THE DETERMINATION OF THE IMPORTANCE OF CELL GROUPING IN THE DIRECT MICROSCOPIC COUNTING OF BACTERIA IN MARKET MILK.

33

By

Ila B.Kennedy

B.A.University of Tennessee, 1923.

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Arts in the Graduate School of the University of Tennessee, 1924.

Knoxville, Tennessee.

OUTLINE OF THESIS.

I. Introduction.

II. Survey of literature.

III. Object of study.

IV. Procedure.

V. Results.

VI. Discussion of results.

VII. Conclusions.

VIII. Bibliography.

Aug. 1924 M.L.B. 1.25

57411

INTRODUCTION

At first thought it might seem that the relation of the plate count on market milk to the direct microscopic count is an unimportant matter. However a close study of this subject brings out the fact that standardization of methods of counting bacteria must precede the successful control of the quality of market milk. This fact should be more fully appreciated. Also it should be remembered in this connection that a careful study of statistics shows definately that where strict bacteriological standards have been established in a city the infant and adult death rates have been decidely lowered. So it is evident that this subject of the correlation of bacterial counting methods, instead of being one of minor importance, is in reality one of fundamental value to public health.

One might inquire why it is necessary to bother with these newer microscopic methods of counting bacteria, when the plate method is so well established. This is a very common question and is eaisly answered. In the first place, the plate method requires from two to seven days to be completed, whibe direct counts of the in from five to thirty minutes. Milk of inferior quality is consumed before the plate method can furnish information designating it as fit or unfit for use. By direct methods the quality of the milk may be determined before the milk has been removed from the cans in the usual procedure of a market milk plant.

Another potent factor which urges the adoption of direct microscopic methods in preference to the plate method is that of the saving of expense and time. The direct methods require very little

apparatus, and one technician can do thousands of counts per day; While the plate method requires much laboratory apparatus,, and comparatively few counts can be made by one worker in a day. From these facts the advantage of grading market milk by the direct counting of bacteria is very obvious. In other words, these newer methods are equal to the requirements, while the plate method does does not have such a possibility.

While the plate method is in vogue at present quite generally where strict bacteriological standards for market milk are established,,still it has never been commercially satisfactory. It is an established fact that many cities have been discouraged in maintaining a grading system for market milk because of the cumbersomeness of the plate method. In contrast to this effect of the use of the plate method it is admitted generally that the direct methods are inexpensive to operate, reliable and prompt in results. It is not an exaggeration to predict that because of the greater efficiency of the direct methods higher standards for market milk will be brought about by their introduction.

If it were a fact that the plate method of counting bacteria had never been introduced in the milk industry, and had never been considered as the standard method of counting bacteria in other foods, the subject of this thesis would be of little interest to people in public health work. However the facts are that physicians, hygienists, bacteriologists, and boards of health are accustomed to the practice of considering the plate count on foods as the standard count and comparing all other methods of bacterial counting with this standard.

It is possible that further progress in milk control demands that city, state, and federal regulations make some direct method of counting bacteria the method of control of quality. This conclusion is quite generally accepted, and for this reason one can easily appreciate how important it is that the relation between the plate and the direct count should be established. This relation once established will make it possible to remodel old codes drawn up with the plate method in mind.

SURVEY OF LITERATURE.

When Robert Koch decribed his pure culture method in his first paper on the cause of tuberculosis he did not have even a faint idea that in a few years this method would become a large factor in the elimination of dirty foods. In Koch's day there was little food control of any kind, and few scientists suggested that there was any relation between good food and a low bacterial content. However today the best authorities on foods agree that there is a very close relationship between the number of bacteria in a food and its quality. This appreciation of the meaning of high bacterial content in many foods has now become so general that great effort has been put forth to correlate the new rapid and accurate methods with the plate method.

While Prescott, Winslow, Slack, Breed and others experimented more or less on the possibilities of the 1/100 cc. pipette in relation to the plate count on various solutions, still the first piece of work dealing with this specific subject was done by Brew(1) in 1914. He says," There appears to be a thoroughly well defined relationship existing between the direct microcopic count and the plate count. This is more apparent in a long series of examinations than in short series, because of wide variations between the results secured by the two methods on single samples."

The bacterial count obtained in milk by the direct microscopic method is equally as good if not a better criterion of its bacterial content than the count made by the plate method. Since the number of bacteria present in a given sample of milk is indicative of the care, and the contaminating influences to which the milk has been subjected the plate method failed in this particular case to reveal

what it is usually supposed to reveal."

