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AUREOMYCIN - KONSERVERINGSBRUG
Manuskript nr. 84

(29)

The effect of chlortetracycline on faecal streptococci in canned ham.

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That antibiotics might have a possible use as an aid to heat processing of canned foods has been indicated by a number of experiments (Curran and Evans (9,10); Andersen and Michener (2); Hounie (14)). The hope that the incorporation of antibiotics would permit a lower heat treatment disappeared, however, when it was found that a number of sporeforming strains surviving the mild heat treatment were not affected much by the antibiotics used (Cameron and Bohrer (7); Adams, Ayres and Tischer (1); Burroughs and Wheaton (5)).

It has later been demonstrated (Leblanc, Dewlin and Stumbo (17); Lewis, Michener, Stumbo and Titus (19)) that certain antibiotics reduce the heat resistance of spores of Cl. botulinum and Cl. sporogenes at normal processing temperatures. However a dependable commercial process does not seem to have been established.

The existing evidence thus indicates that it would be dangerous to use antibiotics as a substitute for heat treatment of canned foods. Certain antibiotics might, however, possibly be used to control some very heat resistant spoilage bacteria ("flat sours" and thermophilic clostridia) which cannot be killed unless the heat treatment is so severe that the quality of the canned product is much damaged. (Hawley (12)).

~~The presence of~~ Faecal streptococci are important spoilage organisms in canned pasteurized hams (Ingram and Hobbs (15); Goldenberg, Sheppey and Robson (11)). They are able to multiply surprisingly fast even at 40-41°F (Goldenberg, Sheppey and Robson (11)), and not published observations by Hessen (13) at The Meat Product Laboratory (Copenhagen) indicate that their multiplication in meat products is not completely inhibited by sodium chloride until the "brine concentration" (i.e. salt-content/

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(salt + water-content)) increases above 10 %. Furthermore the faecal streptococci are very heat resistant. Goldenberg, Sheppey and Robson (11) found that 167°F for some 60 minutes were required to destroy 10^9 faecal streptococci in ham.

This is a more severe heat treatment than common commercial processes resulting in an internal temperature of 154-160°F (Niven (20); Lerche (18)).

Although the initial load of streptococci in a ham is always much lower than the numbers applied by Goldenberg, Sheppey and Robson (11) in their experiments it must be expected that faecal streptococci in ham sometimes survive commercially used processes. That this is the case, , is indicated by a number of publications (Ingram and Hobbs (15); Goldenberg, Sheppey and Robson (11), Lerche (18) and Rievel (21)) and also by more recent observations by Hessen (13) who found that provided preincubation at 86°F was used faecal streptococci could be demonstrated in a rather high proportion of pasteurized hams canned in a number of European countries and in U.S.A.. No other bacteria except for a few bacilli were found. This observation is in agreement with non published experiments in our laboratory showing that a small fraction of cells in cultures of faecal streptococci has a much higher heat resistance than the majority and that surviving cells even if their number is low may eventually grow out if the ham is kept long enough at ordinary storage temperatures (about 40°F).

Almost all other bacteria in pasteurized ham are either killed during the normal processing of the ham such as salmonella (Clarenburg (8)) or kept in check by the salt content and/or the low storage temperature (40°F).

The purpose of the experiments to be described was to test the effect of chlortetracycline on faecal streptococci in chopped ham processed at 150°F for different times and stored under refrigeration (50°F).

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Experimental procedure

Bacterial strains

30 strains of faecal streptococci were used in a preliminary experiment on their sensivity of chlortetracycline. The strains were isolated from a variety of meat products including canned ham. By identification along the lines given by Barnes, Ingram and Ingram (4) 18 strains were found to be Streptococcus faecium and 12 Streptococcus faecalis.

Chlortetracycline

Acronize and chlortetracycline hydrochloride from Lederle Lab. Div. American Cyanamid Co. were used throughout the experiment.

Determination of CTC

Determinations of CTC was carried out by the method described by Kohler and Abbey (16).

Sensitivity to chlortetracycline in tomatobroth

The strains were grown for 24 hrs. in tomatobroth (Sharpe (22)) at 37°C. 0,01 ml was inoculated in tomatobroth to which was added 5, 2, 1, 0,5 and 0,25 ppm chlortetracycline. The lag phase at 37°C was evaluated by nephelometri using an EEL nephelometer. Strains with different sensitivities were selected for experiments with chopped ham.

Effect of CTC on the growth of 4 strains of faecal streptococci in cured, raw ham at temperatures from 39 to 122°F.

Two of the most sensitive and two of the most resistant strains from the preliminary experiment in tomatobroth were selected for experiments with chopped ham.

The strains were Str. faecalis 41 and 129 (isolated from canned hams) and Str. faecium no. 23 (isolated from a canned ham) and 238 (isolated from tank pickle).

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The details in the procedure was as follows.

