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THE BEHAVIOUR OF BACTERIA IN FLUID CULTURES AS INDICATED BY DAILY ESTIMATES OF THE NUMBERS OF LIVING ORGANISMS.

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(With 29 Charts and 1 Text-fig.)

CONTENTS.

		PAGE
Introduction.	The work of previous investigators	134
	The scope of the experiments	136
	Sources of error in these experiments	138
	Method of describing the experiments	139
Section I.	Growth in neutral meat extract medium	139
II.	The influence of the previous rate of transplantation of the culture used for inoculation	141
III.	The relation of growth to the quantity of food substance present	142
IV.	The influence of the numbers inoculated	144
V.	The influence of small differences in numbers in the initial inoculation	145
VI.	The influence of occasional additions of small quantities of food substance	146
VII.	The influence of regular additions of small quantities of food substance	151
VIII.	The effects of diluting cultures with distilled water at various periods of growth	152
IX.	The influence of the incubation temperature	153
X.	The influence of the growth of one species on the growth of others subsequently inoculated into the medium	158
XI.	The influence of varying the reaction of the medium	164
XII.	The influence of the addition of small quantities of *N*/10 hydrochloric acid to the medium	167
XIII.	The effects of cultivating organisms for prolonged periods on media of different reactions	169
XIV.	The influence on growth of the addition of gelatin or agar to meat extract	174
XV.	The effects of adding various quantities of different acids to neutral meat-extract-agar before inoculation	177
XVI.	The influence of the addition of glucose	179
XVII.	The effects of adding living organisms of the same species to growing cultures	185
XVIII.	The distribution of *S. aureus* in meat extract cultures	189
XIX.	Meat extract and pancreas extract compared as media	190
XX.	Further experiments with media made from ox pancreas	195
XXI.	The effects of accidental contamination	197
	The bearing of these experiments on some of the phenomena observed in infective diseases	198
	Summary	200
	References	204

INTRODUCTION.

The work of previous investigators.

Buchner, Longard and Riedlin (1887) working with *V. cholerae* were the first to measure the rate of bacterial growth with any degree of accuracy. They plated cultures at the beginning of each experiment and after two and five hours' incubation and calculated the generation time, making, however, no allowance for "lag." Subsequent observers noticed that under the usual conditions of such experiments there is an initial period of no, or slow, growth—the lag-phase. Müller (1895) was the first to demonstrate the lag-phase; Hehewerth (1901) noted that it varied with the species and age of the culture employed and Rahn (1906) studied it in connection with *B. fluorescens*. Barber (1908), however, who worked with a single bacillus and studied the actual rate of division, observed that the period of lag could be abolished, if the organisms used for the inoculations were derived from rapidly growing cultures. Later observers have confirmed this observation. Lane-Claypon (1909) working with *B. coli*, *B. typhosus* and *B. enteritidis* made observations on the rate of growth of these organisms in broth cultures at temperatures ranging between 20° C. and 42° C. Only in a very few instances were the experiments continued beyond 30 hours' incubation. "The observations show that there are four phases in the bacterial life of a culture: (1) an initial period of slow or no growth; (2) a period of regular growth, the rapidity varying slightly at the same temperature, but differing widely for different temperatures; (3) a period when the numbers remain more or less stationary; (4) a period when the numbers of living bacteria are diminishing."

Period (1) varies between 1–6 hours, according to the temperature. Period (2): "For a given volume of fluid the time during which the bacteria continue to divide at a maximum rate depended upon the insemination and the temperature, being shorter if the inoculations were heavy and at the higher temperatures." Period (3): "After the culture has ceased growing logarithmically the rate of growth slackens gradually, but is still fairly active until the number of bacteria reaches several millions per drop (0·02 c.c.), that is several hundred millions per c.c. At this stage the number of living bacteria appears to remain fairly constant for some time after which it begins to decrease slowly." "There appears therefore to be a maximum number of bacteria which a unit volume of the medium is capable of supporting: this means that for the particular organism the conditions are no longer favourable for increased growth. This may be due to the using up of some constituents of the broth or it may be due to some inhibitory substance produced by the organisms themselves in the process of metabolism. I have not carried out any investigations on these points."

The nature of "lag" has been studied by a number of observers. Coplans (1910), Penfold (1914) and Chesney (1916) have shown that the period of lag differs on different media and under different conditions. Penfold has

enumerated some nine different hypotheses as to the cause of bacterial lag, all of which he regards as inadequate. He makes the suggestion that "the incubation period of infectious disease may partly depend for its existence on bacterial lag." Ledingham and Penfold (1914) published a mathematical analysis of the lag-phase of bacterial growth, and state that the logarithmic or second phase is succeeded by the third phase during which the generation time gradually lengthens till it finally becomes infinite and no further growth occurs. Subsequently the mathematical analysis of Ledingham and Penfold was elaborated by Slator (1916, 1917).

McKendrick and Pai (1911) worked with *B. coli* and kept their cultures at a uniform temperature throughout their experiments. By using for inoculation organisms from cultures 1–3 hours old they eliminated the latent (lag) period. They state that "if there be an unlimited supply of nutriment, an organism reproduces itself by compound interest: in a geometrical progression, *i.e.* 1, 2, 4, 8, etc." "In test tube experiments, however, this simple state of affairs is complicated by the fact that the supply of nutriment is limited, and consequently as time goes on, the rate of multiplication falls off." "Every living organism employs the nutriment which it has absorbed for two objects; first, the maintenance of the individual; and, second, its reproduction. As, however, in the case of those micro-organisms with which we shall deal, the rate of multiplication is very fast, we may, for all practical purposes, consider that the amount of foodstuff utilised for their upkeep is negligible, and assume that the whole of it is employed in reproduction. If we accept this simplifying assumption we may say that organisms in a test tube multiply, by a simple conversion of the available foodstuff into other organisms, and that the rate of multiplication is proportional to the concentration of the foodstuff."

Buchanan (1918) made no experiments, but has published recently a study of the results secured by various authors, and states that "seven relatively distinct periods" of growth may be differentiated: (1) initial stationary phase; (2) lag-phase or positive growth acceleration phase; (3) logarithmic growth phase; (4) phase of negative growth acceleration; (5) maximum stationary phase; (6) phase of accelerated death; (7) logarithmic death phase. His first two phases seem to have been included in the lag-phase of previous workers.

Penfold and Norris (1912) made observations on the relation of concentration of peptone to the generation time, and Salter (1919) determined the rate of growth of *B. coli* for some hours and studied the effects of various dyes. The latter author observes that "a given factor may influence the rate of growth in one phase and not in another. It may cause a lengthening of the leg phase and have no influence on the logarithmic phase or may even stimulate growth during the latter phase."

All these workers have concerned themselves mainly with the earlier phases of growth and very few experiments have been published illustrating

the phases after 24–30 hours' incubation. In all cases media containing peptone were employed.

The scope of the experiments.

The experiments described in this paper were undertaken with the purpose of attempting to estimate the numbers of living organisms, able to multiply and form colonies after subcultivation, present at different times in fluid media during prolonged cultivation under various conditions. Since the events occurring in the earlier stages of growth under certain experimental conditions have been so carefully and so fully dealt with by previous observers, and since the events occurring in the later stages have received little attention, it was considered unnecessary to make many observations on cultures which had been incubated for less than 24 hours.

The bacteria employed. The organism chiefly employed was a strain[1] of *Staphylococcus aureus* obtained from an abscess. In some of the experiments a strain of *B. coli* and in others a strain of *B. pyocyaneus* were used.

Media used. The medium used in most of the experiments was meat extract made from fresh bullock's heart muscle. After the removal of the fat and vessels the meat was passed through a mincing machine and weighed. To every 100 grammes 250 c.c. of distilled water were added, and the fluid gently boiled for 90 minutes. After filtration through filter paper the clear yellow medium was sterilised for 20 minutes on three successive days in a steam steriliser. Neither peptone nor salt were added. In some of the experiments small quantities of $N/10$ soda, $N/10$ hydrochloric acid, agar or gelatin were added.

Method of estimating the number of living[2] organisms present.

In most experiments 5 c.c. of diluted meat extract (1 c.c. of meat extract to which 4 c.c. of sterile distilled water had been added) were measured by means of a sterile pipette into a sterile wide test-tube. This was inoculated with a drop of a freshly prepared emulsion in sterile distilled water of the organism grown for 24 hours at 37° C. on meat extract agar[3]. After violently shaking the culture to distribute the organisms (see Section XVIII) and flaming[4] the mouth of the tube a standard loopful of the fluid was removed and transferred to a tube of meat extract agar, which had been melted and cooled to 45° C.

[1] It was noticed that different strains show not only varying powers of multiplication in the same medium, but remain at a high level for different periods.

[2] If living organisms incapable of forming visible colonies in agar on subculture were present their numbers would not be ascertained by the method employed.

[3] To meat extract prepared in the manner described and made neutral to neutral red by the addition of $N/10$ soda 2 per cent. of washed agar is added. After melting the agar in the autoclave the medium is filtered through cotton-wool and sterilised. No peptone, salt or egg-white are added.

[4] The mouths of all the tubes used were heated in the flame and the other usual precautions against accidental contamination observed.

The loopful of fluid was distributed in the agar partly by thoroughly stirring the agar with the loop and partly by rotating the tube between the hands. The contents of the tube were poured into a Petri dish, and the culture incubated at 37° C. for 24 hours or longer. The colonies growing on the medium were counted with the aid of a dissecting microscope, the Petri dish being placed on a glass plate, ruled with a diamond in the manner shown in Fig. 1 and supported on a large stage fitted with a substage mirror. To facilitate counting a recording machine operated by the finger was made use of. Each colony observed was recorded by pressing the finger and the fatigue of bearing in mind the numbers was thus obviated. The numbers obtained were taken to

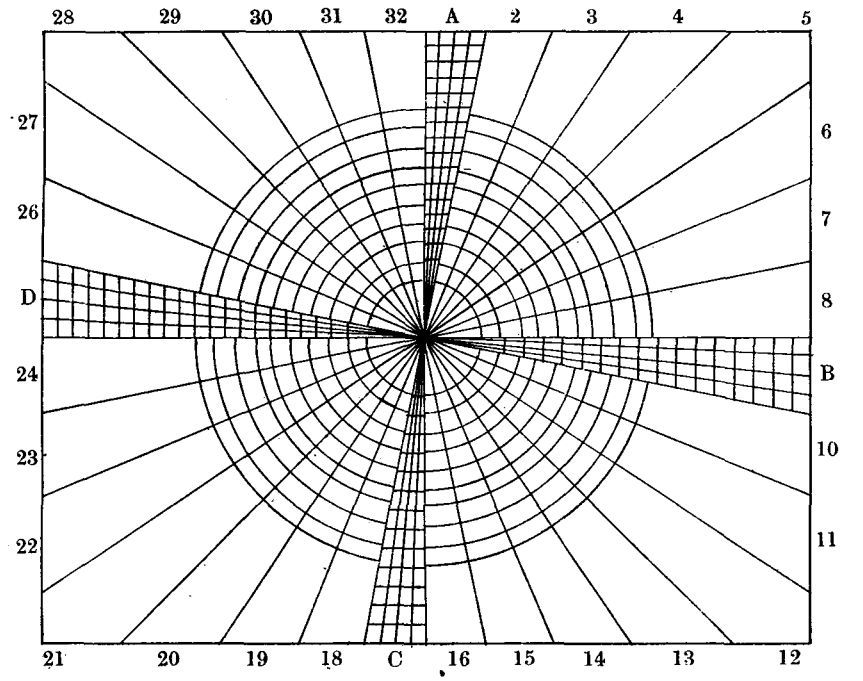

Fig. 1. Counting Plate. ($\times \frac{1}{2}$.)

indicate the number of living organisms capable of growth present in a standard loopful of the culture at the commencement of the experiment.

The standard loop with the wire slightly bent was dipped into the fluid to a certain depth when the tube was tilted towards the horizontal. When used in this manner the loop was found to carry 0·01 c.c.

After varying periods of incubation the cultures were violently shaken to distribute the organisms and standard loopfuls removed. When considerable growth was indicated by slight cloudiness of the medium dilution was found to be necessary before plating. In most cases the loopful was diluted in 5 or 10 c.c. of sterile distilled water, and from this after violent shaking a loopful was transferred to agar at 45° C. The plate cultures thus obtained often

contained very numerous colonies, which, if the manipulations had been carefully performed, were very evenly distributed. In such cases the colonies in at least four of the thirty-two sections into which the ruled counting plate was divided, or an area equivalent to one-eighth of the whole plate, were counted. The sections marked *A*, *B*, *C*, *D* in Fig. 1, subdivided to ensure greater accuracy in counting, were chosen. The numbers counted in these four sections multiplied by eight and the factor for dilution, were taken to represent the living organisms in one loopful of the culture.

Throughout the paper the figures represent the numbers calculated to be present in a standard loopful or 0·01 c.c. of the cultures.

Sources of error in these experiments.

There are several possible sources of error in these experiments of which the most important seem to be the following:

(1) Uneven distribution of the organisms in the culture (see Section XVIII) at the time of taking the sample due to insufficient agitation of the culture before taking the samples or uneven distribution in the diluting fluid due to insufficient agitation before plating. On various occasions several plates were made from the same cultures after different periods of shaking and gave remarkably similar results. It seems probable, therefore, that if the cultures and the dilutions are sufficiently shaken errors from this cause are not of great importance.

(2) Uneven distribution of the organisms owing to unequal breaking up of the clumps formed in the process of multiplication. For varying periods after division organisms such as cocci remain united, and a single colony may represent the descendants of one, two, three, four or perhaps more individuals. Errors from this cause are unavoidable in all experiments of this type. Microscopic examination of the fluid of the culture after shaking usually showed well separated cocci mixed with occasional small clumps consisting of three or four individuals. Also the deep colonies in any given plate were usually of very similar size, apparently indicating that growth had occurred from single organisms or very small clumps. Spontaneous agglutination is said to occur in old cultures. No evidence of the occurrence of this phenomenon was obtained.

(3) Variations in the quantity taken up by the loop. The quantity of fluid taken up by the same loop on different occasions varies, but with careful manipulation the variations are not sufficient to influence materially the broad conclusions. Most of the experiments were carried out in duplicate, a procedure which tends to correct errors due to variations in the quantity carried by the loop. The figures quoted are in many cases the means of readings from two plate cultures made from separate dilutions.

(4) Death of the organisms in the diluting fluid. In all cases cultures were made from the diluting fluid within a few minutes. As almost uniform

results were obtained in cultures made from the diluting fluid at intervals up to 30 minutes this possible source of error may be neglected.

(5) Excessive numbers of colonies on the plates. When very high numbers were suspected plates were prepared from several different dilutions so as to obtain some plates containing suitable numbers for counting.

(6) Contamination of the cultures. When contaminating organisms developed in the cultures the experiments were discontinued.

(7) The failure of living organisms to produce visible colonies within the period of incubation. Plates were counted on several occasions after one, two and three days' incubation, and almost invariably gave approximately identical figures. Only in very exceptional circumstances did the colonies become more numerous after two days' incubation. In most cases twenty-four hours may be considered a sufficient period of incubation.

It is evident that with the many sources of error involved in such experiments results of great accuracy cannot be expected, but the events occurring in the cultures are indicated broadly.

Method of describing the experiments.

Each series of experiments is considered in the following way. First the aim of the experiment is explained, next the composition of the media used and methods employed are stated and then the results are given in the form of tables and charts showing the numbers of organisms calculated as present per standard loop of the culture on each occasion of counting. Finally the sequence of events presumably occurring in the culture is indicated and the general conclusions which appear to be permissible.

Irregularities in the curves have been regarded as due in many cases to errors in manipulation.

SECTION I. *Growth in neutral meat extract medium.*

Several experiments have been carried out at different times in the two years during which the work has been in progress to ascertain the course of events in neutral meat extract cultures of a certain strain of *S. aureus*. In different experiments, provided that the cultures used for inoculating the meat extract had been grown under similar conditions and the number of organisms inoculated was small, very similar results were obtained.

Since the results vary according to the numbers inoculated and the previous history of the culture it is impossible to establish an exact standard with which the results of other experiments can be compared. Consequently every series of experiments devised to ascertain the effects of altered conditions should be accompanied by controls.

In order to keep the strains of organisms alive and vigorous "stock" cultures were grown on meat extract agar, and subcultivated every 8–10 days. Previous to each experiment a subculture was made on the surface of agar and incubated for 18 hours at 37° C. From this subculture an emulsion

in distilled water was prepared, and one drop of the emulsion was used for inoculating each tube containing meat extract 1 c.c., $N/10$ soda 0·08 c.c.[1], distilled water 3·92 c.c. The emulsions were of such strength that each loopful of the medium after inoculation contained less than 10,000 organisms. Immediately after inoculation and the distribution of the organisms by violent shaking a standard loopful of the culture was added to melted agar at 45° C. and a plate culture made in order to ascertain the number of organisms present at the commencement of the experiment. After various periods of incubation at 37° C. standard loopfuls were transferred to distilled water (usually 5 or 10 c.c.), and from these dilutions plate cultures were prepared in the manner previously described. The results of four experiments carried out in November and December 1918 are quoted, and Chart 1 is constructed

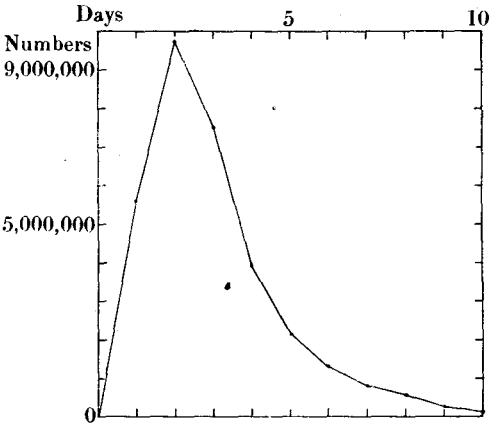

Chart I. Illustrating the numbers of living cocci present daily in a neutral meat extract culture.

from the mean daily counts. This chart may be taken to illustrate the course of events in a culture of *Staphylococcus aureus* under the conditions described[2], and further it may be used to some extent as a rough standard.

		A	*B*	*C*	*D*	Mean
Original numbers:		1784	5660	1392	520	2339
Days.	1	7,632,000	7,680,000	6,208,000	5,920,000	6,610,000
	2	9,280,000	9,872,000	10,606,000	9,248,000	9,751,000
	3	6,656,000	5,840,000	8,656,000	8,905,000	7,514,000
	4	2,992,000	4,016,000	3,120,000	5,632,000	3,940,000
	5	2,312,000	2,560,000	1,600,000	2,280,000	2,188,000
	6	1,608,000	1,376,000	872,000	1,376,000	1,308,000
	7	688,000	732,000	724,000	1,080,000	806,000
	8	—	492,000	508,000	704,000	568,000
	9	—	148,000	200,000	466,000	271,000
	10	—	48,000	68,000	216,000	111,000
	11	—	27,500	32,000	78,000	46,000
	12	—	9,984	—	—	—

[1] It was found that in most preparations of meat extract $N/10$ soda to the extent of 0·08 c.c. to each 1 c.c. of meat extract was required to bring the fluid to the neutral point of neutral red.

[2] The results of the inoculation of different numbers and of organisms grown under other conditions are given elsewhere (Sections IV and V).

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Multiplication proceeds very rapidly during the first day and more slowly in the second, when the maximum number, about 10,000,000 per standard loop, is reached. Later the number of living organisms decreases at first rapidly, but later more slowly, until a low level is reached, which remains fairly constant for a long period, in spite of the diminution of the volume of the fluid by evaporation. In some instances cultures have been examined after 53 days' incubation, when the volume of the fluid had decreased to less than 1 c.c. Even under these circumstances large numbers of the organisms were found to be alive, apparently indicating that the survivors were little influenced by the increasing concentration of their products brought about by evaporation. During this prolonged period of relative constancy in numbers small oscillations are observed in all experiments of this nature. To determine whether such oscillations are the result of faulty technique or are due to occasional periods of slight multiplication and subsequent diminution in numbers would require further investigation.

SECTION II. *The influence of the previous rate of transplantation of the culture used for inoculation.*

The frequency with which the cultures used for inoculation have been subcultivated on agar slopes influences the results sufficiently to modify the appearance of the charts. Three observations are quoted to illustrate this influence.

Experiment (I) was similar in all respects with those quoted in the last section except that the cocci used for inoculation had been transplanted at intervals of three or four days during a period of two months. Experiment (II) was the same in all respects as experiment (I), but was carried out nine months later. Experiment (B) was similar except that the cocci used were transplanted daily for six days.

	Exp. (I)	Exp. (II)	Mean of (I) and (II)	Exp. (B)
Original numbers:	632	5920	3176	5760
Days. 1	5,968,000	6,928,000	6,448,000	9,696,000
2	8,744,000	8,112,000	8,428,000	12,976,000
3	10,448,000	10,240,000	10,344,000	11,248,000
4	9,968,000	9,056,000	9,487,000	2,864,000
5	8,688,000	7,104,000	7,896,000	—
6	7,002,000	4,096,000	5,549,000	1,244,000
7	2,496,000	2,192,000	2,344,000	408,000
8	852,000	1,592,000	1,222,000	272,000
9	296,000	1,092,000	646,000	148,000
10	252,000	114,000	186,000	79,500
11	212,000	70,000	141,000	46,500
12	204,000	56,000	130,000	13,824
13	87,500	42,000	64,000	29,056
14	61,500	24,500	42,100	30,400
15	44,500	—	—	—
16	37,500	—	—	—
17	17,000	—	—	—
18	14,460	—	—	—

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Frequent transplantation over a long period (Expts. I, II) results in the maximum being reached a day later than in the standard, and in the line of decline in numbers lagging about two days behind the standard. Several subcultivations in rapid succession (Expt. B) result in very rapid growth, a high maximum and a very rapid fall in the numbers.

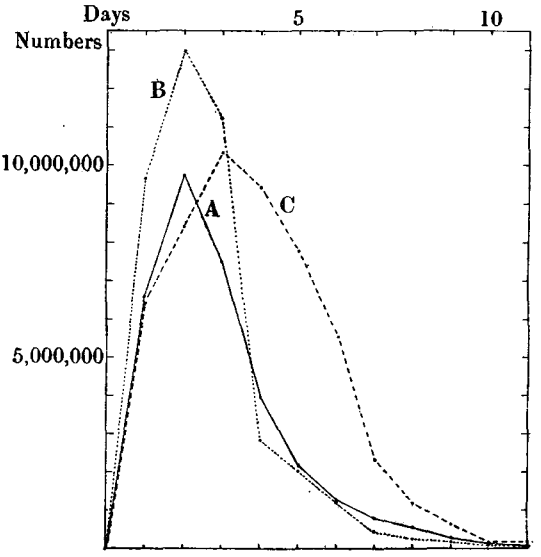

Chart 2. Showing deviations from the "standard" curve, A. Curve B illustrates the type of growth when a culture which has been rapidly transplanted and curve C when a culture which has been subcultivated frequently over a long period is used.

SECTION III. *The relation of growth to the quantity of food substance present.*

In order to ascertain the relation of growth to the proportion of food substance (meat extract) present, five tubes containing the following ingredients were each inoculated with a drop of an emulsion of *Staphylococcus aureus* and incubated at 37° C.

Tube	Meat extract	Distilled water
1	5 c.c.	0 c.c.
2	3·75 c.c.	1·25 c.c.
3	2·5 „	2·5 „
4	1·25 „	3·75 „
5	·5 „	4·5 „

Before incubation the mean number of cocci per standard loopful in each tube was 868.

