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Influence of pH on proliferation of Streptococcus lactis bacteriophage

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INFLUENCE OF pH ON PROLIFERATION OF *STREPTOCOCCUS LACTIS* BACTERIOPHAGE

by

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INTRODUCTION

The production of lactic acid by microorganisms is of great importance in the dairy industry. The various types of cheese, particularly cheddar and cottage, cultured buttermilk and cultured cream all depend upon the production of lactic acid by bacteria. The organisms primarily responsible for this acid production are the lactic streptococci. Variations in the ability of these organisms to produce acid result in an inferior product.

One of the principal causes of slow acid production by lactic streptococcus cultures is bacteriophage. The control of bacteriophage in dairy plants has largely been through selection of cultures, rotation of cultures, strict cleaning and sterilizing of plant equipment and following procedures which minimize the number of bacteriophage particles that may gain entrance from the air into milk and milk products. Despite observing all of these precautions, failures of lactic cultures due to bacteriophage in dairy plants still occur.

The final solution to the bacteriophage problem can come only through an extensive fundamental approach to the problem. An understanding of the fundamental relationships between bacteriophage and host organism then will give a basis for practical approach to the solution of the problem. It is believed that a study on the influence of pH on proliferation

of streptococcus bacteriophage may lead to a partial elucidation of these relationships.

STATEMENT OF PROBLEM

The purpose of the work reported herein was to study the influence of pH on the proliferation of several strains of lactic streptococcus bacteriophage.

REVIEW OF LITERATURE

Twort (1915) made the first observation of a transmissible lytic substance in bacterial cultures. This phenomenon was encountered independently by d'Herelle (1917), who developed a theory to account for the action of the lytic agent, which he termed "bacteriophage." Later d'Herelle (1926) gave an elaborate presentation on bacteriophages and their behavior. Since that time there have been innumerable accounts of bacteriophages active against many species of bacteria. No attempt will be made in this work to review all the material that has accumulated. Much of it, of course, would have no bearing on the present work and the object of this review is to cover only the more pertinent literature.

There are a number of review articles on bacteriophages but unfortunately most of them contain no information on the bacteriophages of the lactic streptococci. For those interested, the reviews of d'Herelle (1926), Hadley (1928), Burnet (1934), Krueger (1936), Delbrück (1942) and (1946) and Fong (1949) may be consulted.

The first report of bacteriophages active against the streptococcus group was that of Hadley and Dabney (1926). The first indication of bacteriophages active against lactic streptococci as used in the dairy industry was in New Zealand. Working in a cheese factory, Whitehead and Wards (1933) observed

failure in activity of a cheese starter transferred from one large can to another. After the first failure in the factory, similar results were obtained with the mother culture of the laboratory upon transferring to large cans in the factory. Failure in activity usually occurred in the second generation. Later Whitehead and Cox (1934) described cases of sudden failure in activity of cheese starters and explained this failure as due to aeration of the milk immediately before inoculation with certain cultures. Actually it was not until 1935 that Whitehead and Cox (1935a) referred to this failure as due to bacteriophage.

In the United States the Iowa Agricultural Experiment Station Report on Agricultural Research (1933) reported that when small amounts of a butter culture that coagulated highly pasteurized or sterile milk very slowly were added to heated milk, the coagulation of this milk by normal butter cultures was greatly delayed. This restraining agent was capable of passing through a bacteria-proof filter and could be destroyed by boiling the filtrate containing it.

Harriman (1934) reported that the slowness of coagulation of certain butter cultures under practical conditions was not due to the source of the milk, the organisms naturally occurring in the milk or contamination from plant equipment. He also showed that bacteria-free filtrates made from the slow cultures were capable of restraining butter cultures when present in amounts as small as one part of filtrate to 20 million parts of milk.

Nelson (1936) and Nelson *et al.* (1939) showed that the inhibitory principle active against *Streptococcus lactis* had the characteristics commonly associated with bacteriophages. This inhibitory principle multiplied on sensitive strains of *S. lactis* and segregation of individual strains of the inhibitory principle was possible. These workers also showed that under proper conditions this principle caused lysis of sensitive organisms.

Since 1939, the major portion of the investigations on lactic streptococcus bacteriophage has been centered in New Zealand, England and in the United States, primarily at the Iowa Agricultural Experiment Station. The Dairy Research Institute, Palmerston North, New Zealand was one of the early leaders in this field and it might be well to review first the work from that institution on the lactic streptococcus bacteriophage.

The early work of Whitehead and Wards (1933) and Whitehead and Cox (1934) describing cases of sudden failure of starter cultures already has been mentioned. Other incidences of sudden culture failure are described by these authors (1935b) and bacteriophage was indicated as being responsible. Whitehead and Cox (1936) recognized the presence of bacteriophage in a starter culture during an investigation of failure supposedly due to aeration. At this time these workers demonstrated plaque formation by the bacteriophage on a yeast whey agar. The bacteriophage was purified and whey filtrates of high titer were

prepared. From this work they found that the optimum temperature for bacteriophage proliferation was about 30° C.; however, proliferation occurred at 20 and 37° C., but more slowly. Likewise they noted the strict specificity of the streptococcal bacteriophage and that it maintained this specificity for the one particular strain of Streptococcus cremoris on which it was active. Hunter (1939) found a marked difference between individual organisms within a pure culture of S. cremoris, as well as variation between cultures, to bacteriophage attack. Attempts by Whitehead and Cox (1936) to produce immune forms from secondary growth organisms did not result in a permanent immunity. Later Whitehead and Hunter (1939a) concluded that streptococcal bacteriophages were common in the cheese making industry and they were able to isolate nine distinct bacteriophage "races" from cheese cultures.

With the increased number of culture failures due to bacteriophage, Hunter and Whitehead (1940) tested a number of chemical disinfectants, to determine time of inactivation of the bacteriophage, as a means of control. Various disinfectants were tried and chlorine was found to be the best suited, less than 1 minute being required for complete inactivation of bacteriophage in the presence of 0.05 per cent chlorine. They also noted that the effect of hydrogen ion was negligible over several days at room temperature when the pH was between 4 and 7.

It was a common practice in New Zealand at this time to inoculate starters at the rate of 0.2 per cent. Whitehead and

Hunter (1939b) found that by increasing this inoculum of single strain cultures to 1.0 to 1.6 per cent the activity of the culture could be maintained from day to day for long periods of time.

Further attempts to eliminate failure of cultures due to bacteriophage action led Whitehead and Hunter (1941a) to discover that the atomization of whey from the whey separator was responsible for many of the spontaneous outbreaks of bacteriophage. From these findings attempts to eliminate air borne bacteriophage led to the use of chlorine sprays, protection of the cultures in the factory and the recommendation of separate rooms, if not separate buildings, for the preparation of the mother culture.

Hunter (1943) found wide differences in optimum temperatures for various strains of bacteriophage attacking strains of S. cremoris. He found that some strains of bacteriophage would be completely inhibited at 37° C., while others would lyse the culture in 5 hr. at 22° C. In general, he concluded that it would be possible to get acid production in the cheese vat if multiple strain cultures were used, even though some of the strains were attacked by bacteriophage.

Later Hunter (1944) found that the evidence of bacteriophage in the cheese vat during the making process was largely dependent upon the amount of initial infection. With small initial infections of bacteriophage in the vat, he was still able to make a good quality cheese. However, with higher concentrations of bacteriophage, the cheese was definitely

"second grade", often having a fermented, yeasty flavor and a weak, pasty body.

Whitehead and Hunter (1941b, 1946) reviewed the work in New Zealand on bacteriophage infection in the cheese industry and concluded that it was the most frequent cause of slow acid development in the cheese vat. These failures could be caused by a gradual build up of bacteriophage concentration in the factory or by improperly sterilized factory or farm equipment (Whitehead and Hunter, 1947) coming in contact with the cheese milk. They advocated strict cleaning and sterilizing and indicated that the possibility of destroying every trace of bacteriophage in a commercial operation was quite remote.

The history of bacteriophage in the cheese making industry of England is not as dramatic as in New Zealand. This is primarily due to the types of cultures that were used in the two countries. In New Zealand single-strain lactic streptococcus cultures were used and an attack of bacteriophage frequently prevented the use of that particular lot of milk for cheese making. In England it was more common to use multiple-strain cultures and thus the cheese maker experienced only slowness in acid development.

During the cheese making season of 1940-41 Meanwell (1941) and Harrison and Wolf (1941) reported the slowness of multiple-strain cultures in England to be due, at least in part, to bacteriophage. Meanwell (1941) suggested the use of a different culture when slowness in the cheese vat was encountered and

stated that every precaution should be taken to avoid contamination of the starter milk. Nichols and Wolf (1942) likewise demonstrated that bacteriophage was responsible for the slowness in the cheese vat during the cheese making season, especially during May and August.

Two cases of slowness were studied by Harrison and Dearden (1941) to determine the possible cause. In each case multiple-strain cultures were in use and the changing of the starters eliminated the trouble. Attempts to isolate bacteriophage were not successful but they did not feel that bacteriophage was completely excluded as the cause of the slowness.

Anderson and Meanwell (1942), investigating the "May slowness" in cheese making, attributed it to bacteriophage. The slowness was found to occur where both single-strain and multiple-strain cultures were used; however it was much more serious where single-strain cultures were used. They indicated that the bacteriophage action was not always specific and some of their whey filtrates were active against more than one strain of the starter organisms. These workers found that a bacteriophage active against one strain of a multiple-strain culture may prevent the growth of the non-susceptible strain although no bacteriophage active against the non-susceptible strain could be isolated from the culture. This particular phenomenon was termed "nascent" bacteriophage activity. However, Nichols and Wolf (1945a) found that this phenomenon was not common in the starter strains studied, but they indicated that it might be used to demonstrate fundamental relationships between strains.

In additional studies, Nichols and Wolf (1944) found bacteriophage to persist for a considerable length of time in cheese known to have shown slowness in the vat and suggested that the infection easily could be carried over from one cheese making season to another. These workers also studied the heat resistance (1945b) of the bacteriophages of cheese starters in milk and found that usually the bacteriophages did not survive 75° C. for 7.5 min. They also showed the practical implications of this in the preparation of milk for starter making.

Nichols (1945) surveyed the incidence of "slowness" and its connection with bacteriophage in England and Wales and outlined methods of control of bacteriophage in the factory. The best method of control was to keep the bulk starter free from bacteriophage contamination. This could be accomplished by destroying air-borne bacteriophage, not allowing milk to stand over night in the vats, thorough cleaning of the vats and rinsing with chlorine solution immediately before use and disinfecting walls, ceilings, floors and drains with chlorine twice weekly during the actual cheese making season. The use of a special starter room also was recommended.

Nichols and Ineson (1947) showed in cheese making experiments that when the dominant strain of a multiple strain starter was lysed by bacteriophage action that slowness occurred but the other strains would take over the acid development and the starter would "recover".

Nichols and Hoyle (1949) recently have attempted to classify the lactic streptococci by their particular bacteriophage patterns. From their work they grouped the lactic streptococci into eleven bacteriophage sensitivity types. The majority of the bacteriophage strains used in the typing were classified by means of anti-phage sera into three groups. It was hoped that these relationships would lead to a basis for selection of various organism types to be used in cheese starters, especially where several different cultures were carried in each plant and rotation of cultures was used as a means of bacteriophage control.

Johns and Katznelson (1941) were the first to report difficulty with bacteriophage active against a cheese starter in Canada. In a particular case cited by these workers, a multiple strain culture that was attacked experienced an abrupt stoppage of acid production. This cessation was as abrupt and complete as if a single strain culture had been used. At this time they felt that many of the so called "starter failures" were due to the action of bacteriophage, as well as many of the reported cases of slow acid development in the cheese making process.

Further reports by Johns (1942) indicated that the bacteriophage was present in the milk just prior to pasteurization. The bacteriophage could not be demonstrated in small samples from individual cows, but 100 ml. samples of the mixed milks were positive. He also reported that in two

plants having trouble with lack of acid production at the cooking temperature (96-100° F.) the cause was the lysis of the dominant strain of the multiple-strain starter.

The early work on lactic streptococcus bacteriophage by Harriman (1934), Nelson (1936) and Nelson et al (1939) has already been mentioned in this review. Following this work there was a considerable delay in the United States before a great deal of further research on this subject was reported in the literature.

Babel (1946) reported the effect of bacteriophage on the cheese making process. He showed that slow acid production due to the presence of bacteriophage usually was apparent at the time of draining the whey or shortly thereafter. The presence of a significant amount of bacteriophage in the cheese milk resulted in an almost complete cessation of acid production when either a single strain or a multiple strain culture was used. Greene and Babel (1948) studied the effect of ultraviolet light on bacteriophage and concluded that irradiation would be of little value in a commercial operation for the destruction of bacteriophage active against S. lactis.

Parmelee et al. (1949) studied lactic streptococcus bacteriophage with the aid of an electron microscope and found the particles to be sperm-shaped and to have an overall length of 220 mμ. The diameter of the head was 70 mμ. and the tail was 30 mμ. wide and 150 mμ. long. Nine different

strains were studied and the size and shape was so nearly alike that differentiation could not be made on this basis.

Collins (1949) found that soluble calcium was necessary for proliferation of a number of strains of bacteriophage active against the lactic streptococci. In his work he found that the calcium could be made unavailable in a defined medium by autoclaving the calcium in the medium or increasing the K_2HPO_4 content to above 0.1 per cent.

Using 49 strains of organisms and 64 bacteriophage strains, Wilkowske (1949) grouped the bacteriophages into seven groups on the basis of their cross-reactions. He also studied plaque size and temperature of inactivation and found considerable variation in both for the different strains of bacteriophage.

Cherry and Watson (1949a), working with only one strain of S. lactis, 122-4, and its homologous bacteriophage, found that lysis took place at pH 6.0, 7.0 and 8.0, but the latent period of virus growth was extended by about 10 minutes at pH 8.0. These workers suggest that pH 5.0 is about the critical level below which lysis does not occur. They also indicated that neutrality was near the optimum for virus adsorption and that adsorption fell off somewhat on either side of neutrality but on the alkaline side the decrease was slow. These workers (1949b) also found that tryptone increased adsorption in a yeast extract glucose medium and that certain electrolytes stimulated cellular lysis as well as virus adsorption.

There are a number of reports from other countries of bacteriophage active against the lactic streptococci. In some cases no actual isolation and characterization of the bacteriophages was accomplished, and in general their work is not as far advanced as that reported from England, New Zealand, the United States and Canada. Maze (1937) in France, Novák (1949) in Czechoslovakia, Yakovlev (1939) in Russia, Mosimann (1946) in Switzerland, Pette (1946) in Holland, Overby (1949) in Denmark and Csiszár and Gura (1949) in Hungary all have reported on some phase of lactic streptococcus bacteriophage, showing the wide distribution of it in the dairy industry.

There are a few reports in the literature regarding the influence of pH on bacteriophages other than those active against the lactic streptococci. These are included, as a matter of interest, but correlation between these and the present work could not be expected because of the individual differences in the species of organisms involved.

Dickinson (1948) found that the bacteriophages active against Pseudomonas pyocyanea were stable at pH 6.5-10.0 but not below 6.5. Also she reported that at pH 2.2-4.0 with phosphate citrate buffer the bacteriophage was inactivated within 3 hr. Wahl and Emerique (1947) reported that the dysentery bacillus bacteriophages C_{16} and S_{13} had a very low critical pH of about 4.0 and that inactivation was very rapid with an increase in the hydrogen ion concentration.

Krueger and Fong (1937), in their work with Staphylococcus aureus, found that by increasing the hydrogen ion concentration there occurred an increase in the lag phase of the organisms without a corresponding increase in the lag phase of bacteriophage production. By increasing the alkalinity there was no pronounced change in the curves of organism growth or bacteriophage proliferation. These workers found that at pH 8.5 the lytic threshold was about 1,000 bacteriophage particles instead of 100 to 140 per cell and the time of lysis was delayed.

Gold and Watson (1950) found that pH of the culture was the determining factor in the lysing of Clostridium madisonii by its homologous bacteriophage. Lysis was inhibited at pH 5.1 and below, but not between 5.1 and 7.6. These workers also indicated that pH interfered with penetration of the virus into the cells. Penetration was completely blocked at pH 5.1 or below and at pH 6.8 and above.

EXPERIMENTAL METHODS

General Procedure for a Representative Trial

In order to simplify and clarify this topic of procedure it may be well as a beginning to describe a complete representative trial and then divide the procedure into the various parts and give more detail following this presentation.

In many of the early determinations, in order to have a complete control, four samples were run at the desired pH as a single experiment. The first sample (organism control) was used to check the normal growth of organisms and the pH changes during the test period. The second sample was used to give a check on the organism growth when the pH was maintained at a constant level. The third sample was inoculated with both organisms and bacteriophage and was used to follow the normal population and pH changes when bacteriophage was present with the organisms. The fourth sample contained both organisms and bacteriophage and this sample was maintained at constant pH to determine the influence of pH on bacteriophage proliferation.

At various intervals an aliquot was withdrawn aseptically and used for the determination of pH, organism count and bacteriophage titer. A relatively large portion was necessary for pH measurement and it could not be returned to the original sample, since the electrodes were not sterile. This necessitated an original substratum of 100 to 150 ml.

After several determinations the first and third samples were discontinued because they proved unnecessary for interpretation of the data obtained on the two other samples.

Preparation of Media

The litmus milk used for enumeration of the bacteriophage was prepared by adding enough aqueous solution of litmus to pasteurized skim milk to impart a light blue color. To this was added 10 per cent by volume of filtered V-8 juice.* The entire lot was mixed thoroughly and then dispensed in 15x125 mm. test tubes in approximately 8 ml. quantities. The litmus milk used as the substratum for the organisms and bacteriophage strains at the various pH levels was prepared the same as above, except the V-8 juice was omitted and the medium was dispensed in 6 oz. screw cap bottles in 100 or 150 ml. quantities. Sterilization was accomplished by heating with steam under 15 pounds pressure for 25 minutes.

In the early work with H1, 1-F4 combination, it was found that a suitable medium other than litmus milk would have to be found that could be adjusted to pH levels lower than 5.2. Two broth media were tried, tomato juice-peptone-peptonized milk broth and V-8 juice-peptone-peptonized milk broth. The tomato juice broth gave bacteriophage-organism populations about equivalent to litmus milk. The V-8 broth gave slightly higher

*A combination of eight vegetable juices made by the Campbell Soup Co., Camden, N. J.

bacteriophage populations and for this reason it was selected for use at the lower pH levels. This also led to the fortification with 10 per cent V-8 juice of litmus milk that was used for bacteriophage enumeration. The added V-8 juice seemed to increase the activity of both the organisms and the bacteriophages. This increased activity eliminated a lot of the trouble with soft "sloppy" coagulated curd and made the reading of the titers more precise. With two combinations used, 0.1 per cent $CaCl_2$ was added to the V-8 broth in order to promote optimum bacteriophage proliferation.