Brew had been associated with Breed previous to 1914, but it was not until 1917 that their first joint paper was published on this particular subject. They(4) say, "It was found to be impossible to get an exact agreement in the results by the two methods as the plate count is not a count of the individual bacteria but rather a count of the number of groups of bacteria as they exist after the groups are more or less broken apart by shaking of samples and dilutions in the process of plating. The microscopic count on the other hand is either a count of the groups of bacteria as they originally existed in the milk or is a count of the individual bacteria ."

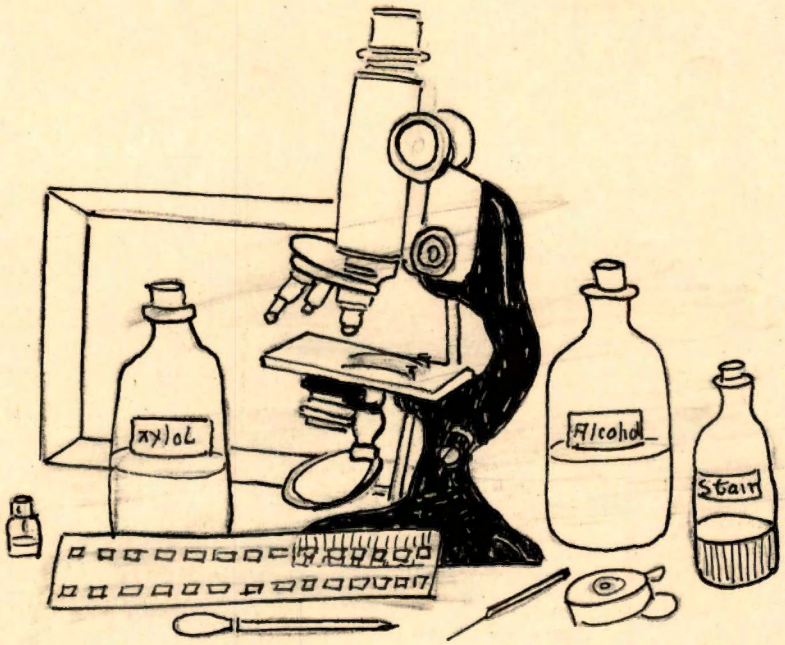
Another piece of work of equal importance was done in 1917 by Brew and Dotterer (8). They summerize their work by saying, "Generally speaking, the groups of bacteria appear to be of small average size in milks containing very few bacteria and to increase in average size as the number of bacteria increase, reaching a maximum average size of 12 to 14 individuals in milks containing very few bacteria, less than 1,000,000 per c.c. As the number of bacteria becomes greater than this the average size of the groups diminishes. Apparently this increase in the average size of the groups in milk containing large numbers of bacteria is due to the increasing predominance of lactic acid bacteria. Indirect data indicate that the average clumps in market milk breaks apart 2.6 times in preparing dilution waters. This varies greatly in individual cases, however , as in 24 instances out of 345 the clumps broke into 7 or more groups each. In one instance the resulting number of groups was 16.5 times the original number."

Further work was done by Breed and Stocking (6) in 1920. In their paper they say, "The average number of individuals in the clumps of bacteria varies between two and six; but at times (when streptococci were present) greatly exceeded these numbers. As the datd indicate the clumps are only very poorly broken apart in the processes ordinarily used in preparing dilution waters, the plate counts did not represent the full number of bacteria present."

"Given unlimeted time, and numerous duplicate samples of the milk,a skilled microscopist can secure reasonably accurate counts of the number of bacteria in an ordinary sample of milk."

Robertson (11) did a piece of work in 1921 which is worthy of consideration here. In his publication he writes,"The ratio of the individual microscopic count to the corresponding agar plate count from the same sample of milk has been determined separately for each period of incubation. The most frequent ratio found when the plates are incubated for two days at 37.5°C the ratio was approximately 4;1, when incubated for five days at 21°C the ratio was found to be approximately 2.5:1, and when incubated for five days at 21°C followed by two days at 37°C the ratio was found to be approximately 2:1. These are the most suitable ratios to use for interpting one count in terms of another.count."

"The individual ratios of the individual microscopic count to the plate count from plates incubated for two days at 37°C are more variable than the ratios between the counts when the plates are incubated for five days at 21°C followed by two days at 37°C or when incubated for five days at 21°C."



