(B4)

13th EUROPEAN MEETING OF MEAT RESEARCH WORKERS

ROTTERDAM, 1967.

THE RELATIONSHIP BETWEEN CARBON DIOXIDE PRODUCTION AND GROWTH OF PURE STRAINS OF BACTERIA ON PORCINE MUSCLE

G.A. Gardner & A.W. Carson

Ulster Curers' Association, Technical Division,
2 Greenwood Avenue, Belfast 4, N. Ireland.

FACTORS WHICH GOVERN the bacteriology of prepacked fresh meats include humidity, temperature, carbon dioxide and oxygen levels (Ingram, 1962).

In an earlier paper (Gardner, Carson & Patton, 1967) we found that the types of bacteria which grew on packaged and unpackaged pork did not differ. There were five groups: Pseudomonas-Achromobacter, Kurthia, Enterobacter-Hafnia, Microbacterium thermosphactum, and lactobacilli. All groups were isolated from pork stored at 16°, but only Pseudomonas-Achromobacter, M. thermosphactum and lactobacilli were found when the storage temperature was 2°. The main difference between packaged and unpackaged pork stored at 16° was an increase in the proportion of M. thermosphactum and at 2° an increase of both M. thermosphactum and lactobacilli.

To evaluate the role each type of bacterium plays in prepacked pork, the experiments presented in this paper were carried out. Growth of pure strains of bacteria, representative of each of the five groups listed above, on sterile pork was examined in both open and gas-tight (sealed) systems. The relative amounts of CO_2 produced by the pork and the bacteria were measured, and data is presented on the effect of CO_2 on bacterial growth.

METHODS AND MATERIALS

Preparation of sterile muscle samples. The method adopted was based on that described by Sharp (1963). Samples of the longissimus dorsi from the loin of chilled pig carcases 24 or 72 h post mortem were exposed using sterile instruments. Sections of muscle (3"-4" long) were flamed for c. 2 min with a Bunsen burner. The whole block was then painted with a saturated alcoholic solution of Crystal Videt and Brilliant Green and allowed to air dry for 30-60 min.

Using sterile instruments, the exterior was partially removed to expose a portion of the main bulk of the muscle. Samples (1-2 g) were excised and transferred to Dobott bottles

containing 2 g of sand, which were previously sterilised and weighed. Only a small section of the muscle was exposed at any one time, thus keeping to a minimum the period that the interior was subject to external contamination.

Organisms. Five organisms were used in these experiments: Pseudomonas Gp.I (Shewan, Hobbs & Hodgkiss, 1960); Microbacterium thermosphactum (Mc Lean & Sulzbacher, 1953); Kurthia zopfii (Breed, Murray & Smith, 1957); Enterobacter sp. (Edwards & Ewing, 1962; Carpenter, Lapage & Steel, 1966), and a Gram-positive, catalase-negative rod which produced H_2O_2 and was classified as a Lactobacillus sp.

All were isolated from prepacked pork which had been stored at 16° for 4 days.

Inoculation of sterile muscled samples. Serial dilutions of basal broth (Gardner, 1966) cultures, of most organisms, incubated for 2-3 days at 22°, were prepared in 0.1% peptone water. The Lactobacillus sp. was cultured in APT broth (Evans & Niven, 1951). The number of organisms in the broth was determined by phase contrast microscopy. From this an inoculum level of approximately 10^4-10^5 organisms was calculated. It was found that only 10-50% of the calculated inoculum was recovered.

Each bottle of muscle was inoculated with 1 (0.02 ml) or 2 (0.04 ml) drops of a suitable dilution, using Astell dropping pipettes. In one experiment a series of 17 such bottles were prepared for each organism. One was analysed for the initial load and the remainder divided into 4 groups. Each group of 4 bottles was further divided. The rubber stoppers of two were sealed with an adhesive, and the stoppers of the remaining two were replaced with cotton wool plugs (open). One group of bottles were used at each sampling time. The sampling times are shown in Table 1.

Table 1 - Sampling times

Storage temp.	Sampling time (days)				
16°	0	1	2	3	4
2°	0	3	7	10	14

Duplicate uninoculated controls were included at each sampling time.

The series inoculated with the 5 organisms and the uninoculated controls were prepared from one longissimus dorsi and is referred to as one experiment. Four such experiments were carried out at each storage temperature, 16° and 2°.

Gas analysis

A sample of 100 µl was taken using a gas tight syringe through the rubber stopper of the closed bottles. Analysis for CO_2, O_2 and N_2 was carried out as previously described (Gardner, Carson & Patton, 1967).