The V-8 broth was prepared by taking 400 ml. of filtered V-8 juice, 10 g. peptonized milk and 10 g. proteose peptone no. 3 and making this up to one liter with distilled water. This gave a clear brown-colored broth with a final pH of 4.8 to 5.1 after sterilization at 15 pounds pressure for 25 minutes.

Tryptone-glucose-extract-milk agar was used for all plate counts. It was prepared by using 24 g. Bacto tryptone-glucose-extract agar (TGE agar) per 1000 ml. of distilled water. This was heated in flowing steam until the agar was melted, dispensed in 100 ml. quantities in 6 oz. screw cap bottles, and sterilized at 15 pounds pressure for 20 minutes. Immediately before using, 1 per cent of sterile skim milk was added aseptically.

Bacteriophage-organisms Combinations

The bacteriophage-organism combinations were selected from the Dairy Bacteriology Laboratories collection at Iowa

State College. Since these cultures and bacteriophage strains had been carried for a considerable length of time in the laboratory, they were considered sufficiently pure for this study and no further purification was made. The sources and designations of the bacteriophage-organism combinations are listed in table 1.

Table 1

Sources and designations of bacteriophage-organism combinations

Designation		Date	Source of
Phage	Culture	obtained	phage
F4	H1,1	3-2-45	Slow vat of cheddar cheese, I. S. C.
F24	W2	7-25-45	Slow vat of cottage cheese, I. S. C.
F43	122,1	9-13-47	Slow culture in market milk lab., I. S. C.
F55	573	4-16-48	Mr. E. B. Anderson United Dairies Ltd., Central Laboratory, Wood Lane, London W. 12, England
F56	712	4-16-48	Mr. E. B. Anderson

The filtrate designated F4 and the culture H1,1 were selected at random; many of the first trials were made with this combination. The cross-reaction determinations by Wilkowske (1949), the nutrition study of the organisms on bacteriophage proliferation of Collins (1949) and the S. lactis-bacteriophage relationship studies by Turner (1948) served partially as the bases for the selection of other combinations. Wilkowske (1949) made cross-reaction determinations of 61 bacteriophages against 49 organism strains.

F55 was active against only one organism strain (573), while most of the other bacteriophage strains available showed additional activity against one or more strains. This particular combination was selected because it was different in this respect from all the others. Collins (1949) and Wilkowske (1949) had information on W2-F24 combination and 712-F56 combination and it was felt that additional work on these would be desirable. The 122,1-F43 combination was selected primarily because a whole series of 122 strains are carried in the Dairy Bacteriology Laboratories of Iowa State College and a number of bacteriophage strains are active against them.

Propagation of the Cultures

All the cultures were carried in fortified litmus milk.* Cultures that were being used were subcultured daily and incubated at 32 to 33° C. for 12 to 16 hr. The cultures that were not in immediate use were subcultured every third day and after the incubation time of 12 to 16 hr. they were stored at 3 to 5° C. in a refrigerator. These cultures then were subcultured daily for three days prior to their use.

Preparation and Storage of Bacteriophage

The bacteriophage preparations used were in the form of whey filtrates. These filtrates were prepared by inoculating

*Contained 10 per cent V-8 juice

150 ml. of sterile skim milk in 6 oz. screw cap bottles with 1 ml. of a freshly coagulated culture of the desired organism. The homologous bacteriophage preparation was added at the rate of 0.1 to 1.0 ml., depending upon the titer, and the mixture was incubated for 6 to 7 hr. at 32° C. After incubation, the skim milk was acidified by adding 5.5 ml. of sterile 10 per cent lactic acid to bring about complete coagulation of the casein. This acidified material then was filtered through coarse sterile filter paper under aseptic conditions. Usually this gave an almost clear solution which then was filtered aseptically through a sterile Selas microporous porcelain filter of #03 porosity. This filtrate was free of cells and contained the bacteriophage at high titer. The filtrates were stored at 3 to 5° C. during the period of use. The titers on most of the filtrates would remain 10^6 or above for several months. Bacteriophage F56 was an exception to this and, in order to maintain a high titer, fresh filtrates had to be prepared about once each month.

Measurement of Bacteriophage Activity

The limiting dilution technique used by Krueger (1930), Harriman (1934), Nelson et al. (1939) and Babel (1946) was used throughout this investigation for enumerating the bacteriophage. The bacteriophage titer was expressed as the most probable number of particles per ml., or the logarithm of this number, based upon the limiting active dilution of the material containing bacteriophage.

The limiting dilution method used herein consisted of making 100-fold dilutions of the bacteriophage suspension in sterile distilled water and dispensing 1 ml. and 0.1 ml. quantities into triplicate tubes of litmus milk fortified with 10 per cent V-8 juice. This in effect amounted to a 10-fold serial dilution. These tubes then were inoculated with 1 drop of a 1 to 10 dilution of a freshly coagulated culture of sensitive organisms (Collins, 1949) and (Wilkowske, 1949) and incubated at 32° C. for 12 to 16 hr. At the end of the incubation period, the last tube that did not show the typical acid, reduction or coagulation of the control (litmus milk inoculated with one drop of the diluted sensitive organism) was recorded. The bacteriophage titer then was obtained by consulting McCrady's tables of most probable number, as adapted by Buchanan and Fulmer (1928).

Determination of Organism Counts

In all trials with litmus milk and with V-8 broth an initial plate count of approximately 100,000 per ml. and a bacteriophage titer of about 10^1 was desired (Turner, 1948). The organism count varied to some extent, due to the specific characteristics of the organism. The count on freshly coagulated cultures would be lower with some strains of organisms than with others, presumably due in part to the tendency toward chain formation. However it will be noticed that for each particular strain of organisms the initial count was

relatively constant in all trials. A portion of the initial bacteriophage titer variation was due to the gradual decrease in titer of the filtrates upon storage.

At various intervals during the test period samples were withdrawn for the plate count and the same dilutions were used for determination of the bacteriophage titer. All organism counts were made by the standard plate count method (American Public Health Association, 1948) on tryptone-glucose-extract-milk agar. The poured plates were allowed to solidify and then were incubated at 32° C. for 48 hr. The plates were counted with the aid of a Quebec colony counter.

Determination and Adjustment of pH

All pH values were determined electrometrically with either a Leeds and Northrup glass electrode potentiometer or a Beckman pH meter (glass electrode). Occasionally a pH value was checked on a Leeds and Northrup potentiometer with a quinhydrone electrode and a saturated calomel cell.

The pH of the bacteriophage-organism culture was taken at various intervals by withdrawing 5 ml. portions with a sterile pipette and placing this in a small beaker. To this portion was added an equal volume of distilled water and the pH taken. The water was added in order to make the sample of sufficient size that the ends of the electrodes could be immersed adequately. Several trials showed that the added water did not change the pH of the milk or the V-8 broth.

Ordinarily the litmus milk had a pH of 6.4 to 6.6, after sterilization, while the V-8 broth had a pH of 4.8 to 5.1. A 10 per cent sterile lactic acid solution was used to adjust the pH to lower levels than the original substrata and a sterile 1N NaOH solution was used to raise the pH.

During the test periods the pH was maintained by the addition of 1N NaOH. In the early trials, 0.05N NaOH was added to the 5 ml. portion withdrawn for pH measurement until the desired pH again was obtained. From this, the necessary amount of 1N NaOH needed to return the pH of the remainder of the bacteriophage-organism culture to the desired level was calculated. This was not too satisfactory because of the lag in neutralization of some of the constituents of the media. This technique was improved by neutralizing one bottle of medium, adding one or two drops of NaOH at a time and recording the pH after each addition. This information then served as a guide to neutralization of the regular trials and was equally as successful as the other method. Even though in some trials the pH was determined as often as every 15 minutes, it still was difficult to maintain a fairly constant pH level.

All pH values referred to in results and indicated on the figures are the pH levels of the trial before any adjustment was made. After this pH was determined, the quantity of base necessary to return the pH to the desired level was added and mixed in by agitation.

EXPERIMENTAL RESULTS

Streptococcus cremoris 573-bacteriophage F55 combination

The population changes for this organism-bacteriophage combination at various pH levels are shown in tables 2 through 9 and the data are summarized in figures 1 and 2. At pH 6.5 (table 5) there is maximum bacteriophage proliferation, along with rapid growth of the organisms. When the pH is lowered from 6.5 to 5.4 (table 4) a slight retardation of bacteriophage proliferation is encountered. With continued lowering of the pH, bacteriophage proliferation declines and at pH 5.0 in V-8 broth (table 2) there is no increase in the bacteriophage population after 20 hr. incubation at 32° C.

The bacteriophage proliferation at pH 7.5 (table 6) follows a pattern similar to that at pH 6.5. At pH 7.6 (table 7 and 8) there is a very definite and pronounced decrease in bacteriophage multiplication. The lag phase of bacteriophage proliferation is increased from an hour or so at pH 6.5 to between 6 and 7 hr. at pH 7.6. At pH 8.0 (table 9) there is a definite decrease in bacteriophage population after 18 hr. incubation at 32° C.

With S. cremoris 573-bacteriophage F55 it is of interest to note the sensitivity of the bacteriophage to the higher pH levels. As shown in table 7, the organism population is still increasing at a rather rapid rate at pH 7.6 while the bacteriophage has barely increased after 11 hr. At pH 8.0

the organism population increased but the bacteriophage numbers decreased. There is a marked difference in bacteriophage proliferation rate at pH 7.5 and 7.6, while the organism population changes are very similar at the two reaction levels. Table 8 shows the population changes at pH 7.6 over an 18 hr. period. In this trial the pH fluctuated over 0.3 pH units. This fluctuation came largely after the 15th hour due to the very rapid production of acid by the organisms. Even though the pH got as low as 7.3 several times, the bacteriophage multiplication was retarded considerably.

In trial 1 of table 4, in which two trials are presented for pH 5.4, the organisms were lysed in 13 hours, whereas in trial 2 there was only an indication in 13 hours that the organism numbers were beginning to decrease. On comparison of these two trials it will be noted that from the 6th to 11th hour of incubation the bacteriophage multiplication was more rapid in trial 1 than in trial 2 and this probably accounts for lysis in one trial and not the other. Lysis did not occur in 20 hr. at pH 5.2 (table 3), although considerable proliferation of bacteriophage occurred.

The bacteriophage proliferation at various pH levels is summarized from typical data in figure 1. The slopes of the lines representing the logarithms of the bacteriophage titers at pH levels of 6.5, 7.5, and 5.4 approximately parallel each other. At pH levels of 7.6 and 5.2 there is an increase in the lag phase and the rate of bacteriophage proliferation is

not as great as at pH 6.5.

A summary of the organism counts of typical trials is presented in figure 2. At the extremes of pH the population curves flatten out and a definite decrease in the number of organisms is noted at pH 5.0. The decrease in organism population at pH 5.0 coincides with the lack of bacteriophage multiplication. Ordinarily there is no increase in bacteriophage numbers when the conditions are not right for organism multiplication.

Table 2

Population changes in the S. cremoris 573-bacteriophage F55 combination during incubation at 32° C. in V-8 broth at pH 5.0

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organism control	0	5.0	4.94	-
	12	5.0	4.99	-
	14	5.0	5.02	-
	16	4.95	5.09	-
	18	4.9	4.95	-
	20	4.9	4.86	-
Organisms maintained at pH 5.0	0	5.0	4.91	-
	12	4.9*	5.13	-
	14	5.0	5.16	-
	16	5.0	4.99	-
	18	4.95*	5.13	-
	20	5.0	4.70	-
Organisms plus phage	0	5.0	5.00	1.40
	12	5.0	5.10	0.95
	14	5.0	4.96	0.95
	16	5.0	4.62	0.95
	18	4.9	4.79	0.95
	20	4.9	4.26	1.40
Organisms plus phage maintained at pH 5.0	0	5.0	5.08	1.40
	12	4.95*	4.68	0.95
	14	4.9*	4.32	0.60
	16	5.0	4.17	1.40
	18	4.95*	4.12	0.95
	20	5.0	3.92	1.40

* 1N NaOH added to bring pH to desired level

Table 3

Population changes in the S. cremoris 573-bacteriophage F55 combination during incubation at 32° C. in litmus milk at pH 5.2 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organism control	0	0	5.2	5.2	5.00	5.00	-	-
	12	12	5.2	5.18	7.30	7.26	-	-
	14	14	5.2	5.18	7.52	7.52	-	-
	16	16	5.1	5.1	7.88	7.78	-	-
	18	18	5.1	5.03**	7.98	8.15	-	-
	20	20	4.9**	4.9	8.15	8.19	-	-
Organisms maintained at pH 5.2	0	0	5.2	5.2	4.94	4.84	-	-
	12	12	5.2	5.19	7.17	7.00	-	-
	14	14	5.2*	5.19	7.55	7.37	-	-
	16	16	5.1	5.12*	7.82	7.51	-	-
	18	18	5.2	5.1*	8.10	8.00	-	-
	20	20	5.15	5.04	8.19	8.11	-	-
Organisms plus phage	0	0	5.2	5.2	4.93	4.79	1.60	1.40
	12	12	5.2	5.2	7.03	7.16	3.98	3.40
	14	14	5.2	5.2	7.34	7.48	5.04	4.65
	16	16	5.15	5.12	7.59	7.80	5.98	5.40
	18	18	5.1	5.03**	7.74	8.00	6.65	4.65
	20	20	5.1	4.9	7.91	8.20	6.65	4.98
Organisms plus phage maintained at pH 5.2	0	0	5.2	5.2	4.88	4.83	1.40	1.65
	12	12	5.2	5.2	7.00	7.24	3.40	3.60
	14	14	5.2	5.19	7.34	7.49	4.18	5.15
	16	16	5.2	5.12*	7.72	7.77	4.98	4.65
	18	18	5.1*	5.1*	7.97	8.13	5.40	5.40
	20	20	5.1	5.02	7.99	8.19	5.65	5.40

* 1N NaOH added to bring the pH to the desired level

** coagulated

Table 4

Population changes in the S. cremoris 573-bacteriophage F55 combination during incubation at 32° C. in litmus milk at pH 5.4 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organism control	0	0	5.4	5.4	4.98	4.69	-	-
	3	3	5.4	5.4	5.59	5.35	-	-
	5	5	5.4	5.4	5.98	5.96	-	-
	7	7	5.4	5.4	6.51	6.42	-	-
	9	9	5.4	5.4	7.11	7.15	-	-
	11	11	5.4	5.3	7.61	7.55	-	-
	13	13	5.3	5.2	7.90	7.83	-	-
Organisms maintained at pH 5.4	0	0	5.4	5.4	5.06	4.92	-	-
	3	3	5.4	5.4	5.53	5.39	-	-
	5	5	5.4	5.4	6.09	5.79	-	-
	7	7	5.4	5.4	6.58	6.42	-	-
	9	9	5.4	5.4 *	7.28	6.97	-	-
	11	11	5.4	5.34 *	7.69	7.53	-	-
	13	13	5.3	5.25	8.08	7.84	-	-
Organisms plus phage	0	0	5.4	5.4	4.99	4.86	0.60	<0.48
	3	3	5.4	5.4	5.60	5.48	1.98	1.65
	5	5	5.4	5.4	5.97	5.88	2.18	2.40
	7	7	5.4	5.4	6.45	6.47	3.40	2.98
	9	9	5.4	5.4	7.34	7.11	5.40	4.65
	11	11	5.4	5.3	7.36	7.46	7.40	6.65
	13	13	5.4	5.25	3.18	7.05	8.65	8.65
Organisms plus phage maintained at pH 5.4	0	0	5.4	5.4	5.06	4.83	1.40	0.60
	3	3	5.4	5.4	5.66	5.46	1.65	1.65
	5	5	5.4	5.4	6.06	5.90	2.65	1.98
	7	7	5.4	5.4	6.60	6.40	3.65	3.65
	9	9	5.4	5.4 *	6.75	6.96	5.98	4.18
	11	11	5.4	5.35 *	3.49	7.51	7.98	6.65
	13	13	5.4	5.25	<2.00	7.33	7.65	7.65

* 1N NaOH added to bring pH to desired level

Table 5

Population changes in the S. cremoris 573-bacteriophage F55 combination during incubation at 32° C. in litmus milk at pH 6.5

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organism control	0	6.5	5.05	-
	1	6.5	5.15	-
	2	6.5	5.57	-
	3	6.5	5.91	-
	4	6.4	6.43	-
	5	6.4	6.98	-
	6	6.4	7.34	-
	7	6.3	7.61	-
	8	6.1	7.82	-
Organisms maintained at pH 6.5	0	6.5	4.99	-
	1	6.5	5.17	-
	2	6.5*	5.53	-
	3	6.4*	6.00	-
	4	6.4*	6.45	-
	5	6.55	7.00	-
	6	6.5	7.34	-
	7	6.4*	7.54	-
	8	6.3	7.78	-
Organisms plus phage	0	6.5	4.99	0.60
	1	6.5	5.17	0.60
	2	6.5	5.58	1.40
	3	6.5	5.77	2.98
	4	6.4	6.34	3.88
	5	6.4	6.69	5.40
	6	6.4	5.32	7.65
	7	6.4	<2.00	7.65
	8	6.4	<2.00	8.30
Organisms plus phage maintained at pH 6.5	0	6.5	4.98	1.18
	1	6.5	5.15	1.40
	2	6.5	5.59	1.65
	3	6.5	6.00	2.30
	4	6.5	6.53	4.65
	5	6.5	7.15	6.18
	6	6.4*	4.13	6.40
	7	6.5	<2.00	6.40
	8	6.5	<2.00	8.30