The small amount of apparatus necessary in the practice of the Breed method of counting bacteria in market milk is well illustrated in the above drawing taken from N.Y.SAgr.Experiment Station Bulletin No.380.

Plate No.1

THE OBJECT OF STUDY.

The object of the work reported in this paper is to show the relation of cell grouping to the accuracy of the Breed method of of direct microscopic counting of bacteria in market milk. This topic is taken up especially in relation to the older method of bacterial estimation, the plate count.

METHODS OF STUDY.

In the following work forty samples were taken from different dairies around Knoxville. Some of these samples were from "grade A" dairies; others were from "grade B" dairies. In other words, the samples were fairly representative of Knoxville milk. Comparative plate and individual Breed counts were made on these samples of milk at different intervals. An initial count was made, and other counts followed at 2, 3, or 4 hour periods. During this time the samples were kept at 12°C and were sampled aceptically.

The plate counts were made on standard agar, using three dilutions and triplicate plates. The incubation of the plates was 37.5°C and the time of incubation was forty eight hours. The results were obtained from the most satisfactory dilutions, that is from plates having between thirty and three hundred colonies. The individual Breed counts were obtained by following out in every detail the Breed method as given in the "A.P.H.A. Standard Methods for Milk Analysis", Fourth Edition, 1923. The Breed group counts were obtained from the Breed individual count slides by counting the number of groups of individuals rather than the individual bacteria.

Another mode of investigating the subject was used. One hundred Breed slides were prepared from the sample of milk, and an individual count was made from each slide. This test was made to show the degree of variation in different counts, made at the same time and under the same conditions. The standard "A.P.H.A." Breed method was used in the work.

A third method of study was carried out to determine the influence of different agar media upon the number of colonies which are able to grow, when a given sample of milk is plated In this experiment standard agar, corn agar, "synthetic" agar, and milk agar were used. These media were made as follows;

Standard Agar.

Agar 15 gm.
Beef extract.. . 3gm.
Peptone.....5gm.
Distilled water..1 liter

"Corn Solubles" Agar.

Agar.....15 gm.
Beef extract...3gm.
Peptone.....5gm.
Water extract from corn..1 liter.

Synthetic Agar.

Magnesium sulphate... .2 gm.
Dibasic potassium phosphste .2 gm.
Sodium cloride..... .2 gm.
Calcium phosphate..... .1 gm.
Succrose.....10 gm.
Distilled water..... 1liter

Milk Agar.

Agar..... 15 gm.
Beef extract... 3 gm.
Peptone.....5gm.
Whole milk..... 1 liter.

Note: The reaction of all of the above media was pH 7.0

A fourth method of attacking this subject was to determine thenormal distribution of bacteria on a representative Breed slide. This was accomplished by making a milk slide according to the usual method and illustrating the distribution of bacteria upon it by a drawing made by the aid of Abbe's "Camera Lucida".

RESULTS.

The following results can be understood from the table headings.

The first forty series of results are represented not only in in Table No.1, but also in the graphs which follow Table No.1

Table No.1

Comparative Bacterial Counts Made by the Plate Method and the Breed Method.

Milk Sample No.	Time of Count	Plate Count	Group Count	Breed Individual Count
I	Initial Count	5,000	0	0
	4 Hr. Count	8,000	0	0
	8 Hr. Count	13,000	0	0
II.	Initial Count	20,000	300,000	3000,000
	4 Hr. Count	48,000	300,000	3,000,000
	8 Hr. Count	78,000	300,000	6,000,000
III.	Initial Count	21,000	300,000	900,000
	4 Hr. Count	28,000	300,000	900,000
	8 Hr. Count	34,000	300,000	900,000
IV.	Initial Count	80,000	900,000	6,600,000
	4 Hr. Count	150,000	2,400,000	9,600,000
	8 Hr. Count	170,000	5,400,000	19,200,000
V.	Initial Count	25,000	210,000	900,000
	4 Hr. Count	30,000	300,000	1,200,000
	8 Hr. Count	42,000	300,000	1,800,000
VI.	Initial Count	22,000	18,000	60,000
	4 Hr. Count	33,000	60,000	150,000
	8 Hr. Count	46,000	60,000	300,000

Table No.1 (Con't.)