Enumeration of bacteria

After sampling for gas analysis, 10 ml of a sterile 0.1% (W/V) peptone water solution was added to each bottle. The bottles were then shaken vigorously using a Griffin Flask Shaker (Griffin & George Ltd.) for 3 min. The sand in the bottle was used to limit variations in recovery of bacteria from the meat. After shaking, serial dilutions in the same diluent were prepared and 3 drops (0.02 ml each) of each dilution were inoculated on to basal medium (Gardner, 1966), using the technique of Davis & Bell (1959). The plates were left for 1-2 hr on the bench, to allow the 'drops' to dry into the medium before being inverted an incubated at 22°. All organisms were enumerated using a low power microscope after 24 hr, except the Lactobacillus sp., which were counted after 48 h.

Each organism had a different colony structure, which could be easily recognised under the low power microscope. However, all plates were examined after 1 week at room temperature for purity.

RESULTS

The growth of bacteria on porcine muscle in open containers

The results of the growth of Pseudomonas sp., M. thermosphactum and Lactobacillus sp. On porcine muscle at 16° and 2° are summarized in Table 2, and for Enterobacter sp. and K. zopfii

in Fig. 1.

There was little difference within the growth curves of each of the five organisms during storage at 16°. In these experiments all the muscles were 72 h post mortem. At the end of the 4 days storage period there were on average equal numbers of Pseudomonas and Enterobacter sp. (c. 40 x 10^9/g). However, there were only 5.6 x 10^9 K. zopfii, 1.8 x 10^9 M. thermosphactum and 0.3 x 10^9 Lactobacillus sp. at this time. These figures relate to counts of pure cultures of the organisms on muscle. Assuming that there is no antagonistic effect between these strains and one accepts that the results of pure culture studies would be in the same order as those of mixed culture studies, the relative importance of each strain confirms our earlier findings (Gardner et al., 1967). The most important bacteria in pork stored aerobically at 16° were Pseudomonas sp., Kurthia sp. and Enterobacter sp. M. thermosphactum and lactobacilli were isolated in some cases, but in only relatively low proportions.

The variation within the growth curves of Pseudomonas, M. thermosphactum and Lactobacillus sp. during storage at 2° was small (Table 2). The growth curves of K. zopfii and Enterobacter sp. are shown in Fig. 1. The growth of K. zopfii was much better in Experiments VI and VIII than in Experiments V and VII. The former muscles were 72 h post mortem and the latter 24 h post mortem at the time of inoculation. From the shape of the curves it would appear that the effect of the 24 h post mortem muscle was to increase the lag phase of growth of this organism by 7 days. Also the growth of Enterobacter sp. was noticeably better on one of the muscles (Experiment VI), which was 72 h post mortem when inoculated. This evidence suggests that the growth of these bacteria on pork is influenced by the age of the meat post mortem.

If one again accepts that the results of pure culture studies at 2° would be in the same order as those of mixed culture, Pseudomonas sp. would be the dominant type on pork

stored at 2°. M. thermosphactum and lactobacilli would represent a small proportion of the flora, and Enterobacter sp. and K. zopfii would be present only in very low numbers. This also confirms our earlier observations (Gardner et al. 1967).

Changes in CO_2 and O_2 in sealed containers

The changes in the levels of CO_2 and O_2 in the headspace of the containers stored at 16° and 2° are given in Table 3. In this table the results are only valuable in giving an approximate picture of gas changes. These changes are quantitatively affected by a number of factors, including weight of meat, bacterial load, and temperature of storage. These will be considered in later sections. In all cases the CO_2 + O_2 level was 22 ± 3%, i.e. a decrease in O_2 was accompanied by a concurrent increase in CO_2. At both temperatures the marked increase in CO_2 production in the inoculated containers over the uninoculated controls began when the bacterial counts were c. 10^8 organisms/g. In the cases of Enterobacter sp., K. zopfii and Lactobacillus sp. at 2° , counts on the meat never reached this level.

The gas changes were brought about by the muscle alone in the uninoculated containers and by the muscle and bacteria in the inoculated containers. The relative amounts of CO_2 produced by each at any one time can be calculated from the following formulae:

$$\text{Carbon dioxide of muscle origin} = CO_2M$$

$$= \frac{CO_2 \text{ in uninoculated container (\%)} \times 10}{\text{Wt. of muscle (g)}}$$

= µl CO_2/ml headspace/g muscle.

$$\text{Carbon dioxide of bacterial origin} = CO_2B$$

$$= \frac{CO_2 \text{ in inoculated container (\%)} \times 10}{\text{Wt. of muscle (g)}} - CO_2M$$

= µl CO_2/ml headspace from the bacteria in 1 g muscle

$$CO_2b = \frac{CO_2B}{\text{No. of bacteria } (\times 10^8) \text{ per g muscle}}$$

= µl CO_2/ml headspace/10^8 organisms.

Carbon dioxide of muscle origin (CO_2M)

The CO_2M values of the porcine muscles used in all experiments are shown in Table 4.