	22 hrs.	40 hrs.	64 hrs.	88 hrs.	112 hrs.	138 hrs.	162 hrs.
Tube 1	11,808,000	18,960,000	23,424,000	25,840,000	22,816,000	19,376,000	16,384,000
2	10,664,000	16,960,000	20,300,000	20,864,000	17,936,000	13,784,000	9,840,000
3	8,880,000	13,288,000	15,760,000	13,696,000	10,960,000	6,904,000	2,448,000
4	6,296,000	9,336,000	9,416,000	5,736,000	732,000	218,000	118,000
5	3,566,000	4,273,000	3,392,000	568,000	57,500	17,750	17,750

	186 hrs.	204 hrs.	234 hrs.	258 hrs.	282 hrs.	306 hrs.	330 hrs.
Tube 1	14,832,000	13,976,000	11,520,000	10,664,000	9,008,000	6,044,000	2,920,000
2	7,713,000	6,984,000	3,920,000	1,024,000	362,000	378,000	204,000
3	992,000	334,000	78,000	23,000	41,000	28,750	26,000
4	68,750	31,750	13,750	12,000	16,250	11,500	10,000
5	15,500	16,750	56,500	57,000	57,750	76,000	71,750

	354 hrs.	372 hrs.	396 hrs.	420 hrs.	444 hrs.	468 hrs.	26 days
Tube 1	1,352,000	446,000	434,000	300,000	182,000	284,000	63,808
2	44,000	32,000	81,000	124,000	127,000	326,000	170,496
3	39,000	84,000	—	140,000	—	142,000	62,848
4	13,750	28,000	26,250	27,500	—	8,464	24,168
5	—	—	—	20,750	—	1,152	736

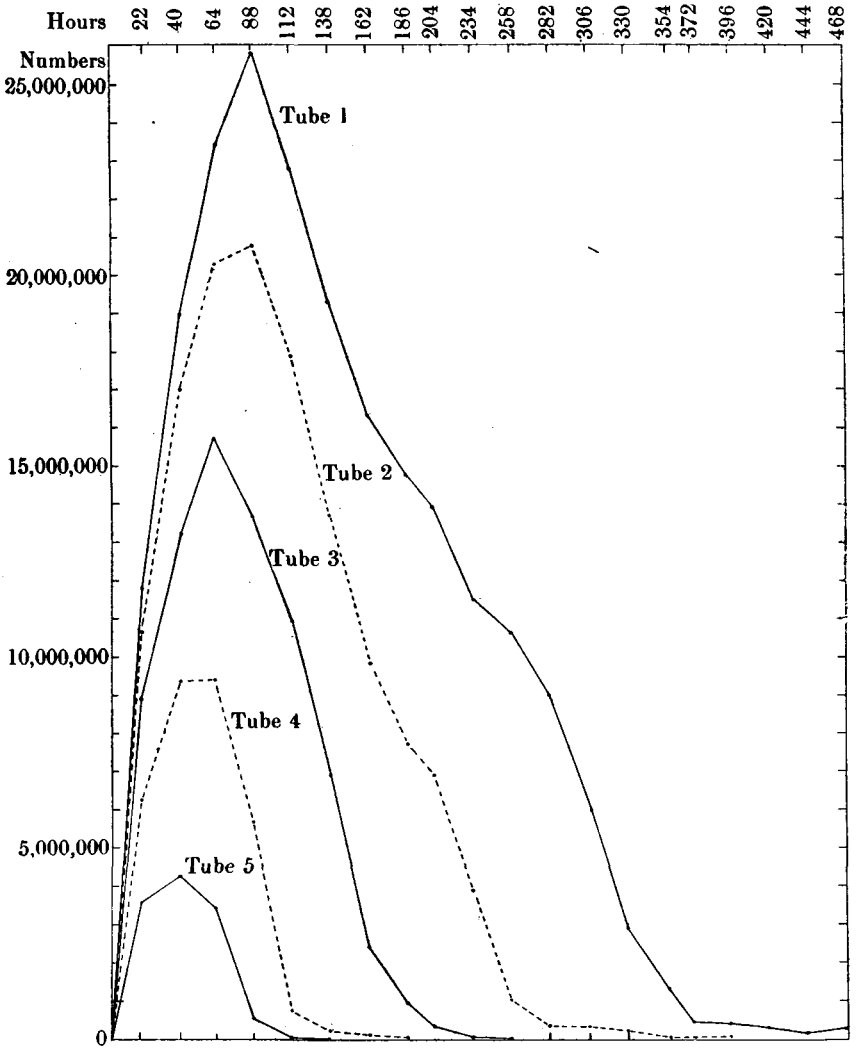

Chart 3. Showing the relation of growth to the proportion of food substance present. Tube 1 contains the largest proportion and tubes 2, 3, 4 and 5 contain $\frac{3}{4}$, $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{16}$ respectively of the amount in tube 1.

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	53 days *	88 days	144 days	209 days
Tube 1	253,725	24,768	29,500	11,744
2	146,816	4,256	12,000	15,360
3	48,896	3,392	4,000	8,576
4	102,912	6,784	32,000	8,768
5	720	360	1,000	128

* Contents of tubes reduced to 1 c.c. or less by evaporation. On 60th, 110th, 121st and 156th days made up to 5 c.c. with distilled water.

It is evident that the greater the proportion of meat extract the greater is the multiplication and the longer the period which elapses before the curve reaches its highest point; in fact the multiplication appears to be proportional to the concentration of meat extract in the culture. The length of the period of rapid decline is also related to the concentration of the meat extract. After the period of rapid decline small numbers of the organisms remain alive for an indefinite time. As in other experiments slight fluctuations in their numbers seemed to occur.

Section IV. *The influence of the numbers inoculated.*

In order to ascertain the extent to which the course of events at 37° C. is influenced by considerable differences in the numbers of cocci inoculated several experiments were carried out of which one is quoted. Each tube contained meat extract 1 c.c., $N/10$ soda 0·08 c.c., distilled water 3·92 c.c.

	Original number	20 hrs.	2 days	3 days	4 days	5 days	6 days
No. 1	25,664,000	27,616,000	28,696,000	22,368,000	15,872,000	8,112,000	1,296,000
2	9,248,000	13,024,000	13,344,000	10,152,000	7,552,000	5,128,000	2,552,000
3	3,216,000	11,566,000	12,848,000	—	5,920,000	2,624,000	936,000
4	1,392	6,208,000	10,606,000	8,656,000	3,120,000	1,600,000	872,000
5	520	5,920,000	9,248,000	8,905,000	5,632,000	2,280,000	1,376,000

	7 days	8 days	9 days	10 days	11 days	23 days
No. 1	488,000	152,000	71,000	41,500	47,500	5
2	2,112,000	1,552,000	1,344,000	800,000	504,000	18,584
3	824,000	728,000	608,000	300,000	248,000	14,376
4	724,000	508,000	200,000	68,000	32,000	16,356
5	1,080,000	704,000	566,000	216,000	78,000	8,704

In this medium with a small inoculation the maximum number of cocci present at any period does not usually exceed 10–12 millions per standard loop. If the initial dose greatly exceeds this figure multiplication proceeds relatively slowly for two days and subsequently there is a very rapid fall in numbers. With an initial dose close to this figure a somewhat similar curve is produced, though the rate of fall is not so rapid. Much smaller initial doses produce the "standard" type of curve.

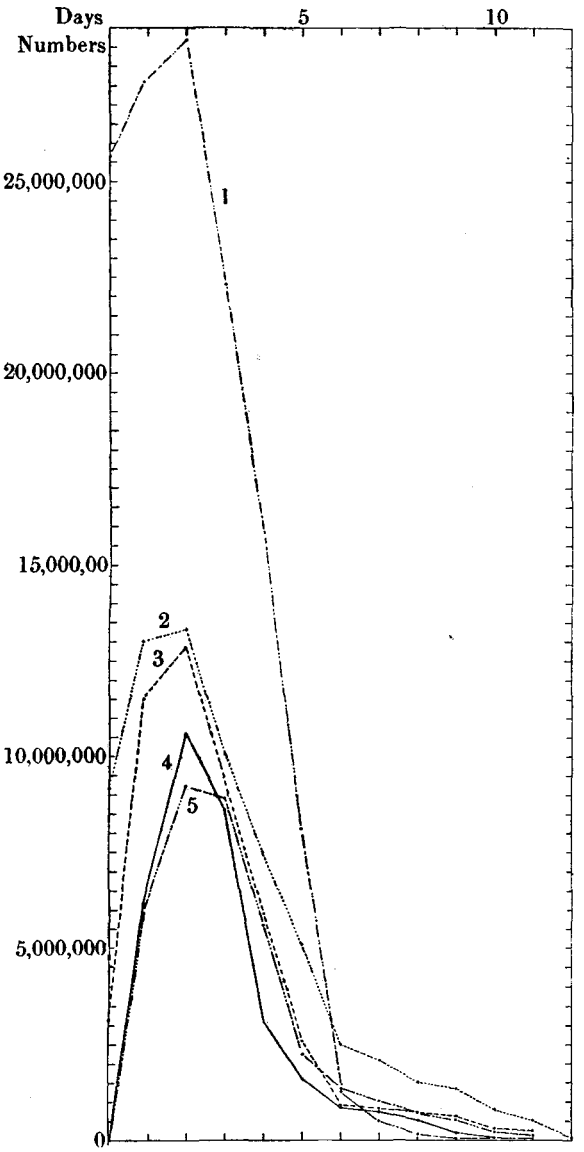

Chart 4. Showing the influence of the dose of organisms inoculated into the medium. Tube 1 contained at the beginning 25 million cocci per loop; tube 2, 9 million; tube 3, 3 million; tube 4, 1392; and tube 5, 520.

SECTION V. *The influence of small differences in numbers in the initial inoculation.*

In most of the experiments quoted in this paper small drops of dilute emulsions of *S. aureus* were used for inoculating the cultures. The experiments of Section V were carried out in order to ascertain to what extent small differences in the numbers inoculated influence counts made after 24 or more

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hours' cultivations at 37° C. An emulsion of moderate strength was first made, and from this dilutions calculated to contain approximately $\frac{1}{10}$, $\frac{1}{100}$ and $\frac{1}{1000}$ of the organisms. It will be seen from the table that after 26 hours' cultivation all four cultures contained nearly equal numbers. By mistake a slightly greater proportion than usual of water was added to the meat extract.

	Original number	2 hrs.	4 hrs.	6 hrs.	8 hrs.	10 hrs.	26 hrs.
Full strength	34,400	56,800	694,000	2,748,000	4,544,000	6,250,000	8,720,000
$\frac{1}{10}$	4,300	6,200	12,750	210,000	1,408,000	3,840,000	8,544,000
$\frac{1}{100}$	420	928	1,775	9,300	34,600	471,000	8,496,000
$\frac{1}{1000}$	59	101	320	2,090	9,760	49,000	7,584,000

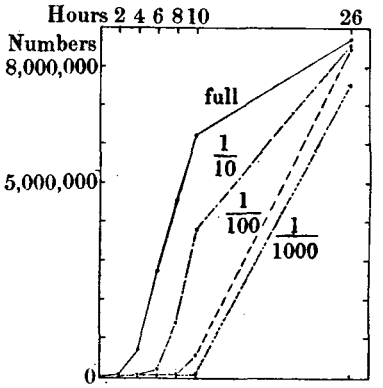

Chart 5. Showing the rate of growth in cultures inoculated with small numbers of cocci.

The variations in numbers usually encountered in emulsions made in the manner generally employed in these experiments are not likely therefore to affect very materially the results in different experiments of the same kind.

Section VI. *The influence of occasional additions of small quantities of food substance.*

Seeing that the concentration of products by evaporation appeared to have little effect on the surviving organisms, a series of experiments, of which one is quoted, was undertaken to ascertain the results of adding fresh food material at different stages of cultivation.

Four tubes, *A*, *B*, *C*, *D*, containing meat extract ·75 c.c., *N*/10 soda 0·06 and distilled water 4·19 c.c. were inoculated with drops of an emulsion of *S. aureus*, and the numbers estimated daily in the usual manner. The tubes were incubated at 37° C. The mean number of cocci per standard loopful at the beginning of the experiment was 816.

In the case of tube *A* no addition was made up to the 12th day and the curve up to that time follows the normal course. On the 12th day ten drops of undiluted meat extract were added resulting in a great rise in the numbers, followed by a gradual decline. On the 23rd day when again a low level had

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been reached two drops of concentrated meat extract (40 c.c. of meat extract evaporated to 1·0 c.c. at 40° C.) were added, and resulted in a rise and subsequent fall in the numbers somewhat similar to that which occurred at the beginning of the experiment.

This experiment appears to show that the fall in numbers is due mainly to exhaustion of food material, and not to accumulation of products.

In the case of tube *B* a drop of undiluted meat extract was added on the 4th and 5th days when the number was declining and caused a retardation in the rate of the fall. On the 10th day six drops of meat extract were added and two drops on each of the following three days. This procedure resulted in a moderate rise like that seen in tube *A*. On the 23rd day when a low level had been reached two drops of the concentrated meat extract given to tube *A* were added, and gave a similar result. On the 37th day the volume of the culture had fallen to 0·53 c.c. owing to evaporation, and sufficient sterile distilled water was added to bring up the volume to 5 c.c. On the 40th day one drop of the concentrated meat extract was added and resulted in a rise in numbers followed by a slow decline. On the 56th and three following days a drop of concentrated meat extract was added and caused a very great increase in the numbers. The experiment was abandoned on the 59th day.

In tubes *C* and *D* growth was allowed to proceed till the 4th day when a decline in numbers was beginning to occur. In the case of tube *C* a drop of meat extract of the same composition as the medium in the tube was added daily from the 4th to the 9th days. This resulted in a slow but steady decline showing that insufficient nutrient material was being supplied daily to keep the numbers at a high level. From the 10th to the 22nd days two drops were added daily, resulting in the rate of decline becoming slower. An irregularity in the curve of unknown causation occurred on the 15th, 16th and 17th days.

In tube *D* a drop of distilled water was added from the 4th to the 9th days and two drops from the 10th to the 23rd days. This slight dilution caused no obvious deviation from the usual course of events.

On the 23rd day two drops of the concentrated meat extract supplied to tubes *A* and *B* were added to *C* and *D* and resulted in considerable rises followed by rapid declines. The rise of *D* was higher than that of *C* and may perhaps be accounted for by some difference in the quantity of material added. Again on the 40th day one drop of the same concentrated meat extract was added to each tube and resulted in a rise followed by a decline. The rise in *C* was greater than in *D*. After a sufficient decline in numbers had taken place a drop of concentrated meat extract (50 c.c. of meat extract evaporated to 2·0 c.c. at 40° C.) was added daily to each tube between the 56th and 64th days, and resulted in a great daily rise in the numbers for five days. Then for four days the numbers remained very high, but with a tendency to remain at approximately the same level. The oscillations in the chart were probably due to irregularities in the drops added and to difficulties in counting such large numbers. On the 64th and 65th days smaller drops

were used. In the case of tube *C* the experiment ceased at this point, but in the case of tube *D* it was continued the smaller drops being added daily by means of a glass tube passed through the cork of the vessel containing the meat extract. In this way drops of approximately equal size were delivered. This procedure resulted in an initial fall in the numbers, and then in the numbers remaining approximately the same for each day's count. The experiment was discontinued on the 75th day.

On the 37th day owing to evaporation each tube contained less than 5 c.c. of fluid. They were made up to 5 c.c. with sterile distilled water.

Tube	Immediate culture	1 day	2 days	3 days	4 days
A	816	4,864,000	5,872,000	6,928,000	5,100,000
B	—	5,024,000	6,000,000	6,696,000	5,168,000 + 1*
C	—	4,016,000	6,160,000	6,352,000	5,264,000 + 1
D	—	4,272,000	6,112,000	6,664,000	4,992,000 + 1 w.
	5 days	6 days	7 days	8 days	9 days
A	3,072,000	742,000	372,000	280,000	140,000
B	4,536,000 + 1	3,408,000	1,054,000	248,000	120,000
C	4,949,000 + 1	4,496,000 + 1	3,848,000 + 1	2,928,000 + 1	2,476,000 + 1
D	3,260,000 + 1 w.	536,000 + 1 w.	344,000 + 1 w.	208,000 + 1 w.	— + 1 w.
	10 days	11 days	12 days	13 days	14 days
A	171,000	75,500	60,500 + 10	2,812,000	2,572,000
B	140,000 + 6	1,786,000 + 2	2,212,000 + 2	2,504,000 + 2	1,984,000
C	2,416,000 + 2	2,200,000 + 2	1,968,000 + 2	1,696,000 + 2	1,740,000 + 2
D	160,000 + 2 w.	82,500 + 2 w.	64,000 + 2 w.	59,000 + 2 w.	77,500 + 2
	15 days	16 days	17 days	18 days	19 days
A	2,088,000	1,056,000	696,000	528,000	312,000
B	600,000	300,000	160,000	104,000	64,000
C	2,008,000 + 2	1,264,000 + 2	1,236,000 + 2	729,000 + 2	780,000 + 2
D	180,000 + 2 w.	102,000 + 2 w.	43,500 + 2 w.	49,500 + 2 w.	32,000 + 2 w.
	20 days	21 days	22 days	23 days	24 days
A	140,000	92,000	65,000	53,000 + 2 c.	7,232,000
B	—	96,000	—	45,000 + 2 c.	5,536,000
C	628,000 + 2	976,000 + 2	1,200,000 + 2	1,304,000 + 2 c.	5,264,000
D	43,500 + 2 w.	224,000 + 2 w.	96,000 + 2 w.	75,500 + 2 c.	7,600,000
	25 days	26 days	27 days	28 days	29 days
A	8,448,000	—	8,336,000	4,536,000	1,720,000
B	6,960,000	7,024,000	8,288,000	3,568,000	1,552,000
C	5,520,000	5,448,000	5,280,000	4,320,000	1,632,000
D	8,112,000	7,888,000	8,440,000	3,536,000	1,472,000
	30 days	31 days	32 days	33 days	34 days
A	1,008,000	742,000	544,000	338,000	254,000
B	1,064,000	888,000	—	436,000	356,000
C	1,502,000	840,000	488,000	292,000	332,000
D	1,312,000	1,240,000	1,008,000	800,000	536,000

* The sign + 1 indicates that a drop of meat extract was added immediately after the sample for estimating the numbers was taken, + 1 c. indicates a drop of concentrated meat extract, and + 1 w. indicates a drop of sterile distilled water.

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Tube	35 days	36 days	37 days	39 days	40 days
A	271,000	228,000	made up to 5 c.c.	12,864	1 drop concentrated meat extract added
B	344,000	276,000	,,	8,192	,,
C	304,000	360,000	,,	12,496	,,
D	548,000	380,000	,,	3,744	,,

	41 days	42 days	43 days	44 days	45 days
A	—	—	—	—	—
B	2,512,000	5,552,000	4,128,000	3,712,000	3,560,000
C	3,936,000	5,280,000	4,928,000	5,008,000	3,768,000
D	3,352,000	2,666,000	2,312,000	2,672,000	2,664,000

	46 days	47 days	48 days	49 days	50 days
A	—	—	—	—	—
B	2,320,000	1,992,000	1,840,000	1,715,000	1,312,000
C	2,096,000	2,448,000	2,072,000	2,112,000	1,408,000
D	2,512,000	1,744,000	416,000	116,000	22,500

	51 days	52 days	53 days	54 days	55 days
A	—	—	—	—	—
B	1,128,000	680,000	556,000	245,000	72,000
C	1,048,000	488,000	268,000	132,000	148,000
D	39,000	70,500	74,000	56,000	12,000

	56 days	57 days	58 days	59 days	60 days
A	—	—	—	—	—
B	49,700 + 1 c.	2,472,000 + 1	9,744,000 + 1	13,792,000 + 1	—
C	43,950 + 1 c.	3,880,000 + 1	11,568,000 + 1	17,360,000 + 1	19,488,000 + 1
D	1,670 + 1 c.	3,584,000 + 1	10,272,000 + 1	17,968,000 + 1	19,152,000 + 1

	61 days	62 days	63 days	64 days	65 days
C	— + 1	24,736,000 + 1	23,892,000 + 1	25,744,000 + 1 s.*	26,118,000 + 1 s.
D	— + 1	26,176,000 + 1	24,160,000 + 1	23,840,000 + 1 s.	25,472,000 + 1 s.

	66 days	67 days	68 days	69 days	70 days
C	22,448,000 + 1 s.	—	—	—	—
D	20,768,000 + 1 s.	— + 1 s.	16,136,000 + 1 s.	14,506,000 + 1 s.	14,064,000 + 1 s.

	71 days	72 days	73 days	74 days	75 days
D	13,232,000	13,008,000	12,850,000	12,960,000	12,736,000

* + 1 s. indicates a smaller drop than previously used. See text, p. 148.

This experiment shows that in the medium used the rapid fall in numbers which follows the initial rise is not due to the accumulation of products, but is caused mainly by the using up of food material, since the addition at any time of small quantities of fresh food material, insufficient in amount to cause appreciable dilution of the products, results in further growth to some extent proportional to the amount of food material added. Moreover it shows that very small daily additions of food material retard the rate of decline and that large daily additions cause a great rise in the numbers, resulting in a high and approximately constant level being maintained at least for some time.

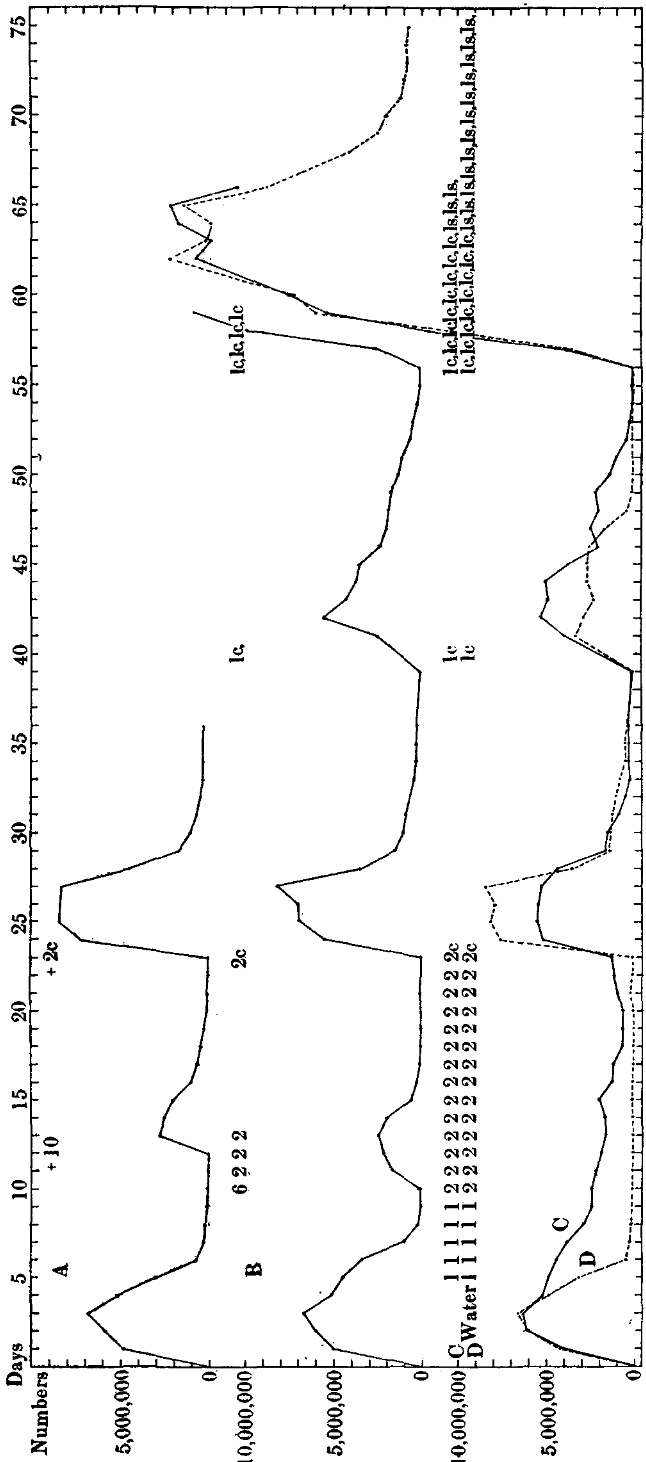

Chart 6. Showing the effects of occasional small additions of food substance to meat extract cultures. +10 = 10 drops of meat extract added; 2c = 2 drops of concentrated meat extract added; 1s = 1 small drop of concentrated meat extract from a special pipette added. In tube *D* drops of water were added.

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SECTION VII. *The influence of regular additions of small quantities of food substance.*

This experiment was carried out in order to ascertain whether it is possible to keep the numbers of bacteria in a culture incubated at 37° C. at a constant level by daily additions of small quantities of food material.

In each of three large test-tubes, *A*, *B* and *C*, 10 c.c. of sterile distilled water were placed. To each two drops of a concentrated meat extract (50 c.c. of meat extract evaporated to 7 c.c. at 40° C.) were added daily up to the 13th day. Approximate uniformity in the size of the drops was secured by keeping the concentrated meat extract in a test-tube closed with a paraffined cork through which a glass tube, drawn out at its inner end, passed. Drops from this tube were used throughout the experiment. Immediately after the first addition of meat extract each tube was inoculated with a drop of an emulsion of *S. aureus*.

The numbers increased daily for five days and then kept approximately level at about 9,000,000 per loop up to the 14th day. On and after that date one drop only was added daily. The numbers fell rapidly during the next three days to 2·5 millions per loop and then remained at this level till the conclusion of the experiment on the 27th day.

Chart 7 illustrating this experiment is compiled from the daily mean of the three tubes *A*, *B* and *C* up to the 12th day, when tube *A* became contaminated; from the daily mean of tubes *B* and *C* to the 21st day, when tube *B* became contaminated. The counts were made, except on the occasion stated in the table, at the same time each day.

Tube	Original number	30 hrs.	2 days	3 days	4 days	5 days
A	3808	2,576,000	3,376,000	4,544,000	6,624,000	9,088,000
B	3136	2,736,000	3,248,000	4,128,000	6,702,000	8,832,000
C	3516	2,272,000	3,904,000	4,976,000	7,024,000	9,744,000
Mean	3487	2,528,000	3,509,000	4,549,000	6,786,000	9,221,000
	6 days	7 days	8 days	9 days, 1 hr. earlier than usual	10 days	11 days
A	9,888,000	9,992,000	9,136,000	9,744,000	10,000,000	10,200,000
B	8,736,000	8,528,000	8,960,000	8,656,000	8,656,000	8,576,000
C	10,184,000	9,496,000	9,112,000	9,776,000	9,818,000	9,600,000
Mean	9,603,000	9,305,000	9,069,000	9,392,000	9,491,000	9,459,000
	12 days	13 days	14 days*	15 days	16 days	17 days
A	9,640,000	—	—	—	—	—
B	8,232,000	8,878,000	8,608,000	8,464,000	6,208,000	2,344,000
C	9,221,000	8,976,000	9,024,000	9,136,000	6,336,000	2,952,000
Mean	9,031,000	8,927,000	8,811,000	8,800,000	6,272,000	2,648,000
	18 days	19 days	20 days	21 days	22 days	23 days
A	—	—	—	—	—	—
B	2,456,000	2,432,000	2,720,000	2,624,000	—	—
C	—	2,040,000	1,924,000	1,984,000	2,240,000	2,368,000
Mean	2,456,000	2,236,000	2,322,000	2,304,000	—	—
	24 days	25 days	26 days	27 days		
C	2,456,000	2,420,000	2,272,000	2,448,000		

* 1 drop only of concentrated meat extract added on and after this day.