Milk Sample No.	Time of Count	Plate Count	Group Count	Breed Individual Count
VII.	Initial Count	4,000	60,000	60,000
	4 Hr.Count	6,000	60,000	90,000
	8 Hr.Count	6,000	60,000	300,000
VIII.	Initial Count	8,000	30,000	90,000
	4 Hr. Count	12,000	60000	210,000
	8 Hr. Count	12,000	60,000	210,000
IX.	Initial Count	30,000	30,000	300,000
	4 Hr. Count	40,000	60,000	420,000
	8 Hr. Count	40,000	90,000	560,000
X.	Initial Count	10,000	30,000	180,000
	4 Hr. Count	30,000	60,000	360,000
	8 Hr.Count	70,000	60,000	300,000
XI.	Initial Count	10,000	9,000	18,000
	4Hr. Count	13,000	18,000	60,000
	8 Hr. Count	16,000	90,000	210,000
XII.	Initial Count	100,000	300,000	3,600,000
	4 Hr. Count	380,000	390,000	12,000,000
	8 Hr. Count	1,030,000	390,000	15,000,000
XIII.	Initial Count	10,000	30,000	199,000
	4 Hr. Count	20,000	60,000	210,000
	8 Hr. Count	40,000	120,000	300,000

Table No.1 (Con't.)

Milk Sample No.	Time of Count	Plate Count	Group Count	Breed Individual Count
XIV.	Initial Count	36,000	0	0
	4 Hr. Count	146,000	0	0
	8 Hr. Count	457,000	0	0
XV.I.	Initial Count	5,000	180,000	360,000
	4 Hr. Count	10,000	180,000	690,000
	8 Hr. Count	22,000	180,000	1,050,000
XVII.	Initial Count	40,000	180,000	2,700,000
	4 Hr.Count	90,000	240,000	3,000,000
	8 Hr.Count	100,000	1,120,000	6,300,000
XVIII.	Initial Count	82,000	240,000	390,000
	4 Hr. Count	96,000	180,000	1,050,000
	8 Hr. Count	143,000	450,000	1,910,000
XIX.	Initial Count	31,000	30,000	217,000
	4 Hr.Count	43,000	60,000	360,000
	8 Hr.Count	98,000	270,000	780,000
XX.	Initial Count	10,000	0	0
	4 Hr.Count	20,000	30,000	60,000
	8 Hr. Count	20,000	90,000	150,000
XXI.	Initial Count	10,000	1,050,000	5,100,000
	4 Hr. Count	30,000	1,500,000	7,200,000
	8 Hr. Count	60,000	1,500,000	7,500,000

Table No.1. (Con't.)

Milk Sample NO.	Time of Count	Plate Count	Group Count	Breed Individual Count
XXII.	Initial Count	72,000	156,000	1,140,000
	4 Hr. Count	96,000	270,000	1,320,000
	8 Hr. Count	120,000	300,000	3,000,000
XXIII.	Initial Count	31,000	150,000	330,000
	2 Hr. Count	53,000	210,000	818,000
	4 Hr. Count	50,000	300,000	1,330,000
XXIV.	Initial Count	730,000	900,000	26,400,000
	2 Hr. Count	890 ,000	1,200,000	48,000,000
	4 Hr. Count	900,000	2,300,000	96,000,000
XXV	Initial Count	300,000	380,000	3,900,000
	4 Hr. Count	360,000	600,000	5,130,000
	8 Hr. Count	420,000	1,320,000	10,500,000
XXVI.	Initial Count	30,000	30,000	198,000
	2 Hr. Count	35,000	60,000	900,000
	4 Hr. Count	45,000	90,000	1,080,000
	6 Hr. Count	60,000	210 , 000	1,780,000
XXVII.	Initial Count	400,000	500,000	1,500,000
	4 Hr.Count	500,000	500, 000	2,600,000
	8 Hr.Count	400,000	500,000	3,000,000
XXVIII.	Initial Count	4,000	0	4,000
	3 Hr. Count	5,000	0	4,000
	6 Hr. Count	7,000	8 ,000	30,000

Table No.1 (Con't.)