* 1N NaOH added to bring pH to desired level

Table 6

Population changes in the S. cremoris 573-bacteriophage F55 combination during incubation at 32° C. in litmus milk at pH 7.5 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organism control	0	0	7.5	7.5	4.74	4.69	-	-
	3	3	7.5	7.5	5.33	5.29	-	-
	5	5	7.39	7.46	6.00	6.23	-	-
	6	-	7.39	-	6.61	-	-	-
	7	7	7.27	7.4	6.98	7.12	-	-
	8	-	7.2	-	7.32	-	-	-
	-	9	-	7.15	-	7.46	-	-
Organisms maintained at pH 7.5	0	0	7.5	7.52	4.52	4.54	-	-
	3	3	7.49	7.53	5.36	5.30	-	-
	5	5	7.39*	7.50	6.16	6.25	-	-
	6	-	7.42*	-	6.76	-	-	-
	7	7	7.53	7.45*	6.95	6.92	-	-
	8	-	7.45	-	7.10	-	-	-
	-	9	-	7.23	-	7.40	-	-
Organisms plus phage	0	0	7.5	7.5	4.72	4.77	0.95	0.60
	3	3	7.49	7.5	5.37	5.30	1.40	1.40
	5	5	7.4	7.5	6.13	6.00	2.98	2.18
	6	-	7.4	-	6.61	-	4.40	-
	7	7	7.32	7.38	6.94	6.85	5.6	5.40
	8	-	7.2	-	6.83	-	7.15+	-
	-	9	-	7.1	-	7.00	-	7.40
Organisms plus phage maintained at pH 7.5	0	0	7.5	7.52	4.74	**	0.60	0.95
	3	3	7.5	7.5	5.34	**	1.40	1.60
	5	5	7.46	7.4**	6.11	**	3.40	1.98
	6	-	7.4*	-	6.74	-	3.88	-
	7	7	7.56	7.49	6.90	**	4.18	5.65
	8	-	7.51	-	7.18	-	5.40	-
	-	9	-	7.03	-	**	-	6.34

* 1N NaOH added to bring pH to desired level

** plates contaminated

Table 7

Population changes in the S. cremoris 573-bacteriophage F55 combination during incubation at 32° C. in litmus milk at pH 7.6 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organism control	0	-	7.58	-	4.59	-	-	-
	3	-	7.58	-	4.95	-	-	-
	5	-	7.51	-	5.61	-	-	-
	6	-	7.51	-	6.11	-	-	-
	7	-	7.42	-	6.48	-	-	-
	8	-	7.38	-	6.93	-	-	-
Organisms maintained at pH 7.6	0	0	7.6	7.6	4.64	4.94	-	-
	3	3	7.6	7.42*	4.88	5.16	-	-
	5	5	7.6	7.58	5.46	5.59	-	-
	6	-	7.53*	-	5.92	-	-	-
	7	7	7.6	7.45	6.30	6.28	-	-
	8	-	7.42	-	6.81	-	-	-
	-	9	-	7.7	-	6.89	-	-
	-	11	-	7.38	-	7.23	-	-
Organisms plus phage	0	-	7.6	-	4.64	-	1.40	-
	3	-	7.58	-	4.81	-	1.40	-
	5	-	7.52	-	5.61	-	<2.00	-
	6	-	7.5	-	6.06	-	2.18	-
	7	-	7.46	-	6.31	-	3.60	-
	8	-	7.32	-	6.89	-	3.98	-
Organisms plus phage maintained at pH 7.6	0	0	7.6	7.6	4.52	4.83	1.40	1.40
	3	3	7.62	7.4*	4.85	5.28	1.40	1.40
	5	5	7.5*	7.62	5.52	5.89	1.60	1.40
	6	-	7.6	-	6.02	-	<1.60	-
	7	7	7.6	7.6	6.21	6.83	2.18	1.60
	8	-	7.5	-	6.72	-	2.48	-
	-	9	-	7.4*	-	7.00	-	2.48
	-	11	-	7.38	-	7.36	-	2.30

* 1N NaOH added to bring pH to desired level

Table 8

Population changes in the S. cremoris 573-bacteriophage P55 combination during longer incubation at 32° C. in litmus milk at pH 7.6 (Trial 3)

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 7.6	0	7.6	5.11	-
	12	7.4**	7.51	-
	15	7.3*	7.65	-
	18	7.54	8.20	-
Organisms plus phage maintained at pH 7.6	0	7.6	4.74	1.40
	12	7.39**	7.20	3.40
	15	7.3*	7.71	5.40
	18	7.6	8.08	5.40

*1N NaOH added to bring pH to desired level

**The pH changed so rapidly after 12 hr., adjustment every 15 to 20 minutes was necessary

Table 9

Population changes in the S. cremoris 573-bacteriophage F55 combination during incubation at 32° C. in litmus milk at pH 8.0

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organism control	0	8.0	4.97	-
	10	7.45	6.50	-
	12	7.25	7.14	-
	14	6.75	7.71	-
	16	5.8	8.59	-
	18	5.1	8.96	-
Organisms maintained at pH 8.0	0	8.0	5.00	-
	10	7.75*	6.18	-
	12	7.6*	6.49	-
	14	8.0	6.67	-
	16	7.8*	6.97	-
	18	8.0	6.72	-
Organisms plus phage	0	8.0	5.08	1.40
	10	7.6	6.27	2.30
	12	7.25	7.04	4.65
	14	6.95	7.28	5.65
	16	6.3	2.47	9.04
	18	6.25	<2.00	9.04
Organisms plus phage maintained at pH 8.0	0	8.0	4.88	1.40
	10	7.65*	6.17	0.85
	12	7.8*	6.44	0.60
	14	7.8*	6.51	1.40
	16	7.7*	6.85	<0.48
	18	8.1	6.47	<0.48

* 1N NaOH added to bring pH to desired level

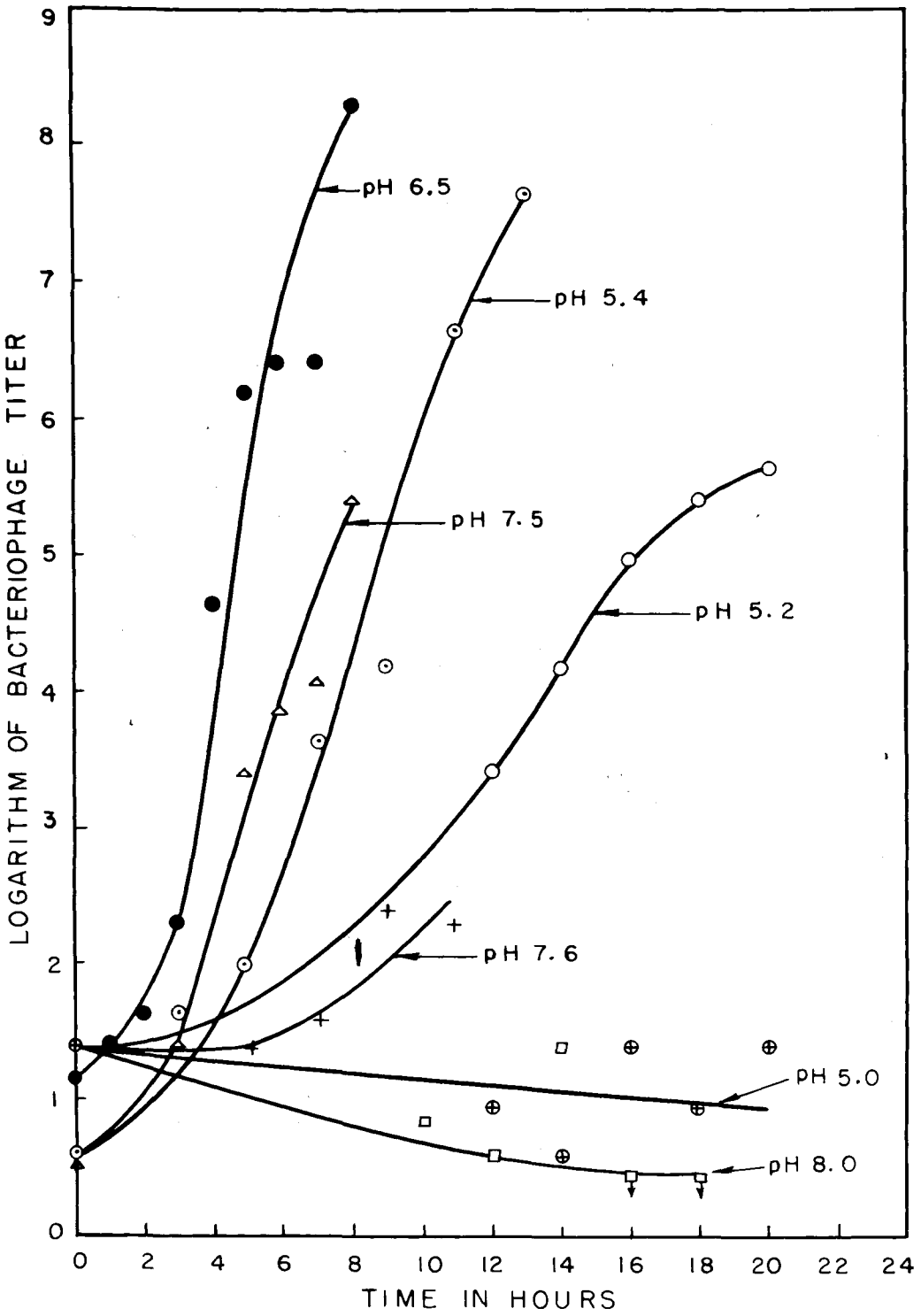

Fig. 1. Summary of typical data of bacteriophage F55 population changes at various pH levels.

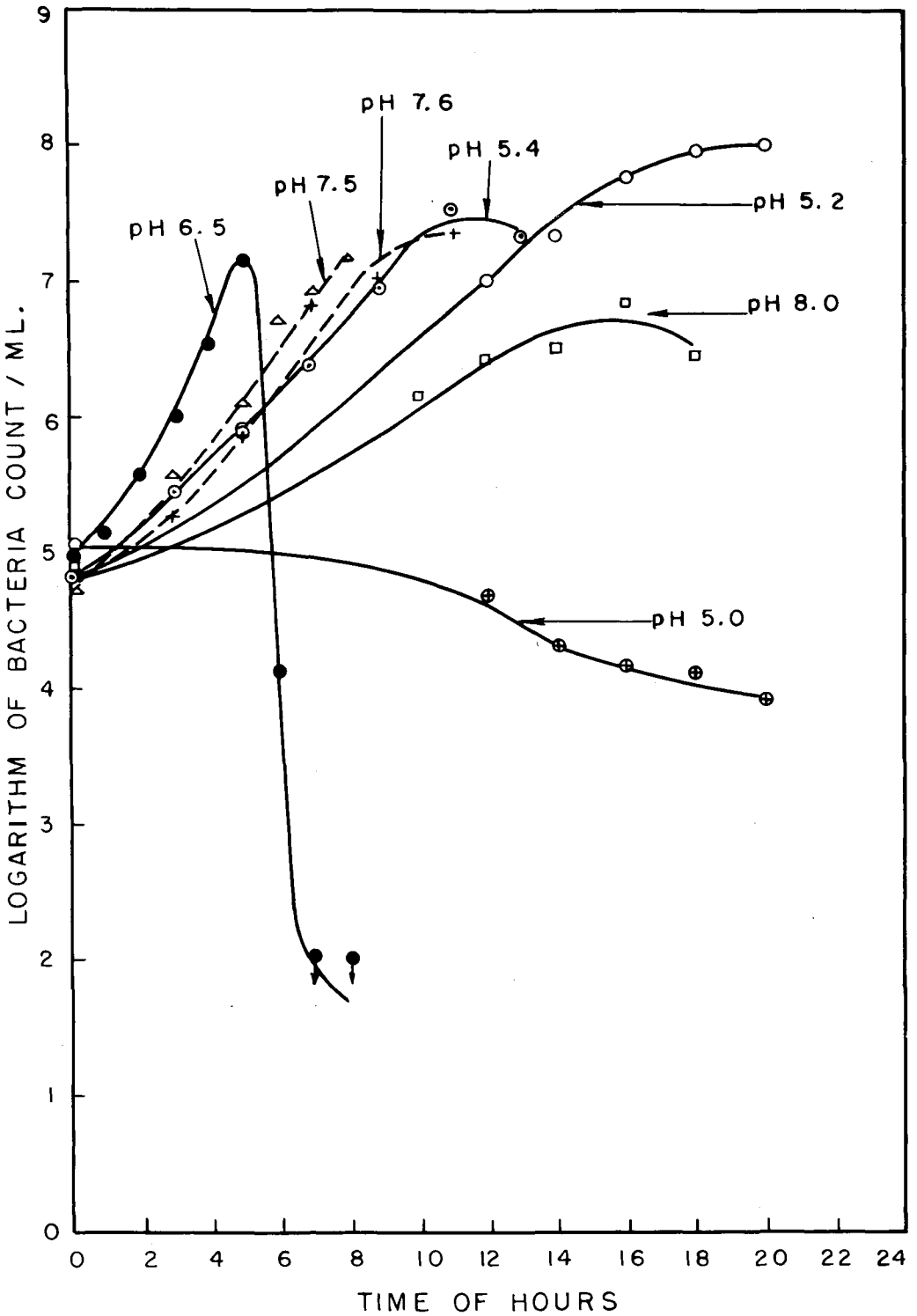

Fig. 2. Summary of typical data of S. cremoris 573 population changes at various pH levels.

Streptococcus lactis W2-bacteriophage F24 combination

The population changes for this organism-bacteriophage combination at the various pH levels are presented in tables 10 through 23 and a summary of typical data is shown in figures 3 and 4. *S. lactis* W2 grows very rapidly and over a pH range of 4.8 to 9.4. The homologous bacteriophage F24 also multiplied over a large pH range. The bacteriophage proliferation was completely inhibited for a 30 hr. incubation period in V-8 broth at pH 4.7 (table 10). At this pH the organism also failed to show growth during the 30 hr. of incubation.

At pH 4.8 (table 11) the bacteriophage proliferation was retarded but there was evidence of increases in bacteriophage numbers after 24 hr. of incubation. The prolonged lag phase of the organism at pH 4.8 also is evident. At pH 5.0 (table 12) the lag phase of the organism as well as of the bacteriophage is decreased as compared to pH 4.8 (table 11). The population of bacteriophage at pH 5.0 is considerably greater than that attained at pH 4.8, even after 48 hr. incubation.

Mass lysis of the organisms occurred during a 12 to 14 hr. incubation period over a pH range of 5.0 to 7.6; compared to pH 6.5, it was delayed 7 to 8 hr. at pH 5.0 (table 12) and 5 to 6 hr. at pH 7.6 (table 13.)

The F24 bacteriophage did not show marked sensitivity to the alkaline reactions; however, bacteriophage populations gradually declined as the pH was increased. At pH 9.1 and 9.4

the lag phase of bacteriophage proliferation was extended by approximately 12 hr. (tables 22 and 23).

With the W2-F24 combination the data indicate that the bacteriophage multiplication occurred over the entire pH range of organism growth. The organisms failed to grow at pH 4.7 (table 10) during 30 hr. incubation and the bacteriophage failed to multiply, actually decreasing in numbers. A difference of 0.1 of a pH unit (from 4.7 to 4.8) made a decided difference in bacteriophage multiplication. At pH 4.8 (table 11) there was only a slight increase in organism numbers and with the organism increase the bacteriophage also increased. When the pH was raised to 5.0 the bacteriophage began to lyse one of the two cultures (table 12) and built up to a high titer in the other culture. At pH 5.2 (table 13) some lysis occurred in less than 12 hr. and appreciable secondary growth had become evident by 18 hr.

Mass lysis occurred at pH 7.6 (table 13) but it was retarded considerably as compared to pH 6.5 (table 14). The final total bacteriophage populations at these two pH levels were essentially the same; however, there was a 4 to 5 hr. delay at pH 7.6 for the bacteriophage population to reach the same level as it did at pH 6.5.

At pH levels of 8.0 and above mass lysis failed to occur during the incubation periods used. There was a longer lag phase of both organism growth and bacteriophage proliferation as the pH was raised through the higher alkaline ranges. In the range of pH 8.0 through 8.8 the organism numbers increased

gradually during the incubation period. At pH 8.6 (table 20) there was very little increase in organism numbers after 12 hr. and bacteriophage reached its maximum level after about 12 to 15 hr. and then declined upon continued incubation. In general, at pH levels of 9.1 and 9.4 there first was a decrease in organism numbers and then a slight increase; it was during this increase that the bacteriophage multiplication took place in some trials (table 22 and 23). Trial 2 at pH 9.4 (table 23) is an exception to this, since there was virtually no increase in the organism population at any time during the incubation period and yet the final bacteriophage titer was quite high. It is possible that this may be accounted for by the high initial bacteriophage population in this trial. Apparently many of the susceptible cells were infected and lysed before organism growth was initiated.

From the data presented, bacteriophage F24 proliferation occurred wherever there was positive growth of its homologus organism and the pH influenced only the rate of bacteriophage proliferation. The proliferation of the bacteriophage is summarized in figure 3. The slopes of the lines representing the logarithms of bacteriophage titers at pH levels of 5.0, 6.5, 7.6 and 8.3 are all quite similar. The lag phase is somewhat longer at pH 7.6 than at pH 6.5 and at pH 5.0 and 8.3 the lag phase is extended up to about 6 hr. The lag phase is further prolonged at pH levels of 4.8, 9.1 and 9.4. By comparing figures 3 and 4 it will be noticed that the increase in bacteriophage population coincides with the increase of organism numbers at pH levels of 9.1 and 9.4.

It is of interest to note the additional time required for lysing the organisms at pH 7.6 (figure 4) as compared with pH 6.5. Even though the total bacteriophage population at pH 7.6 (figure 4) was the same as at pH 6.5, about 4 hr. longer were required to reach this level. Mass lysis also was delayed 5 to 6 hr.