A cured and drained raw ham was obtained from a commercial company. The ham was dipped for 1 minute in boiling tapwater and stored overnight at 32°F. The ham was then trimmed and deboned under very clean conditions and chopped through a sterile meat chopper. To each gram of chopped meat was added approcimately 10^6 cells of each strain from a 24 hrs micro ham culture (Buttiaux and Beerens (6)) grown at 98,6°F. The inoculum was mixed thoroughly into the chopped meat in a sterile master mixer (Tasso mixer). The meat was packed in sterile thermal death time cans (each holding approx. 25 grams). The cans were seamed and incubated in a thermostatically controlled waterbath at 39,2°; 68; 98,6 and 122°F. The temperature of the water did not vary more than 0,2 °F from the stated values. At intervals cans were drawn from the waterbath and cooled in icewater. Samples of the meat in the cans were immediately mixed with sterile 0,9 % saline in a waring blender and dilutions in 0,9 % saline were plated on thallium acetate tetrazolium agar (Barnes (3)).

Effect of CTC on Streptococcus faecalis no. 35 in chopped pasteurized ham

The experiment was carried out as a three factor experiment in three blocks with each factor at three levels. The factors were (i) Concentration of CTC (0; 0,5 and 5 ppm) (ii) Storage time at 40°F after the addition of streptococci and CTC to the chopped ham but before pasteurization (1, 4 and 14 days); (iii) Pasteurization time at 150°F (30, 90 and 270 min.).

The details in the technique were as follows.

A ham which had been cooled overnight after slaughter was dipped for one minute in boiling water. It was then deboned and trimmed under as aseptic conditions as possible. The ham was then chopped in a sterile meat chopper. The lean chopped meat was transferred to the sterile container of a Tasso mixer and approximately 10^6 cells of Str. faecalis was added per gram of meat from a 24 hrs culture grown in micro ham at 98,6°F. 3 % (w/w) sterile NaCl, 100 ppm $NaNO_2$

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(from a sterilized solutinn) and the required amounts of CTC (dissolved in sterile destilled water) was then added. These additives were then mixed into the chopped meat for 20 minutes. The meat was hereafter packed in sterile thermal death time cans (each holding 25 grams). The cans were seamed and kept at 40°F until pasteurization took place.

The pasteurization was performed in a thermostatically controlled waterbath, the temperature of which varied within 150 ±0,2°F. The cans were cooled in running tapwater (about 54°F) for ½ hour and then incubated at 50°F for 22 weeks.

Counts of faecal streptococci were made by surface plating on thalliumacetate tetrazolium agar as above or by dilution technique.

Heat resistance of Str. faecalis no. 35 after a short exposure to high concentrations of CTC

A. A culture grown 24 hrs at 98,6°F in 2 liters of tomatobroth was centrifuged, and the cells were suspended in 20 ml of sterile tomatobroth. The resulting suspension contained about 10^{10} cells per ml. Lean meat from a scolded ham was chopped aseptically. 10 ml of the bacterial suspension was mixed with 50 grams of chopped meat to which had been added 500 ppm of CTC. The inoculated meat was kept overnight at 40°F. Thereafter the CTC was "diluted" away by mixing samples of the meat in a Tasso mixer with chopped meat containing no CTC or streptococci. The same decimal dilution principle as when making bacterial counts was used and the final mixture of meat contained per gram only 10^{-4} grams of the original meat with its content of CTC and streptococci. This means that the concentration of CTC dropped from 500 ppm to 0,05 ppm and the numbers of streptococci was decreased from 10^{9} to 10^{5} per gram.

The meat had then 3 % NaCl and 100 ppm $NaNO_2$ added and was packed in sterile thermal death time cans which were stored for 3 days at 40°F and processed at 150°F for 5, 10, 20, 40 and 80 min. The pasteurized cans were kept for 1 and 7 days at 50°F and surviving streptococci were determined by plating on thalliumacetate tetrazoliumagar. Extra cans were kept 24 hrs at 86°F before they were opened and their content plated on thalliumacetate tetrazoliumagar.

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B. An experiment was made simultaneous with and similar to A but without the addition of CTC.

Results and discussion

Sensitivity to CTC in tomato broth

The preliminary sensitivity test in tomato broth showed an increasing lag period with increasing CTC concentration. 1 ppm prolonged the average lag phase from 0,34 days in the control tubes to 4 days. Inactivation of CTC took place during incubation and from the inactivation curves it could be judged that logarithmic growth on an average started at the time when the CTC concentration had dropped to 0,05 - 0,1 ppm. There was no significant difference in sensitivity between the two groups of Str. faecalis and Str. faecium. There was, however, differences between the individual strains. The most resistant ones passed into logarithmic growth after 2 days in the presence of 1 ppm CTC and the most sensitive were inhibited for more than 5 days by the same concentration. When the concentration of CTC was 1 ppm or above not only the lag phase was influenced but the total number of cells during the stationary phase was decreased considerably, when followed over a period of 5 days. Two of the most resistant strains (showing a lag period of 2 days in the precense of CTC) and two of the most sensitive strains (showing a lag period of 5 days or more) were selected for experiments with chopped raw ham held at temperatures from 39 to 122°F.

Effect of CTC on the growth of 4 strains of faecal streptococci in cured raw ham at temperatures from 39 to 122°F.

No multiplication took place at 39°F for 144 hrs whether CTC was added or not. At the higher temperatures CTC inhibited the growth completely; 1-2 ppm of CTC had almost the same effect as 40 ppm. The inhibitory action of 1 ppm of CTC is shown in fig. 1 and 2. It is seen that CTC at 122°F also had some killing effect on the cells during prolonged incubation.