The increase in CO_2 up to 1 day at 16° and 3 days at 2° is due to its physical release from the freshly cut muscle (Urbin & Wilson, 1961). There appeared to be no difference between the two temperatures. Gardner et al.(1967) reported that the majority of this physical release occurred during the first 5 h from preparation and that there was no difference in the rate of evolution between 16° and 6°.

During subsequent storage of the sterile muscle at 16° there was a gradual increase in CO_2 accompanied by a similar fall in O_2. This is brought about by meat enzyme activity. Andrews, Guthreck, McBride & Schweigert (1952) and Grant (1955) showed the succinic dehydrogenase system to be the most stable of the post mortem respiratory enzyme systems involving molecular oxygen. Urbin & Wilson (1961) demonstrated that this enzyme in bovine tissue was more active at higher pH values. Also, as the temperature fell to 10°, activity at pH values of 8.0, 7.4 and 6.4 was approximately the same. Extrapolation of their results would indicate that the enzyme would not be active at 2°. In our experiments there appeared to be no meat enzyme activity at this temperature.

Carbon dioxide of bacterial origin (CO_2b)

During the storage period at 16° all organisms exceeded a level of 10^8/g muscle, while only Pseudomonas sp. and M. thermosphactum attained this level at 2°. The CO_2b values for these organisms was unaffected by temperature, as seen from the results in Table 5.

Table 5 - Mean CO_2b^* values for Pseudomonas sp. and M. thermosphactum as influenced by growth temperature.

	16° mean	2° mean
Pseudomonas sp.	1.1	1.6
M. thermosphactum	5.7	6.6

* µl CO_2/ml headspace/10^8 organisms

The overall mean CO_2b values of these and other organisms is given in Fig. 2.

A comparison of the CO_2b values for each organism is given in Table 6. The CO_2b values for each bacterium were highly significantly different from any of the others except K. zopfii and M. thermosphactum, which were not significantly different.

The Gram-negative organisms produced much lower amounts of CO_2 than the Gram-positive organisms. Of the Gram-positive strains the catalase-negative Lactobacillus sp. produced more CO_2 than the catalase-positive organisms.

These figures show the relative contributions of each bacterium to the CO_2 of bacterial origin in prepacked pork. Much of the variation in CO_2b values for each organism may be attributed to the inherent inaccuracies of estimating bacterial numbers. Moreover, as all of these values are calculated from counts in the late logarithmic to stationary phase of growth, respiratory enzymes of 'dead' cells may also contribute to the CO_2 increase, but those cells could not be recovered in the viable count. There was a tendency for the CO_2b values of the Gram-positive organisms to increase, as the age of the culture increased. This can be seen from the data in Table 7.

Table 7 - Effect of age of culture on CO_2b value of bacteria growing on porcine muscle at 16°.

	Age of culture (days)		
	2	3	4
Pseudomonas sp.	1.26	1.14	1.06
Enterobacter sp.	1.67	2.96	2.50
M. thermosphactum	4.71	5.75	6.60
K. zopfii	4.98	4.90	8.35
Lactobacillus sp.	0	9.8	12.1

Two other factors which may cause an increase in CO_2b are (a) the CO_2 of the headspace may cause an increase in the bacterial 'respiration', and (b) the pH of the meats may rise due to bacterial activity, thus increasing the rate of meat

respiratory enzymes (Urbin & Wilson, 1961).

Effect of CO_2 on the growth of bacteria on porcine muscle

The effect of CO_2 on the growth of the bacteria on porcine muscle at 16° is shown in Fig. 3. The Gram-negative organisms were inhibited more than the Gram-positive organisms by this gas. The growth of K. zopfii was unaffected, and M. thermosphactum was stimulated by low concentrations of CO_2, but higher levels inhibited both organisms. Carbon dioxide levels up to 16% had only a slight retarding effect on the growth of the Lactobacillus sp.

The effect of CO_2 on the growth of these bacteria at 2° is given in Fig. 4. Only the Pseudomonas sp. and M. thermosphactum grew to levels exceeding 10^8/g, and hence the other organisms were affected only by the CO_2 of muscle origin (3%). At this level the growth of M. thermosphactum and Lactobacillus sp. was stimulated. At higher CO_2 levels there appeared to be little effect on the growth of M. thermosphactum. The Enterobacter sp. was also stimulated by low levels of CO_2 (c. 2%), but a slightly higher concentration (c. 3%) markedly inhibited growth. The growth of K. zopfii and Pseudomonas sp. was noticeably retarded by c. 3% CO_2. Higher levels in the case of Pseudomonas sp. had no increased effect.

There have been many studies on the inhibitory effect of CO_2 on bacterial growth (Valley & Rettger, 1927; Coyne, 1933; Haines, 1933; Scott, 1938). All were concerned with growth in initially high levels of this gas, i.e. the lag and logarithmic phase of the bacterial growth curves. However, in the present work we have been dealing with low levels of CO_2 (3%) at these stages and have also examined the effects of higher CO_2 levels on the late logarithmic and stationary phases of the bacterial growth curve.