Milk Sample No.	Time of Count	Plate Count	Group Count	Breed Individual Count
XXIX.	Initial Count	110,000	570,000	5,250,000
	2 Hr. Count	210,000	630,000	6,660,000
	4 Hr. Count	218,000	840,000	9,000,000
XXX.	Initial Count	131,000	270,000	840,000
	2 Hr.Count	215,000	280,000	1,140,000
	4 Hr.Count	220,000	300,000	1.230,000
XXXI.	Initial Count	40,000	690,000	5,400,000
	4 Hr. Count	190,000	1,080,000	7,050,000
	8 Hr. Count	220,000	1,380,000	8,400,000
XXXII.	Initial Count	144,000	5,190,000	9,120,000
	4 Hr,Count	173,000	3,375,000	21,750,000
	8 Hr.Count	183,000	8,075,000	23,130,000
XXXIII.	Initial count	18,000	69,000	180,000
	4 Hr. Count	15,000	78,000	330,000
	8 Hr. Count	22,000	99,000	450,000
XXXIV.	Initial Count	7,000	360,000	900,000
	4 Hr. Count	10,600	340,000	1,230,000
	8 Hr. Count	16,000	720,000	2,220,000
XXXV.	Initial Count	11,000	480,000	780,000
	2 Hr. Count	24,000	600,000	2,370,000
	4Hr. Count	33,000	600,000	3,000,000
	6 Hr. Count	53,000	2,130,000	4,230,000

Table No.1 (Con't.)

Milk Sample No.	Time of Count	Plate Count	Group Count	Breed Individual Count
XXXVI.	Initial Count	130,000	1,750,000	8,100,000
	2 Hr. Count	220,000	5,700,000	23,400,000
	4 Hr. Count	330,000	9,600,000	31,000,000
	6 Hr. Count	480,000	10,100,000	43,800,000
XXXVII.	Initial Count	2,220,000	5,700,000	14,400,000
	4 Hr.Count	2,290,000	6,000,000	18,000,000
	8 Hr. Count	2,300,000	6900, 000	21,000,000
XXXVIII.	Initial Count	10,000	0	0
	4 Hr.Count	20,000	30,000	60,000
	8 Hr. Count	20,000	90,000	150,000
XXXIX.	Initial Count	48,000	530,000	1,168,000
	2 Hr. Count	76,000	570,000	4,200,000
	4 Hr. Count	28,000	540,000	6,600,000
XL.	Initial Count	10,000	1,050,000	5,100,000
	2 Hr. Count	30,000	1,500,000	7,200,000
	4 Hr. Count	60,000	1,500,000	7,500,000

Table 2(Cont.)

Number of Breed Counts

Individual Breed Field Counts (30 in Number)

26	11-26-57-28-16-19-70-23-16-6-8-22-4-17-2-12-3-18-0-11-28-5-6-0-0-10-12-14-8-14
27	14-76-16-12-19-18-12-23-12-27-10-26-14-8-10-31-24-18-15-19-6-4-17-6-13-15-12-19-23-26
28	19-15-3-44-25-8-10-15-35-16-9-17-15-12-30-22-19-23-29-28-15-17-19-21-16-12-14-11-6-12
29	10-14-13-0-16-15-0-0-0-10-64-13-6-22-6-4-0-12-16-8-8-2-4-6-13-16-12-8-2
30	18-4-6-2-5-5-0-0-4-9-2-4-10-10-5-12-6-8-9-13-15-14-2-0-0-5-9-8-6-3
31	6-9-14-31-53-18-15-18-22-21-17-8-10-9-28-12-4-50-5-22-9-29-13-4-16-18-0-2-0-6
32	12-15-6-8-32-13-0-0-5-20-13-12-6-0-36-17-22-26-2-6-9-7-12-6-18-12-14-13-0-8
33	48-36-134-4-95-73-22-14-136-14-15-135-86-94-28-43-36-97-16-12-10-125-93-86-49-23-36-42-11
34	22-32-23-26-10-16-21-9-17-45-20-23-35-46-18-25-23-9-2-30-11-20-17-25-12-20-23-30-6-12
35	28-32-17-10-11-16-36-5-11-10-22-8-17-18-21-33-6-18-13-12-14-15-10-3-6-9-16-8-14-9
36	8-7-10-4-4-25-4-8-12-6-8-13-14-4-16-10-15-24-4-8-12-4-3-18-16-12-13-11-6-14
37	6-7-20-8-14-12-9-6-15-0-0-2-12-15-8-10-12-8-37-12-43-36-24-19-18-15-44-32-19-6
38	3-9-25-9-6-5-2-2-0-0-24-4-4-6-12-2-0-6-0-0-4-4-8-27-6-3-4-2-0-1
39	12-29-30-4-9-18-37-6-4-20-12-8-4-21-8-4-21-4-6-10-15-19-31-32-18-6-14-26-5-19
40	30-24-43-13-45-32-29-15-19-22-36-42-123-18-44-57-32-26-18-15-17-6-132-14-18-129-25-63-54
41	12-2-4-5-33-17-12-8-15-36-24-4-12-8-15-0-12-16-12-0-6-5-31-12-14-18-43-36-14-32
42	20-9-11-2-5-6-8-4-7-22-2-6-15-39-15-16-8-18-41-59-20-28-9-12-14-13-19-23-26
43	5-4-6-0-14-6-8-11-14-15-19-26-27-8-14-45-52-38-24-17-13-14-33-14-23-36-11-17-6-12
44	2-16-43-15-36-18-16-12-47-16-11-13-21-15-12-13-24-0-8-6-9-6-2-8-0-11-0-6-9-20
45	9-13-1"-152-63-22-19-218-42-27-24-12-20-1-34-8-73-16-4-14-4-25-57-24-19-9-13-4-9-28
46	14-522-25-10-6-2-4-0-9-12-10-17-13-13-13-9-18-19-22-25-4-13-14-19-17-36-12-9-8
47	21-9-30-54-15-21-12-28-19-17-13-26-16-11-18-45-19-20-26-19-4-10-28-15-106-4-19-52-26-12
48	13-4-8-10-14-19-8-17-2-4-6-29-14-16-13-6-8-4-13-4-2-6-6-19-18-23-20-19-16-5
49	0-59-20-4-8-1"-36-19-31-25-18-15-19-6-17-3-5-13-9-8-16-24-6-3-12-0-5-9-2-0
50	3-12-15-23-15-0-6-12-13-0-5-9-2-0-12-17-19-13-26-32-40-46-25-38-12-19-21-23-0-19