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Effect of CTC on Str. faecalis no. 35 in chopped, pasteurized ham

Bacterial cells and CTC was added to the raw chopped ham together with salt and nitrit and the ham was kept at 40°F for 1, 4 and 14 days before pasteurization. During the longest incubation time the count dropped a little (significant at 97,5 % level) but there was no demonstrable effect of CTC. See table 1.

Table 1. Mean bacterial counts before pasteurization (log streptococci per gram of meat)

Preincubation at 40°F / CTC added	1 day	4 days	14 days
0	6,37	6,42	6,29
0,5 ppm	6,40	6,38	6,29
5 ppm	6,38	6,43	6,32

As shown in table 2 the concentrations of CTC did not drop much during the preincubation of the raw ham at 40°F. -

Table 2. Concentrations of CTC in ppm during preincubation of raw cured ham at 40°F

Preincubation at 40°F / CTC added	1 day	4 days	14 days
0,5 ppm	0,430	0,384	0,330
5 ppm	3,64	4,25	3,89

5 ppm of CTC decreased the survival of streptococci during heat processing.

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Table 3. Log surviving streptococci per gram during pasteurization of ham

Amount of CTC added / Heating time at 150°F	0	0,5 ppm	5 ppm
30 min.	3,75	4,17	3,21
90 "	0,40	1,04	0,34
270 "	-0,18	-0,21	-0,25

Table 3 shows the effect which is not very pronounced. 0,5 ppm of CTC does not seem to enhance the killing during pasteurization. There was no effect of the storage time before the pasteurization on the survival.

Table 4 shows the destruction of CTC during the pasteurization. In most cases no CTC could be detected in hams to which had been added 0,5 ppm. The figures in the table reflect the fact that the concentration of CTC decreases during storage at 40°F before pasteurization.

Table 4. The destruction of CTC during pasteurization of ham

CTC added	Storage at 40°F after addition of CTC before pasteurization	CTC found immediately after pasteurization at 150°F for		
		30 min.	90 min.	270 min.
0,5 ppm	1 day	0,19 ppm	<0,1 ppm	<0,1 ppm
	4 days	<0,1	<0,1	<0,1
	14 days	<0,1	<0,1	<0,1
5 ppm	1 day	2,15	1,90	<0,1
	4 days	-	1,28	0,24
	14 days	2,65	1,20	<0,1

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Fig. 4 shows the effect of pasteurization time and CTC on the numbers of streptococci during 22 weeks storage of the pasteurized ham at 50°F. There was no significant changes in the numbers of streptococci during storage when pasteurization had taken place for 90 and 270 min.

It seems, however, that the numbers of survivers after 90 min. pasteurization remain stable on a higher level when CTC was added. The irregularities in the beginning of the storage time might be due to residual amounts of CTC influencing the counts which had to be made by the dilution technique because of the low number of streptococci present. The counts during storage after 270 min. pasteurization have been averaged on fig. 4 because it had no detectable influence whether CTC was added or not.

There was a pronounced influence of CTC during storage when only 30 min. pasteurization had been applied. The number of survivers continued to decrease for about 4 weeks storage when no CTC was added. Thereafter the streptococci multiplied rather fast until their number reached 10^8 - 10^9 per gram of ham. This is a behavior we find regularly when we make heat resistance tests on faecal streptococci in ham. The explanation is not yet known. When 0,5 ppm of CTC was added to the ham the streptococci behaved similarly at the beginning of the storage period but they did not increase in numbers until after 12 weeks of storage. This effect is surprising when it is taken in consideration that no CTC was detectable in these cans after 3-4 weeks storage. When 5 ppm of CTC had been added to the meat there was a tendency to a temporary drop in the numbers of survivers during the first few weeks' storage but the counts remained fairly constant until about 12 weeks of storage. After that time there seemed to be a sligth decrease which continued during the remaining storage period.

The time the ham was kept before pasteurization had a slight effect on the numbers of survivers during storage. The average counts were higher when the ham had been kept for 14 and 4 days after inoculation before pasteurization than when it had been kept for only 1 day. This effect was not detectable when the ham was pasteurized for 270 min.

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When 5 ppm of CTC was added this effect was reversed, so that the lowest counts during storage were obtained in ham kept only one day before pasteurization. These differences in counts, however, did not amount to more than one log cycle.

Residual amounts of CTC

The effects of the time the hams were kept before pasteurization, the length of pasteurization and the storage time after pasteurization were all statistically significant. The interaction between the length of pasteurization and storage after pasteurization could not be regarded significant.

The best estimate of residual CTC during storage can be found from the following equation:

$$X = Y + Z + U$$

Where X is the logaritm of percentage residual CTC

Y represents the pasteurization time; $Y_{30\ min} = 0,1294$

$Y_{90\ min} = -0,1294$

Z represents keeping time before pasteurization; $Z_{1\ day} = 0,0235$

$Z_{4\ days} = 0,0505$

$Z_{14\ days} = -0,0740$

U represents storage time at 50°F after pasteurization

Storage in days	U
0	0,5373
1	0,4547
8	0,3897
15	0,3887
22	0,3128
36	0,1250
57	-0,1772
84	-0,2975
91	-0,4740
120	-0,6137
148	-0,6457

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Residual amounts of CTC of 12 weeks of storage when the length of pasteurization was 90 min. and CTC was added 14 days before pasteurization is thus given by

$$X = -0,1294 - 0,0740 - 0,2975 = -0,5009$$

antilog X = 0,32 % af the amount of CTC added

When these results are considered it must be born in mind that the situation would be quite changed when whole hams are pasteurized. Due to the slow heat penetration the different parts of the ham is exposed to heat treatments which vary very much in severeness. We have found during not published experiments that 90 % of CTC is destroyed in ham during heating at 72°C for 60 min. and in 150 min. at 150°F. A rough estimate of the destruction of CTC in a 6 kg ham heated at 72°C until the geometrical center reach a temperature of 150°F for 30 min. gives as a result that 10-20 % of the added CTC must be present after pasteurization.