Coyne (1932) found that all species of Achromobacter, Flavobacter, Micrococcus, Pseudomonas, Aerobacter, Bacillus and Proteus isolated from fish were able to grow as well in N_2 (containing $< 0.3\%$ O_2) as in air. As the oxygen level never

fell below 1% in our experiments, it would appear that the O_2 is not a limiting factor on growth.

In a previous publication (Gardner et al., 1967) the effect of CO_2 on the composition of the flora of prepacked pork stored at 16° was to increase the proportion of M. thermosphactum with a corresponding decrease in Pseudomonas-Achromobacter sp. Moreover, at a storage temperature of 2° both the proportions of M. thermosphactum and lactobacilli increased, and Pseudomonas-Achromobacter group decreased with increasing CO_2 level. Kurthia sp. were not isolated from pork stored at 2° and Enterobacter-Hafnia sp. were only occasionally found.

Ogilvy and Ayres (1951) demonstrated that CO_2 was a more effective bacterial inhibitor, the lower the temperature. At a level of c. 3% we have shown that Pseudomonas sp. was inhibited more at 2° than 16°. However, at the same CO_2 level growth of M. thermosphactum and lactobacilli was stimulated to a greater extent than at the lower temperature. It would appear that CO_2 has a specific effect on each type of bacterium and that it is more effective in stimulating or inhibiting growth, the lower the growth temperature.

SUMMARY (B4)

The growth of Pseudomonas sp., Enterobacter sp., Kurthia zopfii, Microbacterium thermosphactum and Lactobacillus sp. in pure culture on porcine muscle at 16° and 2° was examined in both 'open' and 'gas-tight' systems. The CO_2 in the headspace of the 'gas-tight' containers was produced by the muscle and by the bacteria. The Gram-negative bacteria produced much lower amounts of CO_2 than the Gram-positive organisms. Data on the effects of CO_2 on the growth of each bacterium at 16° and 2° is presented.

ZUSAMMENFASSUNG

Es wurde die Vermehrung von Pseudomonas sp., Enterobacter sp., Kurthia zopfii, Microbacterium thermosphactum und Lactobacillus sp. als Reinkultur auf Schweineflesch bei 16° und 2° in 'offenen' und 'gasdichten' Systemen untersucht. Das CO_2 im Kopfraum der 'gasdichten' Behälter wurde durch das Fleisch und die Keime gebildet. Die gram-negativen Bakterien erzeugten viel geringere Mengen CO_2 als die gram-positiven Keime. Angaben über die Beeinflussung des Wachstums jedes Keimes durch CO_2 bei 16° und 2° werden erteilt.

RESUME

La multiplication des Pseudomonas sp., Enterobacter sp., Kurthia zopfii, Microbacterium thermosphactum et Lactobacillus sp. en culture pure sur le porc à 16° et 2° était examinée dans des systèmes 'ouverts' et 'étanches au gaz'. Le CO_2 dans le vide laissé dans le haut des boîtes 'étanches au gaz' était produit par le muscle et les bactéries. Les bactéries gram-négatives produisaient des quantités beaucoup plus petites de CO_2 que les germes gram-positifs. Des données sur les effets du CO_2 sur la multiplication de chaque bactérie à 16° et 2° sont présentées.

REFERENCES (B4)

Andrews M.M., Guthneck B.T., McBride B.H. & Schweigert B.S. (1952). Stability of certain respiratory and glycolytic enzyme systems in animal tissues. J. biol. Chem. 194, 715.

Breed R.S., Murray E.G.D. & Smith N.R. (1957). Bergey's Manual of Determinative Bacteriology. 7th ed. London: Bailliere, Tindall & Cox.

Carpenter K.P., Lapage S.P. & Steel K.G. (1966). Biochemical identification of Enterobacteriaceae. In Identification Methods for Microbiologists. Part A. Ed. B.M. Gibbs and F.A. Skinner. London, Academic Press.

Coyne F.P. (1932) The effect of carbon dioxide on bacterial growth with special reference to the preservation of fish. Part 1. J. Soc. chem. Ind., Lond., 51, 119T.

Coyne F.P. (1933). The effect of carbon dioxide on bacterial growth. Proc. roy. Soc. Lond., Ser. B, 113, 196.

Davis J.G. & Bell J.S. (1959) A 'drop technique' for colony counts in microbiology. Lab. Pract., 8, 58.

Edwards P.R. & Ewing W.H. (1962). Identification of Enterobacteriaceae. Minneapolis: Burgess Publishing Co.

Evans J.B. & Niven C.F. (1951). The nutrition of heterofermentative lactobacilli that cause greening of cured meat products. J. Bact., 62, 599.