Table 10

Population changes in the S. lactis W2-bacteriophage F24 combination during incubation at 32° C. in V-8 broth at pH 4.7

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organism plus phage maintained at pH 4.7	0	4.7	5.16	1.40
	24	4.7	4.64	<0.48
	30	4.66	4.18	<0.48

Table 11

Population changes in the S. lactis W2-bacteriophage F24 combination during incubation at 32° C. in V-8 broth at pH 4.8 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organisms maintained at pH 4.8	0	-	4.82	-	5.01	-	-	-
	2	-	4.79	-	5.07	-	-	-
	4	-	4.81	-	5.23	-	-	-
	6	-	4.82	-	5.33	-	-	-
	8	-	4.81	-	5.40	-	-	-
	10	-	4.79	-	5.70	-	-	-
	12	-	4.79	-	5.72	-	-	-
	24	-	-	-	-	-	-	-
Organisms plus phage maintained at pH 4.8	0	0	4.82	4.82	5.11	5.17	0.95	1.40
	2	-	4.79	-	5.22	-	0.60	-
	4	-	4.82	-	5.20	-	1.18	-
	6	-	4.82	-	5.25	-	1.40	-
	8	-	4.80	-	5.41	-	0.85	-
	10	-	4.80	-	5.64	-	0.60	-
	12	-	4.80	-	5.82	-	0.48	-
	24	24	-	4.8	-	5.62	3.65	2.18
	-	30	-	4.8	-	6.12	-	3.88
	-	48	-	4.7	-	-	-	5.65

Table 12

Population changes in the S. lactis W2-bacteriophage F24 combination during incubation at 32° C. in V-8 broth at pH 5.0 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organisms maintained at pH 5.0	0	0	5.0	5.0	5.18	5.17	-	-
	-	2	-	4.99	-	5.24	-	-
	-	4	-	4.98	-	5.39	-	-
	-	6	-	5.0	-	5.66	-	-
	-	8	-	4.99	-	6.25	-	-
	-	10	-	4.91*	-	6.97	-	-
	12	12	4.65*	4.92	7.70	7.56	-	-
	14	-	4.78*	-	8.17	-	-	-
	16	-	4.65*	-	8.42	-	-	-
	18	-	4.62	-	8.51	-	-	-
Organisms plus phage maintained at pH 5.0	0	0	5.0	5.0	5.22	5.12	1.40	1.65
	-	2	-	4.98	-	5.20	-	1.65
	-	4	-	5.02	-	5.41	-	1.65
	-	6	-	5.02	-	5.78	-	1.65
	-	8	-	4.99	-	6.37	-	3.40
	-	10	-	4.91*	-	7.13	-	4.40
	12	12	4.65*	4.92	7.97	7.65	7.15+	6.65
	14	-	4.89*	-	6.77	-	7.15+	-
	16	-	4.98	-	4.48	-	7.15+	-
	18	-	4.94	-	4.45	-	9.04	-

* 1N NaOH added to bring pH to desired level

Table 13

Population changes in the S. lactis W2-bacteriophage F24 combination during incubation at 32° C. in litmus milk at pH 5.2

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 5.2	0	5.2	5.24	-
	12	5.1*	8.04	-
	14	5.05*	8.45	-
	16	5.05*	8.67	-
	18	5.05*	8.61	-
Organisms plus phage maintained at pH 5.2	0	5.2	5.16	0.95
	12	5.2	3.79	7.15+
	14	5.2	3.78	7.60
	16	5.2	4.76	7.40
	18	5.2	5.40	7.40

* 1N NaOH added to bring pH to desired level

Table 14

Population changes in the S. lactis W2-bacteriophage F24 combination during incubation at 32° C. in litmus milk at pH 6.5

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 6.5	0	6.52	5.21	-
	2	6.52	6.07	-
	3	6.5	6.80	-
	4	6.5	7.38	-
	5	6.5	7.83	-
	6	6.38*	7.97	-
	7	6.3*	8.48	-
	8	6.38	8.75	-
Organisms plus phage maintained at pH 6.5	0	6.52	5.28	1.40
	2	6.52	6.13	2.65
	3	6.52	6.84	3.40
	4	6.51	7.41	5.40
	5	6.5	7.69	7.40
	6	6.45*	4.67	8.34
	7	6.5	2.30	9.15+
	8	6.5	<2.00	8.65

* 1N NaOH added to bring pH to desired level

Table 15

Population changes in the S. lactis W2-bacteriophage F24 combination during incubation at 32° C. in litmus milk at pH 7.6

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 7.6	0	7.61	4.98	-
	2	7.61	6.00	-
	4	7.47*	7.15	-
	6	7.63	7.62	-
	8	7.60	8.13	-
	10	6.85	8.57	-
	12	7.53	9.10	-
Organisms plus phage maintained at pH 7.6	0	7.61	5.03	1.18
	2	7.61	5.94	1.40
	4	7.50*	6.89	2.18
	6	7.64	7.80	3.65
	8	7.60	8.11	5.65
	10	6.93	7.93	7.15+
	12	7.4	<3.00	9.15+

* 1N NaOH added to bring pH to desired level

Table 16

Population changes in the S. lactis W2-bacteriophage F24 combination during incubation at 32° C. in litmus milk at pH 8.0 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organisms maintained at pH 8.0	0	0	8.0	8.0	4.95	4.87	-	-
	2	2	8.0	7.95*	5.60	5.60	-	-
	4	4	8.0	7.97*	6.84	5.52	-	-
	6	6	7.75*	7.75*	7.67	6.48	-	-
	8	8	8.05	7.9*	7.66	7.76	-	-
	10	10	7.98	8.1	7.79	7.60	-	-
	12	12	7.89	7.91	7.65	7.86	-	-
Organisms plus phage maintained at pH 8.0	0	0	8.0	8.01	4.94	4.94	0.60	1.18
	2	2	8.0	8.0	5.76	5.63	0.95	0.95
	4	4	8.03	8.0	6.75	6.90	1.98	1.40
	6	6	7.80*	7.85*	7.54	7.52	1.40	2.30
	8	8	8.01	7.9*	7.73	7.83	1.60	3.65
	10	10	7.99	8.08	7.77	7.79	3.65	3.40
	12	12	7.89	7.88	7.89	7.93	4.98	4.40

* 1N NaOH added to bring pH to desired level

Table 17

Population changes in the S. lactis W2-bacteriophage F24 combination during incubation at 32° C. in litmus milk at pH 8.2 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organisms maintained at pH 8.2	0	0	8.2	8.22	5.03	4.88	-	-
	2	2	8.21	8.15*	5.62	5.47	-	-
	4	4	8.23	8.2	6.67	6.43	-	-
	6	6	8.0*	8.06*	7.37	7.38	-	-
	8	8	8.25	8.25	7.55	7.38	-	-
	10	10	8.21	8.25	7.42	7.57	-	-
	12	12	8.20	8.2	7.60	7.73	-	-
Organisms plus phage maintained at pH 8.2	0	0	8.2	8.2	4.93	4.92	1.40	1.65
	2	2	8.2	8.12*	5.62	5.56	0.95	0.95
	4	4	8.2	8.20	6.70	6.52	1.40	0.95
	6	6	8.0*	8.08*	7.36	7.42	1.40	0.95
	8	8	8.3	8.25	7.66	7.54	1.60	1.40
	10	10	8.23	8.25	7.69	7.72	2.54	1.40
	12	12	8.20	8.20	7.65	7.70	3.40	2.65

* 1N NaOH added to bring pH to desired level

Table 18

Population changes in the S. lactis W2-bacteriophage F24 combination during incubation at 32° C. in litmus milk at pH 8.3

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 8.3	0	8.36	5.02	-
	3	8.32	5.87	-
	6	8.38	7.17	-
	8	8.2*	7.61	-
	10	8.33	7.61	-
	12	8.31	7.62	-
	14	8.35	7.81	-
	16	8.35	7.68	-
Organisms plus phage maintained at pH 8.3	0	8.36	4.98	0.95
	3	8.32	6.05	0.60
	6	8.31*	7.29	1.40
	8	8.15*	7.63	1.65
	10	8.29*	7.52	3.65
	12	8.3	7.74	4.30
	14	8.3	7.69	5.98
	16	8.3	7.77	6.18

* 1N NaOH added to bring pH to desired level

Table 19

Population changes in the S. lactis W2-bacteriophage F24 combination during incubation at 32° C. in litmus milk at pH 8.4

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 8.4	0	8.45	5.04	-
	3	8.4	5.67	-
	6	8.4	6.92	-
	8	8.32*	7.39	-
	10	8.49	7.50	-
	12	8.48	7.49	-
	14	8.49	7.30	-
	16	8.4	7.49	-
Organisms plus phage maintained at pH 8.4	0	8.45	5.03	0.60
	3	8.42	5.81	0.60
	6	8.42	7.17	1.40
	8	8.29*	7.49	0.95
	10	8.4	7.68	1.40
	12	8.4	7.61	1.60
	14	8.39*	7.53	3.40
	16	8.45	7.60	3.98

* 1N NaOH added to bring pH to desired level

Table 20

Population changes in the S. lactis W2-bacteriophage F24 combination during incubation at 32° C. in litmus milk at pH 8.6

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 8.6	0	8.65	4.90	-
	12	8.5*	6.91	-
	15	8.59	7.13	-
	18	8.53*	7.28	-
	21	8.7	7.28	-
	24	8.6	7.37	-
Organisms plus phage maintained at pH 8.6	0	8.68	5.00	<0.48
	12	8.48*	7.42	4.04
	15	8.41*	7.42	4.04
	18	8.53*	7.36	3.40
	21	8.67	7.51	3.65
	24	8.51	7.56	3.40

* 1N NaOH added to bring pH to desired level

Table 21

Population changes in the S. lactis W2-bacteriophage F24 combination during incubation at 32° C. in litmus milk at pH 8.8

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 8.8	0	8.86	4.94	-
	12	8.8	6.49	-
	15	8.73*	6.95	-
	18	8.71*	6.93	-
	21	8.86	6.80	-
	24	8.81	6.99	-
Organisms plus phage maintained at pH 8.8	0	8.85	4.98	<0.48
	12	8.8	6.35	1.40
	15	8.75*	6.78	2.98
	18	8.78*	6.95	4.15+
	21	8.8	7.00	4.15+
	24	8.8	7.10	5.15+

* 1N NaOH added to bring pH to desired level

Table 22

Population changes in the S. lactis W2-bacteriophage P24 combination during incubation at 32° C. in litmus milk at pH 9.1

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 9.1	0	9.12	4.96	-
	12	9.1	4.10	-
	15	9.05*	4.87	-
	18	9.09	5.36	-
	21	9.06*	5.59	-
	24	9.1	5.47	-
Organisms plus phage maintained at pH 9.1	0	9.09	5.13	1.40
	12	9.1	4.11	1.40
	15	9.05*	4.88	3.40
	18	9.1 *	4.85	4.15+
	21	9.06*	4.90	4.15+
	24	9.1	4.38	5.18

* 1N NaOH added to bring pH to desired level

Table 23

Population changes in the S. lactis W2-bacteriophage F24 combination during incubation at 32° C. in litmus milk at pH 9.4 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organisms maintained at pH 9.4	0	0	9.4	9.4	5.09	4.96	-	-
	12	12	9.36*	9.28*	4.01	2.78	-	-
	-	14	-	9.28*	-	3.23	-	-
	15	-	9.36*	-	4.37	-	-	-
	-	16	-	9.4	-	3.67	-	-
	18	18	9.38*	9.3*	4.87	4.45	-	-
	-	20	-	9.43	-	4.85	-	-
	21	-	9.4	-	4.86	-	-	-
	-	22	-	9.3*	-	5.09	-	-
	24	24	9.3	9.4	5.01	5.33	-	-
Organisms plus phage maintained at pH 9.4	0	0	9.41*	9.4*	5.09	4.94	1.40	3.40
	12	12	9.38	9.31*	3.84	3.00	1.60	3.40
	-	14	-	9.32*	-	2.70	-	3.65
	15	-	9.38*	-	4.05	-	3.65	-
	-	16	-	9.39	-	3.00	-	4.15+
	18	18	9.39	9.31*	3.98	3.08	4.15+	4.40
	-	20	-	9.41	-	2.78	-	5.40
	21	-	9.4	-	3.87	-	4.15+	-
	-	22	-	9.38	-	2.70	-	4.48
	24	24	9.4	9.38	3.71	3.11	4.98	5.98

* 1N NaOH added to bring pH to desired level

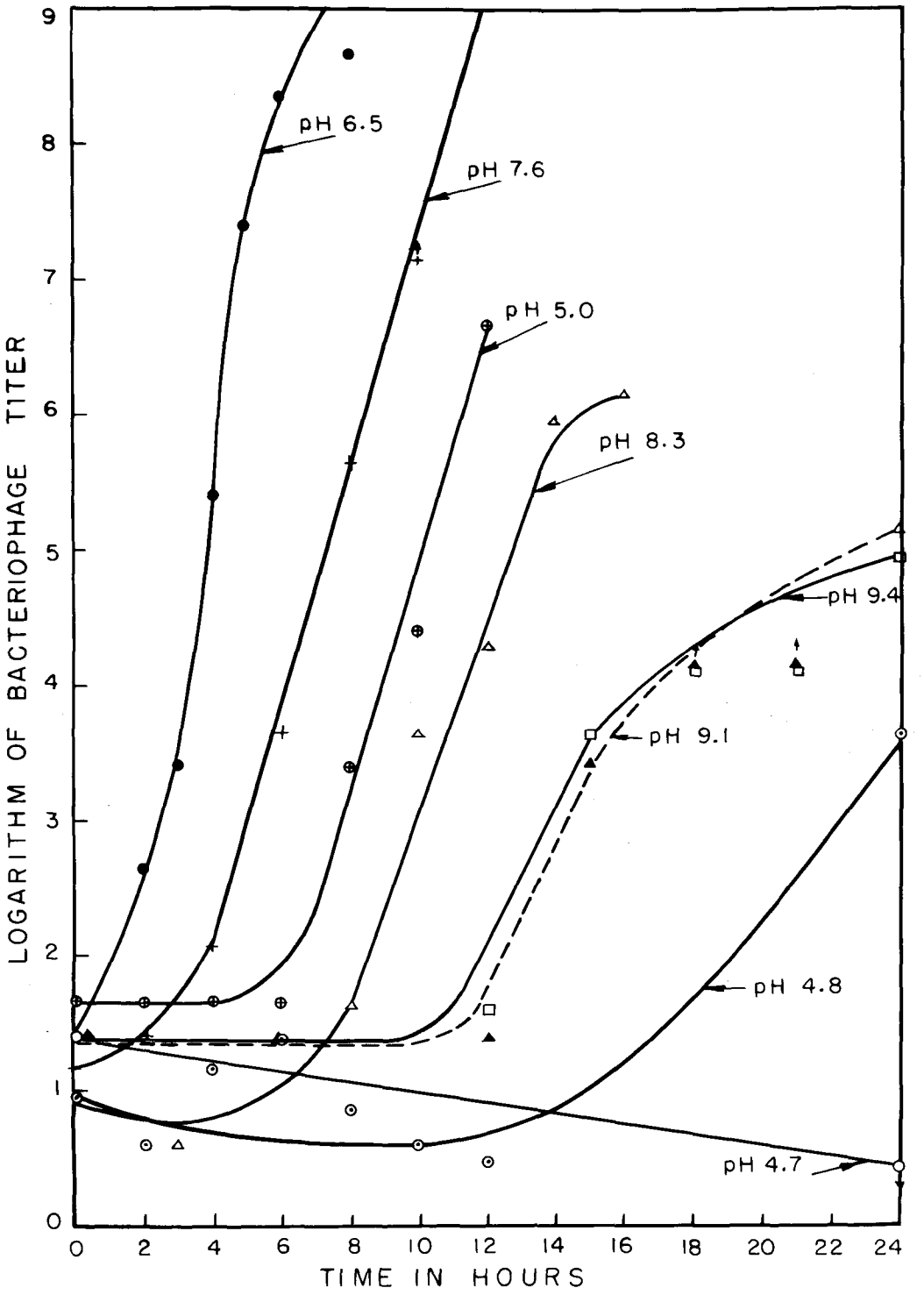

Fig. 3. Summary of typical data of bacteriophage F24 population changes at various pH levels.

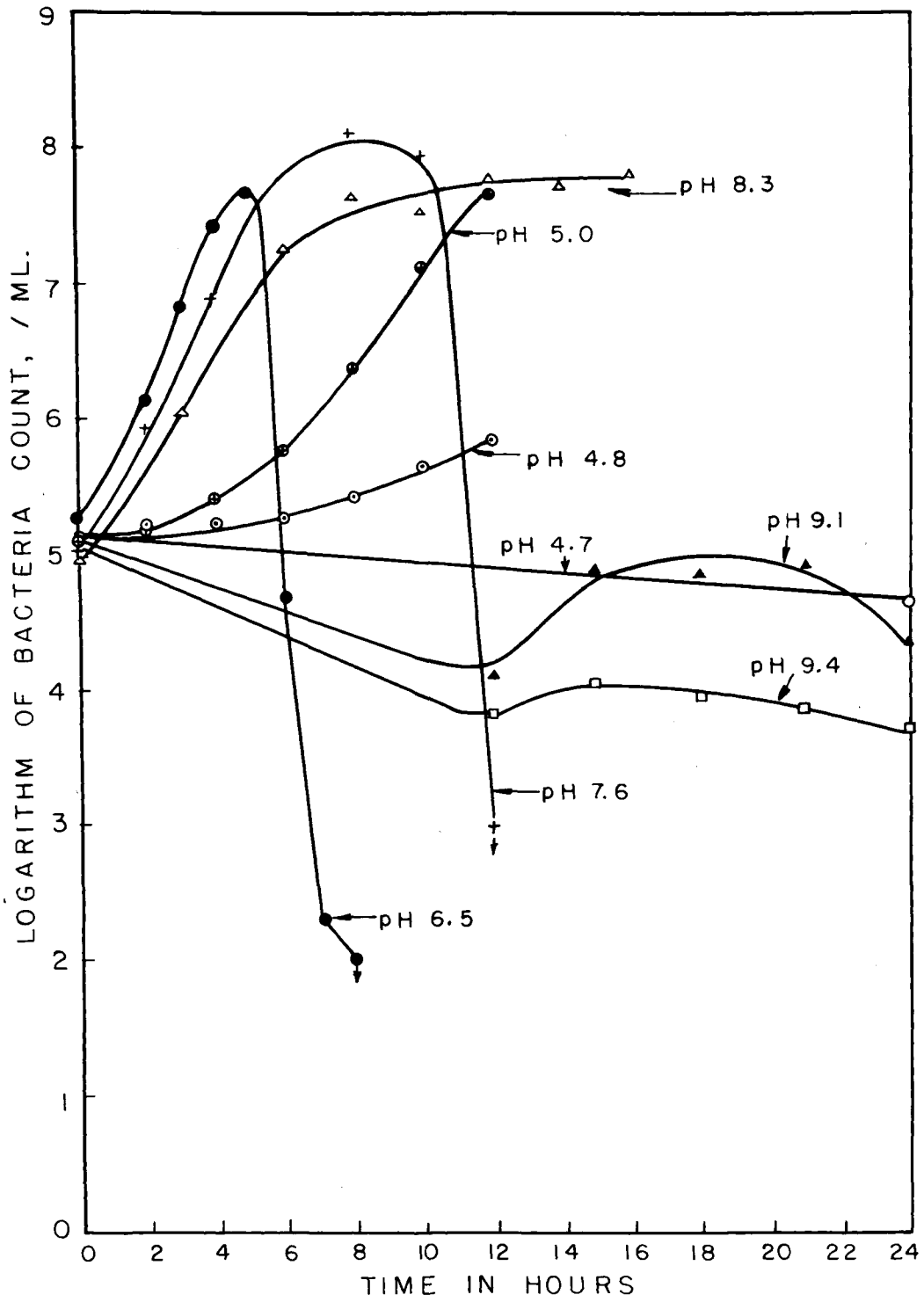

Fig. 4. Summary of typical data of S. lactis W2 population changes at various pH levels.