By subcultures made at short intervals on certain days it was shown that after each addition of food material the numbers increase for a few hours and then decrease. Consequently estimations made every twenty-four hours indicate the general effect of the additions.

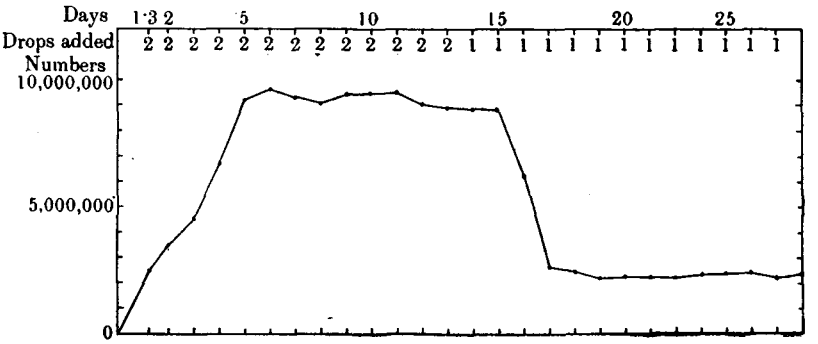

Chart 7. Showing the effects of regular small additions of food substance to meat extract cultures.

It is evident from this experiment that by suitable small regular additions of food material a concentration of *Staphylococci* within certain desired limits could be maintained in such a fluid culture medium for a long period of time. Accumulation of the products may gradually inhibit growth, but on this point the experiment gives little evidence.

SECTION VIII. *The effect of diluting cultures with distilled water at various periods of growth.*

In one series of experiments three tubes *A*, *B*, *C* each containing meat extract 1 c.c., *N*/10 soda 0·08 c.c. and distilled water 3·92 c.c. were inoculated with a drop of an emulsion of a *Staphylococcus* culture and incubated at 37° C. At the commencement of the experiment about 4400 cocci were present per standard loopful. After 24 hours' incubation the numbers in each tube were counted. Then 1 c.c. of distilled water was added to tube *B*, and 2·5 c.c. to tube *C* and the numbers present in each estimated immediately. Tube *A* was used as a control. The cultures were incubated at 37° C. for 11 days and counts made daily.

A second similar series of tubes *D*, *E*, *F* made from another sample of meat extract were incubated for four days. On that date after the numbers present in each tube had been counted 1 c.c. of sterile distilled water was added to tube *E* and 2·5 c.c. to tube *F*, and the numbers present in each estimated immediately. These cultures were incubated subsequently for seven days and counts made daily.

Tube	Number present originally	1 day before dilution	After dilution	2 days	3 days
A	4,400	5,440,000	—	9,772,000	11,344,000
B	—	5,195,000	4,944,000	8,176,000	9,664,000
C	—	5,456,000	3,376,000	6,880,000	8,256,000
D	—	5,840,000	—	9,600,000	10,960,000
E	—	5,600,000	—	9,088,000	10,552,000
F	—	5,744,000	—	9,854,000	10,528,000

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Tube	4 days before dilution	After dilution	5 days	6 days	7 days
A	10,032,000	—	6,686,000	5,744,000	4,496,000
B	8,912,000	—	5,600,000	4,720,000	3,488,000
C	6,880,000	—	4,656,000	3,216,000	2,720,000
D	6,944,000	—	3,288,000	2,772,000	2,048,000
E	6,784,000	5,504,000	2,384,000	2,144,000	1,584,000
F	6,480,000	4,784,000	2,176,000	1,772,000	1,048,000

	8 days	9 days	10 days	11 days
A	3,088,000	2,488,000	1,048,000	560,000
B	2,944,000	2,256,000	788,000	204,000
C	1,696,000	1,280,000	588,000	272,000
D	844,000	520,000	256,000	66,500
E	612,000	432,000	170,000	47,000
F	568,000	196,000	—	—

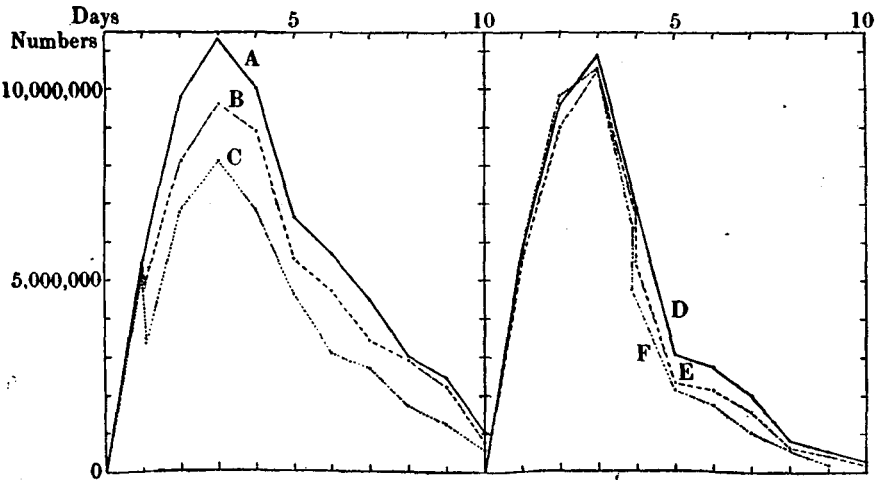

Chart 8. Showing the effects of diluting cultures at different times during incubation.

Though some of these cultures have been diluted no food material has been removed, and each culture contains the same amount of food at different dilutions. If growth goes on as usual each tube should contain at any given time the same number of organisms, though in each standard loopful the number should be proportional to the dilution. This seems to be the case and it may be concluded that moderate dilution with distilled water at any stage of incubation has little effect on multiplication.

SECTION IX. *The influence of different incubation temperatures on the growth of* S. aureus.

In the first of the experiments quoted three tubes, *A*, *B*, *C*, each containing meat extract 1 c.c., *N*/10 soda 0·08 c.c. and distilled water 3·92 c.c. were sterilised by boiling, and inoculated with a drop of an emulsion of a *Staphylococcus* culture, which had been transplanted frequently. Tube *A* was cultivated

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at 37° C., tube *B* at 27° C. and tube *C* at 17·0° C. Daily counts were made for the first 18 days, but subsequently only tube *C* was counted daily.

Tube	Number originally present	1 day	2 days	3 days	4 days
A at 37° C.	632	5,968,000	8,744,000	10,448,000	9,968,000
B at 27° C.	—	5,528,000	10,918,000	12,568,000	14,288,000
C at 17° C.	—	2,064	73,850	3,120,000	8,128,000

	5 days	6 days	7 days	8 days	9 days	10 days
A	8,688,000	7,002,000	2,496,000	852,000	296,000	152,000
B	15,918,000	15,664,000	13,568,000	11,488,000	7,248,000	3,584,000
C	12,400,000	14,512,000	17,072,000	18,272,000	17,592,000	16,304,000

	11 days	12 days	13 days	14 days	15 days	16 days
A	212,000	204,000	87,500	61,000	44,500	37,500
B	1,896,000	1,286,000	906,000	432,000	516,000	480,000
C	16,176,000	16,200,000	16,128,000	15,988,000	15,760,000	15,256,000

	17 days	18 days	19 days	20 days	21 days	22 days
A	17,000	14,460	—	—	—	11,424
B	444,000	360,000	—	—	—	—
C	14,096,000	13,664,000	12,000,000	11,488,000	10,592,000	10,134,000

	23 days	24 days	25 days	26 days	27 days	28 days
A	—	—	—	—	6,016	—
B	—	—	—	—	39,040	—
C	9,664,000	9,324,000	8,192,000	8,064,000	6,304,000	6,544,000

	29 days	30 days	31 days	32 days	33 days	34 days
C	5,816,000	5,912,000	5,535,000	4,464,000	3,456,000	2,656,000

	35 days	36 days	37 days	38 days	39 days	40 days
C	2,056,000	1,704,000	1,452,000	1,160,000	1,090,000	976,000

	41 days	42 days	43 days	44 days	45 days	46 days
C	976,000	914,000	844,000	662,000	295,000*	262,000

* Culture made up to 5 c.c. with distilled water.

It will be seen that the highest maximum was attained and the numbers remained greatest for the longest period in the culture grown at 17° C., and the least maximum was attained and the numbers fell most quickly in the culture grown at 37° C. As this was contrary to expectations a similar experiment was carried out six months later.

In this case six cultures were prepared. Two of them were incubated at 33° C., two at 27° C. and two at 8 to 10° C. The culture used for inoculation had not been so frequently transplanted as the one used in the experiment just quoted. For the cultures grown at 33° C. and 27° C. the figures given in the table represent the mean for the two tubes used at each temperature, but the results of each of the tubes incubated at 8 to 10° C. are quoted separately, since the results in the two tubes were somewhat different. After the 26th day the results in one only of the tubes incubated at 33° C. are quoted to illustrate the fluctuations which sometimes occur at this period (see p. 141).

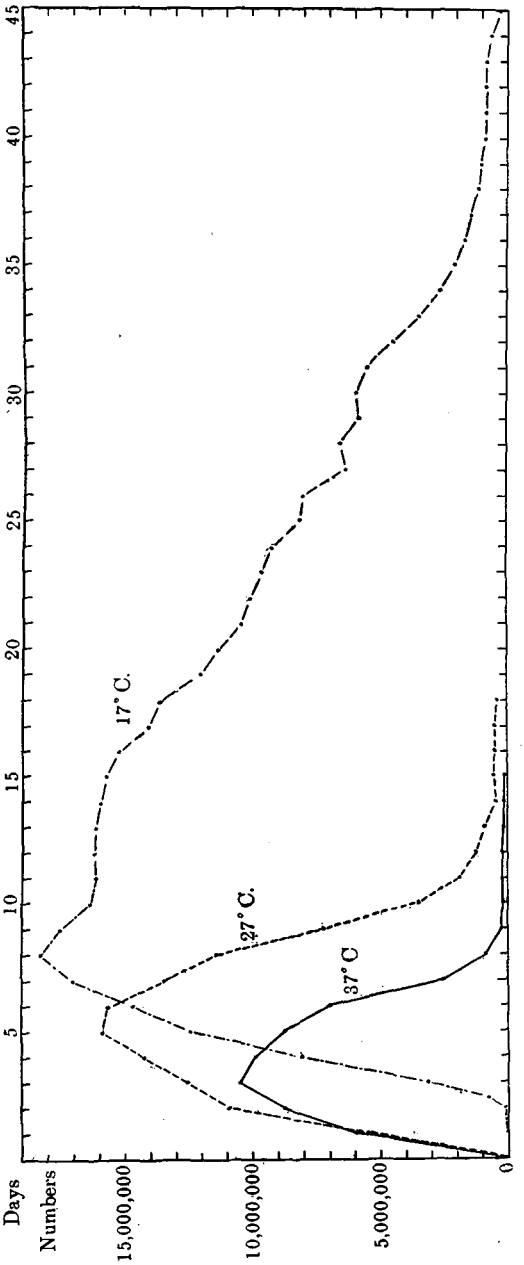

Chart 9. Showing the results of incubating meat extract cultures at 37° C., 27° C. and 17° C.

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	Number originally present	1 day	2 days	3 days	4 days	5 days
Culture at 33° C.	2024	7,360,000	9,632,000	10,288,000	10,610,000	6,410,000
„ 27° C.	—	6,528,000	13,104,000	17,647,000	18,671,000	18,184,000
„ (1) 8–10° C.	—	2,300	1,904	1,728	1,592	1,448
„ (2) „	—	2,304	2,104	—	2,016	1,984

	6 days	7 days	8 days	9 days	10 days
Culture at 33° C. 2024	4,880,000	3,704,000	2,280,000	1,536,000	914,000
„ 27° C. —	15,968,000	15,488,000	16,072,000	12,712,000	6,680,000
„ (1) 8–10° C. —	1,240	1,152	936	802	536
„ (2) „ —	1,712	1,648	1,392	1,336	1,236

	11 days	12 days	13 days	14 days	15 days
Culture at 33° C. 2024	870,000	693,000	558,000	496,000	154,000
„ 27° C. —	5,946,000	3,525,000	2,365,000	2,412,000	2,012,000
„ (1) 8–10° C. —	398	297	312	253	262
„ (2) „ —	1,062	997	940	939	—

	17 days	19 days	21 days	23 days	26 days
Culture at 33° C. 2024	65,500	9,744	4,192	6,744	24,448*
„ 27° C. —	1,312,000	834,000	932,000	612,000	—
„ (1) 8–10° C. —	164	155	120	54	64
„ (2) „ —	752	728	440	379	290†

	28 days	31 days	35 days	39 days	41 days
Culture at 33° C. 2024	10,016	4,032	15,424	19,328	74,500
„ 27° C. —	276,000	360,000	260,000	270,000	165,000
„ (1) 8–10° C. —	14	26	21	19	—
„ (2) „ —	281	136	47	47	26

	44 days	47 days	52 days	65 days
Culture at 33° C. 2024	65,000	53,500	10,500	—
„ 27° C. —	—	—	—	62,800
„ (1) 8–10° C. —	—	—	—	14
„ (2) „ —	7	—	—	—

* One culture only quoted on and after this date.

† Culture contaminated by a mould.

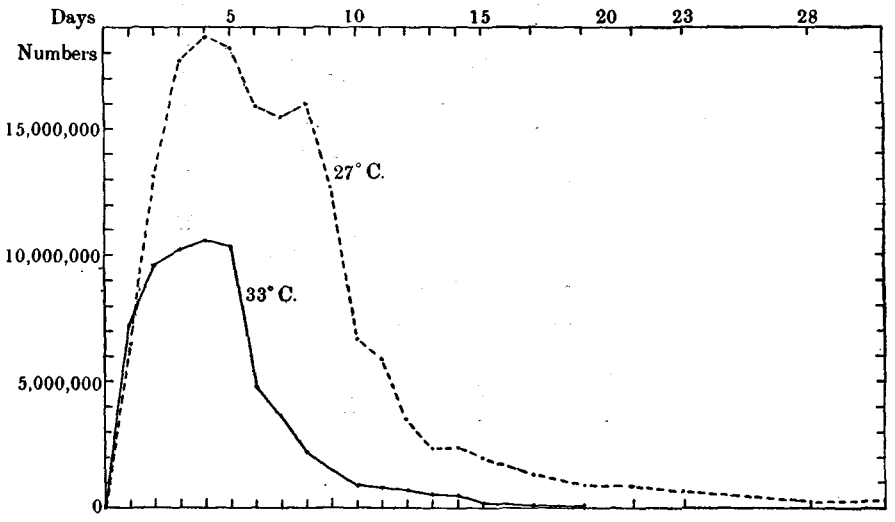

Chart 10. Showing the results of incubating meat extract cultures at 33° C. and 27° C.

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The results obtained with cultures grown at 33° C. and at 27° C. confirm the results of the first experiment.

If we regard the rate of multiplication within the first few days as indicating the specially favourable conditions for growth then a temperature between 37° C. and 27° C. may be regarded as the most suitable for the growth of the *S. aureus*. On the other hand if we regard the maximum numbers attained at any period of incubation as indicating the most favourable conditions then a temperature about 17° C. may be regarded as the most suitable for the growth of the coccus.

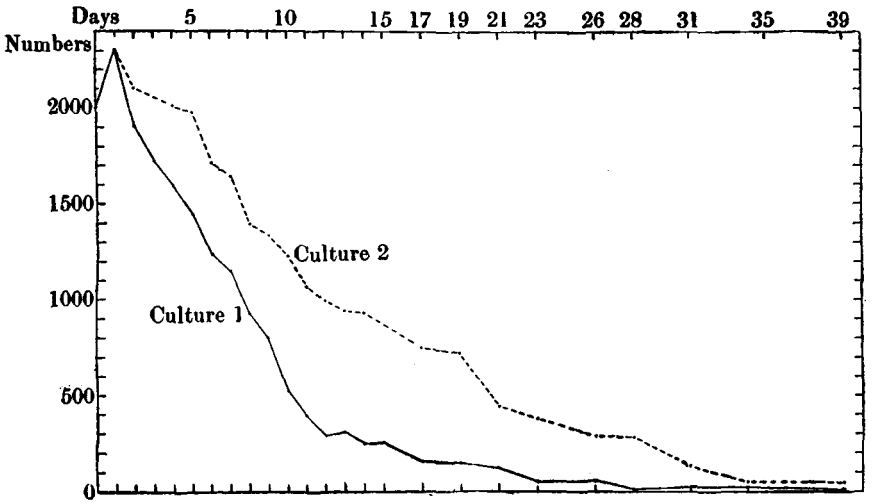

Chart 11. Showing the daily counts in two cultures incubated at 8 to 10° C.

At 8 to 10° C. very slight multiplication, if any, occurs during the first 24 hours' incubation and subsequently the numbers steadily decline for at least 60 days. The experiment was not continued long enough to decide whether at this temperature the organisms ultimately die out.

This unexpected result led to the carrying out of a series of experiments at lower temperatures. Two tubes of the same medium inoculated with *S. aureus* were incubated at about − 1° C., two at about − 6° C., and two at − 10° to − 12° C., and the numbers counted daily. The mean for the two tubes kept at each temperature is recorded in the tables. Occasionally some of the tubes were frozen. These were thawed very slowly before samples were taken for counting.

	Number originally present	1 day	2 days	3 days	4 days	5 days	6 days
At − 1° C.	5264	3876	3120	2612	2228	1936	1552
„ − 6° C.	4480	1592	1044	944	800	620	364
„ − 11° C.	4528	1468	925	484	311	196	75

	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days	15 days	16 days	17 days	18 days	19 days	20 days
At − 1° C.	1000	660	332	181	104	35	14	5	4	1	1	1	0	0
„ − 6° C.	110	26	14	6	1·5	·5	0	0	0	0	0	0	0	0
„ − 11° C.	9	2	0	0	0	0	0	0	0	0	0	0	0	0

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From the 21st to the 28th days all the tubes were incubated at 37° C. but no signs of growth occurred and no organisms were found on subculture. It may therefore be assumed that the cultures had completely died out.

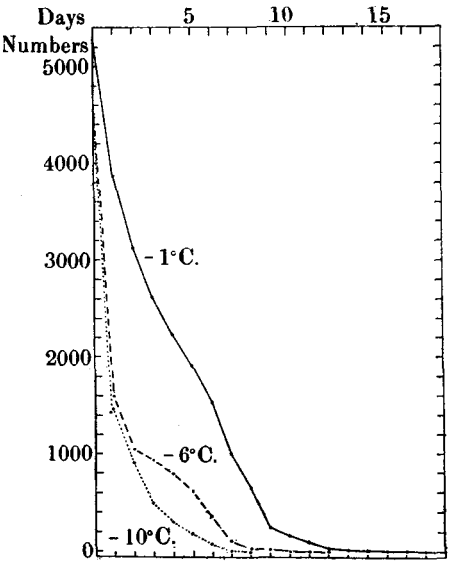

Chart 12. Showing the results of keeping meat extract cultures at low temperatures, −1° C., −6° C. and −10° C.

It will be seen that in each culture the numbers rapidly declined and eventually died out. At − 10° C. the cocci were dead on the 9th day, at − 6° C. on the 13th day and at − 1° C. on the 19th day.

It seems possible that at some temperature between 10° C. and 17° C. the numbers might remain approximately constant.

Section X. *The influence of the growth of one species on the growth of others subsequently inoculated into the medium.*

Some of the experiments described in previous sections appear to indicate that the fall in numbers is due mainly to the exhaustion of the food supply. The following experiments were undertaken in the hope of throwing some further light on this subject. 21 tubes each containing meat extract 1 c.c., *N*/10 soda 0·08 c.c. and water 3·92 c.c. were prepared in the usual manner. Seven of the tubes were inoculated with an emulsion of *S. aureus*, seven with an emulsion of *B. coli*, and seven with an emulsion of *B. pyocyaneus*. All these cultures were incubated at 37° C. for sixteen days, and during that time from one culture of each organism, acting as a control, counts were made daily in order to ascertain the course of events in each series. At the end of that time a slight daily fall in numbers was occurring in each culture, and it was presumed that the greater part of the food supply had been exhausted.

On the 16th day the six cultures of each organism were divided into two groups, *A* and *B*, three cultures in each group. Those of group *A* were not sterilised, but those of group *B* were sterilised by boiling. Then a drop of an emulsion of *S. aureus* was added to cultures A_1 and B_1, a drop of an emulsion of *B. coli* to cultures A_2 and B_2, and a drop of an emulsion of *B. pyocyaneus* to cultures A_3 and B_3 of each series, and the number of organisms present in each culture immediately estimated. Daily counts were made for the next seven days to ascertain to what extent multiplication of the added organisms took place.

	Number originally present	1 day	2 days	3 days	4 days
Staphylococci ...	5920	6,928,000	8,112,000	10,240,000	9,056,000
B. coli	3408	3,217,000	6,256,000	11,712,000	9,440,000
B. pyocyaneus ...	3232	13,568,000	24,784,000	18,252,000	18,206,000
		5 days	6 days	7 days	8 days
Staphylococci ...		7,104,000	4,096,000	2,192,000	1,592,000
B. coli		5,216,000	3,504,000	2,232,000	2,032,000
B. pyocyaneus ...		11,808,000	10,528,000	9,536,000	7,664,000
		9 days	10 days	11 days	12 days
Staphylococci ...		1,096,000	114,500	70,000	56,000
B. coli		1,968,000	1,312,000	1,247,000	896,000
B. pyocyaneus ...		6,416,000	6,288,000	6,016,000	6,592,000
		13 days	14 days	15 days	16 days
Staphylococci ...		42,000	24,500	25,000	21,000
B. coli		944,000	688,000	512,000	316,000
B. pyocyaneus ...		6,272,000	5,040,000	—	4,896,000

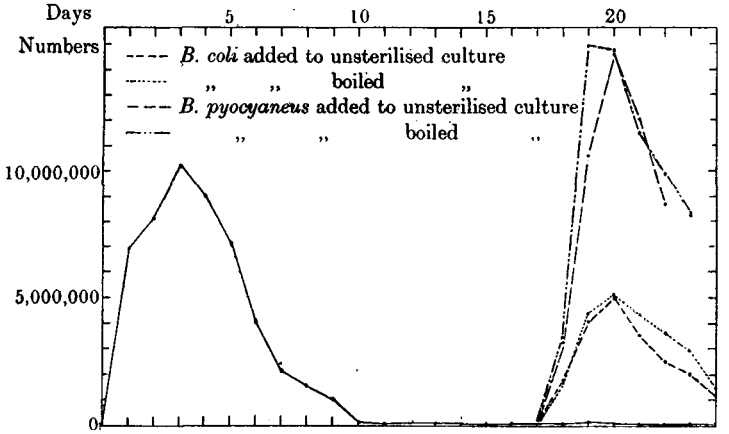

Chart 13. Showing the rate of multiplication of added organisms in meat extract in which *S. aureus* had been growing for 17 days.

In the sterilised groups (*B*) multiplication of the added bacteria took place in every case, whether the added organisms belonged to the species previously growing in the medium or not. After a culture had been sterilised

by boiling some food was therefore available for freshly added organisms, even though they belonged to the same species and strain.

In the unsterilised groups (*A*) multiplication of the added bacteria took place in all cases with two exceptions, when *B. coli* and *B. pyocyaneus* were added respectively to cultures in which the same species had been growing.

It is difficult to compare with any degree of accuracy the extent of multiplication in comparable examples of the two groups, *A* and *B*, since in the

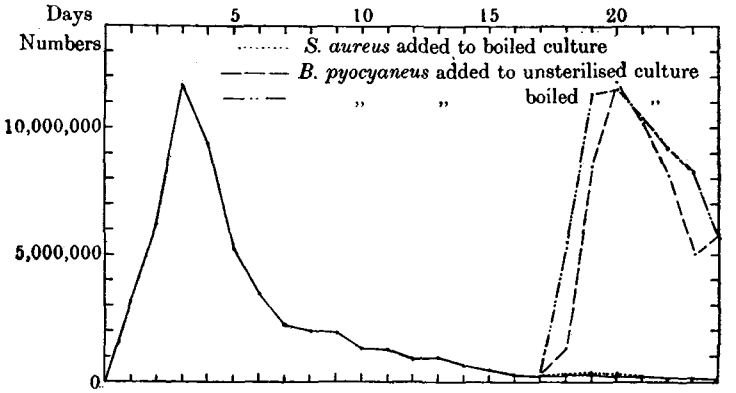

Chart 14. Showing the rate of multiplication of added organisms in tubes in which *B. coli* had been growing for 17 days.