Gardner G.A. (1966). A selective medium for the enumeration of Microbacterium thermosphactum in meat and meat products. J. appl. Bact., 29, 455.

Gardner G.A., Carson A.W. & Patton J. (1967). Bacteriology of prepacked pork with reference to the gas composition within the pack. J. appl. Bact., 30 (2), in press.

Grant N. (1955). The respiratory enzymes of meat. I. Identification of the active enzymes. Food Res., 20, 250.

Haines R.B. (1933). The influence of carbon dioxide on the rate of multiplication of certain bacteria, as judged by viable counts. J. Soc. chem. Ind., Lond., 52, 13T.

Ingram M. (1962). Microbiological principles in prepacking meats. J. appl. Bact., 25, 259.

McLean R.A. & Sulzbacher W.L. (1953). Microbacterium thermosphactum spec. nov., a non-heat resistant bacterium from fresh pork sausage. J. Bact., 65, 428.

Ogilvy W.S. & Ayres J.C. (1951). Post-mortem changes in stored meats. V. Effect of atmospheres containing carbon dioxide in prolonging the storage life of cut-up chicken, Food Tech., Champaign, 5, 97.

Scott W.J. (1938). The growth of micro-organisms on ox muscle. III. The influence of 10% carbon dioxide on rates of growth at -1°. J. coun. sci. ind. Res., Aust., 11, 266.

Sharp J.G. (1963). Aseptic autolysis in rabbit and bovine muscle during storage at 37°. J. Sci. Fd. Agric., 14, 468.

Shewan J.M., Hobbs G. & Hodgkiss W. (1960). A determinative scheme for the identification of certain genera of Gram-negative bacteria with special reference to the Pseudomonadaceae. J. appl. Bact., 23, 379.

Urbin M.C. & Wilson G.D. (1961). The post mortem oxygen requirements of bovine tissue. J. Fd. Sci., 26, 314.

Valley G. & Rettger L.F. (1927). The influence of carbon dioxide on bacteria. J. Bact., 14, 101.

Table 2 - Growth of Pseudomonas sp., M. thermosphactum and Lactobacillus sp. on sterile porcine muscle at 16° and 2°. (B4)

Log_{10} viable count/g

	Pseudomonas sp.				M. thermosphactum				Lactobacillus sp.			
Experiment No.++	I	II	III	IV	I	II	III	IV	I	II	III	IV
Storage time at 16° (days)												
0	3.97+	3.30	3.81	3.69	4.11	4.45	3.92	3.79	3.80	3.69	4.49	3.99
1	7.38	7.50	*	8.16	7.35	7.69	*	7.47	6.38	6.66	7.67	6.27
2	9.96	10.1	9.95	10.16	8.40	8.86	8.70	8.87	7.84	8.26	8.62	7.80
3	10.31	10.57	10.79	10.51	8.73	9.01	9.06	9.16	8.03	8.32	8.72	ø
4	10.51	10.56	10.70	10.53	8.80	9.33	9.48	9.06	8.1	8.46	8.63	ø
Experiment No.++	V	VI	VII	VIII	V	VI	VII	VIII	V	VI	VII	VIII
Storage time at 2° (days)												
0	4.0	3.86	3.46	3.78	4.19	3.60	3.49	3.87	4.15	4.27	3.50	4.10
3	5.07	5.59	5.88	5.28	5.25	5.05	4.91	5.06	4.76	4.99	4.02	4.57
7	8.72	8.96	8.12	7.97	6.93	7.65	6.26	6.97	5.86	6.56	5.25	5.94
10	9.57	10.17	9.61	10.08	8.84	8.82	7.58	8.30	7.24	7.51	6.40	6.74
14	10.11	10.35	10.28	10.27	9.06	9.29	8.37	9.20	7.77	8.06	7.51	7.56

+Each result represents the average of duplicates.

*Not done

øMixed culture

++The muscles used in Experiments V and VII were 24 h post mortem. The remainder were 72 h post mortem.

Table 3 - Changes in CO_2 and O_2 levels (%) in sealed containers of inoculated porcine muscle stored at 16° and 2° (Each result represents the average of 8 determinations).