When the results are compared with the destruction rate of CTC in raw beef (Weiser, Goldberg, Cahill, Kunkle and Deathrage (23)) it seems that CTC is considerably more stable in cooked ham than in raw beef.

Heat resistance of Str. faecalis no. 35 after a short exposure to high concentrations of CTC

Fig. 6 ~~and 7~~ shows the survival curves for streptococcus faecalis which had been kept at 40°F overnight in meat containing 500 ppm of CTC and then for 3 days in meat containing 0,05 ppm of CTC. The survival curves did not differ from the curves obtained for streptococci which had not been exposed to CTC. However, when the pasteurized cans were incubated at 86°F overnight there was a more rapid multiplication of streptococci in the control cans. This was especially pronounced when the cans after pasteurization were kept for 7 days at 50°F before the incubation at 86°F ~~(see fig. 7).~~

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The deviating shape of some of the survival curves after 10-40 min. heating cannot be explained but they are often found when heat resistance test are made on faecal streptococci.

Acknowledgements

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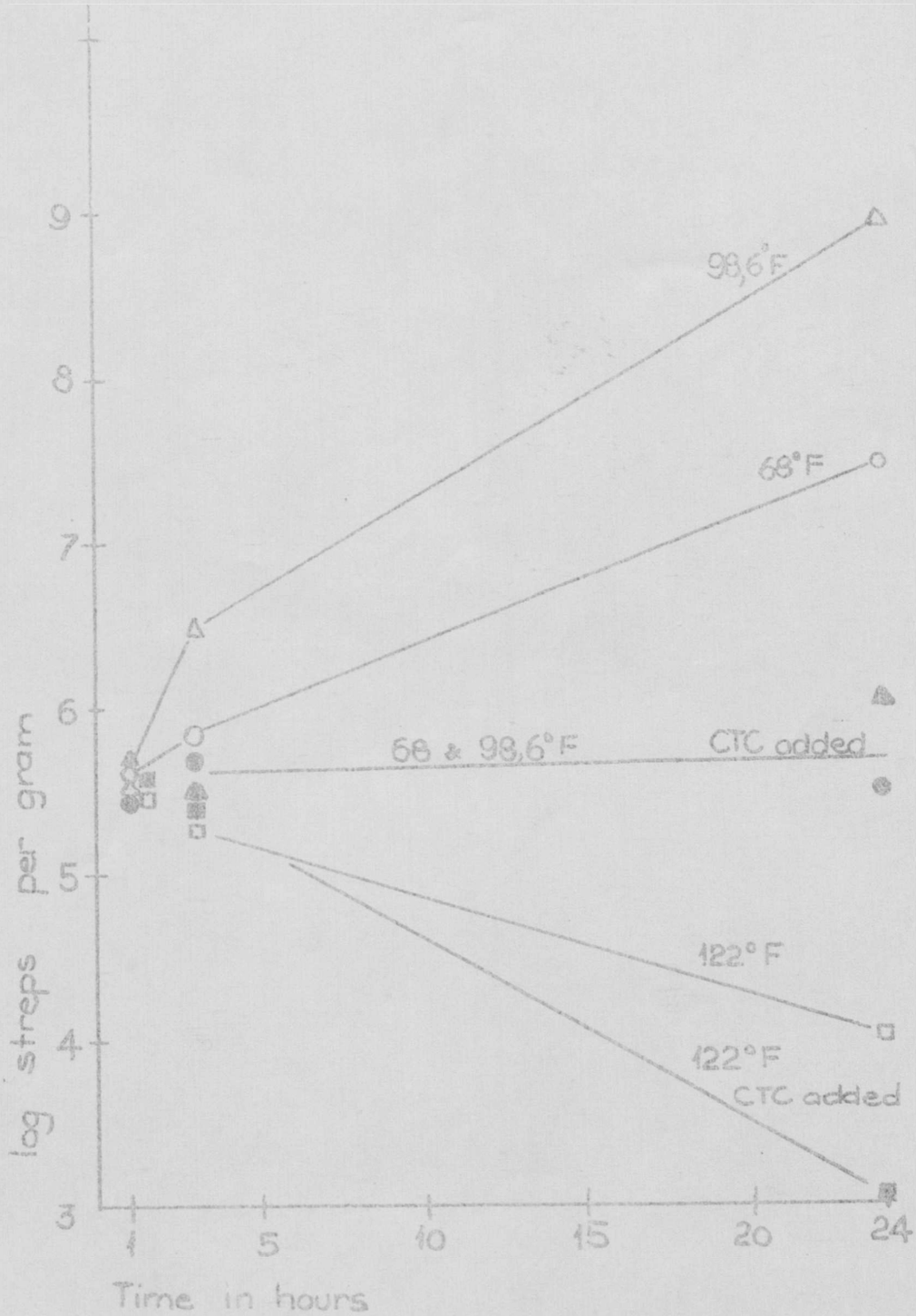