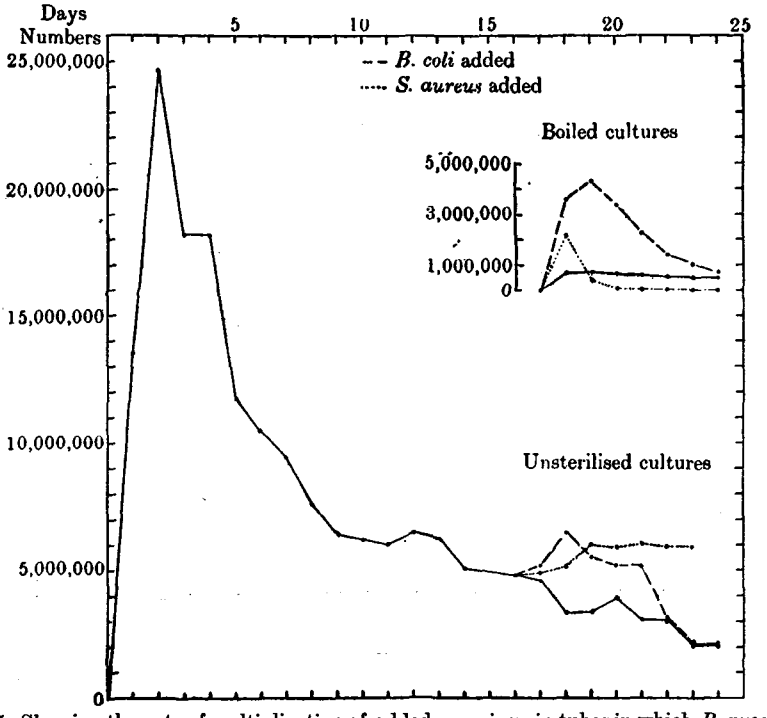

Chart 15. Showing the rate of multiplication of added organisms in tubes in which *B. pyocyaneus* had been growing for 17 days.

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			17th day after inoculation	18th day	19th day	20th day	21st day	22nd day	23rd day	24th day
COCCUS CULTURE										
Unsterilised	Coccus added	A_1	24,000	53,000	111,500	59,500	28,500	21,800	20,184	16,128
	B. coli ,,	A_2	27,000	1,840,000	4,096,000	4,992,000	3,536,000	2,464,000	2,096,000	1,168,000
	B. pyocyaneus ,,	A_3	29,000	3,248,000	10,688,000	14,656,000	12,048,000	8,640,000	8,208,000	6,616,000
Sterilised	Coccus added	B_1	2,560	44,500	95,000	32,500	2,000	1,008	1,872	2,296
	B. coli ,,	B_2	3,072	1,824,000	4,400,000	5,024,000	4,384,000	3,600,000	2,912,000	1,560,000
	B. pyocyaneus ,,	B_3	6,144	3,424,000	14,896,000	14,720,000	11,520,000	9,984,000	8,320,000	—
***B. COLI* CULTURE**										
Unsterilised	Coccus added	A_1	452,000	492,000	648,000	668,000	580,000	576,000	416,000	376,000
	B. coli ,,	A_2	288,000	312,000	256,000	188,000	172,000	71,500	41,856	21,366
	B. pyocyaneus ,,	A_3	324,000	1,392,000	8,524,000	11,808,000	10,144,000	8,144,000	5,008,000	5,876,000
Sterilised	Coccus added	B_1	2,176	172,000	212,500	134,000	19,500	1,664	536	—
	B. coli ,,	B_2	2,912	296,000	328,000	296,000	280,000	328,000	208,000	39,425
	B. pyocyaneus ,,	B_3	6,048	5,584,000	11,328,000	11,584,000	10,448,000	9,264,000	8,320,000	5,760,000
***B. PYOCYANEUS* CULTURE**										
Unsterilised	Coccus added	A_1	4,972,000	5,136,000	6,080,000	5,984,000	6,080,000	5,936,000	5,968,000	—
	B. coli ,,	A_2	5,232,000	6,513,000	5,568,000	5,288,000	5,248,000	3,376,000	3,248,000	3,008,000
	B. pyocyaneus ,,	A_3	4,640,000	3,312,000	3 424,000	3,968,000	3,088,000	3,072,000	2,062,000	2,096,000
Sterilised	Coccus added	B_1	2,312	2,200,000	440,000	27,500	16,500	6,350	4,704	5,728
	B. coli ,,	B_2	2,784	3,624,000	4,320,000	3,488,000	2,320,000	1,424,000	1,040,000	728,000
	B. pyocyaneus ,,	B_3	5,888	712,000	744,000	656,000	628,000	576,000	—	576,000

unsterilised series, *A*, a gradual decline in the numbers of the organisms originally present was doubtless proceeding, and the counting of controls, which would have shown the extent of the decline during this period of the experiment, was not continued after the 16th day. A rough comparison was obtained in the following manner. In each example of the sterilised group of cultures, *B*, the highest figure recorded was divided by the number introduced, while in comparable examples of the unsterilised group, *A*, the number at the time the fresh organisms were added (group *B*, column 1, 17th day, subtracted from comparable culture, group *A*, column 1) was subtracted from the highest figure recorded and the result divided by the number introduced. The figures so obtained indicate very roughly the extent to which multiplication took place in each case.

Cultures originally inoculated with		Multiplication of added		
		S. aureus	*B. coli*	*B. pyocyaneus*
S. aureus	(unsterilised, A_1)	×35	(A_2) ×1617	(A_3) ×2381
	(sterilised, B_1)	×37	(B_2) ×1635	(B_3) ×2424
B. coli	(unsterilised, A_1)	×100	(A_2) ×8?	(A_3) ×1916
	(sterilised, B_1)	×97	(B_2) ×112	(B_3) ×1915
B. pyocyaneus	(unsterilised, A_1)	×480	(A_2) ×461	(A_3) ×0
	(sterilised, B_1)	×951	(B_2) ×1551	(B_3) ×126

These figures seem to indicate that the *Staphylococci* added to tubes of media, whether sterilised or unsterilised, in which the same organism had been growing multiplied to a small and nearly equal extent; when added to sterilised or unsterilised tubes in which *B. coli* had been growing multiplication in both cases was about three times as great, and when added to tubes in which *B. pyocyaneus* had been growing multiplication was greater, 14 times as great in the unsterilised and 27 times in the sterilised.

B. coli added to tubes, sterilised or unsterilised, in which *S. aureus* had been growing multiplied to a considerable and equal extent, and it multiplied to nearly the same extent in the sterilised tube in which *B. pyocyaneus* had been growing, but to only one-quarter of the extent in the unsterilised tube. When it was added to the unsterilised tube in which *B. coli* had been growing there was little or no multiplication, but when added to the sterilised tube moderate multiplication occurred.

B. pyocyaneus multiplied to a great and equal extent in sterilised or unsterilised tubes in which *S. aureus* had been growing and to a less, but equal extent, in sterilised or unsterilised tubes in which *B. coli* had been growing. In the unsterilised tube in which *B. pyocyaneus* had been growing there was no multiplication, but there was moderate multiplication in the sterilised tube.

It will be noticed that when *S. aureus* or *B. coli* had been growing in the medium boiling in either case did not increase the food value of the medium for the other two species. The food value for added *S. aureus* was not increased by boiling a culture in which *S. aureus* had been growing, though in the case

of *B. coli* the food value of the medium for added *B. coli* seemed to be increased by boiling the medium in which that organism had been growing. On the other hand after the growth of *B. pyocyaneus* boiling caused added organisms to grow more freely than they did on the unsterilised medium.

The growth of any of these organisms in the medium seems to remove a portion of the food substance used by other species since in no case was the growth of an added species nearly so considerable in extent as in its primary culture. If it is assumed that the increase in numbers is proportional to the food supply the growth of *Staphylococcus* removes about half the food originally available for *B. coli* and for *B. pyocyaneus*; the growth of *B. coli* removes most of the food available for *Staphylococci*, and about half of that available for *B. pyocyaneus*.

Cocci added to unsterilised coccus cultures appear to make use of some material, which those already present do not seem to have utilised. This does not appear to be the case when *B. coli* or *B. pyocyaneus* are added to their own cultures.

The results obtained in the experiments just quoted were controlled by another series of experiments.

Sixty tubes each containing meat extract 1 c.c., *N*/10 soda 0·08 c.c. and distilled water 3·92 c.c. were prepared. Twenty were inoculated with *S. aureus*, twenty with *B. coli*, and twenty with *B. pyocyaneus* and incubated at 35° C. Of each series two were incubated for one day, two for two days, two for three days, two for five days and two for seven days. One of each of the pairs just mentioned was boiled, the other not sterilised, and to each 4 c.c. of 2 per cent. agar in distilled water at 50° C. were added, and plates poured. After the medium had set three streaks of heavy emulsions of *S. aureus*, *B. coli* and *B. pyocyaneus* were made across each plate, and the results recorded after 48 hours' incubation at 37° C.

Cultures in which *S. aureus* was growing.

Period of growth	Unsterilised series			Boiled series		
	Streaks of cocci	*B. coli*	*B. pyocyaneus*	Cocci	*B. coli*	*B. pyocyaneus*
7 days	0	+ +	+ +	0	+ +	+ +
5 ,,	0	+ +	+ +	✱?	+ +	+ +
3 ,,	0	+ +	+ +	+	+ +	+ +
2 ,,	0	+ +	+ +	+	+ +	+ +
1 day	✱?	+ +	+ +	+	+ +	+ +

Cultures in which *B. coli* was growing.

Period of growth	Unsterilised series			Boiled series		
	Streaks of cocci	*B. coli*	*B. pyocyaneus*	Cocci	*B. coli*	*B. pyocyaneus*
7 days	0	0	+ +	✱	+ +	+ +
5 ,,	0	0	+ +	+	+ +	+ +
3 ,,	✱?	0	+ +	+	+ +	+ +
2 ,,	✱?	0	+ +	+	+ +	+ +
1 day	✱?	0	+ +	+	+ +	+ +

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Cultures in which *B. pyocyaneus* was growing.

Period of growth	Unsterilised series: Streaks of cocci	Unsterilised series: *B. coli*	Unsterilised series: *B. pyocyaneus*	Boiled series: Cocci	Boiled series: *B. coli*	Boiled series: *B. pyocyaneus*
7 days	0	+ +	0	0	+ +	+ +
5 ,,	✱?	+ +	0	0	+ +	+ +
3 ,,	+	+ +	✱?	+	+ +	+ +
2 ,,	+	+ +	✱?	+ +	+ +	+ +
1 day	+	+ +	✱	+ +	+ +	+ +

0 = no visible colonies. ✱? = doubtful growth. ✱ = very slight growth. + = moderate growth. + + = abundant growth.

It will be seen that *B. coli* and *B. pyocyaneus* grew well on the medium in which *S. aureus* had been growing, and that on the same medium after boiling a few colonies of the cocci grew.

S. aureus grew poorly and *B. pyocyaneus* grew well on the medium in which *B. coli* had been growing, but while *B. coli* itself grew well on this medium after boiling there was little or no growth on the example which had not been boiled.

S. aureus grew moderately and *B. coli* grew well on the medium in which *B. pyocyaneus* had been growing, and *B. pyocyaneus* itself grew well on it after it had been boiled.

Taking into consideration the fact that agar seems to inhibit growth to a slight extent and that very small surface colonies are difficult to see, the results of this series of experiments are in general agreement with the results of the more exact series just quoted.

Section XI. *The influence of varying the reaction of the medium.*

The experiments hitherto described were carried out in meat extract neutralised to Neutral Red. The experiments quoted in this section, which were amongst the first to be undertaken, were made in order to ascertain the effects of varying the reaction of the medium by the addition of $N/10$ hydrochloric acid or $N/10$ soda on the growth of *Staphylococci* and *B. coli* at 37° C. It will be noticed that the proportion of meat extract differs in these experiments from that used in most of the previous experiments. The addition of certain quantities of both acid and soda caused a precipitate to form. The medium was approximately neutral to neutral red when 0·1 $N/10$ soda was added.

Tube	Meat extract (1 part meat to 4 parts water)	$N/10$ HCl	Distilled water	Precipitate
1	2·5 c.c.	·5 c.c.	1·3 c.c.	Slight
2	2·5	·4	1·4	Present
3	2·5	·3	1·5	,,
4	2·5	·2	1·6	Slight
5	2·5	·1	1·7	None
6	2·5	—	1·8	,,
		$N/10$ soda		
7	2·5	·2	1·6	,,
8	2·5	·4	1·4	,,
9	2·5	·6	1·2	Slight
10	2·5	·8	1·0	Present
11	2·5	1·0	·8	,,
12	2·5	1·2	·6	,,
13	2·5	1·6	·2	,,
14	2·5	1·8	—	,,
15	2·5	2·0	—	,,

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After inoculation the cultures contained 376 *S. aureus* and 68 *B. coli* per loopful.

Tube	15 hours		48 hours		72 hours	
	Cocci	*B. coli*	Cocci	*B. coli*	Cocci	*B. coli*
1	29	1	226	0	6	0
2	52	0	—	0	1	0
3	304	0	146,170	0	—	5
4	491,200	9,450	6,680,000	71,000	6,824,000	440,000
5	4,616,000	3,264,000	10,560,000	6,688,000	12,040,000	12,248,000
6	7,088,000	5,440,000	14,328,000	13,072,000	15,424,000	14,864,000
7	7,824,000	7,232,000	17,704,000	12,832,000	19,816,000	12,168,000
8	6,928,000	4,272,000	15,990,000	8,304,000	12,288,000	5,600,000
9	2,952,000	3,728	14,496,000	5,592,000	9,176,000	4,968,000
10	58,000	0	10,592,000	0	7,192,000	0
11	1,144	0	9,504,000	0	6,224,000	0
12	130	0	9,408,000	0	7,104,000	0
13	0	0	24	0	5,424,000	0
14	0	0	7	0	1,408,000	0
15	0	0	1	0	0	0

Tube	94 hours		120 hours		168 hours	
	Cocci	*B. coli*	Cocci	*B. coli*	Cocci	*B. coli*
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	296,000	0	4,356,000	0	8,840,000	0
4	8,144,000	316,000	6,504,000	336,000	5,424,000	236,000
5	11,440,000	11,272,000	6,736,000	8,240,000	5,525,000	3,232,000
6	14,736,000	11,424,000	10,860,000	4,704,000	7,752,000	2,712,000
7	11,520,000	8,536,000	8,080,000	4,888,000	4,752,000	2,416,000
8	—	6,072,000	3,424,000	4,024,000	2,944,000	1,688,000
9	6,136,000	4,864,000	4,592,000	4,336,000	1,328,000	2,576,000
10	5,824,000	0	5,224,000	0	2,488,000	0
11	6,240,000	0	4,928,000	0	2,896,000	0
12	5,808,000	0	4,448,000	0	2,424,000	0
13	7,168,000	0	5,344,000	0	2,464,000	0
14	7,352,000	0	5,240,000	0	2,768,000	0
15	0	0	0	0	0	0

Tube	216 hours		16 days		36 days*	
	Cocci	*B. coli*	Cocci	*B. coli*	Cocci	*B. coli*
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	10,360,000	0	864,000	0	392,000	0
4	4,808,000	976,000	408,000	444,000	400,000	192,000
5	1,616,000	2,224,000	496,000	100,000	257,000	116,000
6	4,636,000	1,204,000	544,000	68,000	960,000	106,000
7	2,760,000	2,004,000	404,000	27,500	760,000	12,000
8	1,223,000	1,480,000	228,000	76,000	—	106,000
9	—	—	72,000	—	136,000	—
10	—	0	52,000	0	98,000	0
11	2,324,000	0	144,000	0	—	0
12	1,896,000	0	72,000	0	20,250	0
13	1,668,000	0	96,000	0	6,500	0
14	840,000	0	6,500	0	8,250	0
15	0	0	0	0	0	0

* Cultures much reduced by evaporation.

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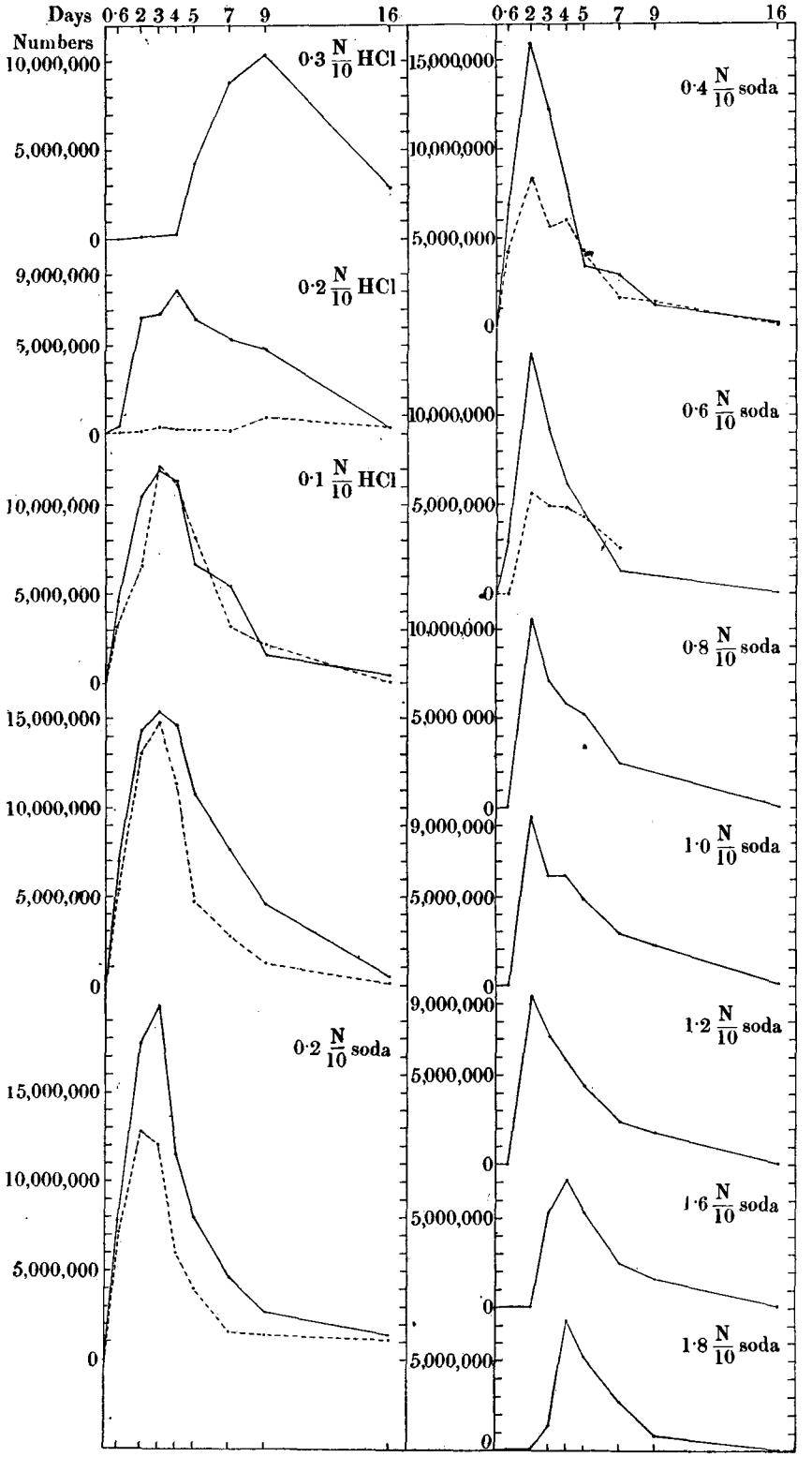

Chart 16. Showing the course of events in cultures of *S. aureus* and *B. coli* at different reactions.

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It will be seen that with a small inoculation of cocci when the medium contains ·5 c.c. or ·4 c.c. $N/10$ hydrochloric acid the organisms decrease in number and are dead on the 4th day. With ·3 c.c. $N/10$ hydrochloric acid added to the medium there is at first a slight decrease in the numbers and then a slow rise for four days, followed by a great increase, the numbers reaching a maximum about the 9th day. With 0·2 c.c. $N/10$ hydrochloric acid added there is only a very slight increase in 15 hours, followed by a rapid increase during the next day. The maximum is reached on the 4th day. With 0·1 c.c. $N/10$ hydrochloric acid added the curve resembles that obtained with unneutralised meat extract, the maximum being reached on the 4th day. The subsequent fall in numbers is moderately rapid.

The effect of adding increasing quantities of $N/10$ hydrochloric acid up to 0·3 c.c. is to retard the growth during the earlier stages of incubation though subsequently rapid growth takes place and a high maximum is reached. Considerable numbers of the cocci survive for a long time. With small primary inoculations the addition of more than 0·3 c.c. $N/10$ hydrochloric acid results in the death of the cocci in a few days.

When 0·2 c.c. $N/10$ soda is added the maximum is reached on the third day and is followed by a rapid fall in numbers. With additions varying between 0·4 and 1·2 c.c. of $N/10$ soda there is a progressive decrease in the height reached by maxima, the rate of growth in the early stages is progressively retarded, and the rate of decrease in numbers seems to be retarded also. With 1·6 c.c. and 1·8 c.c. $N/10$ soda added the rate of growth in the early stages is markedly retarded, and the maxima are not reached till the fourth day. With 2·0 c.c. $N/10$ soda no growth occurs.

In this series of experiments *B. coli* seemed to be more sensitive to the reaction of the medium, especially on the alkaline side, than *S. aureus*, but this may be due partly to the very small number inoculated. In the case of *S. aureus* certain other experiments seem to indicate that the larger the primary inoculation the wider is the range of reaction in which multiplication takes place.

In the case of *B. pyocyaneus* growth does not occur if more than 0·2 c.c. of $N/10$ hydrochloric acid or more than 1·6 c.c. soda are added.

SECTION XII. *The influence of the addition of small quantities of $N/10$ hydrochloric acid.*

The two series of experiments here quoted were undertaken to ascertain more precisely than in previous experiments the effects of additions of small quantities of $N/10$ hydrochloric acid. Each tube contained 5 c.c. of *undiluted* meat extract. To tube 1, which acted as a control, no acid was added. To tube 2 0·2 c.c. $N/10$ hydrochloric acid was added and caused the medium to become opalescent; to tube 3 0·3 c.c. $N/10$ hydrochloric acid was added and the fluid became opalescent and a precipitate formed; to tube 4 0·4 c.c.

$N/10$ hydrochloric acid was added and a considerable precipitate formed to tube 5 0·7 c.c. $N/10$ hydrochloric acid was added and a still greater pre cipitate formed. In both series of experiments a drop of an emulsion c *S. aureus* was added to each tube, but while in the first series the mediur

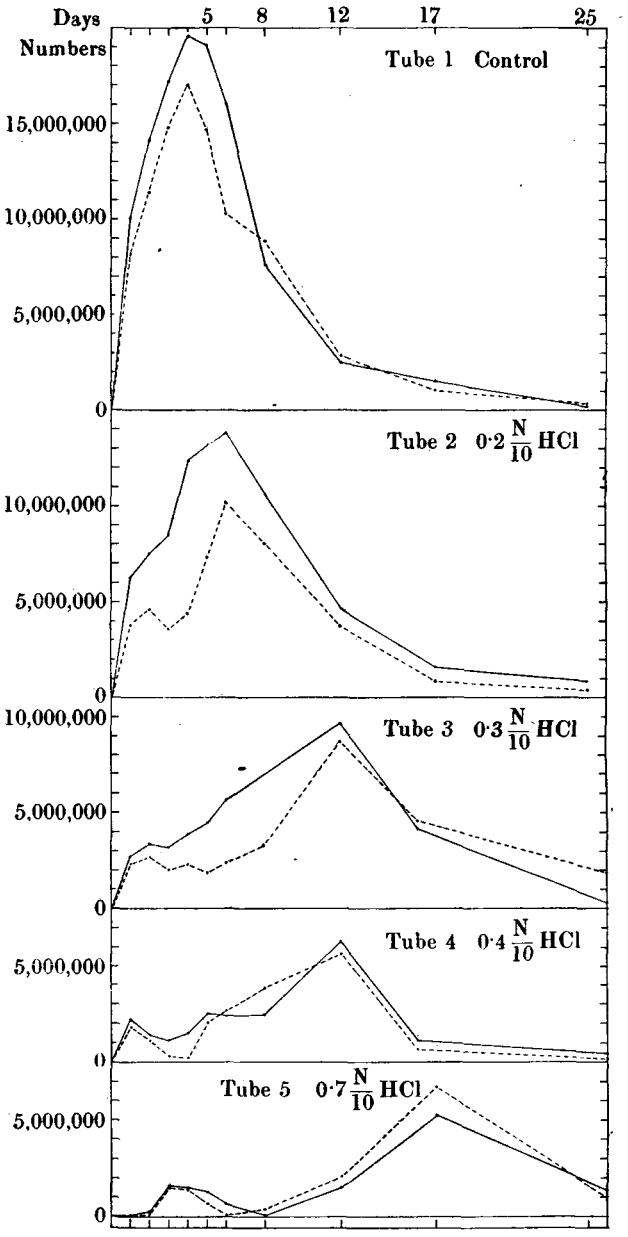

Chart 17. Showing the influence of the addition of various quantities of $N/10$ HCl to meat extract cultures. Series I = continuous line, Series II = broken line.

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after inoculation contained 168 organisms per loop, in the second series it contained only 13 per loop.