Table No.2.

This Table Illustrates the Normal Variation in Making Fifty Breed Counts on the Same Sample of Milk.

Individual Breed Counts of Fields (30 in Number).

Number of Breed Counts.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	A
1	6	4	16	42	14	35	12	4	2	18	8	33	20	21	20	16	63	13	18	32	14	8	16	10	84	16	13	14	6	2	6
2	10	26	13	6	16	8	25	15	13	16	11	6	20	24	10	15	28	19	2	13	0	12	0	16	12	15	23	12	3	16	6	1
3	10	4	0	4	4	10	9	20	6	16	0	6	19	26	25	18	0	25	50	9	12	31	2	27	13	4	18	8	6	3	
4	5	9	10	37	13	19	24	6	4	6	7	10	8	20	29	8	8	41	14	15	10	16	8	5	2	21	10	29	4	8	
5	30	33	55	40	36	4	10	46	1	35	2	1	10	26	19	14	33	22	15	10	20	17	26	23	26	2	24	35	44	27	26
6	6	42	17	7	56	16	14	4	4	38	19	4	31	10	18	37	13	22	20	41	14	19	8	13	12	42	2	2	32	12	
7	6	20	17	42	13	14	6	0	9	14	21	2	8	17	13	4	6	10	6	15	20	30	17	13	36	8	13	18	6	31	
8	22	30	4	9	23	10	15	38	11	38	26	29	22	16	33	20	31	22	17	14	18	20	49	10	18	11	13	15	21	15	
9	60	28	81	51	46	243	32	43	30	68	66	16	19	93	13	68	31	223	2	76	83	36	93	68	49	33	87	6	4	98	106
10	0	0	12	42	12	20	40	38	30	25	39	35	16	19	93	20	18	15	6	52	26	96	38	36	16	23	22	10	47	48	
11	12	16	15	29	9	2	6	4	0	10	25	5	12	47	24	130	6	11	17	5	149	5	8	6	23	2	38	29	14	20	
12	4	13	25	8	9	13	24	10	0	6	11	18	8	166	63	9	23	43	42	17	26	7	2	23	13	6	4	8	0	30	
13	11	4	3	29	15	18	4	9	12	7	16	23	8	0	19	33	28	36	23	19	32	29	10	21	15	6	26	4	13	23	
14	5	28	35	78	26	36	21	28	30	33	21	26	21	16	14	9	66	19	12	14	16	29	25	18	28	37	22	14	16	28	
15	15	21	4	16	26	11	13	37	12	24	19	15	9	83	21	38	22	29	21	27	22	10	38	29	18	230	21	42	13	14	
16	21	20	28	8	0	3	29	7	13	8	8	11	15	20	10	7	12	46	14	22	35	26	19	24	18	23	16	30	82	21	
17	14	54	52	10	18	9	13	8	18	4	7	14	43	5	24	22	13	22	22	38	11	20	10	8	11	29	6	17	36	6	
18	6	10	28	16	31	50	9	39	8	16	11	18	57	26	10	6	25	1	26	89	320	11	26	27	75	28	14	34	26	24	
19	28	14	42	16	9	5	39	35	25	46	21	65	95	16	37	43	22	60	37	23	42	60	37	83	29	46	19	36	21	14	
20	14	53	14	124	6	10	12	20	235	34	33	196	14	8	18	26	14	23	25	18	21	19	2	9	5	11	14	10	5	6	
21	20	11	19	15	31	6	22	20	17	9	12	31	22	10	25	7	6	16	15	22	2	22	6	11	10	9	10	14	6	8	
22	22	19	13	14	23	50	2	21	37	522	56	84	324	38	32	2	16	18	2	28	18	28	4	16	17	8	14	15	16	2	
23	23	102	11	27	41	28	29	39	19	36	37	15	29	12	36	25	31	54	14	206	15	32	7	9	13	20	29	16	14	8	1
24	51	0	16	6	12	14	10	12	15	45	15	16	27	14	19	21	18	12	9	13	11	10	13	11	23	1	13	13	20	14	
25	16	15	20	19	44	0	0	14	23	31	12	14	3	18	30	5	7	15	14	10	13	29	7	13	2	15	8	6	12	13	