Storage at 16° (days)	Uninoculated		Inoculated organism: Pseudomonas sp.		M. thermosphactum		Enterobacter sp.		K. zopfii		Lactobacillus sp.	
	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2
1	2.9	17.6	3.5	18.1	3.1	17.9	3.0	18.2	3.6	17.6	2.6	19.6
2	4.2	18.9	14.8	7.7	7.9	16.0	9.8	13.3	12.6	9.6	3.9	18.4
3	4.2	17.4	19.1	3.1	12.6	10.9	19.3	4.9	17.9	3.3	6.3	16.5
4	4.8	16.3	18.4	2.9	16.3	8.8	19.9	3.7	18.1	4.2	10.6	13.4
Storage at 2° (days)												
3	2.3	19.2	1.9	19.2	2.1	19.3	1.9	19.8	1.8	19.5	2.0	19.5
7	2.3	19.0	2.2	18.4	2.2	19.2	2.0	19.4	2.2	19.3	2.3	19.2
10	2.0	18.7	9.0	10.7	4.8	16.7	1.7	18.8	2.5	18.5	2.4	18.1
14	2.0	19.0	17.8	1.4	11.0	12.4	2.5	18.3	2.5	18.7	2.9	18.6

Table 4 - CO_2M values of stored sterile muscle.* (B4)

Storage at 16° (days)	Experiment I	II	III	IV	Av.	Storage at 2° (days)	Experiment V	VI	VII	VIII	Av.
1	11	12	10	19	13	3	14	13	16	13	14
2	19	16	18	21	18	7	11	14	18	14	14
3	18	18	15	28	20	10	10	13	15	12	13
4	22	18	14	36	22	14	13	13	15	13	14

Each result is an average of duplicate determinations.

* µl CO_2/ml headspace/g muscle.

Table 6 - Significant differences between mean CO_2b values of each organism. (B4)

	Probability (%)			
	Enterobacter sp.	K. zopfii	M. thermosphactum	Lactobacillus sp.
Pseudomonas sp.	< 0.1	< 0.1	< 0.1	< 0.1
Enterobacter sp.		< 0.1	< 0.1	< 0.1
K. zopfii			> 50.0	< 0.1
M. thermosphactum				< 0.1

Fig. 1 Growth of Enterobacter sp. and K. zopfii on porcine muscle at 16° for 4 days and 2° for 14 days