Tube		5 hours		1st day		2nd day	
		Series I	Series II	Series I	Series II	Series I	Series II
1	—	3200	190	10,112,000	8,192,000	14,168,000	11,432,000
2	0·2 c.c. *N*/10 HCl	1968	88	6,368,000	3,856,000	7,584,000	4,616,000
3	0·3 „	688	37	2,784,000	2,392,000	3,432,000	2,780,000
4	0·4 „	244	28	2,216,000	1,808,000	1,432,000	1,112,000
5	0·7 „	33	6	298	4	228,000	100,000

Tube		3rd day		4th day		5th day	
		Series I	Series II	Series I	Series II	Series I	Series II
1	—	17,256,000	14,872,000	19,609,000	17,088,000	19,120,000	14,752,000
2	0·2 c.c. *N*/10 HCl	8,496,000	3,640,000	12,416,000	4,456,000	13,209,000	7,368,000
3	0·3 „	3,192,000	2,048,000	3,944,000	2,304,000	4,472,000	1,920,000
4	0·4 „	1,136,000	362,000	1,544,000	203,000	2,560,000	2,096,000
5	0·7 „	1,640,000	1,608,000	1,556,000	1,516,000	1,372,000	676,000

Tube		6th day		8th day		12th day	
		Series I	Series II	Series I	Series II	Series I	Series II
1	—	16,016,000	10,320,000	7,648,000	8,968,000	2,556,000	2,908,000
2	0·2 c.c. *N*/10 HCl	13,824,000	10,200,000	10,648,000	8,064,000	4,732,000	3,808,000
3	0·3 „	5,768,000	2,472,000	—	3,336,000	9,740,000	8,704,000
4	0·4 „	2,392,000	2,632,000	2,440,000	3,896,000	6,336,000	5,768,000
5	0·7 „	716,000	75,000	25,000	392,000	1,515,000	2,118,000

Tube		17th day		25th day	
		Series I	Series II	Series I	Series II
1	—	1,560,000	1,020,000	284,000	300,000
2	0·2 c.c. *N*/10 HCl	1,664,000	909,000	992,000	442,000
3	0·3 „	4,175,000	4,624,000	367,900	1,828,000
4	0·4 „	1,144,000	702,000	494,000	186,000
5	0·7 „	5,200,000	6,768,000	1,490,000	918,000

In passing down the series it will be seen that the type of curve gradually changes from a "standard" with one peak to a curve with two peaks, separated by an interval in which the numbers are small. The second series containing the smaller initial dose seems to be the most influenced.

SECTION XIII. *The effects of cultivating organisms for prolonged periods on media of different reactions.*

Three series of tubes containing media of the following compositions were prepared:

Series	Meat extract	Distilled water	
I	2·5 c.c.	1·5 c.c.	0·3 c.c. *N*/10 HCl
II	2·5	1·8	—
III	2·5	0	1·8 c.c. *N*/10 NaOH

Series I and III correspond to tubes 3 and 14 of Section XI, the most acid and most alkaline of that series in which satisfactory growth took place. A tube of each series was inoculated with an emulsion of the *S. aureus*, and

incubated at 37° C. and subcultures into similar tubes were made weekly for ten weeks. Then subcultures from each were grown for 24 hours at 37° C. on solid media made by adding agar to fluids of the composition mentioned. Consequently the cocci in Series I had been growing on an acid medium, those of Series II on a nearly neutral medium and those of Series III on an alkaline medium for ten weeks. They are referred to subsequently as acid, neutral and alkaline acclimatised cocci respectively. In order to ascertain the effects of this acclimatisation tubes of the same composition as Series I, II, and III were inoculated with emulsions of the cocci from each of the agar cultures. The whole series of experiments therefore included nine cultures, namely:

				Immediate count of numbers
Acid acclimatised cocci inoculated into	acid	meat extract,	*A* I	82
	neutral	„	*A* II	—
	alkaline	„	*A* III	—
Neutral „	acid	„	*B* I	—
	neutral	„	*B* II	992
	alkaline	„	*B* III	—
Alkaline „	acid	„	*C* I	—
	neutral	„	*C* II	—
	alkaline	„	*C* III	360

Tube	1 day	2 days	3 days	4 days	5 days	6 days
A I	122,500	2,928,000	2,560,000	2,640,000	4,088,000	6,544,000
II	5,280,000	10,192,000	10,256,000	9,344,000	9,760,000	8,432,000
III	2,256,000	2,248,000	1,528,000	680,000	276,000	220,000
B I	4,830	1,240,000	1,760,000	2,416,000	2,376,000	1,680,000
II	7,616,000	12,080,000	11,184,000	11,088,000	11,520,000	11,966,000
III	5,616,000	2,784,000	2,156,000	1,264,000	554,000	472,000
C I	65	544,000	1,752,000	2,176,000	2,584,000	744,000
II	9,840,000	12,688,000	13,664,000	14,176,000	13,844,000	14,480,000
III	7,240,000	5,168,000	3,336,000	1,776,000	980,000	864,000

	7 days	8 days	9 days	10 days	11 days	12 days
A I	10,752 000	8,768,000	6,616,000	5,456,000	4,464,000	2,056,000
II	4,460,000	1,056,000	1,840,000	1,560,000	1,456,000	488,000
III	240,000	328,000	252,000	336,000	312,000	220,000
B I	944,000	560,000	732,000	3,820,000	5,584,000	9,280,000
II	9,936,000	7,856,000	4,592,000	2,576,000	1,960,000	1,104,000
III	220,000	204,000	132,000	80,000	46,500	10,500
C I	556,000	340,000	252,000	264,000	1,184,000	7,071,000
II	13,456,000	8,720,000	2,680,000	1,184,000	960,000	2,000,000
III	560,000	492,000	196,000	70,500	53,000	10,000

	13 days	14 days	15 days	16 days	17 days	18 days
A I	1,992,000	2,384,000	220,000	160,000	324,000	404,000
II	256,000	180,000	380,000	784,000	996,000	1,088,000
III	148,000	56,000	40,500	26,000	6,000	4,500
B I	9,680,000	8,768,000	7,376,000	5,728,000	4,336,000	208,000
II	380,000	220,000	3,248,000	5,632,000	6,544,000	6,240,000
III	12,500	9,500	13,500	17,000	18,000	8,000
C I	9,880,000	11,250,000	11,936,000	10,456,000	8,768,000	4,304,000
II	1,832,000	1,784,000	1,528,000	1,200,000	704,000	296,000
III	107,000	96,000	66,000	24,000	5,500	500

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Tube	19 days	20 days	21 days	22 days	23 days	24 days *
A I	448,000	268,000	280,000	292,000	292,000	3,424,000
II	852,000	564,000	308,000	556,000	780,000	2,252,000
III	13,500	17,350	5,888	2,720	2,336	1,896,000
B I	168,000	93,000	148,000	116,000	80,000	4,944,000
II	768,000	161,000	36,000	—	25,500	2,400,000
III	1,500	172	5	9	1	1,645,000
C I	2,936,000	1,148,000	592,000	176,000	124,000	4,416,000
II	132,000	152,000	180,000	232,000	184,000	4,206,000
III	—	544	2,384	2,816	3,568	3,384,000

	25 days	26 days	27 days	28 days	29 days	30 days
A I	4,736,000	5,264,000	4,096,000	3,680,000	2,216,000	784,000
II	2,144,000	1,384,000	904,000	1,216,000	998,000	880,000
III	2,184,000	1,704,000	1,360,000	198,000	20,500	9,500
B I	6,320,000	5,096,000	3,944,000	1,816,000	800,000	520,000
II	3,632,000	5,256,000	4,328,000	2,125,000	576,000	170,000
III	2,720,000	1,756,000	1,328,000	668,000	100,000	33,500
C I	5,200,000	4,576,000	1,968,000	912,000	348,000	204,000
II	4,720,000	4,136,000	1,856,000	772,000	418,000	304,000
III	2,224,000	1,840,000	1,268,000	1,080,000	616,000	248,000

* After the samples for counting had been removed, the contents of the tubes were made up to 5 c.c. with sterile distilled water, and one drop of a concentrated meat extract (50 c.c. concentrated to 2 c.c. by evaporation at 40° C.) added.

It will be noticed that in the case of the *acid acclimatised coccus* in the acid medium (*A* I) moderate growth occurred in 24 hours and considerable multiplication in two days. During the next two days no further multiplication took place, but subsequently rapid multiplication occurred, the maximum numbers being reached on the 7th day. After this there was a rapid decline. In the neutral medium (*A* II) there was rapid multiplication during the first two days. During the next four days a high level was maintained followed by a rapid fall. A small secondary rise commenced on the fifteenth day. In the alkaline medium (*A* III) moderate multiplication occurred in the first 24 hours followed by a slow decline to a very low level.

In the case of the *neutral acclimatised coccus* in the acid medium (*B* I) very little multiplication occurred in the first 24 hours. During the next three days slow multiplication took place followed by a slow fall in numbers during the next five days. After this rapid multiplication occurred the maximum being reached on the 13th day. A rapid fall in numbers followed. In the neutral medium (*B* II) a high level was reached on the second day, and maintained for four days. After a rapid fall in numbers a marked secondary rise occurred, commencing on the 14th day. In the alkaline medium (*B* III) rapid multiplication occurred in the first 24 hours followed by a fall in the numbers to a low level.

In the case of the *alkali acclimatised coccus* in the acid medium (*C* I) after a moderate primary multiplication which reached its maximum on the 5th day, a fall in numbers occurred followed by a rapid and great multiplica-

tion reaching its maximum on the 15th day. In the neutral medium (*C* II) a high level was reached on the second day, and maintained for five days. This was followed by a rapid fall and a small secondary rise commencing on

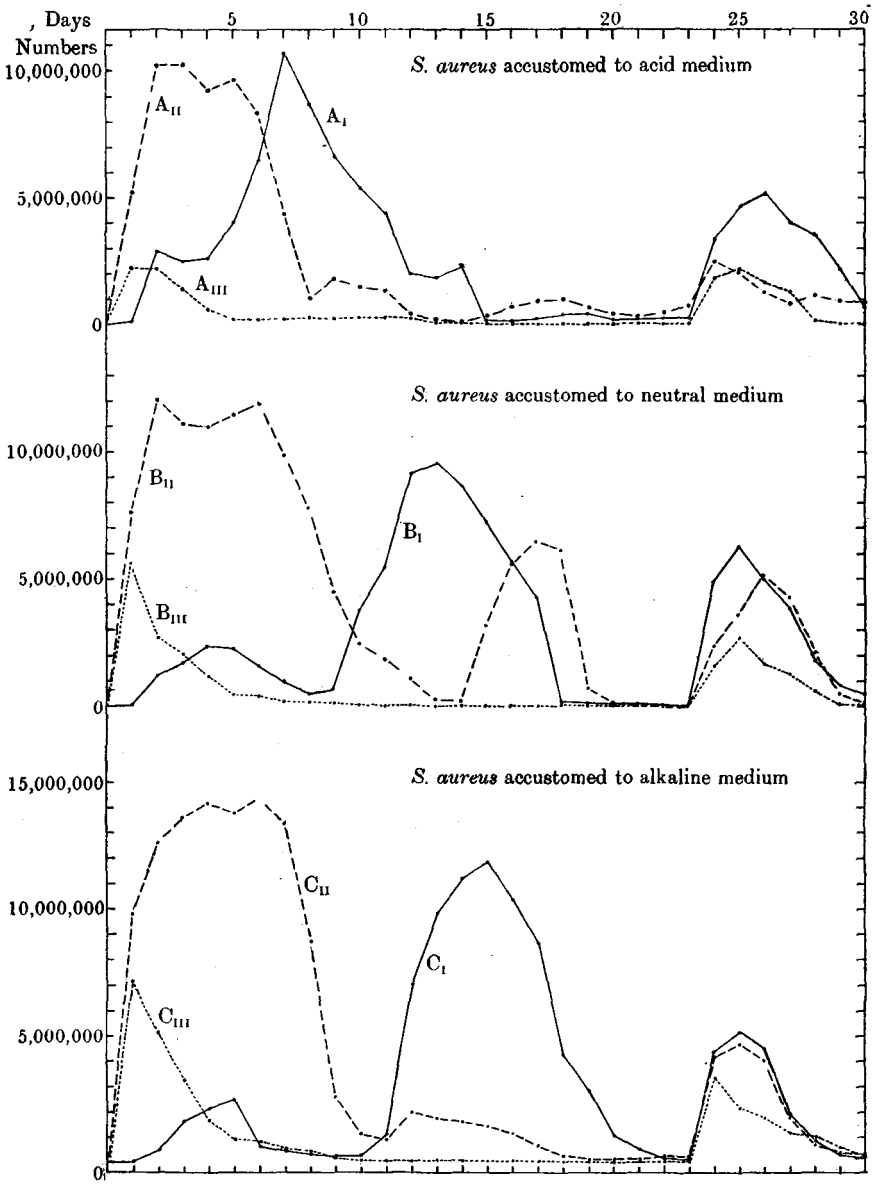

Chart 18. Showing the rate of multiplication of acid, neutral and alkali acclimatised cocci in acid, neutral and alkaline media respectively.

the 12th day. In the alkaline medium (*C* III) rapid multiplication occurred in the first 24 hours followed by a somewhat rapid fall to a low level.

The addition of further food material on the 23rd day caused considerable

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multiplication in all the cultures, which was most marked in the acid media (*A* I, *B* I, *C* I).

The influence of the previous treatment is perhaps best seen in Chart 19, in which the growths of the three treated strains are compared on similar media.

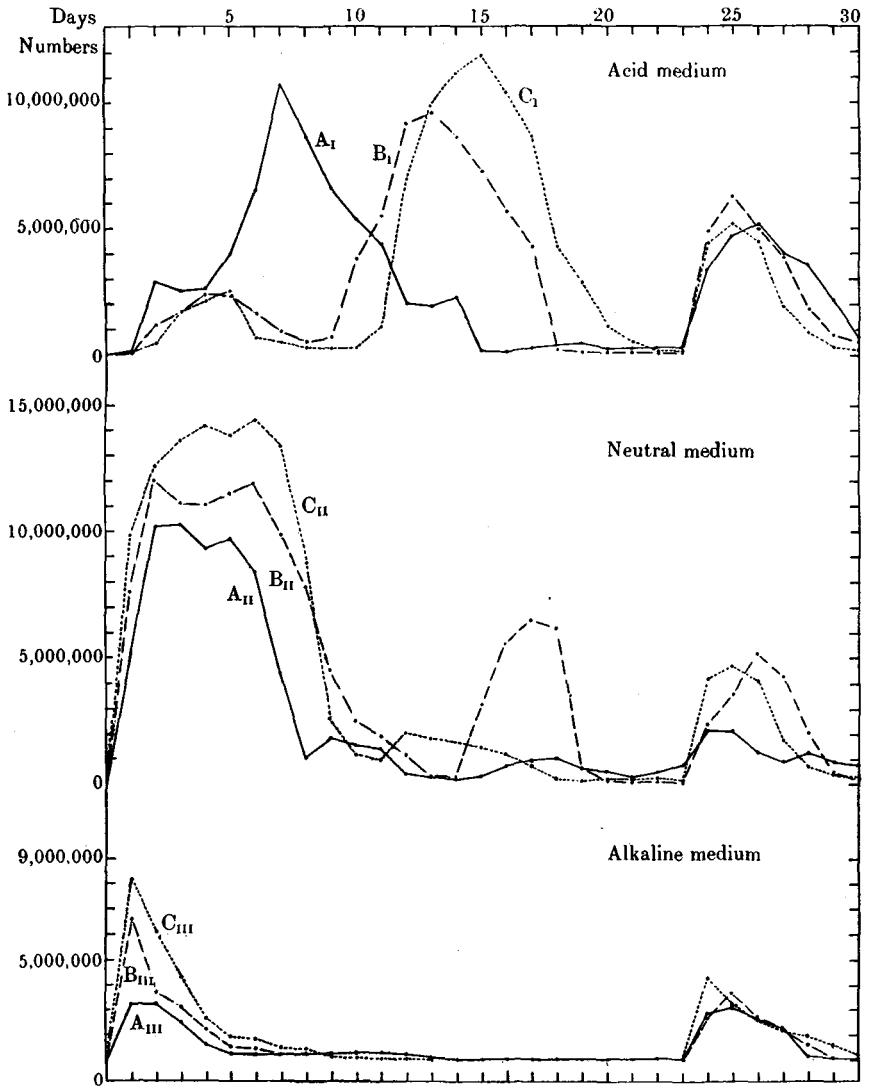

Chart 19. Comparing the rate of multiplication on acid, neutral and alkaline meat extract respectively of acid, neutral and alkali acclimatised cocci.

It will be seen that on the acid medium all three strains show a small primary rise followed after a fall in numbers by a great secondary rise. In the case of the acid acclimatised coccus the secondary rise reaches its maximum on the 7th day, in the neutral acclimatised coccus on the 13th day and in

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the alkali acclimatised coccus on the 15th day. In the neutral medium there is also a primary and a secondary rise, but the former is much greater than the latter. In the case of the acid acclimatised coccus the primary rise is least in height and duration and in the alkali acclimatised coccus greatest both in height and duration. In the alkaline medium a primary rise only occurs, and subsequently the numbers fall to a very low level. The rise is least in the acid acclimatised coccus, and greatest in the alkali acclimatised coccus.

In comparing these experiments with others previously quoted it should be remembered that the organisms have not only been acclimatised to growth in media of different reactions, but also to continuous growth in fluid meat extract medium.

Section XIV. *The influence on growth of the addition of gelatin or agar to meat extract.*

In order to ascertain the influence of the addition of small quantities of gelatin or agar to meat extract five series of tubes containing media of the following composition were prepared and sterilised in the autoclave.

Series	Meat extract	$N/10$ soda	Gelatin (20 %) in distilled water	Agar (2 %) in distilled water	Distilled water
A	1	0·26	2	—	1·74
B	0	0·18	2	—	2·82
C	1	0·08	—	—	3·92
D	1	0·08	—	0·5	3·42
E	0	0	—	0·5	4·5

All the series were made neutral to neutral red by the addition of $N/10$ soda. It will be noticed that Series *A* contains both meat extract and gelatin, Series *B* gelatin alone, Series *C* acts as a control, Series *D* contains meat extract and agar, and Series *E* agar alone.

Four tubes of each series were prepared and numbered *A* 1, 2, 3, 4, etc. To the first tube of each series a drop of a strong emulsion of *S. aureus* was added; the 2nd, 3rd and 4th tubes received such dilutions of the emulsion that approximately $\frac{1}{100}$, $\frac{1}{10{,}000}$, and $\frac{1}{100{,}000}$ of the dose was added.

In order to avoid confusion three experiments only of each series are illustrated in Chart 20. It will be noticed that the appearances in the charts are affected in each series only to a very slight degree by variations in the initial dose of the organisms. Series *C*, the controls, show "standard" curves. In Series *B*, containing gelatin only, multiplication occurs, but only to about one-fifth the extent in Series *C*. On the other hand in Series *A*, containing both meat extract and gelatin, multiplication occurs to twice the extent it does in Series *C*, the maximum figures being greater by one-third than the maxima of *B* and *C* together. In Series *D*, agar and meat extract, multiplication occurs to only half the extent it does in Series *C*, but the fall in the

Tube	Immediately after inoculation	6·5 hours	24 hours	30 hours	54 hours	72 hours	96 hours
A 1	—	2,856,000	8,658,000	11,280,000	14,096,000	15,808,000	16,464,000
2	—	194,000	6,320,000	9,472,000	13,288,000	15,000,000	16,484,000
3	—	1,520	—	—	—	—	—
4	—	21	3,952,000	5,988,000	10,848,000	14,864,000	15,502,000
B 1	—	1,116,000	1,752,000	2,096,000	1,568,000	1,320,000	288,000
2	—	41,600	1,664,000	1,720,000	1,856,000	1,656,000	432,000
3	—	328	1,368,000	1,798,000	2,096,000	2,004,000	332,000
4	—	3	931,000	1,656,000	2,120,000	1,952,000	336,000
C 1	78,048	2,472,000	6,620,000	7,744,000	9,200,000	9,568,000	7,424,000
2	872	57,600	5,744,000	7,376,000	8,604,000	9,344,000	8,260,000
3	7	248	5,166,000	6,522,000	9,088,000	9,088,000	7,232,000
4	0·8	1	2,800,000	5,984,000	8,500,000	—	6,670,000
D 1	—	1,832,000	4,848,000	5,100,000	5,232,000	3,568,000	—
2	—	44,000	3,520,000	4,944,000	4,932,000	3,776,000	—
3	—	?	2,464,000	4,624,000	5,166,000	3,904,000	—
4	—	6	1,528,000	4,688,000	4,848,000	4,688,000	—
E 1	—	75,136	2,850	—	—	0	0
2	—	145	0	—	—	0	0
3	—	7	0	—	—	0	0
4	—	0	0	—	—	0	0

Tube	5 days	6 days	7 days	8 days	9 days	10 days	11 days	13 days	14 days
A 1	17,120,000	17,096,000	10,496,000	7,792,000	5,924,000	5,166,000	4,208,000	244,000	232,000
2	17,280,000	17,344,000	8,672,000	7,968,000	6,152,000	5,832,000	4,240,000	592,000	232,000
3	—	—	—	—	—	—	—	—	—
4	16,716,000	16,604,000	13,288,000	11,536,000	9,296,000	8,528,000	4,320,000	744,000	520,000
B 1	196,000	228,000	188,000	228,000	148,000	—	—	—	82,000
2	240,000	212,000	192,000	148,000	228,000	—	—	—	44,500
3	320,000	172,000	164,000	172,000	204,000	—	—	—	43,000
4	276,000	184,000	184,000	250,000	224,000	—	—	—	66,000
C 1	3,856,000	704,000	324,000	160,000	224,000	—	—	—	83,000
2	5,936,000	1,080,000	308,000	272,000	232,000	—	—	—	42,000
3	—	—	260,000	152,000	144,000	—	—	—	145,000
4	5,120,000	1,664,000	460,000	244,000	114,000	—	—	—	67,000
D 1	2,368,000	1,456,000	1,628,000	1,136,000	1,144,000	736,000	284,000	29,500	—
2	2,920,000	2,200,000	1,648,000	1,520,000	1,588,000	1,312,000	932,000	136,000	—
3	2,512,000	1,944,000	1,632,000	1,600,000	1,696,000	1,440,000	792,000	104,000	—
4	3,120,000	2,304,000	1,416,000	1,326,000	1,392,000	1,112,000	420,000	46,000	—

numbers is much more prolonged. In Series *E*, agar alone, no multiplication took place, and the cocci died out rapidly.

From experiments of this nature, of which three were carried out, it is evident that *S. aureus* can grow in gelatin alone, and that the addition of gelatin to meat extract results in a medium, which is very favourable to multiplication. *S. aureus* cannot grow on agar alone, and the addition of agar to meat extract checks multiplication, but causes the decline in numbers to be slower.

Organisms belonging to other groups may react differently under such experimental conditions.

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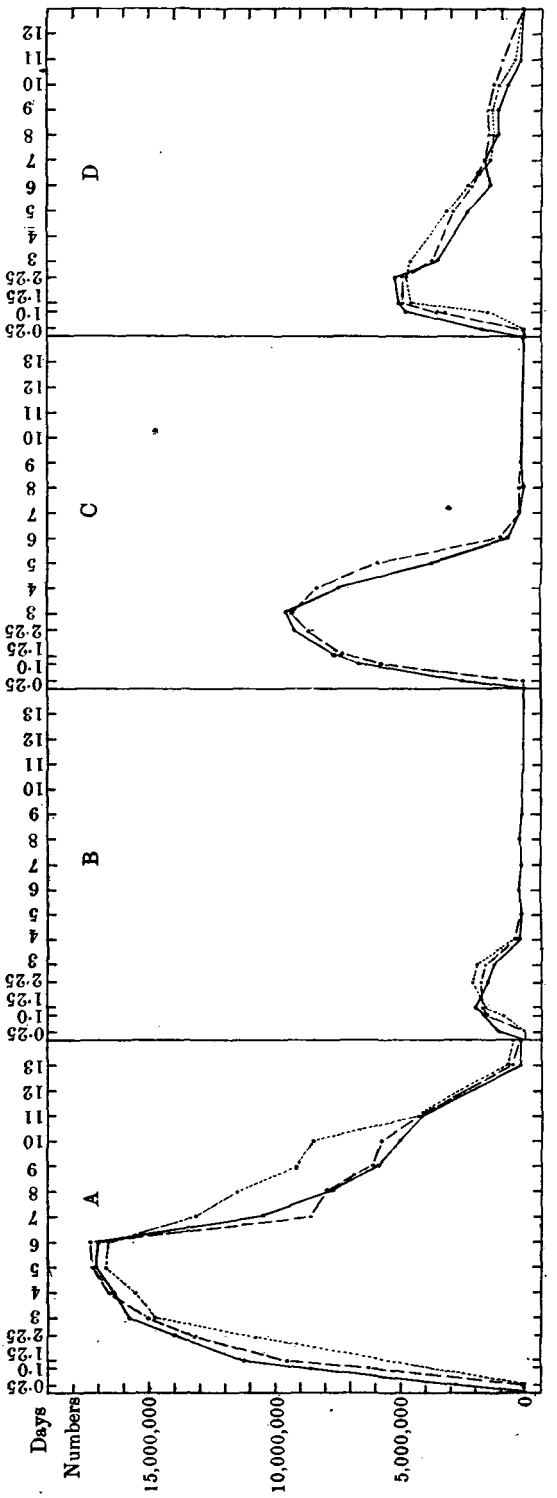

Chart 20. Showing the rate of multiplication of *S. aureus* in *A*, gelatin and meat extract; *B*, gelatin only; *C*, meat extract only; and *D*, agar and meat extract.

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SECTION XV. *The effects of adding various quantities of different acids to neutral meat extract agar before inoculation.*

The experiments described in this section were carried out only once, and not repeated. They are described here as they are related to those recounted in the last section and exhibit some interesting features.