Streptococcus lactis 712-bacteriophage F56 combination

Tables 24 through 37 show the population changes for 712-F56 combination at the various pH levels and a summary of typical data is presented in figures 5 and 6. *S. lactis* 712 was not as sensitive to the acid reactions as were the other organisms used. It grew quite well at pH 4.7 in V-8 broth (table 24). Another interesting characteristic of this organism was the large colonies produced on TGEM agar. These colonies were several times larger than colonies from other strains.

In order to promote proliferation of F56 in the V-8 broth, 0.1 per cent of $CaCl_2$ had to be added to the medium (tables 28 and 29). Similar results are reported by Collins (1949) for this bacteriophage, using a defined medium. With the conditions used in this work and also under the conditions used for propagating bacteriophage and preparing filtrates in the Dairy Bacteriology laboratories, it was not possible to build up a high titer for this particular bacteriophage. Usually a titer of 10^6 was obtained but occasionally the titer would be lower.

Bacteriophage F56 was sensitive to the low pH levels and very little, if any, multiplication occurred below pH 5.0 (tables 24, 25 and 26). At pH 5.0 in V-8 broth with added $CaCl_2$, bacteriophage multiplication took place. Mass lysis took place at pH 5.1 at about the 6th hour (table 29); however, there was not as complete lysing of the cells as at pH 6.5

(table 33). This might possibly be because the cycle was not followed as far. This same characteristic also was noted at pH levels of 5.4 (table 31), 5.7 (table 32) and 7.5 (table 34).

When the pH of litmus milk was raised to 8.3 (table 35), it caused a retarding of the bacteriophage multiplication and this inhibiting effect became greater with an increase in pH. At pH 8.6 (table 37) there actually was a decrease in bacteriophage numbers after 24 hr. incubation.

Typical data of bacteriophage population changes are summarized in figure 5. Of particular interest are the definite lag phases in bacteriophage proliferation at pH levels of 5.0, 5.6, 8.3 and 8.4. The slopes of the lines representing the logarithms of the bacteriophage titer give an indication of the rate of bacteriophage proliferation. Marked differences occur at pH levels of 8.3, 8.4 and 5.0 as compared to pH 6.5, which is taken to be nearer optimum for bacteriophage multiplication.

Figure 6 shows the organism population changes and, in general, they follow similar patterns until the pH is raised to 8.6, at which time the curve flattens out considerably. At pH 5.1 the time of mass lysis is increased approximately 3 hr. over lysis at pH 6.5 in the example on the graph, but in the first trial at pH 5.1, mass lysis occurred a little earlier (table 29).

Table 24

Population changes in the S. lactis 712-bacteriophage F56 combination during incubation at 32° C. in V-8 broth plus $CaCl_2$ at pH 4.7

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 4.7	0	4.7	5.10	-
	2	4.68*	5.55	-
	4	4.69	6.02	-
	6	4.54*	6.34	-
	8	4.71	7.63	-
	10	4.56*	8.25	-
	12	4.42	8.83	-
Organisms plus phage maintained at pH 4.7	0	4.7	5.10	0.60
	2	4.68*	5.39	<0.48
	4	4.69	5.82	0.60
	6	4.58*	6.67	0.95
	8	4.70	7.43	0.60
	10	4.61*	7.94	<0.48
	12	4.52	8.72	<1.20

* 1N NaOH added to bring pH to desired level

Table 25

Population changes in the S. lactis 712-bacteriophage F56 combination during incubation at 32° C. in V-8 broth plus $CaCl_2$ at pH 4.8

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 4.8	0	4.8	5.09	-
	2	4.81	5.37	-
	4	4.81	6.03	-
	6	4.81	6.85	-
	8	4.79*	7.45	-
	10	4.7*	8.21	-
	12	4.6	8.70	-
Organisms plus phage maintained at pH 4.8	0	4.8	5.11	0.95
	2	4.81	5.47	0.60
	4	4.81	6.05	<0.48
	6	4.81	6.94	<0.48
	8	4.71*	7.53	<0.48
	10	4.7*	8.47	1.40
	12	4.6	8.72	<0.48

* 1N NaOH added to bring pH to desired level

Table 26

Population changes in the S. lactis 712-bacteriophage F56 combination during incubation at 32° C. in V-8 broth plus $CaCl_2$ at pH 4.9 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organisms maintained at pH 4.9	0	0	4.9	4.89	5.22	5.22	-	-
	2	2	4.93	4.89	5.41	5.48	-	-
	4	4	4.93	4.9	6.14	6.33	-	-
	6	6	4.91	4.9	7.18	7.25	-	-
	8	8	4.86*	4.8*	7.73	7.95	-	-
	10	10	4.82*	4.71*	8.65	8.59	-	-
	12	-	4.65	-	8.80	-	-	-
Organisms plus phage maintained at pH 4.9	0	0	4.9	4.9	5.11	5.32	1.40	2.98
	2	2	4.93	4.9	5.42	5.53	1.65	2.65
	4	4	4.91	4.9	6.27	6.28	<0.48	2.65
	6	6	4.90	4.9	7.20	7.67	1.40	3.40
	8	8	4.85*	4.8*	7.74	7.95	<0.48	2.98
	10	10	4.85*	4.75*	8.70	8.74	0.95	3.40
	12	-	4.6	-	8.88	-	<0.48	-

* 1N NaOH added to bring pH to desired level

Table 27

Population changes in the S. lactis 712-bacteriophage F56 combination during incubation at 32° C. in V-8 broth plus $CaCl_2$ at pH 5.0

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 5.0	0	5.0	5.23	-
	2	5.0	5.46	-
	4	5.0	6.33	-
	6	5.0	7.24	-
	8	4.85*	8.11	-
	10	4.7*	8.81	-
Organisms plus phage maintained at pH 5.0	0	5.0	5.18	2.65
	2	5.0	5.51	2.65
	4	5.0	6.36	2.65
	6	5.0	7.30	3.65
	8	4.88*	8.05	3.98
	10	4.71*	8.79	5.15+

* 1N NaOH added to bring pH to desired level

Table 28

Population changes in the S. lactis 712-bacteriophage F56 combination during incubation at 32° C. in V-8 broth at pH 5.1

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 5.1	0	5.1	5.09	-
	2	5.12	5.44	-
	4	5.1	6.23	-
	6	5.02*	7.05	-
	8	5.0*	7.73	-
	10	4.54*	8.55	-
	12	4.4	8.65	-
Organisms plus phage maintained at pH 5.1	0	5.08	5.16	0.60
	2	5.1	5.47	<0.20
	4	5.1	6.29	<0.20
	6	5.0*	7.00	0.60
	8	5.01*	7.54	<0.20
	10	4.6*	8.79	1.40
	12	4.5	9.06	0.95

* 1N NaOH added to bring pH to desired level

Table 29

Population changes in the S. lactis 712-bacteriophage F56 combination during incubation at 32° C. in V-8 broth plus $CaCl_2$ at pH 5.1 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organisms maintained at pH 5.1	0	-	5.1	-	5.19	-	-	-
	2	-	5.01*	-	5.52	-	-	-
	4	-	5.08	-	6.50	-	-	-
	6	-	5.01*	-	7.65	-	-	-
	8	-	4.82*	-	8.42	-	-	-
	10	-	4.23	-	9.05	-	-	-
Organisms plus phage maintained at pH 5.1	0	0	5.1	5.12	5.15	5.24	1.40	3.40
	2	2	5.0*	5.11	5.84	5.74	0.95	2.98
	4	4	5.12	5.13	6.30	6.75	3.40	3.40
	6	6	5.09	5.1	7.31	7.66	3.98	5.15+
	8	8	5.11**	4.98*	4.72	7.30	4.40	5.15+
	10	10	5.10	5.25**	4.72	5.15	4.98	7.04

* 1N NaOH added to bring pH to desired level

** Culture cleared

Table 30

Population changes in the S. lactis 712-bacteriophage F56 combination during incubation at 32° C. in litmus milk at pH 5.2

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 5.2	0	5.2	5.17	-
	2	5.2	5.56	-
	4	5.2	6.26	-
	6	5.13*	6.94	-
	8	5.1*	7.54	-
	10	5.1*	8.10	-
	12	5.08	8.41	-
Organisms plus phage maintained at pH 5.2	0	5.25	5.15	1.40
	2	5.2	5.63	<0.48
	4	5.2	6.28	1.40
	6	5.13*	6.97	1.40
	8	5.1*	7.66	1.40
	10	5.12*	7.51	5.65
	12	5.21	6.02	5.98

* 1N NaOH added to bring pH to desired level

Table 31

Population changes in the S. lactis 712-bacteriophage F56 combination during incubation at 32° C. in litmus milk at pH 5.4

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 5.4	0	5.4	5.19	-
	2	5.4	5.71	-
	4	5.4	6.29	-
	6	5.4	7.22	-
	8	5.4	7.76	-
	10	5.3*	8.18	-
	12	5.28	8.65	-
Organisms plus phage maintained at pH 5.4	0	5.4	5.32	1.40
	2	5.4	5.43	0.95
	4	5.4	6.32	1.40
	6	5.38	7.10	1.40
	8	5.35*	7.67	5.40
	10	5.32*	6.64	3.98
	12	5.35	4.82	6.40

* 1N NaOH added to bring pH to desired level

Table 32

Population changes in the S. lactis 712-bacteriophage F56 combination during incubation at 32° C. in litmus milk at pH 5.7

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 5.7	0	5.7	5.14	-
	2	5.7	5.67	-
	4	5.7	6.91	-
	6	5.7	7.74	-
	8	5.6*	8.31	-
	10	5.41*	8.76	-
	12	5.61	9.33	-
Organisms plus phage maintained at pH 5.7	0	5.73	5.10	1.40
	2	5.73	5.70	0.95
	4	5.73	6.92	1.40
	6	5.71	5.35	1.40
	8	5.65*	3.20	5.40
	10	5.72	3.08	3.98
	12	5.72	3.90	6.40

* 1N NaOH added to bring pH to desired level

Table 33

Population changes in the S. lactis 712-bacteriophage F56 combination during incubation at 32° C. in litmus milk at pH 6.5

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 6.5	0	6.5	5.20	-
	1	6.5	5.41	-
	2	6.5	5.88	-
	3	6.5	6.45	-
	4	6.45*	7.14	-
	5	6.45*	7.79	-
	6	6.4*	8.03	-
	7	6.39*	8.27	-
	8	6.39	8.77	-
Organisms plus phage maintained at pH 6.5	0	6.5	5.18	1.40
	1	6.5	5.57	1.40
	2	6.5	5.89	1.65
	3	6.5	6.48	2.40
	4	6.45*	7.20	4.40
	5	6.47*	6.16	5.40
	6	6.5	2.90	5.65
	7	6.5	2.30	5.98
	8	6.5	<2.00	5.98

* 1N NaOH added to bring pH to desired level

Table 34

Population changes in the S. lactis 712-bacteriophage F56 combination during incubation at 32° C. in litmus milk at pH 7.5

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 7.5	0	7.5	5.19	-
	2	7.5	5.91	-
	4	7.43*	6.96	-
	6	7.43*	7.20	-
	8	7.35*	8.03	-
	10	7.3	8.50	-
Organisms plus phage maintained at pH 7.5	0	7.52	5.13	2.65
	2	7.52	6.00	3.40
	4	7.45*	6.93	4.15+
	6	7.48*	5.88	6.65
	8	7.52	2.95	6.40
	10	7.52	3.00	6.65

* 1N NaOH added to bring pH to desired level

Table 35

Population changes in the S. lactis 712-bacteriophage F56 combination during incubation at 32° C. in litmus milk at pH 8.3 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organisms maintained at pH 8.3	0	0	8.3	8.2*	5.15	5.22	-	-
	2	2	8.29	8.26*	5.44	5.44	-	-
	4	4	8.2*	8.26*	6.26	6.17	-	-
	6	6	8.29*	8.32	6.80	6.85	-	-
	8	8	8.30	8.29	7.06	7.15	-	-
	10	10	8.17*	8.3	7.36	7.49	-	-
	12	12	8.38	8.22*	8.04	7.89	-	-
	-	14	-	8.19*	-	8.09	-	-
	-	16	-	8.28	-	8.26	-	-
Organisms plus phage maintained at pH 8.3	0	0	8.28	8.2*	5.22	5.13	2.65	2.40
	2	2	8.24*	8.25*	5.46	5.41	2.40	2.40
	4	4	8.25*	8.30	6.53	6.07	2.40	1.65
	6	6	8.31	8.26*	6.84	6.71	2.65	1.40
	8	8	8.26*	8.25*	7.29	7.07	3.40	2.65
	10	10	8.2*	8.3	7.74	7.51	3.65	2.40
	12	12	8.31	8.2*	7.97	7.95	3.98	3.40
	-	14	-	8.22*	-	8.14	-	3.40
	-	16	-	8.35	-	8.18	-	3.40

* 1N NaOH added to bring pH to desired level

Table 36

Population changes in the S. lactis 712-bacteriophage F56 combination during incubation at 32° C. in litmus milk at pH 8.4

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 8.4	0	8.32*	5.06	-
	2	8.41	5.31	-
	4	8.36	5.79	-
	6	8.42	6.41	-
	8	8.39	6.83	-
	10	8.41	7.28	-
	12	8.42	7.58	-
	14	8.3*	7.70	-
	16	8.45	7.89	-
Organisms plus phage maintained at pH 8.4	0	8.3*	5.10	1.65
	2	8.38*	5.27	1.40
	4	8.32*	6.04	1.65
	6	8.42	6.42	1.40
	8	8.36*	6.97	2.65
	10	8.4	7.34	2.40
	12	8.39	7.59	2.65
	14	8.29*	7.85	2.40
	16	8.45	7.88	2.98

* 1N NaOH added to bring pH to desired level

Table 37

Population changes in the S. lactis 712-bacteriophage F56 combination during incubation at 32° C. in litmus milk at pH 8.6

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms plus phage maintained at pH 8.6	0	8.68	4.76	1.40
	12	8.68	6.38	<0.48
	15	8.6	7.09	0.95
	18	8.49*	7.55	1.40
	21	8.55*	7.67	0.95
	24	8.68	7.67	<0.48

* 1N NaOH added to bring pH to desired level

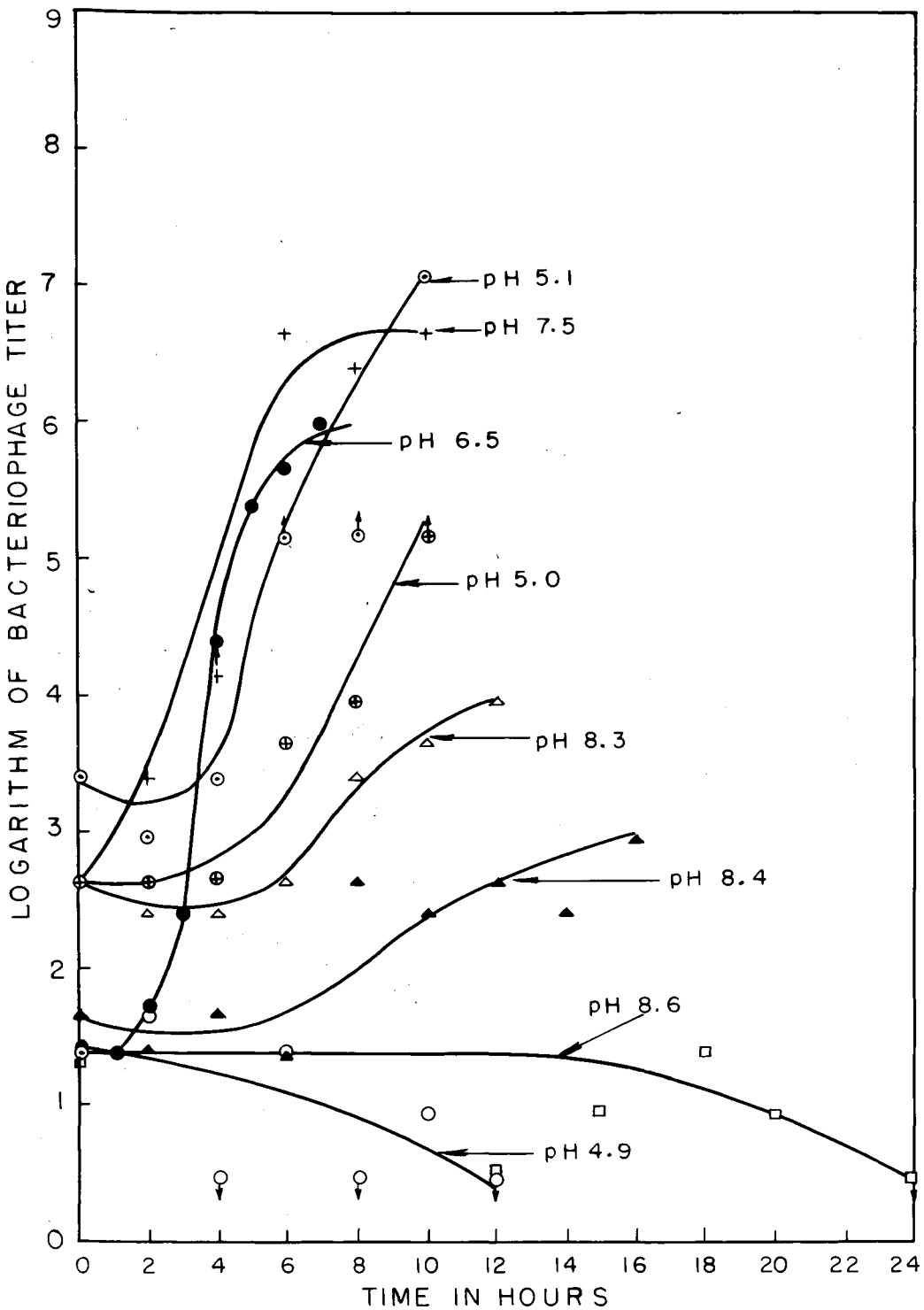

Fig. 5. Summary of typical data of bacteriophage F56 population changes at various pH levels.