Multiplication of Str. faecalis no 129 in cured raw ham without and with 1 ppm CTC

Fig. 1

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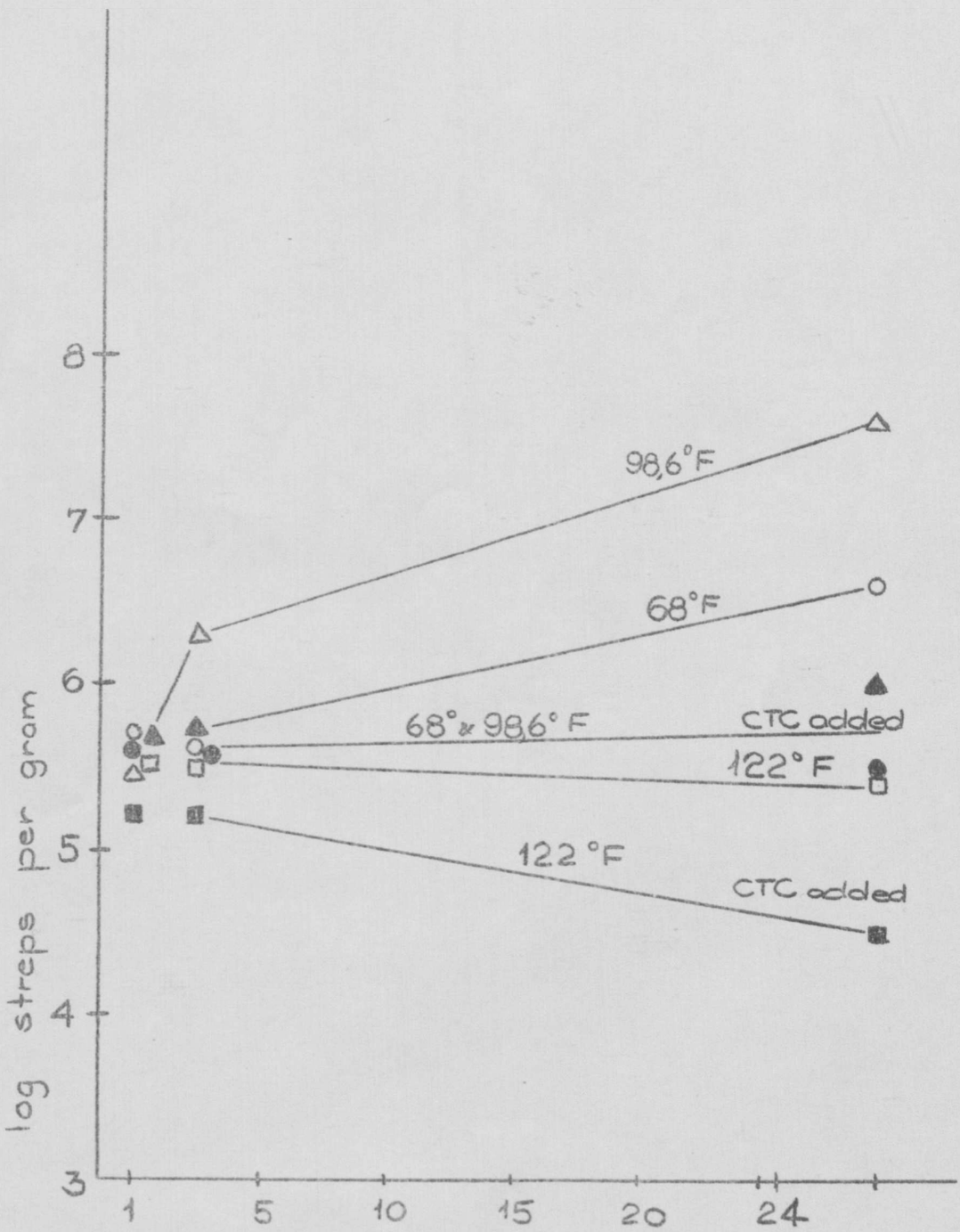

Multiplication of Str. faecium no 73 & 238 in cured raw ham without and with 1 ppm CTC (Average curves)