The medium, consisting of meat extract (1 vol. of meat extract to 2·5 vols. of water) and agar (2 per cent.), was cleared with egg-white, neutralised to neutral red and sterilised on three occasions in the steamer. While hot 9 c.c. portions were pipetted into large test-tubes. To a second series sufficient salt was added to make a concentration of 0·9 per cent. Neither series contained peptone.

At the commencement of the experiment the agar in the tubes was melted and cooled to 45° C. To different tubes 0·25, 0·5, 0·75, 1·0, 1·5, 2·0, 3·0, 4·0 and 5·0 c.c. of *N*/10 solutions of the following acids[1], hydrochloric, orthophosphoric, lactic, butyric, isobutyric and a mixture of glutaminic and aspartic, were added, and the contents of the tubes mixed by rotation. Then one drop of an emulsion of *S. aureus* in distilled water was added, the presence or absence of a precipitate noted, the tubes rotated and plates poured. After 24 hours' incubation at 37° C. the colonies present on each plate were counted.

Quantity of acid added	Medium without salt		0·9 % salt added	
	Colonies	Condition of medium	Colonies	Condition of medium
		N/10 hydrochloric acid		
0·25 c.c.	1946	Clear	1018	Clear
0·5	1720	„	1725	Slight opalescence
0·75	1990	„	1550	Clear
1·0	0	Marked precipitate	1116	„
1·5	0	„ „	1890	„
2·0	1988	Clear	1527	„
3·0	0	Marked precipitate	1076	Slight precipitate
		N/10 orthophosphoric acid		
0·25	1704	Slight opalescence	491	Very slight opalescence?
0·5	1995	Clear	1960	„ „
0·75	1775	„	1297	„ „
1·0	1918	„	928	Clear
2·0	1	Precipitate	0	Precipitate
3·0	1521	Slight opalescence	664	Clear
4·0	0	Precipitate	1675	„
5·0	0	„	0	Precipitate
		N/10 lactic acid		
0·25	1908	Clear	1564	Clear
0·5	1843	„	1586	Slight opalescence
0·75	1786	Slight precipitate	1313	„ „
1·0	1835	Clear	1491	„ „
2·0	1889	„	0	Precipitate
3·0	0	Precipitate	0	„
4·0	0	„	0	„
5·0	0	„	0	„

[1] These solutions were made up by Mr F. W. Foreman.

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Quantity of acid added	Medium without salt: Colonies	Medium without salt: Condition of medium	0·9 % salt added: Colonies	0·9 % salt added: Condition of medium
		N/10 butyric acid		
0·25	1939	Clear	1383	Clear
0·5	1663	„	916	„
0·75	794	Slight opalescence	1863	Slight opalescence
1·0	1545	Clear	252	Precipitate
1·5	0	Precipitate	1	„
2·0	—	—	0	„
		N/10 isobutyric acid		
0·25	1846	Slight opalescence	1692	Very slight opalescence
0·5	1989	„ „	1260	„ „
0·75	1831	Clear	1753	„ „
1·0	0	Precipitate	0	Precipitate
1·5	1868	Clear	0	„
2·0	0	Precipitate	0	„
		N/10 glutaminic and aspartic acids (calculated as if all glutaminic)		
0·25	2134	Very slight opalescence	1826	Clear
0·5	1847	Slight precipitate	1867	Slight opalescence
0·75	1804	„ „	1708	„ precipitate
1·0	1282	Precipitate	1817	Clear
2·0	0	„	0	Precipitate
3·0	0	„	1778	Clear
4·0	0	„	0	Precipitate
5·0	0	„	0	„

In the series without salt it will be noticed that in the case of hydrochloric, orthophosphoric and isobutyric acids growth occurred when the medium remained clear after the addition of the acid, but not when the addition of the acid caused a precipitate. In the case of butyric acid the addition of 0·75 c.c. caused a slight opalescence, and the number of colonies was greatly reduced, but in the case of lactic acid though the addition of 0·75 c.c. caused a precipitate little reduction in the number of colonies occurred. In the case of glutaminic acid growth occurred when quantities up to 1·0 c.c. were added in spite of the production of a precipitate.

In the series containing 0·9 per cent. of salt most of the tubes to which hydrochloric acid was added remained clear and growth occurred in all of them. In the case of orthophosphoric acid a well marked precipitate formed on the addition of 2·0 c.c. and at that point no growth occurred. In the tubes to which 1·0 and 3·0 c.c. were added the number of colonies was small. In the case of lactic, butyric and isobutyric acids inhibition of growth was caused by the addition of smaller quantities than in the series to which no salt was added. In the case of glutaminic acid a precipitate was formed on the addition of 0·75 and 2·0 c.c. At the latter point only was growth inhibited.

SECTION XVI. *The effects of the addition of glucose.*

Several series of experiments were undertaken to ascertain the effects of the addition of glucose to meat extract media. The results were not uniform, and therefore the experiments are quoted in the order in which they were carried out.

Series I. Four tubes containing meat extract 1 c.c., *N*/10 soda 0·08 c.c., glucose (5 per cent.) in distilled water 1 c.c. and distilled water 2·92 c.c. were prepared, and each was inoculated with a drop of an emulsion of *S. aureus* in distilled water and incubated at 37° C.

Tube	Number at beginning of experiment	1 day	2 days	3 days	4 days	5 days
1	4416	4,000,000	3,288,000	1,120,000	116,000	2,500
2	—	3,888,000	3,776,000	1,744,000	316,000	7,000
3	—	3,632,000	3,648,000	1,712,000	352,000	12,000
4	—	4,060,000	3,376,000	1,536,000	160,000	4,000

	6 days	7 days	8 days	12 days	13 days	14 days
1	62	3	1	0	0	0
2	74	1	0	0	0	0
3	22	0	0	0	0	0
4	73	1	0	0	0	0

All the members of this series behaved in a very uniform manner (Chart 21), and all the cultures were dead by about the 9th day. These cultures differed in appearance from those to which glucose was not added. After 24 hours' incubation there was a copious, granular deposit in the glucose cultures, whereas in those without glucose the fluid was cloudy, and the sediment smaller in quantity and finely divided. Microscopically the organisms in the former were in groups, while in the latter they were separate.

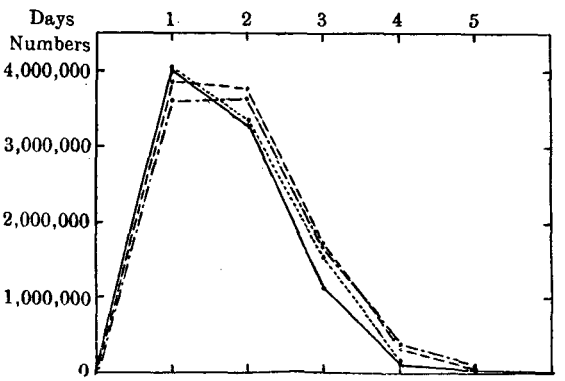

Chart 21. Showing the rate of multiplication in meat extract to which 1 % glucose was added.

In Series II the first three tubes contained different proportions of glucose, the fourth tube contained no glucose and acted as a control, and the last three tubes were of the same composition as tube 3, but additions were made to them daily. To tube 5 was added daily one drop of mixture *A*, to tube 6 one drop of mixture *B* and to tube 7 one drop of mixture *C*.

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In making up mixtures *A* and *B* concentrated meat extract obtained by evaporating 40 c.c. of meat extract to 2 c.c. was made use of.

Mixture *A* consisted of 1 c.c. concentrated meat extract and 1 c.c. distilled water
„ *B* „ 1 c.c. „ „ and 1 c.c. glucose (5 %) in distilled water
„ *C* „ 1 c.c. distilled water and 1 c.c. „ „ „

Tube	Meat extract	Distilled water	*N*/10 soda	Glucose (5 %) in distilled water	
1	1 c.c.	0·92	0·08	3·0	
2	1	1·92	0·08	2·0	
3	1	2·92	0·08	1·0	
4	1	3·92	0·08	0	
5	1	2·92	0·08	1·0	1 drop of mixture *A* added daily
6	1	2·92	0·08	1·0	„ „ *B* „ „
7	1	2·92	0·08	1·0	„ „ *C* „ „

Each tube was inoculated with one drop of an emulsion of *S. aureus* in distilled water, and incubated at 37° C.

Tube	No. at beginning of experiment	21 hours	45 hours	3 days	4 days	5 days	6 days	7 days
1	5660	4,816,000	2,168,000	352,000	101,000	70,270	98,432	440,000
2	—	5,024,000	2,552,000	696,000	48,000	8,080	1,784	7,392
3	—	5,152,000	3,264,000	1,152,000	83,000	4,368	3,000	808
4	—	7,680,000	9,872,000	5,840,000	4,016,000	2,560,000	1,376,000	732,000
5	—	4,720,000	3,686,000	1,132,000	234,000	69,000	644,000	2,040,000
6	—	4,962,000	4,296,000	1,848,000	321,000	69,000	42,000	179,700
7	—	4,928,000	3,600,000	1,748,000	180,000	40,000	7,500	203

	8 days	9 days	10 days	11 days	12 days	13 days	14 days	15 days
1	234,000	14,500	1,000	2,040	6,240	8,836	7,232	2,544
2	52,700	240,000	268,000	200,000	27,500	1,032	—	79
3	175	5	1	0	0	0	0	0
4	492,000	148,000	48,000	27,500	9,984	9,696	12,736	39,040
5	1,256,000	988,000	76,000	228,000	556,000	496,000	340,000	248,000
6	1,016,000	816,000	124,000	348,000	504,000	684,000	1,056,000	1,144,000
7	42	118	1,064	31,250	64,000	71,500	28,000	3,984

	16 days	17 days	18 days	19 days	20 days	21 days	22 days	23 days
1	1,904	4,544	8,960	2,000	2,064	1,728	5,776	10,880
2	81	66	82	96	90	81	74	49
3	0	0	0	0	0	0	0	0
4	—	—	—	—	—	—	—	—
5	508,000	856,000	1,176,000	268,000	244,000	224,000	145,000	82,000
6	628,000	808,000	664,000	596,000	604,000	716,000	568,000	620,000
7	960	138	9	11	12	33	354	1,496

	24 days	25 days	26 days	27 days	28 days	29 days	30 days	31 days
1	10,596	11,712	14,688	15,488	14,816	16,566	8,400	32,040
2	28	18	17	20	23	35	34	48
3	0	0	0	0	0	0	0	0
4	—	—	—	—	—	—	—	—
5	66,500	51,500	72,000	102,000	166,000	198,000	350,000	548,000*
6	992,000	760,000	708,000	712,000	448,000	348,000	429,000	500,000†
7	4,704	10,272	19,648	21,344	16,128	7,728	488	22

* 9 drops of mixture *A* added.

† 10 drops of mixture *B* added. After the 32nd and 31st days respectively no additions were made to tubes 5 or 6.

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	32 days	33 days	34 days	35 days	36 days	37 days	38 days	39 days
1	15,488	3,686	480	53	20	2	0	0
2	38	31	9	1	1	0	0	0
3	0	0	0	0	0	0	0	0
4	—	—	—	—	—	—	—	—
5	1,032,000‡	1,648,000	2,816,000	2,856,000	3,176,000	3,003,000	2,216,000	1,144,000
6	796,000	2,024,000	2,650,000	3,040,000	3,224,000	2,968,000	3,392,000	1,672,000
7	1	0	0	0	0	0	0	0

	40 days	41 days	43 days	44 days	47 days
1	0	—	0	0	0
2	0	—	0	0	0
3	0	—	0	0	0
4	—	—	—	—	—
5	196,000	13,500	0	0	0
6	308,000	2,500	0	0	0
7	0	—	0	0	0

‡ 10 drops of mixture *A* added.

From the tables it will be seen that culture 3 behaved in the same manner as the cultures of Series I. On the other hand culture 1 containing three times as much glucose showed several oscillations in numbers, and small numbers of organisms were alive up to the 37th day. The same phenomenon was exhibited in culture 2, but to a lesser extent. In all three cultures death of the organisms ultimately occurred. In culture 5 to which concentrated meat extract was added daily considerable oscillations occurred, but the figures remained throughout at a moderately high level. The addition of larger quantities of concentrated meat extract on the 31st and 32nd days caused a decided rise in the numbers. Subsequently no additions were made and the culture died. Culture 6 to which concentrated meat extract together with glucose was added daily followed a very similar course to culture 5. These two cultures seem to show that if in the presence of glucose small quantities of food material are added daily, whether with or without glucose, the cultures remain alive for prolonged periods. The addition of larger quantities of food material causes considerable multiplication to take place, but in the absence of further additions of food material the organisms die. With daily small additions of glucose as in culture 7 the organisms may remain alive for 30 days and small oscillations in the numbers occur (Chart 22).

Series III. In this series the proportion of glucose varied in all the tubes, which contained media of the following compositions:

Tube	Meat extract	*N*/10 soda	Distilled water	Glucose (25 %) in distilled water
1	1 c.c.	0·08	1·92	2
2	1	0·08	2·92	1
3	1	0·08	3·42	0·5
4	1	0·08	3·72	0·2
5	1	0·08	3·82	0·1
6	1	0·08	3·9	0·02
7	1	0·08	3·92	0

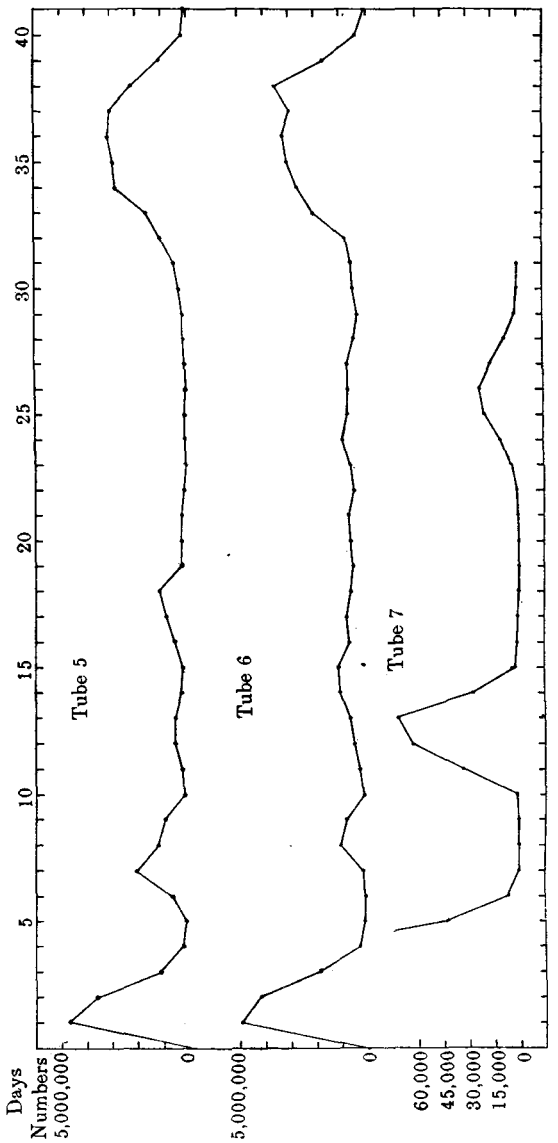

Chart 22. Showing multiplication in tubes of meat extract containing 1 % glucose. Up to the 30th day daily additions were made of meat extract to tube 5, meat extract and glucose to tube 6, and glucose to tube 7. On the 31st and 32nd days large additions were made to tube 5, and on the 31st day to tube 6. Subsequently no additions were made. Tube 7 is illustrated on a larger scale in order to show more clearly the remarkable late fluctuations.

Each tube was inoculated with a drop of an emulsion of *S. aureus* in distilled water and incubated at 37° C. The organisms had been subcultured daily on agar for four days.

Tube	No. at beginning of experiment	23 hours	47 hours	67 hours	4 days	5 days
1	5760	5,616,000	1,260,000	600,000	46,500	8,400
2	—	6,160,000	1,414,000	128,000	10,000	824
3	—	7,137,000	3,052,000	298,000	12,000	1,264
4	—	7,796,000	4,904,000	1,192,000	8,000	208
5	—	8,656,000	5,592,000	2,408,000	660,000	1,906
6	—	8,832,000	7,424,000	3,536,000	1,720,000	296,000
7	—	9,696,000	12,976,000	11,248,000	2,864,000	868,000

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Tube	6 days	7 days	8 days	9 days	10 days	11 days
1	2,708	228	36	1	0	0
2	63	0	0	0	0	0
3	190	0	0	0	0	0
4	22	0	0	0	0	0
5	77	8	—	—	384	0
6	55,808	26,176	26,200	26,288	7,536	3,760
7	1,244,000	408,000	272,000	148,000	79,500	46,500

	12 days	13 days	14 days	17 days	22 days
1	0	—	—	0	0
2	0	—	—	0	0
3	0	—	—	0	0
4	0	—	—	0	0
5	0	—	—	0	0
6	856	1,248	888	568	14
7	13,824	29,056	30,400	45,440	48,128

Even when the quantity of glucose present is very small the numbers begin to fall after 24 hours' incubation, instead of rising as they do in cultures without glucose (Chart 23).

With increasing quantities of glucose the maximum attained diminishes, and the rate of the subsequent fall, at least from the 2nd to the 4th day, increases.

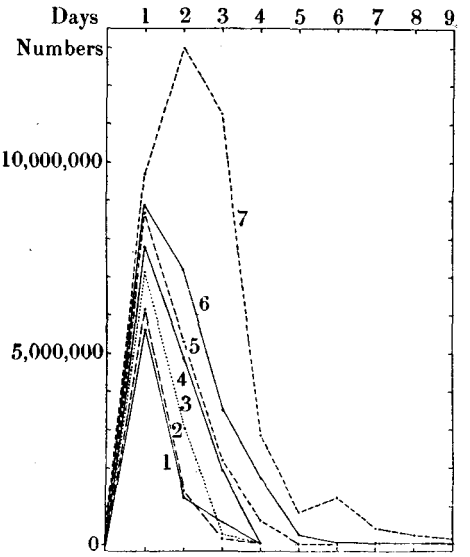

Chart 23. Showing the rate of multiplication in meat extract to which different quantities of glucose have been added. Tube 6 contains 0·1 % glucose; tube 5, 0·5 %; tube 4, 1 %; tube 3, 2·5 %; tube 2, 5 %; tube 1, 10 %; tube 7 contains no glucose and acts as a control.

Series IV was a repetition of part of Series II in order to ascertain the effect of small daily additions of food material, or glucose, or both, to cultures containing glucose.

Four tubes each containing meat extract 1·0 c.c., *N*/10 soda 0·08 c.c., distilled water 2·92 c.c. and glucose (5 per cent. in distilled water) 1·0 c.c.

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were prepared, sterilised by boiling, inoculated with an emulsion of *S. aureus* in distilled water, and incubated at 33° C.

To tube I were added daily two drops of concentrated meat extract (100 c.c. meat extract evaporated to 10 c.c. and 10 c.c. of distilled water), to tube II two drops of concentrated meat extract with glucose (100 c.c. meat extract evaporated to 10 c.c. and 10 c.c. of 25 per cent. glucose in distilled water), and to tube III one drop of 5 per cent. glucose in distilled water. To tube IV which acted as a control no additions were made.

Tube	No. at beginning of experiment	22 hours	48 hours	76 hours	4 days	5 days
I	2024	5,392,000	2,176,000	2,688,000	2,120,000	576,000
II	—	4,896,000	2,152,000	2,563,000	968,000	188,000
III	—	6,608,000	2,036,000	616,000	412,000	224,000
IV	—	5,824,000	2,640,000	2,024,000	1,048,000	2,184,000

	6 days	7 days	8 days	9 days	10 days	11 days
I	356,000	160,000	244,000	232,000	784,000	2,784,000
II	144,000	64,000	84,000	1,188,000	2,816,000	4,312,000
III	640,000	648,000	450,000	176,000	—	224
IV	568,000	428,000	200,000	28,000	6,500	432

	12 days	13 days	14 days	15 days	17 days	19 days
I	3,792,000	5,952,000	4,504,000	4,744,000	952,000	228,000
II	3,688,000	3,824,000	3,176,000	2,112,000	1,176,000	756,000
III	154	27	17	8	10	41
IV	87	9	4	6	30	32

	21 days	23 days	26 days	28 days	31 days	35 days
I	1,752	223	161	154	13	77
II	576,000	940,000	788,000	612,000	185,000	68,000
III	2	18	29	7	30	6
IV	100	103	233	176	248	152

	39 days	41 days	44 days	48 days	52 days
I	0	0	0	0	0
II	103,000	29,500	0	0	0
III	12	0	0	0	0
IV	148	319	275	98	110

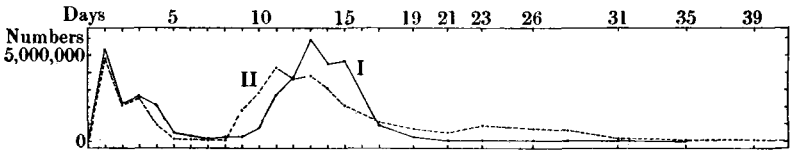

Chart 24. Showing the rate of multiplication in tubes of meat extract to which 1 % glucose was added, and to which daily additions of meat extract (I) and meat extract and glucose (II) were made.

In tube I, with daily additions of meat extract, after the primary rise the numbers fell to a moderately low level and then rose again to nearly the same level as in the primary rise. Subsequently a rapid fall to a low level

occurred and the culture was dead by the 39th day. In tube II with daily additions of meat extract and glucose the course of events was similar, but the fall after the secondary rise was slower, and the culture was dead on the 44th day. In tube III with daily additions of glucose the secondary rise was very slight, but very small numbers remained alive till the 39th day. In tube IV to which no addition was made there was a slow fall after the primary rise, and small oscillations subsequently. The culture was still alive on the 52nd day.

Series I, cultures 1, 2, 3, 4, Series II, culture 3, Series IV, culture 4, were similar in composition and Series III, culture 4, contained the same ingredients in the same proportions. The cultures in Series I, II and III behaved in the same manner, the organisms being dead in about seven to ten days, but in the cultures of Series IV the organisms after reaching a very low level on the 17th day, multiplied to a small extent and were still alive on the 52nd day. This perhaps indicates that if some of the organisms survive the critical period life may be prolonged for a very considerable time. The experiments described in Series II, culture 5, and in Series IV, culture 1, were very similar and indicate that in spite of the presence of glucose the daily addition of small quantities of meat extract prolongs the life of the culture; similarly Series II, culture 6, and Series IV, culture 2, show that in spite of the presence of glucose the daily addition of small quantities of meat extract and glucose prolongs the life of the culture. Series II, culture 7, and Series IV, culture 3, show that the daily addition of small quantities of glucose does not result in the rapid death of the culture.

SECTION XVII. *The effects of adding living organisms of the same species to growing cultures.*

Several series of experiments, of which four are quoted, were carried out in order to ascertain the effects of adding at different times varying numbers of *S. aureus* to cultures already growing in meat extract media. As it is necessary in such experiments to estimate the numbers at frequent intervals a low concentration of meat extract was employed so as to avoid very prolonged observations, and the errors liable to be introduced in counting large numbers. The strain of *S. aureus* employed had been isolated recently from pus.

Exp. 1. In the first experiment five tubes, each containing meat extract 0·5 c.c., *N*/10 soda 0·04 c.c. and distilled water 4·46 c.c., were employed. All the tubes received at the same time a primary inoculation of a drop of an emulsion of the coccus in distilled water, and all except tube *A*, which was used as a control, received at some time a second inoculation of a drop of a freshly prepared emulsion of the same coccus. The tubes were kept in an incubator at 37° C., but were taken out for a short time on each occasion on which subcultures were made from them.

Tube	Count before incubation	Cocci added 1·75 hours	Cocci added 3·5 hours	6·25 hours	Cocci added 6·5 hours	8 hours	Cocci added 8·5 hours
A	2216	—	—	863,000	—	2,092,000	—
B	2552	1592	—	786,000	—	2,300,000	—
C	2592	—	1465	818,000	—	2,276,000	—
D	2320	—	—	836,000	2554	2,695,000	—
E	3004	—	—	848,000	—	2,384,000	1394
Mean	—	—	—	830,000	—	2,349,000	—

	15·75 hours	17 hours	20 hours	22 hours	26 hours	44 hours	68 hours
A	7,840,000	6,608,000	5,784,000	5,672,000	5,344,000	488,000	220,000
B	7,304,000	6,224,000	5,816,000	5,698,000	5,624,000	447,000	211,000
C	7,976,000	7,120,000	6,040,000	6,024,000	5,480,000	556,000	208,000
D	7,176,000	7,192,000	5,564,000	5,660,000	5,280,000	592,000	242,000
E	7,656,000	6,872,000	6,232,000	5,696,000	5,296,000	536,000	187,500
Mean	7,590,000	6,803,000	5,887,000	5,750,000	5,405,000	524,000	213,000

It will be seen that in this experiment the addition of small numbers of cocci at different times between 1·75 and 8·5 hours after the beginning of incubation exerted no influence which could be determined by the methods employed.

The slow rate of fall in numbers between the 15th and 26th hours was probably due to cooling of the tubes owing to frequent removals from the incubator in order to prepare dilutions for subcultures.

Exp. 2. In this series six tubes were used containing a medium of the same composition as in Exp. 1. In order to maintain an approximately equal temperature during the whole period the tubes were kept throughout in a water-bath at 37° C.