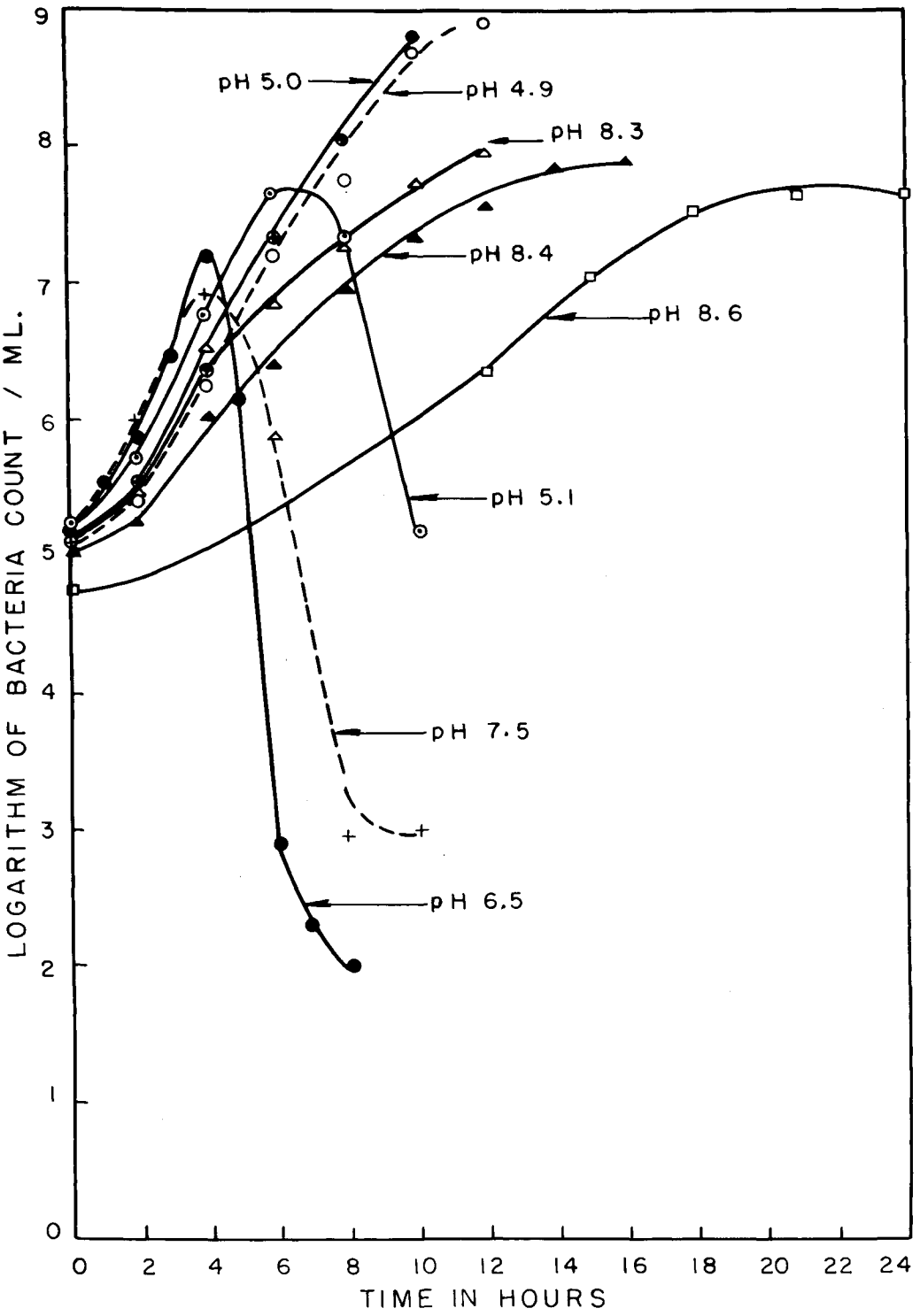

Fig. 6. Summary of typical data of S. lactis 712 population changes at various pH levels.

Streptococcus cremoris H1,1-bacteriophage F4 combination

The population changes for H1,1-F4 combination are shown in tables 28 through 48 and a summary of typical data is presented in figures 7 and 8. This particular bacteriophage multiplied over the pH range from 5.2 to 9.4 Very high titers were obtained at pH levels of 5.4, 6.3 and 6.5 (tables 42, 43 and 44).

Table 38 shows a slight but retarded increase in bacteriophage numbers at pH 5.0 in V-8 broth. Similar results are shown in table 39. The V-8 broth used in securing the data for table 38 contained 0.1 per cent of $CaCl_2$, while the other trial (table 39) was run without $CaCl_2$. Also by comparison of table 43 with table 44 at pH levels of 6.3 and 6.5, respectively, indications are that added $CaCl_2$ was not necessary for multiplication of this particular strain of bacteriophage.

Bacteriophage proliferation occurred in litmus milk at pH 5.2 (table 40) but was greatly retarded as compared with pH 6.5 (table 44). Of interest at pH 5.2 (table 40) is the large numbers of organisms that were maintained from 12 to 24 hr. while the bacteriophage numbers increased relatively little during this period, after reaching fairly high numbers at about 12 hr. The organism numbers compare favorably to the numbers of the control where no bacteriophage was present.

In trial 2 at pH 5.3 (table 41), mass lysis of the organisms took place between 6 and 8 hr., while in trial 1

mass lysis did not occur in 24 hr. This possibly may be explained by the slight variation of the pH in the two trials. In trial 1 the pH was dropping before the bacteriophage population had really begun to increase. At pH 5.2 (table 40) the sensitivity of the bacteriophage to pH levels in this range is shown and it is possible that the slightly lower pH in trial 1 (table 41) accounts for the extended incubation period without lysis occurring.

The organisms of this combination show more sensitivity to the alkaline reactions than does the bacteriophage. At pH 8.2 (table 45) the organisms growth is greatly retarded, while the bacteriophage built up to a rather high titer. Of course the maximum organism population may have been missed due to the 12 hr. lapse from the time of the initial count to the next count. However this is doubtful, since there was no great fluctuation in the pH during that time. A similar situation also is shown at pH 8.6 (table 46).

An interesting but at the present unexplained phenomenon occurred with this combination at high pH levels. On several occasions considerable bacteriophage proliferation would take place at one pH and not at a lower pH. When the data were tabulated it was found that the organisms did not always show positive growth in a 24 hr. period. There is no logical explanation for this since all procedures were standardized and very closely controlled. At pH levels of 9.1 and 9.4 (tables 47 and 48) there was a decrease in number of organisms

after the original counts were made. If during the incubation period the organisms became adjusted and showed evidence of growth, the bacteriophage numbers increased very rapidly.

Figure 7 summarizes typical data on the bacteriophage population changes for this combination. Of particular interest are the prolonged lag phases at pH levels of 8.6, 9.1 and 9.4. In each case, when bacteriophage proliferation begins, the increase is almost as rapid as at pH 6.5 for a few hours but it then tapers off more abruptly and at a much lower total population.

A summary of the organism population changes is shown in figure 8. Again the higher pH levels (8.6, 9.1 and 9.4) are of interest. In each case there is a decrease in number of organisms followed by an increase. This increase in number of organisms is correlated closely with the bacteriophage proliferation (figure 7). At pH 8.6 there is an indication that mass lysis occurred between the 18th and 22nd hours. This is not the typical abrupt and complete lysis that occurs at lower pH levels but a more gradual one and not nearly so complete on a percentage basis.

Table 38

Population changes in the S. cremoris H1,1-bacteriophage F4 combination during incubation at 32° C. in V-8 broth plus $CaCl_2$ at pH 5.0

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 5.0	0	5.0	4.76	-
	2	4.99	4.69	-
	4	4.99	4.57	-
	6	4.99	4.00	-
	8	4.99	3.28	-
	10	4.92*	2.30	-
	12	5.02	2.48	-
	24	-	-	-
Organisms plus phage maintained at pH 5.0	0	5.0	4.67	1.98
	2	5.0	4.81	1.98
	4	5.0	4.65	1.30
	6	5.0	4.09	1.65
	8	5.0	2.70	1.65
	10	4.98	2.30	0.95
	12	4.96	2.30	1.30
	24	-	-	3.40

* 1N NaOH added to bring pH to desired level

Table 39

Population changes in the S. cremoris H1,1-bacteriophage F4 combination during incubation at 32° C. in V-8 broth at pH 5.0

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organism control	0	5.0	4.54	-
	6	5.0	4.53	-
	8	5.0	4.54	-
	10	4.95	4.52	-
	12	4.95	4.53	-
	14	4.95	4.32	-
	24	4.95	- *	-
Organisms maintained at pH 5.0	0	5.0	4.51	-
	6	5.0	4.58	-
	8	5.0	4.51	-
	10	4.95	4.53	-
	12	4.95	4.30	-
	14	4.95	4.25	-
	24	4.95	- *	-
Organisms plus phage	0	5.0	4.39	1.40
	6	5.0	4.38	1.40
	8	5.0	4.60	1.60
	10	4.95	4.38	1.60
	12	4.95	4.49	1.18
	14	4.95	4.37	1.65
	24	4.95	- *	2.65
Organisms plus phage maintained at pH 5.0	0	5.0	4.34	1.40
	6	5.0	4.48	1.40
	8	5.0	4.28	1.88
	10	5.0	4.36	1.60
	12	5.0	4.59	1.40
	14	5.0	4.31	1.40
	24	5.0	- *	1.40

* Plate count was not made

Table 40

Population changes in the S. cremoris H1,1-bacteriophage F4 combination during incubation at 32° C. in litmus milk at pH 5.2 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organisms maintained at pH 5.2	0	0	5.2	5.2	4.51	4.64	-	-
	6	-	5.2	-	4.99	-	-	-
	8	8	5.2	5.2	4.90	7.08	-	-
	10	-	5.2	-	5.15	-	-	-
	12	12	5.2	5.15*	6.09	7.41	-	-
	14	-	5.2	-	6.60	-	-	-
	-	18	-	5.2	-	7.58	-	-
	-	20	-	5.2	-	7.51	-	-
	-	21	-	5.2	-	7.61	-	-
	-	22	-	5.2	-	7.60	-	-
	-	23	-	5.2	-	7.72	-	-
	24	24	4.9	5.2	7.32	7.64	-	-
Organisms plus phage maintained at pH 5.2	0	0	5.2	5.2	4.49	4.61	1.48	1.65
	6	-	5.2	-	5.15	-	1.60	-
	8	8	5.2	5.2	5.30	7.06	2.88	3.98
	10	-	5.2	-	5.70	-	3.95	-
	12	12	5.2	5.15*	6.34	7.40	6.40	5.65
	14	-	5.2	-	6.89	-	6.18	-
	-	18	-	5.2	-	7.61	-	6.40
	-	20	-	5.15*	-	7.52	-	7.18
	-	21	-	5.2	-	7.44	-	6.65
	-	22	-	5.15*	-	7.50	-	7.18
	-	23	-	5.2	-	7.47	-	6.65
	24	24	4.9	5.2	6.89	6.95	6.98	6.30

* 1N NaOH added to bring pH to desired level

Table 41

Population changes in the S. cremoris H1,1-bacteriophage F4 combination during incubation at 32° C. in litmus milk at pH 5.3 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organism control	0	0	5.3	5.35	4.56	4.59	-	-
	6	6	5.3	5.35	5.94	5.96	-	-
	8	8	5.2	5.35	6.51	6.61	-	-
	10	10	5.2	5.25	7.11	7.09	-	-
	12	12	5.1**	5.15	7.46	7.44	-	-
	14	14	5.05	5.05	7.45	7.51	-	-
	24	24	4.9	4.8	7.51	7.47	-	-
Organisms maintained at pH 5.3	0	0	5.3	5.3	4.67	4.37	-	-
	6	6	5.3	5.3	5.79	5.74	-	-
	8	8	5.25*	5.3	6.59	6.26	-	-
	10	10	5.25*	5.25*	7.17	6.83	-	-
	12	12	5.2*	5.25*	7.47	7.26	-	-
	14	14	5.25*	5.15*	7.67	7.53	-	-
	24	24	4.9	5.0	7.87	7.82	-	-
Organisms plus phage	0	0	5.3	5.3	4.49	4.32	2.40	0.95
	6	6	5.3	5.3	5.49	5.79	2.40	5.15+
	8	8	5.2	5.3	6.08	2.30	2.98	6.15+
	10	10	5.2**	5.3	6.98	<2.00	4.40	7.15+
	12	12	5.1**	5.3	7.20	<2.00	4.65	7.15+
	14	14	5.1	5.25	7.37	<2.00	4.65	8.40
	24	24	4.9	5.3	7.45	<2.00	6.40	8.65
Organisms plus phage maintained at pH 5.3	0	0	5.3	5.3	4.58	4.46	2.40	0.60
	6	6	5.3	5.3	5.80	5.51	2.40	5.15+
	8	8	5.2*	5.4	6.48	2.00	4.40	6.15+
	10	10	5.25*	5.4	7.15	<2.00	4.88	7.15+
	12	12	5.2*	5.35	7.52	<2.00	5.65	7.15+
	14	14	5.3	5.35	7.55	<2.00	7.98	8.30
	24	24	4.9	5.35	7.73	<2.00	8.40	8.65

* 1N NaOH added to bring pH to desired level

** Coagulated

Table 42

Population changes in the S. cremoris H1,1-bacteriophage F4 combination during incubation at 32° C. in litmus milk at pH 5.4

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organism control	0	5.3	3.98	-
	1	5.4	4.56	-
	2	5.4	4.70	-
	3	5.4	5.10	-
	4	5.4	5.65	-
	5	5.4	5.54	-
	6	5.4	6.04	-
	7	5.4	6.58	-
	8	5.4	6.99	-
Organisms maintained at pH 5.4	0	5.25	4.16	-
	1	5.4	4.37	-
	2	5.45	4.83	-
	3	5.45	5.25	-
	4	5.45	5.51	-
	5	5.45	6.04	-
	6	5.45	6.39	-
	7	5.45	7.03	-
	8	5.4	7.28	-
Organisms plus phage	0	5.3	4.33	2.65
	1	5.4	4.51	1.65
	2	5.4	4.80	4.40
	3	5.4	5.30	3.40
	4	5.4	5.38	4.98
	5	5.4	5.32	5.98
	6	5.4	5.64	6.98
	7	5.4	<2.00	9.15+
	8	5.4	<2.00	8.65
Organisms plus phage maintained at pH 5.4	0	5.3	4.18	1.65
	1	5.4	4.49	2.40
	2	5.45	4.82	4.40
	3	5.4	5.26	3.40
	4	5.4	5.62	4.18
	5	5.4	6.05	5.40
	6	5.4	5.95	7.40
	7	5.4	2.00	8.40
	8	5.4	<2.00	9.15+

Table 43

Population changes in the S. cremoris H1,1-bacteriophage F4 combination during incubation at 32° C. in V-8 broth plus $CaCl_2$ at pH 6.3

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 6.3	0	6.3	4.52	-
	1	6.2*	4.75	-
	2	-	-	-
	3	6.15*	5.04	-
	4	6.15*	5.30	-
	5	6.2*	5.89	-
	6	5.8*	6.86	-
	7	5.65*	7.12	-
	8	5.25	6.78	-
Organisms plus phage maintained at pH 6.3	0	6.3	4.53	1.65
	1	6.0*	4.68	1.65
	2	-	-	-
	3	6.25*	5.15	2.98
	4	6.15*	5.30	3.65
	5	6.2*	5.85	3.88
	6	5.85*	6.92	5.98
	7	5.65*	7.53	8.98
	8	5.8	2.60	9.98

* 1N NaOH added to bring pH to desired level

Table 44

Population changes in the S. cremoris M1,1-bacteriophage F4 combination during incubation at 32° C. in litmus milk at pH 6.5

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 6.5	0	6.5	4.24	-
	1	6.5	4.37	-
	2	6.5	4.97	-
	3	6.5	5.47	-
	4	6.5	6.09	-
	5	6.45*	6.09	-
	6	6.41*	7.23	-
	7	6.45*	7.30	-
	8	6.45	7.50	-
Organisms plus phage maintained at pH 6.5	0	6.5	4.25	<0.48
	1	6.5	4.33	0.60
	2	6.5	5.01	<0.48
	3	6.5	5.67	2.40
	4	6.5	6.46	3.65
	5	6.45*	6.61	5.98
	6	6.41*	4.57	7.98
	7	6.41*	<2.00	9.15+
	8	6.5	<2.00	9.65

* 1N NaOH added to bring pH to desired level

Table 45

Population changes in the S. cremoris H1,1-bacteriophage F4 combination during incubation at 32° C. in litmus milk at pH 8.2

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 8.2	0	8.2	3.86	-
	12	8.1*	3.28	-
	15	8.2	3.34	-
	18	8.23	3.67	-
	20	8.19	3.92	-
	22	8.11*	4.63	-
	24	8.2	4.67	-
Organisms plus phage maintained at pH 8.2	0	8.2	3.79	2.40
	12	8.05*	3.73	6.15+
	15	8.2	3.30	6.15+
	18	8.21	3.30	6.15+
	20	8.18	3.30	6.15+
	22	8.11*	3.00	6.15+
	24	8.2	3.51	6.65

* 1N NaOH added to bring pH to desired level

Table 46

Population changes in the S. cremoris H1,1-bacteriophage F4 combination during incubation at 32° C. in litmus milk at pH 8.6

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 8.6	0	8.61	3.93	-
	12	8.59	3.15	-
	15	8.59	3.32	-
	18	8.56	3.90	-
	20	8.5*	3.48	-
	22	8.6	3.79	-
	24	8.57	3.92	-
Organisms plus phage maintained at pH 8.6	0	8.62	3.86	1.40
	12	8.5*	3.66	<0.48
	15	8.59	4.38	0.60
	18	8.59	4.37	3.98
	20	8.58	3.78	5.65
	22	8.58	2.85	6.40
	24	8.52	3.04	6.40

* 1N NaOH added to bring pH to desired level

Table 47

Population changes in the S. cremoris H1,1-bacteriophage F4 combination during incubation at 32° C. in litmus milk at pH 9.1 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organisms maintained at pH 9.1	0	0	9.12	9.1	4.38	4.75	-	-
	12	12	8.95*	9.1	3.72	3.11	-	-
	15	15	9.1	9.05*	3.54	3.23	-	-
	18	18	9.01*	9.08	3.62	3.26	-	-
	21	21	9.2	9.08	3.53	3.11	-	-
	24	24	9.2	9.05	3.15	3.67	-	-
Organisms plus phage maintained at pH 9.1	0	0	9.1	9.1	4.39	4.75	1.60	0.95
	12	12	8.95*	9.1	3.57	3.46	1.98	1.30
	15	15	9.1	9.05	3.76	3.59	1.98	1.40
	18	18	9.07*	9.06	3.79	3.97	1.98	1.65
	21	21	9.08*	9.05	3.43	4.07	1.65	2.40
	24	24	9.2	9.0	3.11	4.29	1.98	5.65