Fig 2

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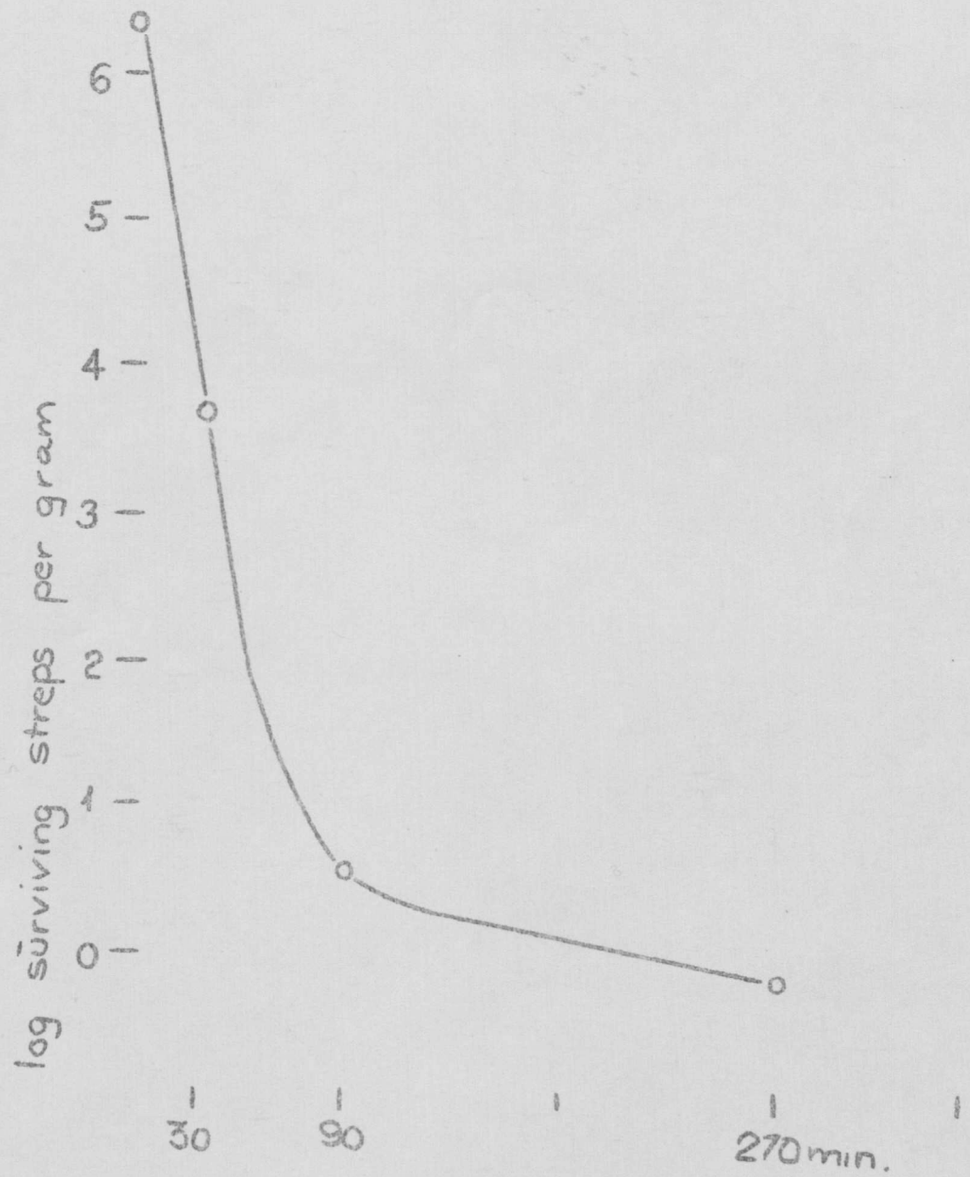
During pasteurisation of the ham at 150°F there was a ripidly increasing kill of the cells but the order of death was not strictly semilogaritmic because a small fraction of cells showed an increased resistance. This is shown on fig. 3.



Survival of Str. faecalis no 35 in ham

Fig. 3.