Tube	Count before incubation	0·5 hour	Cocci added 0·5 hour	1·5 hours	Cocci added 1·5 hours	2·5 hours
A	2896	2752	—	4112	—	9,984
B	2672	2768	3712	7824	—	12,960
C	2656	2920	—	4008	2624	10,944
D	2808	2776	—	4248	—	10,744
E	2720	2712	—	4002	—	11,080
F	2744	2800	—	4320	—	9,854
Mean	—	—	—	—	—	10,927

	Cocci added 2·5 hours	5 hours	Cocci added 5 hours	6·5 hours	7·75 hours	Cocci added 7·75 hours
A	—	2,680,000	—	1,160,000	2,912,000	—
B	—	292,000	—	1,216,000	3,016,000	—
C	—	320,000	—	1,104,000	2,856,000	—
D	1808	292,000	—	1,228,000	2,720,000	—
E	—	342,000	1502	1,368,000	3,188,000	—
F	—	246,000	—	1,196,000	2,776,000	1672
Mean	—	293,000	—	1,212,000	2,911,000	—

	10 hours	12 hours	14 hours	16 hours	17·5 hours
A	4,904,000	5,508,000	6,298,000	6,144,000	5,808,000
B	4,960,000	5,932,000	6,040,000	6,008,000	5,208,000
C	4,600,000	6,060,000	6,650,000	5,948,000	5,768,000
D	5,186,000	6,443,000	7,050,000	6,816,000	6,634,000
E	4,736,000	6,280,000	6,699,000	6,600,000	6,146,000
F	4,104,000	5,982,000	6,891,000	6,080,000	5,820,000
Mean	4,738,000	6,034,000	6,604,000	6,266,000	5,596,000

In this experiment also the addition of small numbers of cocci seemed to have very little influence on the numbers present at various times, except in the early stages of tube *B* before rapid multiplication had begun.

Exp. 3. In this experiment four tubes were used, each containing meat extract 0·25 c.c., *N*/10 soda 0·02 c.c. and distilled water 4·73 c.c., and the tubes were kept throughout in the water-bath at 37° C. Half an hour after the commencement of incubation a small number of cocci were added to tube *B*, and a large number to tube *D*. A small number was added to tube *C* after 4·5 hours.

Tube	Count before incubation	0·5 hour	Cocci added 0·5 hour	2·5 hours	3 hours	3·5 hours
A	905	897	—	1,600	2,676	4,024
B	923	1047	2,369	3,828	4,328	4,996
D	922	943	19,089	21,440	23,968	37,312
C	794	805	—	1,152	2,028	2,336

	4 hours	4·5 hours	Cocci added 4·5 hours	5·5 hours	6·5 hours	7·75 hours
A	5,984	20,000	—	63,000	114,500	428,000
B	7,768	16,600	—	53,500	112,000	336,000
D	50,944	201,500	—	600,000	980,000	1,972,000
C	3,640	10,800	2784	38,000	82,000	284,000

	8·75 hours	9 hours	10·25 hours	11·25 hours	12·25 hours	28 hours
A	606,000	1,128,000	2,128,000	2,696,000	3,264,000	1,716,000
B	594,000	828,000	1,544,000	2,748,000	3,124,000	1,708,000
D	2,424,000	2,572,000	3,216,000	3,772,000	3,064,000	1,564,000
C	548,000	717,000	1,488,000	2,680,000	3,316,000	1,604,000

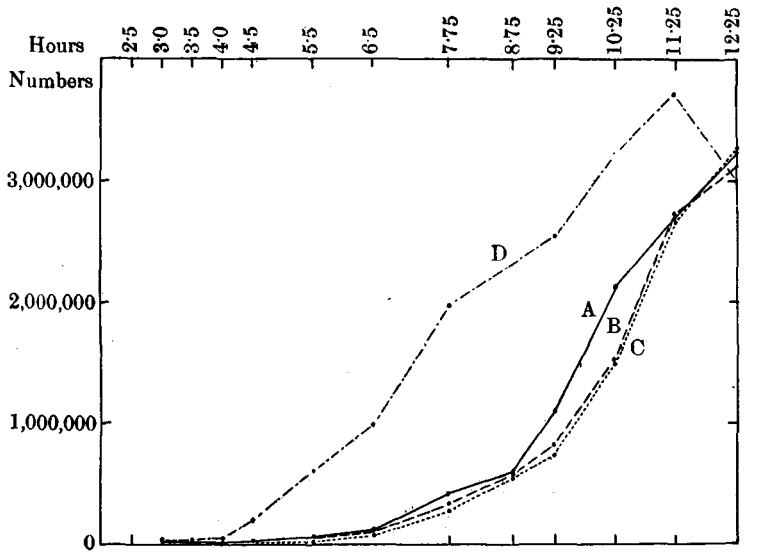

Chart 25. Showing the results of adding small numbers of cocci to growing cultures in tube *B* after 0·5 hour and to tube *C* after 4·5 hours, and larger numbers to tube *D* after 0·5 hour's incubation at 37° C., as compared with the control tube *A*.

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It will be seen that in tube *D*, to which a large number of cocci were added within the lag period, the increase in numbers was most rapid. Little difference in the rate of growth in the other three tubes, the control *A*, and the tubes *B* and *C*, to which small numbers were added, could be detected. The experiment seems to indicate that while the addition of small numbers has an inappreciable influence on the numbers present at any period of growth, the addition of a large number, at any rate within the lag period, exerts a considerable influence.

Exp. 4. In this experiment three tubes, containing a medium of the same composition as in Exp. 3, were employed, and a large number of cocci added to tube *B* 0·75 hour, and to tube *C* 4 hours after the commencement of incubation.

Tube	Count before incubation	0·75 hour	Cocci added 0·75 hour	3 hours	4 hours	Cocci added 4 hours
A	1504	1372	—	2,592	18,400	—
B	1568	1696	27,872	30,656	307,200	—
C	1512	1536	—	2,632	20,200	9212
	5 hours	6 hours	8 hours	9 hours	10 hours	11 hours
A	35,200	138,000	604,000	1,320,000	3,992,000	4,868,000
B	1,008,000	2,076,000	3,992,000	4,928,000	5,380,000	5,496,000
C	45,600	164,800	1,348,000	3,528,000	4,800,000	5,252,000
	12 hours	13 hours	14 hours	27 hours	52 hours	
A	5,212,000	5,386,000	5,396,000	928,000	432,000	
B	5,600,000	5,624,000	5,424,000	1,008,000	536,000	
C	5,480,000	5,420,000	5,312,000	882,000	480,000	

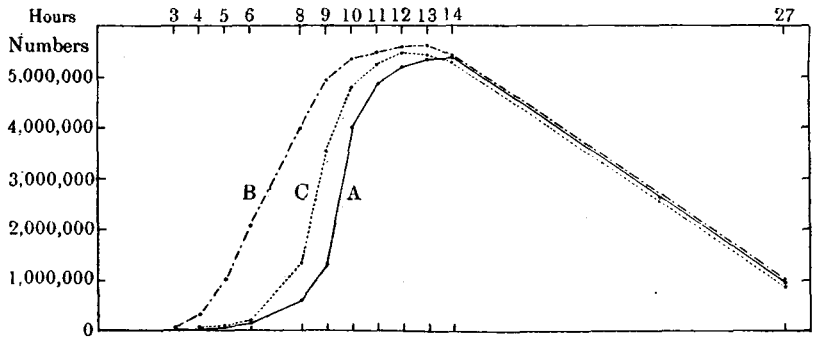

Chart 26. Showing the results of adding large numbers of cocci to growing cultures in tube *B* after 0·75 hour's and in tube *C* after 4 hours' incubation at 37° C., as compared with the control tube *A*.

It will be seen that in both *B* and *C* the numbers rose more rapidly than in the control tube *A*, showing that the addition of large numbers, at least within the first few hours of incubation, accelerates the rise in numbers.

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SECTION XVIII. *The distribution of* S. aureus *in meat extract cultures.*

The experiments described in this section were undertaken in order to ascertain roughly the distribution of *S. aureus* in meat extract cultures after various periods of incubation at 37° C. Large tubes containing meat extract 2 c.c., *N*/10 soda 0·16 c.c. and distilled water 7·84 c.c. were employed. The strain of *S. aureus* used had been isolated freshly from pus.

(*A*) In one series the cultures were shaken once daily, as in most of the experiments described in this paper. Two samples were taken daily with a small pipette, containing 0·01 c.c. up to a diamond mark, the first from the upper part of the culture with as little disturbance as possible, and the second after thorough shaking. After dilution subcultures were made in the usual manner.

	Immediately after inoculation	44 hours	47 hours	70 hours	94 hours	118 hours	142 hours	166 hours	190 hours	214 hours
Before shaking	616	8,112,000	10,216,000	9,408,000	8,016,000	5,744,000	2,576,000	856,000	322,000	170,000
After shaking	—	9,600,000	12,416,000	10,816,000	9,504,000	7,424,000	3,112,000	976,000	405,000	215,000

It will be seen that 24 hours after each shaking about 15 per cent. of the living organisms had fallen towards the bottom of the tube, the majority of them probably forming the deposit.

(*B*) In another series of experiments the cultures were left undisturbed in the incubator until the time of examination.

Six tubes inoculated at the same time and incubated at 37° C. were employed. Samples from the upper part of the first culture tube before and after shaking were examined after 18 hours' incubation, from the second, third, fourth. fifth and sixth tubes after 2, 3, 4, 5 and 6 days' incubation respectively.

	Tube I		Tube II	Tube III	Tube IV	Tube V	Tube VI
	After inoculation	18 hours	48 hours	72 hours	96 hours	120 hours	144 hours
Before shaking	584	7,680,000	8,156,000	7,792,000	7,208,000	2,416,000	1,680,000
After shaking	—	8,880 000	10,736,000	10,976,000	8,992,000	3,132,000	2,080,000

It will be seen that after 48 hours' incubation about 25 per cent. of the living organisms had fallen to the bottom, but that subsequently the proportion at the bottom remained about the same.

(*C*) Effects of centrifugalisation. Cultures consisting of meat extract 1 c.c., *N*/10 soda 0·08 c.c. and water 3·92 c.c. were prepared in centrifugal tubes. After 22 hours' incubation at 37° C. a sample was taken from the upper part of a culture, the tube centrifugalised for one hour, and another sample taken from the top. The first sample showed 7,904,000 colonies, and the second 125,000 colonies, indicating that 98·5 per cent. of the organisms occurring

near the surface had been driven down. The upper 3 c.c. of the fluid, which were quite clear, were pipetted off, and again incubated for 25 hours. Subcultures from the top before shaking showed 6,848,000 colonies, and after shaking 8,176,000 colonies. The cocci remaining in the upper layers therefore multiplied in the same manner as cocci inoculated into fresh medium, and about 16 per cent. settled to the bottom.

Another culture incubated for 48 hours was centrifugalised for 3 hours. Before centrifugalisation subcultures from the top showed 11,232,000 colonies, and after, when the fluid was clear, 2344 colonies, or a reduction of 99·98 per cent. The upper 3 c.c. were pipetted off and incubated for 24 hours at 37° C. Before shaking the top showed 706,000 colonies, and after shaking 768,000 colonies.

Section XIX. *Meat extract and pancreas extract compared as media.*

In order to ascertain whether the mode of preparation of the extract influences the rate of growth of the organisms six equal portions from one heart were weighed out, ground up with sterile sand and triturated with 2·5 c.c. of distilled water to each gramme of meat. Six portions of bullock's pancreas were prepared in the same way. The heart preparations were labelled *H* 1, 2, 3, 4, 5, 6 and the pancreas preparations *P* 1, 2, 3, 4, 5, 6, and were treated in the following manner.

H 1 and *P* 1. Boiled immediately after preparation for 10 minutes, boiled next day, filtered through filter paper and again boiled.

H 2 and *P* 2. Autoclaved immediately after preparation for 20 minutes, filtered next day, and again autoclaved.

H 3 and *P* 3. 2 per cent. chloroform added and incubated for 24 hours at 37° C., boiled and filtered and again boiled.

H 4 and *P* 4. 2 per cent. chloroform added and incubated for 24 hours at 37° C., autoclaved and filtered and again autoclaved.

H 5 and *P* 5. Incubated for 24 hours at 37° C. without chloroform, boiled and filtered and again boiled.

H 6 and *P* 6. Incubated for 24 hours at 37° C. without chloroform, autoclaved and filtered and again autoclaved.

In the preparations labelled *H* 5, 6 and *P* 5, 6 putrefactive organisms had grown, and before sterilisation the preparations were turbid and foul smelling.

From each extract two tubes were prepared, in one the extract was diluted with distilled water (*e.g. H* 1) and in the other neutralised to neutral red with *N*/10 soda and diluted (*e.g. H* 1 A). The composition of these tubes is given in the following table. It will be noticed that the pancreas extract when incubated for 24 hours at 37° C., whether with or without chloroform, requires a relatively large quantity of soda to neutralise it.

Two such sets of media were prepared; one was inoculated in the usual manner with *S. aureus* and the other with *B. coli.*

	Meat extract	$N/10$ soda	Distilled water		Pancreas extract	$N/10$ soda	Distilled water
H 1	1·0 c.c.	0	1·0	*P* 1	1·0 c.c.	0	1·0
1 A	1·0	0·05	0·95	1 A	1·0	0·05	0·95
H 2	1·0	0	1·0	*P* 2	1·0	0	1·0
2 A	1·0	0·05	0·95	2 A	1·0	0·05	0·95
H 3	1·0	0	1·0	*P* 3	1·0	0	1·0
3 A	1·0	0·075	0·925	3 A	1·0	0·25	0·75
H 4	1·0	0	1·0	*P* 4	1·0	0	1·0
4 A	1·0	0·1	0·9	4 A	1·0	0·375	0·625
H 5	1·0	0	1·0	*P* 5	1·0	0	1·0
5 A	1·0	0·1	0·9	5 A	1·0	0·225	0·775
H 6	1·0	0	1·0	*P* 6	1·0	0	1·0
6 A	1·0	0·125	0·875	6 A	1·0	0·35	0·65

Except in the modified form quoted later (p. 195), these experiments have not been repeated and therefore too much stress cannot be laid on them, but attention may be called to the following points.

Meat extract. There is no appreciable difference between the results obtained with fresh meat extract sterilised by boiling (*H* 1, 1 A) and by autoclaving (*H* 2, 2 A). The curve is higher and more prolonged with meat extract incubated with chloroform and sterilised by boiling (*H* 3, 3 A), the unneutralised sample (*H* 3) showing a curve like that produced when small quantities of $N/10$ hydrochloric acid have been added to fresh meat extract. In the samples of meat extract incubated with chloroform, and sterilised by autoclaving (*H* 4, 4 A) the neutralised specimen (*H* 4 A) produces a curve similar to *H* 3 A, but in the unneutralised specimen (*H* 4) the curve though prolonged is relatively low. In the samples incubated without chloroform and sterilised by boiling (*H* 5, 5 A) the curves resemble those produced with fresh extract, but the maximum growth in the whole series was obtained with similar samples sterilised by autoclaving (*H* 6, 6 A). In the two samples incubated without chloroform growth of putrefactive organisms had occurred.

Pancreas extract. Growth was considerably greater in the sample of fresh pancreas extract sterilised by autoclaving (*P* 2, 2 A) than in the sample sterilised by boiling (*P* 1, 1 A). By far the greatest multiplication took place in the unneutralised specimen of extract incubated with chloroform and sterilised by boiling (*P* 3), but in the neutralised specimen of the same sample it was not so great. In the samples incubated with chloroform and sterilised by autoclaving (*P* 4, 4 A) the results most closely resemble those obtained with fresh autoclaved samples (*P* 2, 2 A). It is of interest to note that multiplication was very slight during the first day in the neutralised specimen (*P* 4 A). The least growth in this series was obtained with samples incubated without chloroform (*P* 5, 5 A, 6, 6 A).

It is evident that under all the conditions of these experiments, except those in which putrefactive organisms had grown (*H* 5, 6, *P* 5, 6), pancreas extract is a much better medium for the growth of *Staphylococci* than meat

Cultures inoculated with *Staphylococcus*.

	20 hours	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	37 days	Fluid in tube, c.c.
H 1	7,488,000	12,336,000	9,856,000	3,968,000	948,000	644,000	628,000	1,032,000	1,508,000	1,736,000	1,660,000	·13
1 A	9,792,000	14,848,000	10,528,000	2,032,000	972,000	868,000	1,064,000	1,192,000	896,000	720,000	1,236,000	·41
2	8,424,000	12,944,000	9,760,000	3,600,000	3,284,000	2,392,000	936,000	1,144,000	971,000	1,576,000	1,184,000	·24
2 A	10,752,000	13,472,000	9,264,000	8,304,000	2,432,000	—	512,000	536,000	596,000	1,168,000	3,376,000	·28
3	8,296,000	8,976,000	8,432,000	11,696,000	15,984,000	12,992,000	9,288,000	4,336,000	3,984,000	4,240,000	4,585,000	·51
3 A	10,192,000	12,112,000	14,032,000	14,832,000	13,232,000	4,968,000	2,624,000	2,624,000	2,063,000	1,856,000	4,328,000	·07
4	7,976,000	5,816,000	5,936,000	6,704,000	6,816,000	6,584,000	5,404,000	5,824,000	3,776,000	3,152,000	5,936,000	·24
4 A	7,992,000	13,280,000	12,512,000	12,896,000	12,368,000	5,264,000	1,916,000	3,288,000	4,208,000	3,584,000	3,152,000	·21
5	8,264,000	12,656,000	10,592,000	5,504,000	3,288,000	2,128,000	2,372,000	2,704,000	2,496,000	2,208,000	2,448,000	·05
5 A	9,752,000	18,224,000	12,144,000	5,968,000	3,496,000	2,708,000	2,096,000	1,904,000	1,632,000	1,424,000	2,472,000	·09
6	7,216,000	20,624,000	20,144,000	21,056,000	16,848,000	8,176,000	4,784,000	3,816,000	2,762,000	2,608,000	7,456,000	·38
6 A	8,856,000	19,136,000	19,392,000	9,312,000	6,112,000	3,120,000	1,616,000	1,856,000	1,611,000	1,712,000	7,505,000	·44
P 1	15,600,000	18,720,000	18,048,000	16,496,000	12,640,000	7,184,000	4,944,000	3,000,000	1,600,000	—	3,312,000	·48
1 A	14,892,000	16,096,000	15,660,000	17,776,000	14,144,000	9,120,000	6,944,000	5,184,000	2,704,000	—	3,216,000	·43
2	17,024,000	24,656,000	23,712,000	23,684,000	14,136,000	10,304,000	7,048,000	11,760,000	11,536,000	—	5,982,000	·41
2 A	20,608,000	26,920,000	30,624,000	26,244,000	14,720,000	6,200,000	3,056,000	6,048,000	5,120,000	—	—	·41
3	40,416,000	51,984,000	39,520,000	22,288,000	13,872,000	11,648,000	7,856,000	13,856,000	10,800,000	—	8,912,000	·19
3 A	31,888,000	29,168,000	21,750,000	12,800,000	7,456,000	6,048,000	5,840,000	5,440,000	9,044,000	—	6,656,000	·1
4	13,888,000	18,112,000	26,782,000	22,384,000	11,248,000	6,560,000	4,304,000	5,920,000	2,304,000	—	2,400,000	·55
4 A	194,000*	17,114,000	32,096,000	30,832,000	11,968,000	9,696,000	5,248,000	8,992,000	7,584,000	—	5,904,000	·4
5	5,472,000	11,024,000	10,544,000	9,856,000	8,144,000	7,520,000	4,592,000	6,704,000	5,536,000	—	2,080,000	·48
5 A	8,512,000	8,272,000	8,388,000	9,216,000	8,000,000	7,440,000	5,616,000	4,944,000	5,088,000	—	4,176,000	·76
6	2,304,000	5,088,000	8,480,000	10,752,000	10,816,000	10,752,000	5,080,000	2,848,000	5,792,000	—	2,688,000	·75
6 A	2,780,000*	6,880,000	6,752,000	7,408,000	7,840,000	4,800,000	5,328,000	3,632,000	4,288,000	—	3,488,000	·52

* Colonies very small after 24 hours' growth.

extract. The rate of growth was greatest in unneutralised pancreas extract incubated with chloroform and sterilised by boiling (*P* 3). On the other hand, an inhibition of multiplication for the first 24 hours took place in the neutralised specimen incubated with chloroform and sterilised by autoclaving (*P* 4 A). Further investigations on this subject are being carried on.

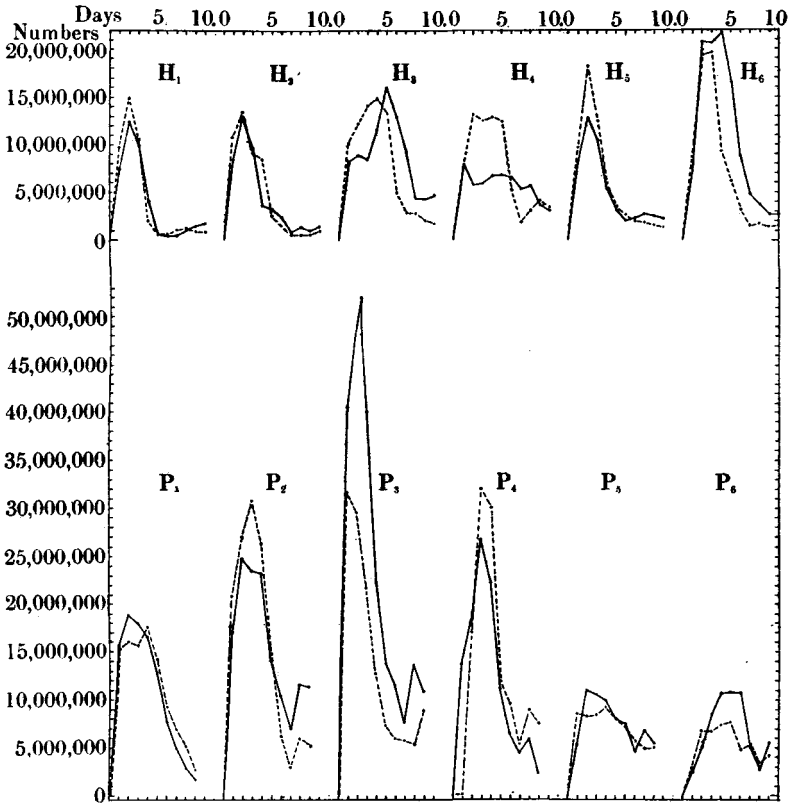

Chart 27. Showing the rate of multiplication of *S. aureus* in samples of meat extract (*H*) and pancreas extract (*P*) prepared in different ways. The continuous line indicates unneutralised and the broken line neutralised samples.

In spite of great reduction in the quantity of the fluid in each tube by the removal of samples and evaporation, in all cases the organisms were alive on the 37th day.

In many features the results with *B. coli* closely resemble those with *Staphylococci.*

Meat extract. With fresh meat extract the curves are similar whether sterilisation was by boiling (*H* 1, 1 A) or by autoclaving (*H* 2, 2 A). Meat extract incubated with chloroform and boiled (*H* 3) gives a moderately high figure with less of the delay noticed in the cultures sown with *Staphylococci.* Extract incubated with chloroform and autoclaved (*H* 4, 4 A) gives

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Cultures inoculated with *B. coli*.