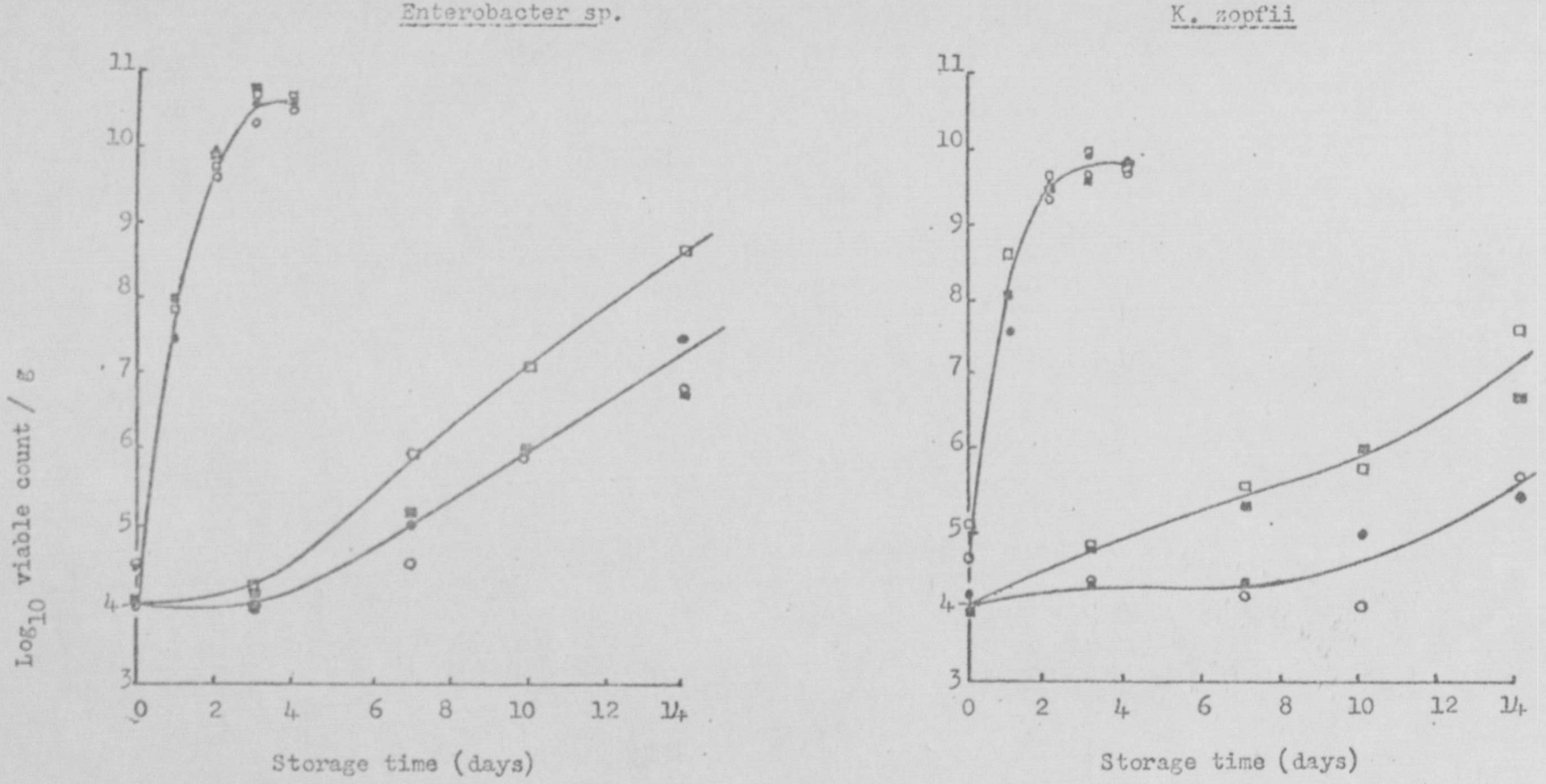
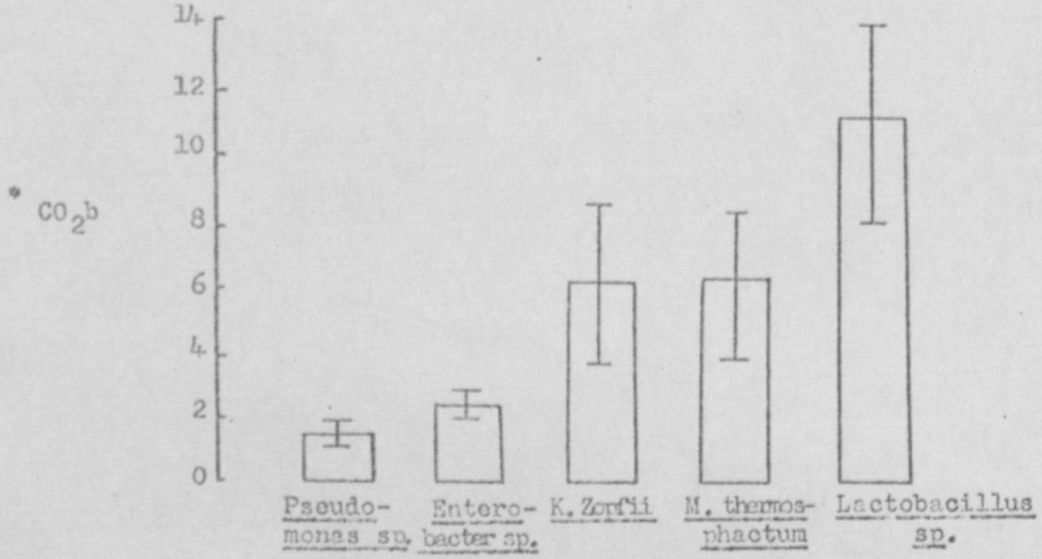
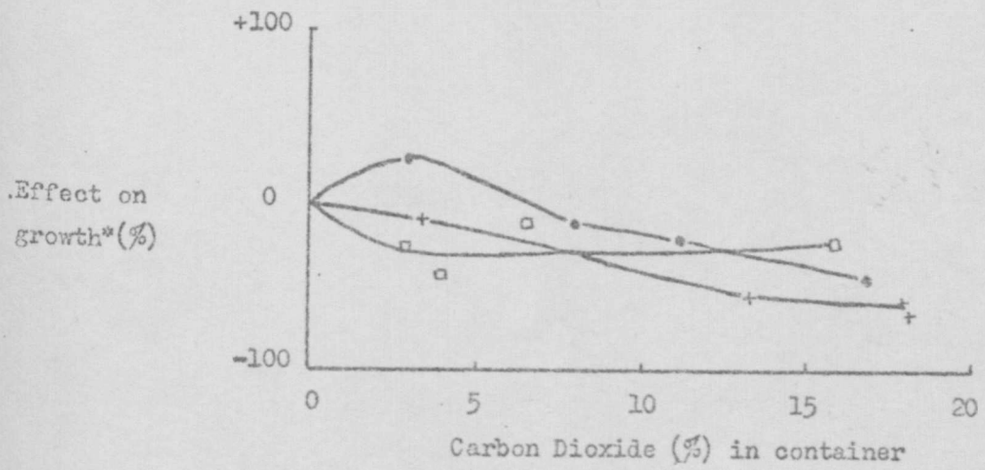