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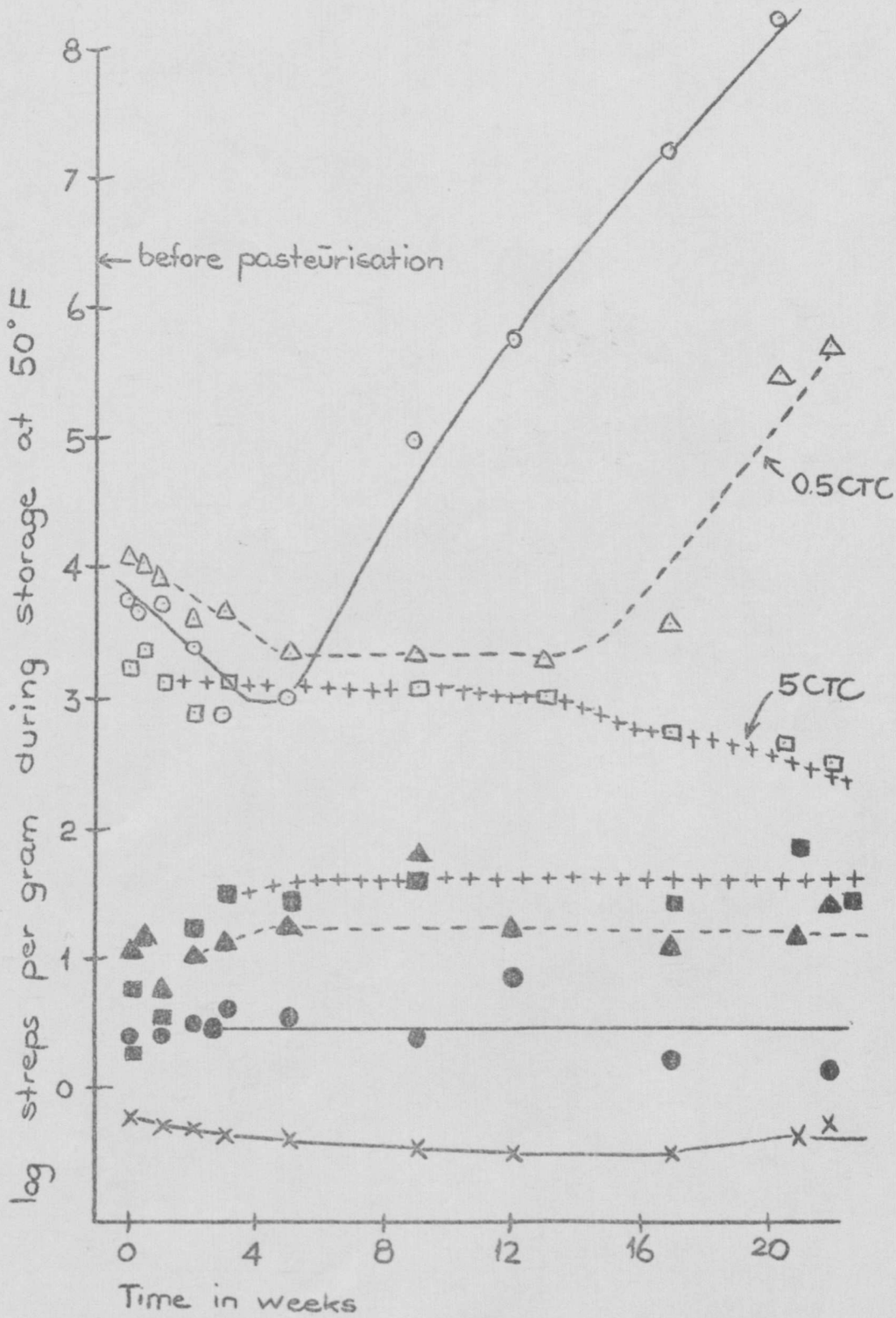
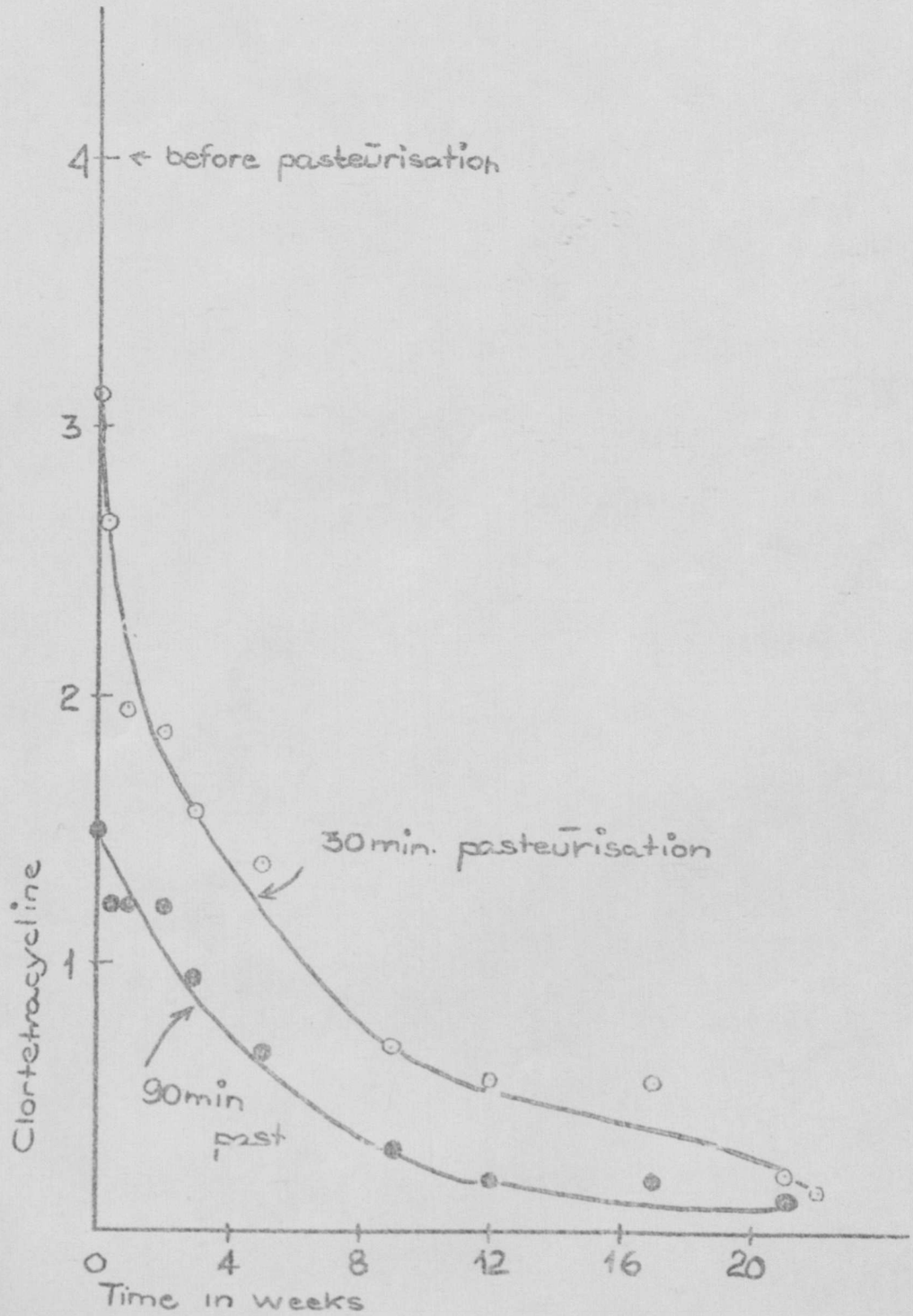


Numbers of streptococci in ham stored at 50° F after pasteürisation at 150° F. Open signs = 30 min. pasteurisation. Closed signs = 90 min. pasteürisation. Crosses: 270 m. pasteurisation (averaged values) Circles: No C.T.C. Triangels: 0,5 p.pm C.T.C. Squares: 5 ppm. C.T.C.