	26 hours	2 days	3 days	4 days	5 days	6 days	7 days	9 days	11 days	15 days	Fluid in tube, c.c.
H 1	5,904,000	10,592,000	10,176,000	8,112,000	4,640,000	3,136,000	2,176,000	3,104,000	2,896,000	1,088,000	·47
1 A	7,088,000	12,128,000	9,248,000	5,072,000	3,936,000	3,238,000	2,096,000	2,032,000	2,016,000	800,000	·73
2	5,440,000	11,472,000	6,528,000	1,904,000	1,488,000	—	992,000	768,000	760,000	288,000	·6
2 A	8,176,000	12,080,000	6,592,000	3,632,000	2,672,000	2,720,000	1,856,000	2,566,000	1,536,000	992,000	·66
3	5,056,000	12,760,000	9,248,000	8,896,000	3,952,000	2,960,000	2,784.000	3,056,000	1,968,000	688,000	·65
3 A	6,288,000	6,904,000	10,176,000	3,872,000	2,976,000	1,888,000	2,080,000	1,904,000	1,264,000	144,000	·75
4	2,400,000	4,912,000	7,008,000	9,200,000	6,512,000	4,368,000	3,872,000	5,200,000	6,560,000	10,096,000	·22
4 A	7,840,000	7,084,000	3,856,000	2,512,000	808,000	2,720,000	2,544,000	2,192,000	2,096,000	600,000	·62
5	4,656,000	12,624,000	10,352,000	4,704,000	3,424,000	4,000,000	2,048,000	2,096,000	1,280,000	1,507,000	·43
5 A	4,960,000	11,360,000	7,152,000	6,352,000	4,432,000	2,288,000	2,144,000	3,360,000	1,120,000	504,000	·43
6	4,016,000	9,648,000	11,296,000	8,640,000	6,576,000	5,536,000	4,800,000	4,240,000	3,968,000	5,152,000	·31
6 A	5,568,000	10,514,000	7,936,000	4,928,000	4,080,000	4,014,000	4,400,000	4,848,000	2,912,000	4,112,000	·54
P 1	5,088,000	14,064,000	17,024,000	15,776,000	10,544,000	6,834,000	8,128,000	7,904,000	4,576,000	4,240,000	·73
1 A	6,624,000	15,264,000	15,696,000	12,120,000	11,312,000	8,960,000	9,344,000	5,238,000	4,416,000	2,720,000	·7
2	7,248,000	17,808,000	17,888,000	16,912,000	12,768,000	8,594,000	7,936,000	9,360,000	9,600,000	8,805,000	·15
2 A	7,390,000	17,440,000	18,736,000	19,420,000	12,912,000	10,720,000	7,840,000	11,792,000	—	—	—
3	19,820,000	7,258,000	3,904,000	3,400,000	5,248,000	4,096,000	3,456,000	9,792,000	7,600,000	3,488,000	·1
3 A	16,848,000	7,280,000	2,064,000	1,520,000	2,048,000	2,720,000	1,520,000	8,688,000	6,304,000	7,808,000	·62
4	12,688,000	15,168,000	9,552,000	4,416,000	3,200,000	1,904,000	2,096,000	8,400,000	4,800,000	6,176,000	·58
4 A	16,048,000	8,544,000	4,016,000	2,288,000	2,240,000	2,016,000	1,968,000	6,237,000	4,512,000	4,896,000	·68
5	5,008,000	4,288,000	3,056,000	1,232,000	976,000	928,000	1,344,000	1,952,000	5,132,000	2,576,000	·73
5 A	4,032,000	3,000,000	2,304,000	1,152,000	1,088,000	1,306,000	1,552,000	2,544,000	4,896,000	3,618,000	·53
6	5,488,000	640,000	1,536,000	1,360,000	1,072,000	1,072,000	736,000	1,680,000	1,216,000	704,000	·72
6 A	4,752,000	336,000	1,112,000	1,168,000	688,000	1,040,000	544,000	1,072,000	960,000	336,000	·48

ow figures with, in the case of the unneutralised sample (*H* 4), considerable delay in reaching the maximum. Good growths were obtained in the samples (*H* 5, 6) in which putrefactive organisms had grown. *Pancreas extract. B. coli* grows better in fresh pancreas extract than in meat extract, and better in the autoclaved (*P* 2) than in the boiled sample (*P* 1). Pancreas extract incubated with chloroform and boiled (*P* 3) gives the most rapid growth of the whole series, but growth in the autoclaved sample (*P* 4) is not better

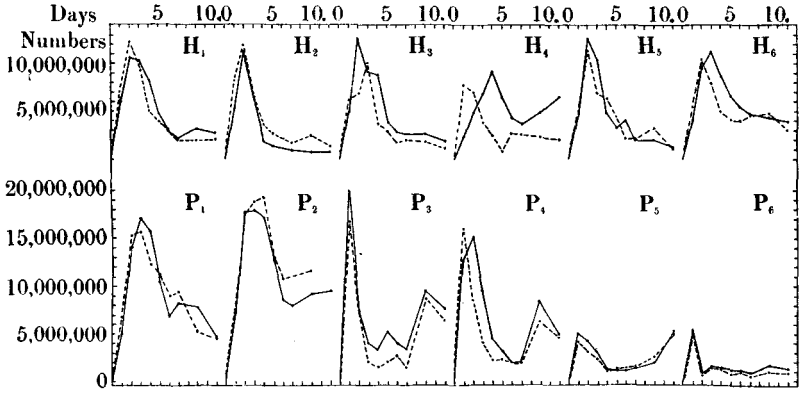

Chart 28. Showing the rate of multiplication of *B. coli* in samples of meat extract (*H*) and pancreas extract (*P*) prepared in different ways. The continuous line indicates unneutralised and the broken line neutralised samples.

than in the fresh extract. In the neutralised specimen (*P* 4 A) no early inhibition, such as occurred with *Staphylococci*, took place; in fact very rapid early growth occurred. Multiplication was comparatively very small in the samples (*P* 5, 6) in which putrefactive organisms had grown.

As in the case of *S. aureus* unneutralised pancreas extract incubated with chloroform and boiled (*P* 3) is the medium which gives the most rapid growth, but in the case of *B. coli* its superiority over pancreas extracts treated in other ways is not so marked.

SECTION XX. *Further experiments with media made from incubated ox pancreas.*

To 100 grms. of fresh, finely minced ox pancreas 250 c.c. of distilled water and 5 c.c. of chloroform were added, and the mixture incubated for 24 hours at 37° C. After thorough shaking the contents of the flask were divided into two portions. One portion, *P* 3, was steamed for 20 minutes, filtered, and the filtrate boiled. The other portion, *P* 4, was autoclaved, filtered and the filtrate again autoclaved. 10 c.c. of the former required 3·0 c.c. of *N*/10 soda, and 10 c.c. of the latter 3·2 c.c. *N*/10 soda to bring the reaction to the neutral point of neutral red.

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From each medium a series of tubes containing different quantities of $N/10$ soda and of distilled water were prepared.

	P 3	*N*/10 soda	Distilled water		*P* 4	*N*/10 soda	Distilled water
A	1	0	4	*A*	1	0	4
B	1	0·1	3·9	*B*	1	0·1	3·9
C	1	0·2	3·8	*C*	1	0·2	3·8
D	1	0·3	3·7	*D*	1	0·3	3·7
E	1	0·4	3·6	*E*	1	0·4	3·6
F	1	0·5	3·5	*F*	1	0·5	3·5

Each tube was inoculated with a drop of an emulsion in distilled water of *S. aureus* recently isolated from pus. The cultures were incubated at 37° C., plates prepared daily, and the colonies counted in the usual manner.

It will be noticed that the dilution of the pancreas extract is greater than in the experiments quoted in the last section.

	4 hours	24 hours	48 hours	3 days	4 days
P 3 *A*	39,712	29,644,000	43,328,000	42,048,000	31,498,000
B	16,032	26,624,000	42,528,000	41,334,000	35,904,000
C	4,592	25,376,000	40,320,000	39,712,000	33,472,000
D	2,112	22,880,000	41,608,000	41,728,000	37,120,000
E	1,968	5,136	19,328,000	35,904,000	38,464,000
F	1,024	3,696	317,000	12,576,000	15,056,000

	5 days	7 days	9 days	11 days	14 days
P 3 *A*	28,864,000	15,296,000	9,536,000	3,936,000	2,480,000
B	26,048,000	18,048,000	11,008,000	6,144,000	4,608,000
C	23,744,000	19,752,000	15,040,000	7,832,000	6,048,000
D	35,520,000	21,638,000	16,512,000	7,104,000	3,248,000
E	40,960,000	34,432,000	31,464,000	24,128,000	10,656,000
F	17,664,000	14,848,000	12,480,000	11,968,000	5,568,000

	4 hours	24 hours	48 hours	3 days	4 days
P 4 *A*	2296	10,544,000	20,800,000	23,936,000	26,816,000
B	1896	11,184,000	33,952,000	37,616,000	40,576,000
C	1480	134,000	27,392,000	32,256,000	37,192,000
D	1592	506	100,500	20,288,000	28,192,000
E	1432	10	1	0	0
F	1296	8	0	0	0

	5 days	7 days	9 days	11 days	14 days
P 4 *A*	30,784,000	21,642,000	16,512,000	13,696,000	6,752,000
B	43,072,000	32,832,000	25,728,000	19,072,000	12,715,000
C	40,064,000	34,600,000	25,088,000	16,960,000	9,472,000
D	34,652,000	32,320,000	24,384,000	19,264,000	8,672,000
E	0	0	0	0	0
F	0	0	0	0	0

Cultures were made from the tubes immediately after inoculation and the mean number of organisms found was 1520.

Though during the first few hours multiplication was greatest in the earlier specimens in the *P* 3 series after 24 hours' cultivation there was little difference between the results in *P* 3 *A*, *B* and *C*, and in Chart 29 the con-

tinuous line, x, represents the daily mean of these three cultures. The numbers in *P* 3 *D* fell more slowly during the earlier stages of the decline. In *P* 3 *E*, which was slightly alkaline, multiplication was slow during the first 24 hours, and the maximum was not attained until the fifth day. In *P* 3 *F* multiplication was very slow during the first 48 hours and a relatively low maximum was reached about the fifth day.

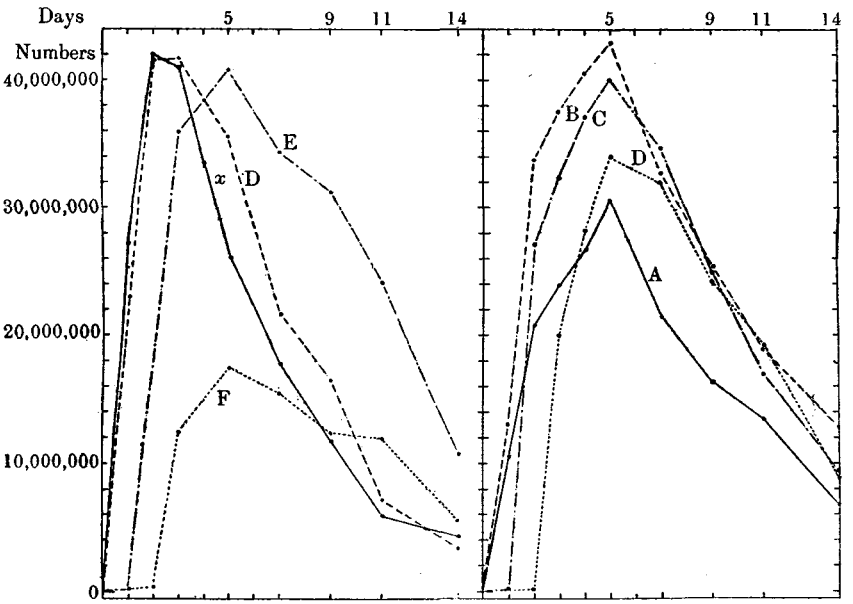

Chart 29. Showing on the left the results of growing cocci in *P* 3 medium. The line x represents the mean of the three cultures *A*, *B*, *C* in which the reaction was acid. In *D* the reaction was neutral and in *E* and *F* slightly alkaline. On the right are shown the results of growing cocci in *P* 4 medium. *A* was distinctly acid, *B* less acid and *C* still less acid. *D* was nearly neutral.

In the *P* 4 series multiplication took place in the first four tubes, *A*, *B*, *C* and *D*, only, and all reached their maximum about the fifth day, the highest figures being attained in cultures *B* and *C*. In *P* 4 *C* multiplication was very slow during the first 24 hours, and in *P* 4 *D* during the first 48 hours. In the alkaline cultures *P* 4 *E* and *F* no multiplication occurred, and the organisms soon died.

In both these media *Staphylococci* multiply to an extraordinary extent provided that the quantity of soda added is not sufficient to bring the reaction beyond the neutral point of neutral red.

SECTION XXI. *The effects of accidental contamination.*

Though experiments in which accidental contamination occurred were soon abandoned some features worthy of record were observed. When cultures of *S. aureus* became contaminated with certain aerobic, spore-bearing bacilli

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a sudden and unexpected decrease in the numbers of the cocci was noticed, even when the contaminating organisms were so few as to produce but one or two colonies on the agar subcultures. On the other hand on one occasion a great and unexpected increase in the numbers of coccus colonies was observed when the culture became contaminated with a small, diphtheroid bacillus. Instances have occurred in which the growth of a streptothrix or a mould did not appear to exert any influence on the growth of the cocci. It is evident, therefore, that the introduction into the medium of an organism of a different species may exert a great influence on the growth of *S. aureus*.

The bearing of these Experiments on some of the Phenomena observed in Infective Diseases.

It has been suggested by Penfold (1914) that the "incubation period of infectious diseases may partly depend for its existence on bacterial lag." Some of the experiments described in this paper suggest the possibility that certain of the phenomena observed in connection with infective diseases depend, at least to some extent, on the available supply of food for the organisms.

It is evident that pathogenic bacteria living in the body cannot multiply without food, and that this food must in most cases be derived from the tissues. Though several factors doubtless operate in checking the multiplication of bacteria after they have gained entrance into the body their capacity to multiply must depend on their ability to procure food.

If the food is derived directly from healthy, living tissue, the supply is almost unlimited, but if it is a breakdown product then possibly the enzymes of the injured tissue as well as the enzymes of the bacteria play a part in its manufacture, and food in sufficient quantities to keep up a high rate of multiplication fails, unless the agents concerned in its production are acting efficiently. In a localised suppuration a variation in reaction or other result of local changes may produce conditions such as to inhibit partially the action of the enzymes.

Speculations such as these are suggested by certain resemblances between some of the phenomena observed in infective diseases and events in cultures.

(1) In disease an incubation period of longer or shorter duration invariably occurs. Its length probably depends on the virulence of the organisms and the size of the initial dose. In cultures the "lag" (or incubation period) can be abolished by using for inoculation rapidly multiplying organisms accustomed to the medium. In diseases associated with certain classes of organisms the incubation period may be greatly reduced experimentally by repeatedly passing the organism through the same species of animal. By this means the organism becomes accustomed to the medium (animal body) and its virulence, or capacity to grow in the tissues, is increased. In cultures, if the medium is not very suitable, many of the organisms die and a large inoculation is required to ensure growth. The form of the curve of growth depends to some extent on the numbers introduced (Section IV) and their source (Sections II, XIII).

Experimentally in animals a dose of a certain size is required, unless the organism is highly virulent, in order to secure the production of disease, and the course of the resulting disease depends to some extent on the size of the dose.

(2) In diphtheria the bacilli growing in the tonsillar crypts may be compared to organisms growing in test-tubes, and the numbers present at any time on the surface of the tonsils can be estimated roughly. Rapid multiplication of diphtheria bacilli occurs after the incubation period and for a longer or shorter time very great numbers are present. Subsequently the numbers decline until a low level has been reached. In carriers, who show few or no symptoms, the same phenomenon occurs, so that the process is independent of susceptibility or immunity to the toxins. The normal course of events in meat extract cultures is similar.

(3) The low level just mentioned often persists with slight variations for weeks or even months in diphtheria convalescents and carriers, and in the same manner small numbers of organisms remain alive in cultures and apparently exhibit slight oscillations in numbers. It often happens that one or more negative cultures from the tonsils are followed by positive cultures and this may happen on several occasions before the three consecutive negative cultures required for release are recorded.

(4) In typhoid, diphtheria and other carriers relapses occasionally occur, when the specific organisms again become numerous. These relapses are often preceded by slight deviations from normal health, in which catarrh is a prominent feature. These ailments which are often associated at the sites of infection with local changes, possibly resulting in an increase of material available as food to the specific organisms, appear to be due to intercurrent infections.

In cultures temporary multiplication may be induced by occasional small additions of food material.

(5) While some intercurrent infections appear to cause multiplication of the diphtheria bacilli in the tonsils of carriers, others, such as those associated with streptococci, may apparently cause their extermination. In cultures some accidental contaminations produce similar effects.

(6) Regular small additions of food material to cultures result in a high level being maintained or, if the additions are not sufficiently large, in the rate of decline being very slow. In some carriers large numbers of diphtheria bacilli persist for very long periods of time in the throat or nose, and perhaps in them there exist circumstances producing analogous results. Shearer (1917) showed that in nasal secretion "there is present some body, which greatly accelerates the growth of meningococcus on artificial culture medium," and Kligler (1919) investigated "saline washings of the nose of apparently healthy individuals." He states that "there were marked individual and specific differences. A given organism grew in the washings of one individual and not in those of another. Evidently the nasal secretions of some individuals contain substances, which actively stimulate growth."

(7) It is not uncommon to observe in both mild clinical cases and carriers a sudden fall from great numbers ending in the rapid disappearance of the diphtheria bacilli from the surface of the tonsils. In cultures, if the number of organisms inoculated is relatively large, the maximum is soon reached and the decline in numbers is very rapid owing to the speedy exhaustion of the food supply. The surprisingly rapid disappearance of the bacilli in the class of case mentioned may be due partly to the rapid exhaustion of the available food in the tonsillar crypts.

(8) The sequence of events in two individuals apparently infected from the same source may be very different. Whereas in one the symptoms may reach their full height in a few days, in the other the incubation period may be longer and the symptoms may not reach their height for several days. Presumably in the latter the early free multiplication of the organisms has been checked. Similar phenomena are noticed when equal doses of organisms are inoculated into neutral and slightly acid meat extract cultures.

(9) The acclimatisation experiments illustrate in cultures the phenomenon observed in streptococci of increase of virulence or capacity to grow in one species of animal (acid medium) simultaneously with loss of virulence for another species (alkaline medium).

Experiments on local immunity such as those carried out by Cobbett and Melsome (1896) on the ears of rabbits with *Streptococcus erysipelatus* might decide to what extent the exhaustion of food supply is a factor in conferring temporary local immunity. These workers showed that "an absolute local immunity had been conferred upon the parts directly affected by the first attack, unless the interval had been long enough to permit of the entire disappearance of all inflammatory thickening." On second inoculation they "could get no evidence of the invasion of these ears by streptococci." Transitory inflammation could, however, be produced by the inoculation of killed cocci or their poisonous products, showing that local resistance to the organisms is independent of local resistance to their toxins.

The speculations contained in this paragraph suggested themselves from time to time during the course of the work, but no attempt was made to prove them by animal experiments. Should they stimulate further research on the factors influencing the increase and decline in numbers of pathogenic bacteria in the tissues they will have fulfilled their purpose.

SUMMARY.

1. In dilute neutral meat extract cultures (without salt or peptone) inoculated with relatively small numbers of *S. aureus*, taken from agar cultures grown for 18 hours at 37° C. and incubated at 37° C., multiplication proceeds rapidly during the first day and more slowly on the second, when the maximum number, about 10,000,000 per standard loop (0·01 c.c.), is reached. Later the number of living organisms decreases at first rapidly, but later more slowly, until a low level is reached, which remains fairly constant or falls very slowly

for a long period. During the period of relative constancy small oscillations are observed. The curve produced on plotting out the daily counts may be regarded as a "standard."

2. The frequency with which the culture used for inoculation has been transplanted on agar slopes influences the growth on neutral meat extract. Several transplantations in rapid succession result in very rapid growth, a high maximum and a very rapid fall in the numbers. Less frequent transplantation over a long period seems to cause the maximum to be reached later than in the standard and the period of decline to be postponed.

3. In one series of experiments (Section III) the proportion of meat extract was varied in the different tubes employed. These experiments show that the greater the proportion of meat extract the greater is the multiplication, and the longer the period which elapses before the curve reaches its highest point, in fact the extent of multiplication appears to be closely related to the amount of meat extract present in the culture. The length of the period of rapid decline is also related to the amount of meat extract present.

4. The form of the curve of growth is influenced by the number of cocci inoculated. With a small inoculation into dilute neutral meat extract the maximum number of cocci present in the medium at any period does not usually exceed 10 to 12 millions per standard loop. If the initial dose greatly exceeds this figure multiplication proceeds relatively slowly for two days and subsequently there is a very rapid fall in the numbers. With an initial dose close to this figure a somewhat similar curve is produced, though the rate of fall is not so rapid. Much smaller doses produce "standard" types of curves.

5. Provided the numbers inoculated are small (50,000–50 per drop) the results after 24 hours' incubation in different experiments of the same kind are not materially affected.

6. If after the numbers have reached a low level small drops of concentrated meat extract, insufficient to cause appreciable dilution, are added to the culture further multiplication occurs, to some extent proportional to the amount of food material added. The fall in numbers, which follows the initial rise, is not due therefore to the accumulation of products, but seems to be caused mainly by the using up of food material.

7. By small regular additions of food material (concentrated meat extract) a definite concentration of *Staphylococci* can be maintained in a meat extract medium for a long period of time, and probably by suitable additions any desired concentration could be maintained. Accumulation of the products may gradually inhibit growth, but on this point the experiment gives little evidence.

8. Moderate dilution with distilled water at any stage of incubation has little effect. Events occur in the usual sequence, but the number of organisms in each standard drop is proportional to the dilution.

9. The incubation temperature has a great influence on the course of events in meat extract cultures of *S. aureus*. At 37° C. multiplication during the first 24 hours is very rapid, the maximum is attained on the second or third

day, and the numbers fall very rapidly. At 27° C. the maximum is attained on the fifth or sixth day, and is considerably greater than that attained at 37° C. The fall is rapid. At 17° C. multiplication is very slow during the first 48 hours, but is subsequently rapid, and the maximum, which is higher than that attained at 27° C., is reached on the eighth day. The decline in numbers is slow.

At 8 to 10° C. very slight multiplication, if any, occurs during the first 24 hours and subsequently the numbers steadily decline for at least 60 days.

At lower temperatures the numbers fall rapidly and the cultures die. At − 1° C. the organisms were dead by the 19th day, at − 6° C. by the 13th day, and at − 10° C. by the 9th day.

10. If organisms such as *S. aureus*, *B. coli* or *B. pyocyaneus* are allowed to grow in meat extract medium at 37° C. until the numbers have reached a low level, and the tubes are then inoculated with the species originally present little or no multiplication takes place, but if one of the other organisms is inoculated multiplication of the added organisms occurs. If the cultures are sterilised by boiling before inoculation with fresh organisms the original strain or the others, when added, multiply. Boiling, therefore, appears to liberate some food for added organisms belonging to the strain which was originally present.

The growth of any of these organisms in the medium seems to remove most of the food for that species as well as a portion of the food substance used by other species, since in no case was the growth of the added species nearly so considerable in extent as in its primary cultures.

11. The effect of adding increasing quantities of $N/10$ hydrochloric acid up to 0·3 c.c. to each 5 c.c. of the medium is to retard the growth of the cocci during the earlier stages of incubation, though subsequently rapid growth takes place, and a high maximum is reached. With small inoculations of cocci the addition of more than 0·3 c.c. $N/10$ hydrochloric acid results in the death of the organisms within a short time.

With additions of $N/10$ soda varying between 0·4 and 1·2 c.c. there seems to be a progressive decrease in the height reached by the maxima, the rate of growth in the early stages is retarded, and the rate of decrease in numbers seems to be retarded. With the addition of 1·6 or 1·8 c.c. $N/10$ soda the rate of growth in the early stages is markedly retarded. With the addition of 2·0 c.c. $N/10$ soda no growth occurs.

B. coli seems to be more sensitive than *S. aureus*, especially to the addition of alkali.

12. More precise experiments with $N/10$ hydrochloric acid show that with the addition of increasing amounts of the acid the type of curve gradually changes from a "standard" with one peak to a curve with two peaks, separated by an interval in which the numbers are small.

13. By continuous growth in acid, neutral and alkaline meat extract the capacity of *S. aureus* to multiply when transplanted into media of different

reactions is altered. When transferred into an acid medium all strains show a small primary rise followed, after a fall in the numbers, by a great secondary rise. In the case of the acid acclimatised cocci the secondary rise reached its maximum on the 7th day, in the neutral acclimatised cocci on the 13th day, and in the alkali acclimatised cocci on the 15th day. In the neutral medium there is also a primary and a secondary rise, but the former is much greater than the latter. In the case of the acid acclimatised cocci the primary rise was least in height and duration, and in the case of the alkali acclimatised cocci greatest both in height and duration. In the alkaline medium a primary rise only occurs and subsequently the numbers fall to a very low level. The rise was least in the acid acclimatised cocci and greatest in the alkali acclimatised cocci.

In comparing these experiments with others previously quoted it should be remembered that the organisms have been acclimatised to growth not only in media of different reactions, but also to continuous growth in fluid meat extract medium.

14. *S. aureus* can multiply to a small extent in neutral gelatin solution (8 per cent.). On a medium consisting of gelatin and meat extract the greatest multiplication takes place, much higher figures being obtained than the maxima of growth on gelatin solution and meat extract respectively added together. In agar solution (0·8 per cent.) alone no multiplication takes place, and the cocci quickly die. In a medium consisting of agar and meat extract the maximum reached is lower than in meat extract, but the decline in numbers is slower.

15. When certain quantities of various acids are added to warm meat extract agar precipitates are formed, though little or no precipitate may be produced by lesser or even slightly greater quantities. In some instances no growth occurred in plates poured from those tubes in which a precipitate had formed.

16. The addition of glucose to the extent of 1 per cent. to dilute meat extract results in most cases in *S. aureus* multiplying rapidly during the first day. Subsequently the numbers decline and the culture dies. With increasing quantities of glucose the maximum figure attained diminishes, and the rate of the subsequent fall, at least from the second to the fourth day, increases. Even with a very small quantity of glucose the numbers begin to fall after 24 hours' incubation, instead of rising as they do in cultures without glucose.

If to cultures containing 1 per cent. glucose daily additions of small quantities of concentrated meat extract or of concentrated meat extract with glucose are made oscillations in the numbers occur, but the cultures remain alive and with large additions multiplication may take place. The death of the organisms is not hastened by small daily additions of glucose.

17. The addition at different times of small numbers of the cocci to growing cultures of *S. aureus* has no appreciable influence, but the addition of large numbers exerts a considerable influence.

18. In meat extract cultures of *S. aureus* incubated at 37° C. about 15 per cent. of the living organisms sink to the bottom after each daily shaking. If the tubes are left undisturbed about 25 per cent. sink to the bottom.

19. Meat extract incubated with chloroform for 24 hours at 37° C. and sterilised by boiling seems to be a slightly better medium than fresh meat extract sterilised by boiling or autoclaving immediately after preparation. Pancreas extract is a better medium than meat extract. The multiplication of cocci is greatest in pancreas extract incubated with chloroform for 24 hours at 37° C. and sterilised by boiling.

20. Organisms accidentally contaminating cultures of *S. aureus* may cause, according to their species, a sudden decline or a rapid increase in the number of the cocci.

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