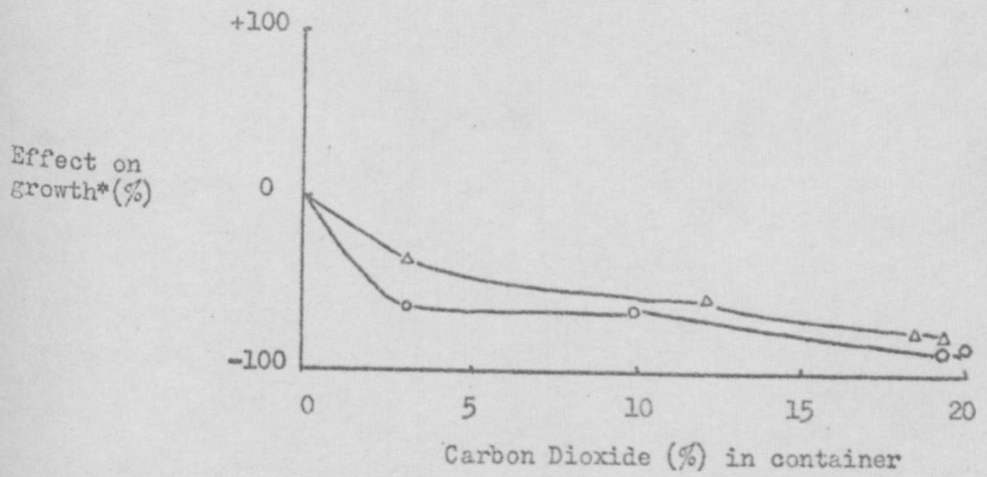
Fig 2 *Mean CO_2b values and standard deviations of bacteria growing on Porcine muscle in sealed containers.*



* μl CO_2/ml headspace / 10^8 organisms

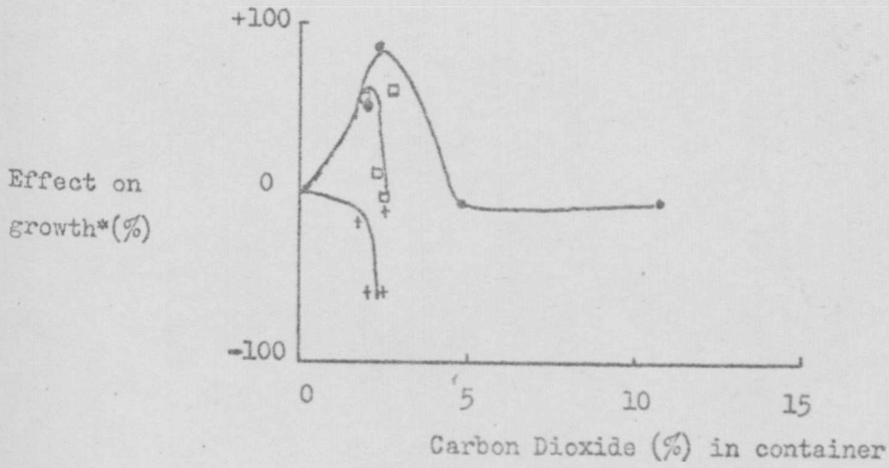
Fig. 3 The effect of CO_2 on the growth of Pseudomonas sp (△), Enterobacter sp (○), M. thermosphactum (•), K. zopfii (+) and Lactobacillus sp (□) on porcine muscle at 16°.

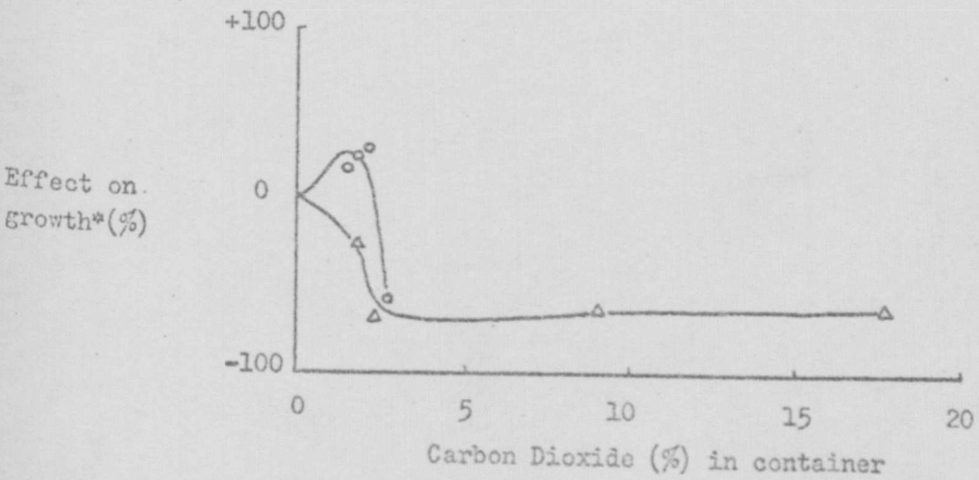




*Viable counts in the sealed containers as a percentage of the open containers. Each point represents the average of 4 determinations.

Fig. 4 The effect of CO_2 on the growth of Pseudomonas sp (Δ), Enterobacter sp (o), M. thermosphactum (•), K. zopfii (+) and Lactobacillus sp (□) on porcine muscle at 2°.





* Viable counts in the sealed containers as a percentage of the open containers. Each point represents the average of 4 determinations.