* 1N NaOH added to bring pH to desired level

Table 48

Population changes in the S. cremoris H1,1-bacteriophage F4 combination during incubation at 32° C. in litmus milk at pH 9.4 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organisms maintained at pH 9.4	0	0	9.4	9.4	4.37	4.84	-	-
	12	12	9.22*	9.4	4.00	2.60	-	-
	15	15	9.34*	9.31*	4.14	2.95	-	-
	18	18	9.31*	9.31*	4.23	2.30	-	-
	21	21	9.39	9.31*	4.38	2.48	-	-
	24	24	9.4	9.32	4.72	3.08	-	-
Organisms plus phage maintained at pH 9.4	0	0	9.4	9.4	4.38	4.64	1.98	0.95
	12	12	9.2*	9.4	3.54	2.70	2.18	0.95
	15	15	9.32*	9.3*	3.88	2.78	1.65	0.95
	18	18	9.41	9.32*	3.67	2.60	2.40	0.60
	21	21	9.35*	9.36*	4.07	2.00	4.65	0.60
	24	24	9.4	9.35	3.80	2.30	5.65	1.40

* 1N NaOH added to bring pH to desired level

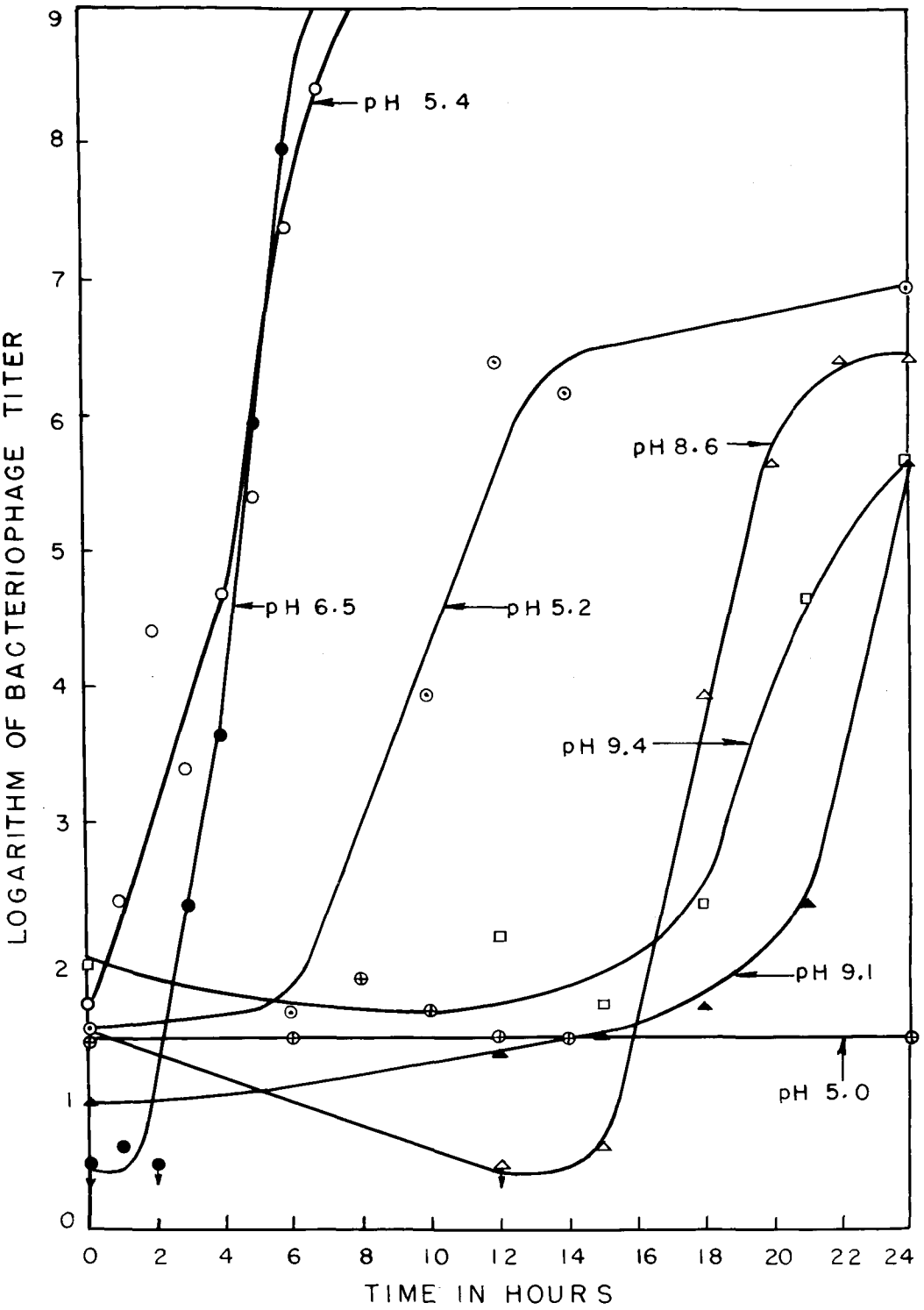

Fig. 7. Summary of typical data of bacteriophage F4 population changes at various pH levels.

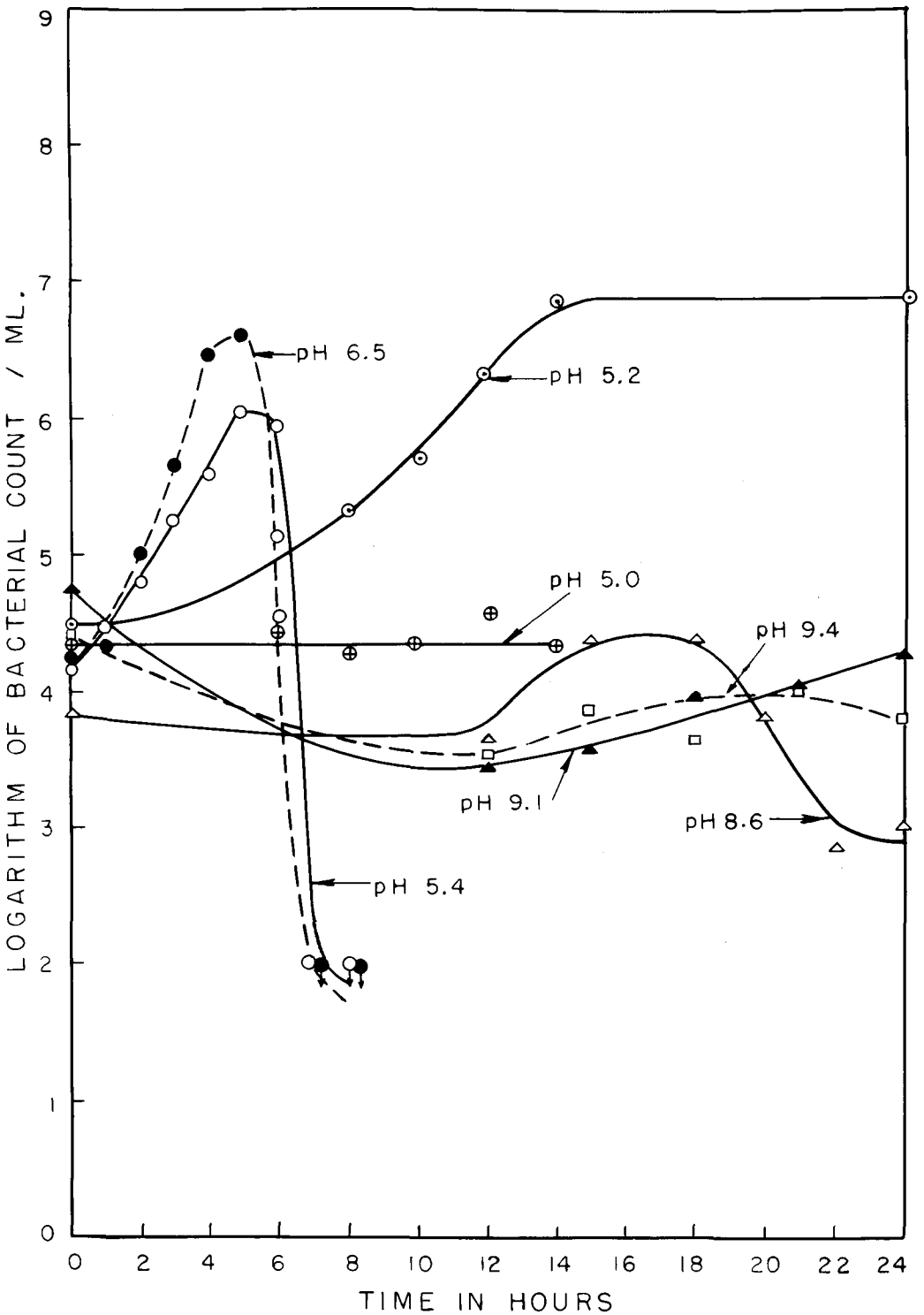

Fig. 8. Summary of typical data of S. cremoris H1,1 population changes at various pH levels.

Streptococcus cremoris 122,1-bacteriophage F43 combination

The population changes at various pH levels for 122,1-F43 combination are presented in tables 49 through 57 and a summary of typical data is shown in figures 9 and 10. The bacteriophage was more sensitive to the acid conditions than was the organism. At pH 4.8 (table 49) there is no definite multiplication of bacteriophage during a 24 hr. incubation period, while there is a substantial increase in the number of organisms.

At pH 5.0 in V-8 broth with added $CaCl_2$ the bacteriophage multiplication was very rapid and in trial 2 (table 50) the organisms were lysed at 12 hr. In trial 1 the bacteriophage increased somewhat more slowly and the titer at the end of 12 hr. incubation was considerably lower than in trial 2. Added $CaCl_2$ was necessary for optimum bacteriophage proliferation for this strain, although table 51 possibly shows a slight increase in bacteriophage population in V-8 broth without added $CaCl_2$ during a 24 hr. period. By comparing data of table 51 with that of table 50, in which $CaCl_2$ was added to the V-8 broth, there is a significant difference in final population of bacteriophage at 12 hr. The effect of $CaCl_2$ on proliferation of this strain is also demonstrated in tables 52 and 53 at pH 5.2. although a difference in basal medium also may have been a factor.

There is very little retardation in bacteriophage proliferation at pH 5.0 (table 50) or pH 5.2 (table 52) as compared to pH 6.5 (table 54). However, when the reaction is changed to pH 8.0 and above there is a significant retarding of organism growth accompanied by a similar retardation in bacteriophage proliferation.

In table 55 at pH 8.0 there appears to be an increase in bacteriophage numbers without an increase in organisms as determined by the plate count.

In figure 9 the prolonged lag phases at pH levels of 8.4 and 8.6 are of interest. In each case there was a decrease in the numbers of organisms followed by a slight increase at about 18 hr. and a concurrent increase in bacteriophage population. Even at pH levels nearer the optimum for bacteriophage proliferation, there is a lag phase of several hours for this strain.

Figure 10 shows typical data on the changes in organism populations at various pH levels. As can be seen from this figure, mass lysis is delayed more than 1 hr. at pH 5.2 as compared to pH 6.5 and about 5 hr. at pH 5.0. At pH 4.8 the organisms continued to increase in numbers up to 20 hr., indicating that they were not inhibited by the acid reaction. At pH levels of 8.4 and 8.6 there was a decrease in organism numbers, indicating that the alkaline reaction was inhibitory to them at least for 16 to 18 hr., after which slight growth took place and apparently put the cells in a condition to support bacteriophage multiplication.

Table 49

Population changes in the S. cremoris 122,1-bacteriophage F43 combination during incubation at 32° C. in V-8 broth plus $CaCl_2$ at pH 4.8

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 4.8	0	4.82	4.77	-
	12	4.79	7.08	-
	15	4.75*	7.43	-
	18	4.71*	7.85	-
	20	4.75*	8.14	-
	22	4.7*	8.13	-
	24	4.62	8.24	-
Organisms plus phage maintained at pH 4.8	0	4.82	4.88	<0.48
	12	4.78	7.13	1.40
	15	4.72*	7.45	1.40
	18	4.71*	8.01	<0.48
	20	4.74*	8.36	<0.48
	22	4.7*	8.35	0.48
	24	4.6	8.24	<0.48

* 1N NaOH added to bring pH to desired level

Table 50

Population changes in the S. cremoris 122,1-bacteriophage F43 combination during incubation at 32° C. in V-8 broth plus $CaCl_2$ at pH 5.0 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organisms maintained at pH 5.0	0	0	5.0	5.0	4.85	5.05	-	-
	2	2	4.99	4.99	5.24	5.40	-	-
	4	4	4.99	4.99	5.92	5.85	-	-
	6	6	4.99	4.99	6.43	6.48	-	-
	8	8	4.96*	4.96*	7.00	6.96	-	-
	10	10	4.96*	4.95*	7.39	7.61	-	-
	12	12	5.0	4.85	7.89	7.96	-	-
Organisms plus phage maintained at pH 5.0	0	0	5.0	5.0	4.81	4.91	1.40	1.98
	2	2	5.0	4.99	5.42	5.47	1.40	1.65
	4	4	5.0	4.99	5.92	5.87	1.40	2.65
	6	6	5.0	4.99	6.33	6.37	1.65	2.65
	8	8	4.96*	4.95*	6.90	7.02	3.40	3.98
	10	10	4.96*	4.90*	7.41	7.40	5.04	7.15+
	12	12	5.0	4.90**	7.18	<2.00	6.15+	8.15+

* 1N NaOH added to bring pH to desired level

** Culture cleared

Table 51

Population changes in the S. cremoris 122,1-bacteriophage F43 combination during incubation at 32° C. in V-8 broth at pH 5.0

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 5.0	0	5.0	4.87	-
	2	5.0	5.09	-
	4	5.0	5.86	-
	6	5.0	6.42	-
	8	5.0	6.95	-
	10	4.91*	7.38	-
	12	4.93*	7.69	-
	24	4.2	7.56	-
Organisms plus phage maintained at pH 5.0	0	5.0	4.84	2.65
	2	5.0	5.19	2.40
	4	5.0	5.92	3.40
	6	5.0	6.34	3.18
	8	5.0	6.90	3.30
	10	4.91*	7.50	3.65
	12	4.92*	7.86	3.04
	24	4.2	7.76	4.98

* 1N NaOH added to bring pH to desired level

Table 52

Population changes in the S. cremoris 122,1-bacteriophage F43 combination during incubation at 32° C. in litmus milk at pH 5.2

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 5.2	0	5.2	4.66	-
	2	5.2	5.23	-
	4	5.2	5.96	-
	6	5.2	6.59	-
	8	5.18	7.37	-
	10	5.17	7.66	-
	12	5.11**	7.95	-
Organisms plus phage maintained at pH 5.2	0	5.2	4.69	2.65
	2	5.2	5.21	1.98
	4	5.2	6.00	2.40
	6	5.19	6.68	5.65
	8	5.18	5.12	6.15+
	10	5.18	2.00	6.15+
	12	5.13	2.00	7.15+

** Milk coagulated

Table 53

Population changes in the S. cremoris 122,1-bacteriophage F43 combination during incubation at 32° C. in V-8 broth at pH 5.2

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 5.2	0	5.2	4.89	-
	2	5.12*	5.37	-
	4	5.21	6.02	-
	6	5.21	6.68	-
	8	5.12*	7.29	-
	10	5.16*	7.88	-
	12	5.12	8.18	-
Organisms plus phage maintained at pH 5.2	0	5.2	5.03	1.88
	2	5.19	5.42	0.95
	4	5.19	5.98	1.40
	6	5.18	6.76	1.40
	8	5.12*	7.24	1.98
	10	5.16*	7.71	<0.48
	12	5.2	8.26	<0.48

* 1N NaOH added to bring pH to desired level

Table 54

Population changes in the S. cremoris 122,1-bacteriophage F43 combination during incubation at 32° C. in litmus milk at pH 6.5

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 6.5	0	6.51	4.72	-
	1	6.51	4.76	-
	2	6.51	5.38	-
	3	6.5	5.95	-
	4	6.41*	6.60	-
	5	6.5	7.03	-
	6	6.4*	7.62	-
	7	6.4*	7.77	-
	8	6.42	7.93	-
Organisms plus phage maintained at pH 6.5	0	6.51	4.77	2.40
	1	6.51	4.93	2.40
	2	6.51	5.39	2.40
	3	6.5	5.99	2.65
	4	6.4*	6.35	2.40
	5	6.5	7.03	6.18
	6	6.41*	3.51	7.15+
	7	6.52	3.11	9.15+
	8	6.52	2.78	8.65

* 1N NaOH added to bring pH to desired level

Table 55

Population changes in the S. cremoris 122,1-bacteriophage F43 combination during incubation at 32° C. in litmus milk at pH 8.0

Culture	Time (hr.)	pH	Log of bacteria count/ml.	Log of phage titer/ml.
Organisms maintained at pH 8.0	0	8.0	4.75	-
	2	8.0	4.75	-
	4	8.0	3.64	-
	6	7.96*	3.04	-
	8	8.04	3.08	-
	10	8.05	3.45	-
	12	8.0	3.88	-
Organisms plus phage maintained at pH 8.0	0	8.0	4.82	2.65
	2	8.0	4.85	2.65
	4	7.96*	3.68	2.65
	6	8.0	2.85	3.40
	8	7.98	2.70	3.65
	10	7.98	2.48	3.98
	12	7.95	<2.00	5.65

* 1N NaOH added to bring pH to desired level

Table 56

Population changes in the S. cremoris 122,1-bacteriophage F43 combination during incubation at 32° C. in litmus milk at pH 8.4 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organisms maintained at pH 8.4	0	0	8.45	8.4	4.63	5.07	-	-
	12	12	8.31*	8.23*	2.00	4.06	-	-
	15	15	8.41	8.4	2.48	4.61	-	-
	18	18	8.4	8.38	3.34	4.61	-	-
	21	21	8.4	8.32*	4.01	5.17	-	-
	24	24	8.36	8.4	5.06	4.99	-	-
Organisms plus phage maintained at pH 8.4	0	0	8.45	8.41	4.38	4.85	1.98	1.40
	12	12	8.3*	8.24*	3.36	2.70	1.98	1.40
	15	15	8.41	8.4	2.85	3.11	2.65	1.65
	18	18	8.4	8.38	2.30	3.30	3.40	2.98
	21	21	8.35*	8.35*	3.08	3.52	3.65	3.40
	24	24	8.4	8.4	3.15	3.46	4.40	3.98

