazier (1961), the inhibition varying with temperature and the test medium.

Staphylococci are extremely tolerant of salt, and many of the selective media recommended for their cultivation contain high concentrations of sodium chloride, e.g. the mannitol–salt agar of Chapman (1945) and staphylococcus medium 110 (Chapman, 1946). It is therefore clear that *Staph. aureus* and associated types (coagulase-negative staphylococci and micrococci) can flourish in salt concentrations considerably in excess of those present in bacon at any stage of the curing process. The salt content of the Wiltshire (4·14 %) or of the 'hygienic' bacon (2·71 %) would in no way restrict the growth of staphylococci.

The effects of curing ingredients and pH on the survival of staphylococci were studied by Lechowich, Evans & Niven (1956). They showed that in a basal medium containing salt, nitrate and nitrite, anaerobic tolerance to nitrite was in the range 100–200 parts per million (p.p.m.) and growth was prevented at pH 5·60. The nitrite content and pH of the bacon in the present study would not therefore be inhibitory in this sense. Likewise the work of Castellani & Niven (1955) showed that *Staph. aureus* could tolerate 800 p.p.m. NO_2 under anaerobic conditions at pH 6·55. Tarr (1941, 1942) studied the effect of NO_2 at 200 p.p.m. on a wide range of organisms and found the bacteriostatic effect increased markedly with decrease in the pH of the medium. At pH 5·60, 200 p.p.m. NO_2 gave partial inhibition of *Staph. aureus*.

Staphylococci are widely distributed in nature; the very high frequency with which they occur in the nasal passage of humans is shown by the work of several investigators. Thus Miles, Williams & Clayton-Cooper (1944) obtained incidences of between 19·0 and 65·0 %. More recently, Ravenholt, Eelkema, Mulhern & Watkins (1961) found that 32·0 % of 318 workers in 15 meat-handling plants in the U.S.A. carried coagulase-positive staphylococci on their skin. Obviously then, a considerable proportion of all people handling food are liable to infect it with staphylococci either directly or indirectly. The danger increases in cases of respira-

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tory infection, causing coughing and sneezing. In addition, food handlers with infected cuts or skin infections like boils and pimples will heavily contaminate food. It is not unreasonable to assume that vacuum packaging with its attendant handling predisposes bacon to potential contamination with staphylococci. Hansen & Riemann (1962) have suggested that full security against staphylococci growing in vacuum packs would be realized if prophylactic and compositional control of the product were implemented. By prophylactic control they meant bacteriological control, judicious selection of personnel and handling of the product under aseptic conditions. By composition control they included the effects of concentration of curing ingredients and water activity. Vacuum-packaged bacon invites abuse by storage at ambient temperature (Kitchell & Ingram, 1963). This was demonstrated forcibly by Thatcher, Robinson & Erdman(1962), who showed that staphylococci reached 10^8 to 10^9 per g. in bacon stored anaerobically at 37° C. and that toxin was produced even though the product was organoleptically acceptable.

Cured meats have a bacterial flora that is quite different from that of fresh meats. This is due to the selective bacteriostatic activity of the curing salts. The major selective action is due to sodium chloride. However, one of the most important groups of bacteria that is not inhibited by any palatable salt concentration is the staphylococcus group which includes food-poisoning types. Thus staphylococci grow on cured products, being halotolerant, whereas salmonellas and clostridia do not. The tolerance of staphylococci to nitrite is much lower under anaerobic conditions (Castellani & Niven, 1955) and it seems probable that at the low oxygen tension and low pH likely in a vacuum pack, sufficient nitrite might often be present to prevent their growth although this was not observed in the present investigation.

Although the total bacterial count of the 'hygienic' bacon was about one-tenth that of the Wiltshire bacon initially, total numbers reached about the same level in both bacons during storage and yet the staphylococcus was repressed in the Wiltshire but not in the 'hygienic' bacon. Thus suggests that it may not be simply a question of numbers as suggested by Eddy & Ingram (1962), but rather types of organisms which exercise the inhibitory effect.

From the above observations it appears that coagulase-positive staphylococci can survive in vacuum-packaged bacon at temperatures much lower than the optimum (37° C.) for this organism. However, to cause food poisoning, staphylococci must multiply sufficiently to produce toxin and little is known about the optimum conditions for toxin production (Wilson & Miles, 1964). Thatcher *et al.* (1962) showed that only a small amount of enterotoxin was produced in vacuum-packaged bacon at 37° C. even though the count of staphylococci had reached 10^9 to 10^{10} per g. McCoy & Faber (1966) studied the influence of food micro-organisms on staphylococcal growth and toxin production in meat and concluded that staphylococcal numbers cannot be used as an index of toxin formation. Therefore the aim should be to prevent contamination of food in the first instance. However, prevention and control are fraught with difficulty because of the ubiquitous nature of the organism, by the fact that the infected food is not altered in appearance, taste or smell, and by the heat-resistant nature of the toxin. Prevention must

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depend greatly upon such factors as general hygiene, personal cleanliness and the protection of food during preparation and storage.

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