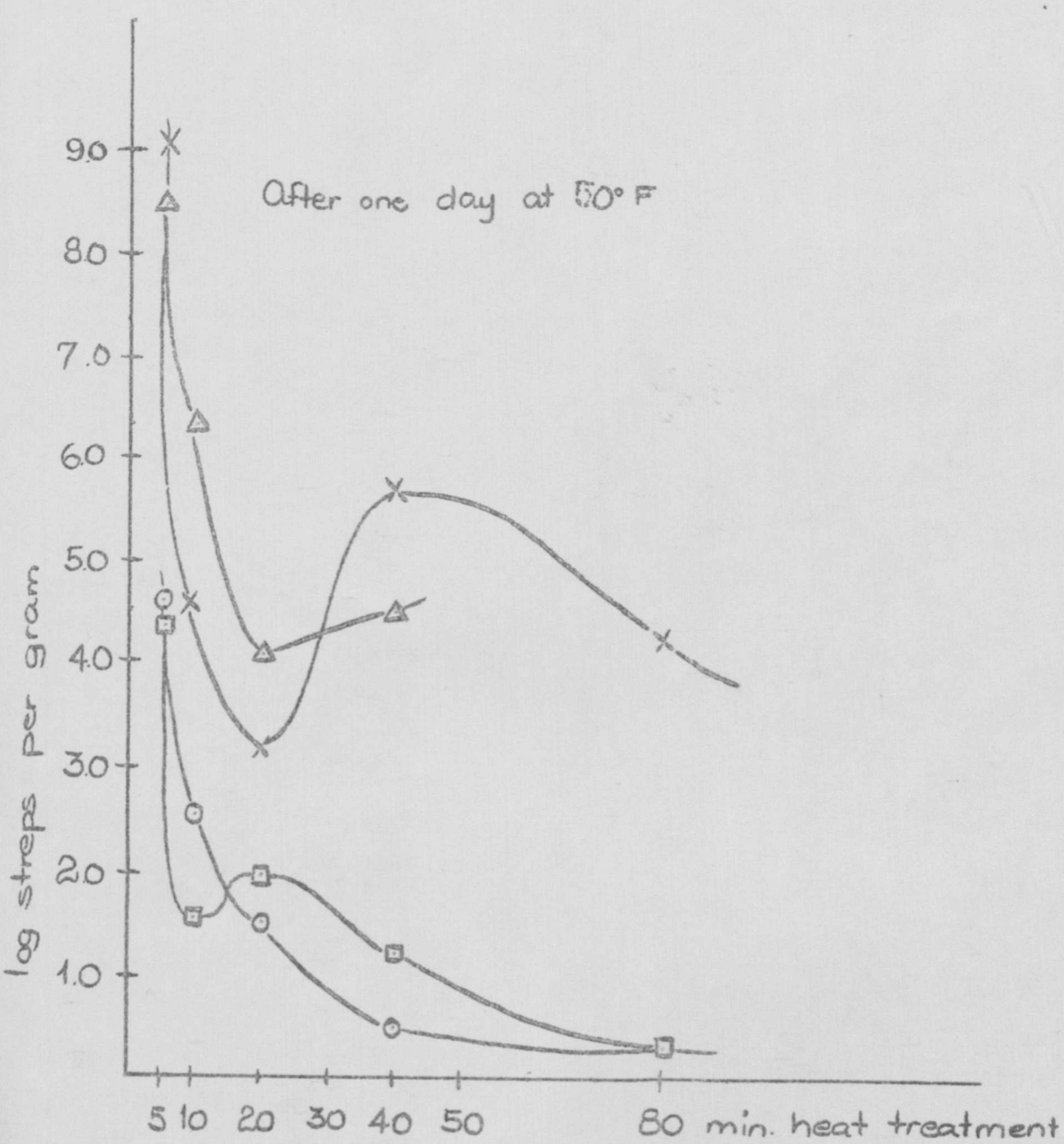
Fig. 4.

The dissappearance of CTC during storage is shown in fig 5. Only the curves for 5 ppm of CTC are shown because no detectable amounts of CTC was found after a few weeks storage when initial amounts was 0,5 ppm



Decrease in CTC during storage of pasteurised ham at 50° F

Fig. 5



Survival and growth of Str. faecalis no 35 in ham pasteurised at 50° F.

⊡ Streptococci exposed to CTC before pasteurisation } counts made on cans stored one day at 50° F after pasteurisation

○ Streptococci not — — — — }

× Streptococci exposed to CTC before pasteurisation } counts made on cans which were stored after pasteurisation for one day at 50° F and overnight at 86° F

△ Streptococci not — — — — }

Fig 6.