* 1N NaOH added to bring pH to desired level

Table 57

Population changes in the S. cremoris 122.1-bacteriophage F43 combination during incubation at 32° C. in litmus milk at pH 8.6 (Trials 1 and 2)

Culture	Time (hr.)		pH		Log of bacteria count/ml.		Log of phage titer/ml.	
	1	2	1	2	1	2	1	2
Organisms maintained at pH 8.6	0	0	8.65	8.6	4.57	4.80	-	-
	12	12	8.5*	8.45*	4.89	3.28	-	-
	15	15	8.58	8.58	4.75	3.85	-	-
	18	18	8.54*	8.55	5.19	4.17	-	-
	21	21	8.6	8.5*	5.00	4.83	-	-
	24	24	8.55	8.54	5.46	5.03	-	-
Organisms plus phage maintained at pH 8.6	0	0	8.63	8.6	4.47	4.81	1.98	1.40
	12	12	8.4*	8.45*	<2.00	2.30	1.65	1.40
	15	15	8.61	8.6	3.08	2.95	1.65	1.65
	18	18	8.6	8.57	3.40	2.48	2.18	2.98
	21	21	8.6	8.53*	2.60	2.00	1.98	3.40
	24	24	8.55	8.58	2.60	<2.00	3.98	3.65

* 1N NaOH added to bring pH to desired level

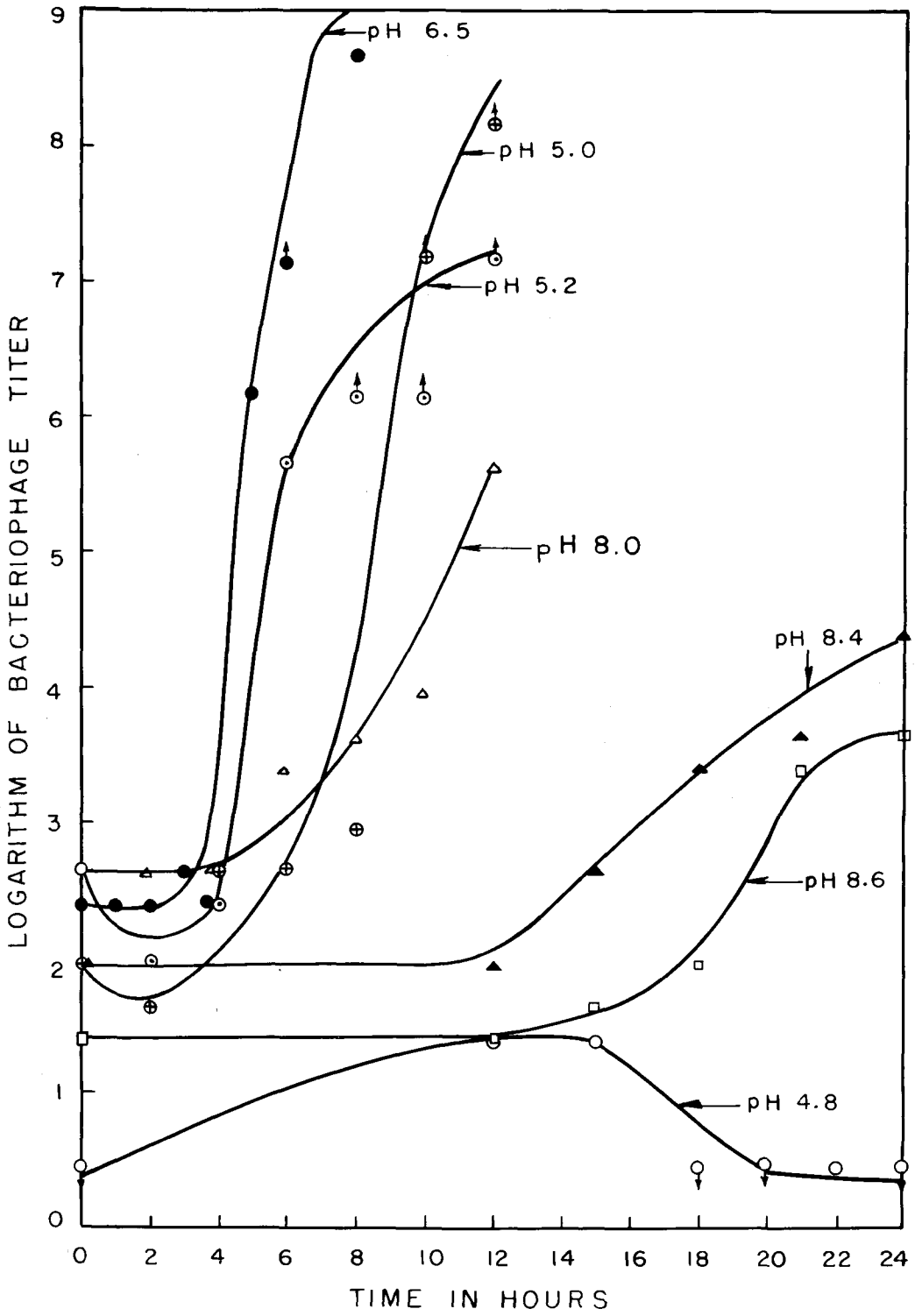

Fig. 9. Summary of typical data of bacteriophage F43 population changes at various pH levels.

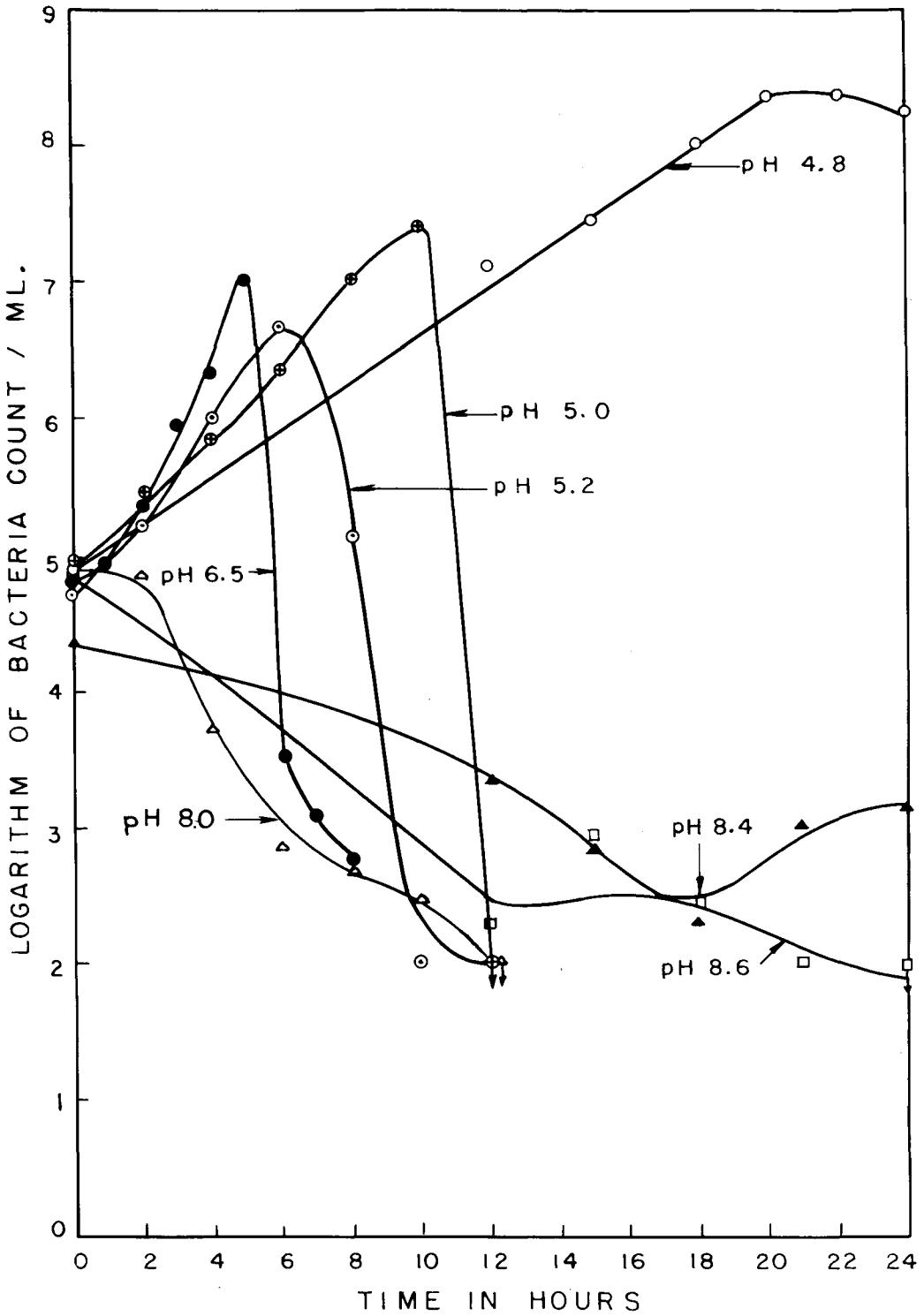

Fig. 10. Summary of typical data of S. cremoris 122,1 population changes at various pH levels.

DISCUSSION

The optimum pH for lactic streptococcus bacteriophage proliferation was slightly on the acid side of neutrality, at about pH 6.5. From the study of five strains of bacteriophage the data indicate that considerable proliferation occurs over a pH range of 5.4 to 7.5 for all strains and extends beyond this range for some strains. At pH 5.4 there was very little difference in the rate of bacteriophage proliferation as compared to pH 6.5 for these strains. The lag phases were increased slightly but high final bacteriophage titers were obtained with all strains. For strains F24, F4 and F43 the titers were in the range of 10^8 to 10^9 or above at pH ranges of 5.4 to 7.5, while for strains F55 and F56 the values were somewhat lower being in the range of 10^6 to 10^8. With F43 and F24, proliferation comparable to that at pH 6.5 occurred as low as pH 5.0 and F24 increased slightly at pH 4.8 after a very long lag phase.

At pH 7.5 rapid proliferation of all five strains of bacteriophage occurred, although F55 was retarded somewhat, as was evident by the increased lag phase. There was no close relationship among the strains in their ability to proliferate under alkaline conditions. While F55 increased very little at pH 7.6, and then only after an extended lag phase, F24 caused mass lysis of the susceptible cells at this level. Strains

F56 and F43 were able to proliferate at pH 8.4 and 8.6, respectively. F24 and F4 did not show the sensitivity to alkaline reactions that the other strains did, each multiplying at pH 9.4. Proliferation of F4 at pH 9.1 and pH 9.4 depended upon growth of the organism, as determined by the plate count, which occurred in some instances and not in others. The reasons for the variability were not apparent from the data available.

Because F24 proliferated over the entire range of organism growth (pH 4.8 to pH 9.4), it is quite probable that this represents the extremes at which lactic streptococcus bacteriophage can multiply, since these pH levels approximate the limits of growth for the S. lactis species. F55 could easily represent the other extreme, since its proliferation was confined to such a narrow range of pH (5.2 to 7.6). In view of these data, the probability of obtaining strains vastly different in their response to pH does not seem likely.

Under the conditions of this experiment bacteriophage proliferation was dependent upon growth of the organisms. However, with 122,1-F43 on one occasion at pH 8.0 it appeared that proliferation occurred without organism increases. The organisms evidently were in the right physiological state for bacteriophage proliferation but their increase, as determined by the plate count, was inhibited by the presence of the bacteriophage. Further study is needed to elucidate certain aspects of the organism-bacteriophage relationships at these higher

pH levels where consistent results are not always obtained. Unquestionably factors other than pH play a considerable role in bacteriophage proliferation at these higher pH levels.

Strain F24 proliferated over the entire pH range of organism growth. F56 failed to increase below pH 5.0 or above pH 8.4, while its host organism, 712, grew rapidly at pH 4.7 and also above pH 8.4. Organisms H1,1 and 573 failed to exhibit definite increases in number of organisms at pH 5.0, but H1,1 did, upon occasion, show very slight and temporary increases at pH 9.1 and 9.4 and when these increases occurred bacteriophage proliferation took place.

Lysis of the cells occurred whenever there were large bacteriophage increases. When mass lysis occurred at the higher pH levels it was not the typical abrupt lysing of the cells but rather a gradual process taking place over several hours. Mass lysis occurred with the W2-F24 combination from pH 5.0 to pH 7.6; F43 also caused mass lysis at pH 5.0. These data are in direct contrast with those from combination 573-F55 for which neither bacteriophage nor organism increased in numbers at pH 5.0 and both were retarded considerably at pH 7.6. Mass lysis for 573-F55 combination occurred at pH 5.4 but not at pH levels as high as 7.5.

All the data on these five strains of bacteriophage were obtained at incubation temperatures of 32° C. It is possible that other temperatures would give different results. Turner (1948) found 32° C. to be optimum for bacteriophage and organism multiplication and variation from this temperature increased the

lag phase of both organisms and bacteriophage. His results applied over a temperature range of 21 to 35° C., or slightly above. Probably with the combinations used in the present work some differences in results could be expected as a result of a variation of the incubation temperature within the 21 to 35° C. range.

The data presented fail to show any correlation between the type of organism and bacteriophage response to pH. Organism W2 and 712 are classified as S. lactis, while H1,1, 573 and 122,1 are S. cremoris. The "cremoris type" usually is considered more fastidious and more sensitive to adverse conditions and one might expect a similar situation in the homologus bacteriophage strains; the data show that this is not true. With H1,1-F4 combination the bacteriophage was able to multiply at pH 9.4 and with the 122,1-F43 combination, F43 multiplied at pH 5.0, while proliferation of F56 on S. lactis 712 was greatly retarded at pH levels of 5.0 and 8.4.

When inoculations of one drop of a 1-to-10 dilution of a freshly coagulated culture were used in 8 ml. of litmus milk, the "lactis type", W2 and 712, brought about coagulation in 10 hr. or less. The "cremoris type", 573, and 122,1, were only slightly slower, requiring 10 to 12 hr. to form a firm, solid curd. On the other hand, H1,1 required at least 15 hr. for coagulation and then the curd was very soft.

These five strains of bacteriophage were chosen from a large collection with the idea of selecting strains as different as possible. Both very rapid and rather slow growing

organisms and their homologous bacteriophages were represented. Two strains came from England, while the other three were isolated at Iowa State College, indicating widely separated sources. Also each strain was from a separate serological grouping, as determined by Wilkowske (1949). The wide variation of these five strains in their response to pH at various levels and their calcium requirement indicates quite a difference among bacteriophages of the lactic streptococci.

The occasional failure of a seemingly normal culture can be explained in part by the data presented. The organisms may lower the pH so rapidly that mass lysis does not occur but still high concentrations of bacteriophage may be built up, especially if the bacteriophage is capable of some proliferation in the lower pH ranges. As a result of a situation like this the culture would coagulate and appear normal, but when used as an inoculum in milk with a pH near the optimum for bacteriophage proliferation, lysis of the susceptible cells would occur in a few hours. This whole problem becomes a question of the balance between the time the medium reaches a pH low enough to prevent rapid bacteriophage proliferation and the time of mass lysis. With some combinations a large inoculation of organisms is necessary in order to lower the pH quickly enough to that point where mass lysis can not occur. Otherwise the bacteriophage would lyse the organisms before there was much change in the pH. Parmelee (1950) has found that as few as 200 bacteriophage particles of a strain not used in this study, when introduced into 300 ml. of milk inoculated with 1 per cent of a freshly coagulated culture

of sensitive organisms were capable of preventing normal coagulation by causing mass lysis. The ability of certain strains of bacteriophage to multiply at low pH levels and build up to high concentrations, if not lysing the organisms, make it difficult in commercial operations for certain strains of organisms to overgrow the bacteriophage.

SUMMARY AND CONCLUSIONS

Five strains of lactic streptococcus bacteriophage and the homologous organisms were studied over a wide pH range to determine the influence of reaction on bacteriophage proliferation.

The maximum and minimum pH at which bacteriophage proliferation occurred varied for each strain studied. Strains F24 and F4 multiplied at pH 9.4 and the F24 strain as low as pH 4.8. The other three strains were between these extremes. The optimum pH for bacteriophage proliferation was slightly on the acid side of neutrality, probably at about pH 6.5, but considerable proliferation occurred over a pH range of 5.4 to 7.5.

In general bacteriophage proliferation failed to occur at pH levels where organism growth was inhibited. In one trial at pH 8.0 with 122,1-F43 it appears that the bacteriophage increased and no increase in numbers of organisms occurred. With combinations 122,1-F43, 712-F56 and 573-F55, growth of the organisms occurred beyond the pH ranges of bacteriophage proliferation.

Mass lysis occurred with W2-F24 combination at pH 5.0 and pH 7.6 with indications that mass lysis may occur at pH levels as high as 8.0 and as low as 4.9 or below under proper conditions. For the other combinations the pH range for mass lysis was somewhat narrower.

In the 573-F55 combination there was a sharp cut-off in bacteriophage proliferation between pH 7.5 and pH 7.6. There was very little difference in organism growth at these two pH levels.

With the H1,1-F4 and 573-F55 combinations there was bacteriophage proliferation at pH 5.2 but very little at pH 5.0. Growth of these two organism strains at pH 5.0 was questionable.

Strain F56 multiplied very slowly after a prolonged lag phase at pH 8.4 and strain F43 followed a similar pattern at pH 8.6.

The growth of organism 712 was abundant at pH 4.7 but its homologous bacteriophage proliferated very little below pH 5.0.

The growth of H1,1 at pH 9.4 was erratic and the same was true of proliferation of bacteriophage F4. If the organism became adapted to the highly alkaline conditions and definitely increased, the bacteriophage proliferation was very rapid.

There was very little correlation between the sensitivity of the organisms to high or low pH levels and the sensitivity of the homologous bacteriophage.

That the extremes in response to pH are represented in the five strains of bacteriophage studied is quite probable, since the proliferation of F24 occurred from pH 4.8 to pH 9.4, which approximates the limits for growth of the S. lactis species. Proliferation of F55 over a narrow pH range could easily represent the other extreme.

The use of large inoculum in mother cultures as a means of bacteriophage control will not eliminate the trouble when bacteriophage strains are capable of multiplying at low pH ranges and building up high concentrations. When the bacteriophage of the mother culture is subjected to more nearly optimum pH levels, after proliferation has been stopped by lowering of pH before mass lysis has occurred, mass lysis of the susceptible organisms will occur in a few hours.

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