An illustration of the distribution of bacteria over a representative film of the Breed Method of counting bacteria in market milk. The distribution was determined by counting and recording all organisms found in each of the 3,025 fields.

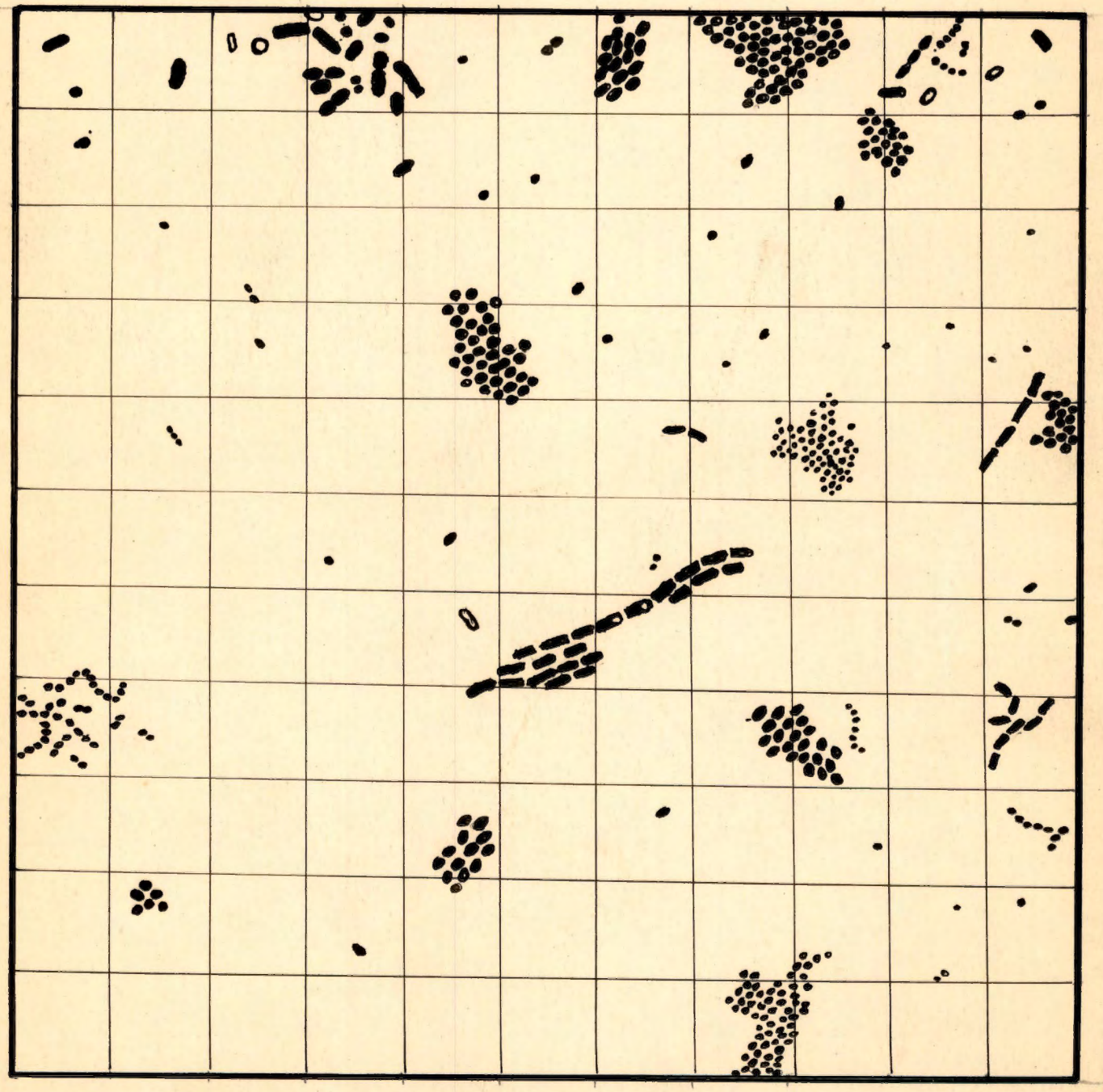
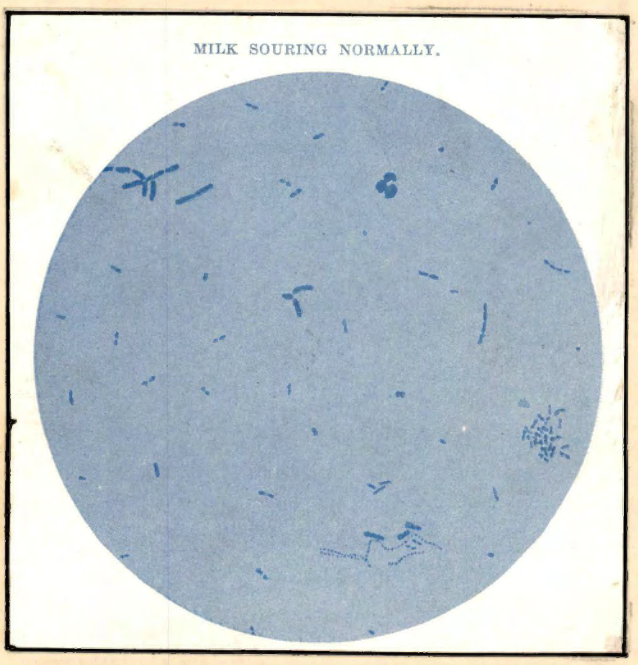


Plate No. 2



The above illustration is from N. Y. State Agr. Experiment Sta. Bulletin 373. It illustrates the great variability in the number of bacteria which may form a group in market milk.

Plate No. 3

where the counts are low. Graphs, 2, 4, 5, 12, 15, 18, 19, 22, 24, 25, 29, 31, 32, 35, 39, and 40 are representative of high individual counts. One will observe in these counts that there is no relation between the Breed count and the plate count. Even in counts which are rather low as illustrated by graphs, 11, 13, 14, 18, 20, 23, 26, 27, 28, 33, and 34, it is evident that there is no constant relation. From the foregoing data it appears that it is possible for milk to double in the total number of bacteria present without much increase in the plate count. This increase is taken up by the enlargement of groups. This point is strongly indicated in graphs, 2, 3, 4, 5, 7, 8, 13, 15, 16, 17, 18, 19, 22, 23, 24, 27, 29, 31, 32, 33, 34, 35, 36, 37, 39, and 40.

From the illustration reproduced here from Bulletin 373 by Brew of the New York Agricultural Experiment Station certain facts may be easily observed. Specific bacteria grow in groups characteristic only of themselves. This illustration was not used by Brew to emphasize this fact, however it does accidentially bring it out very strongly. This is cited merely as an accidental bit of evidence.

In the counts of the one hundred slides 50 of which are tabulated in tables 11 and 12, there is a wide range of variation. This variation ranges from an average of six organisms to fifty organisms. It seems evident from this data that great discrepancies exist in all Breed counts. Even when the greatest precautions are takenin making Breed slides it is difficult to reduce these apparent inaccuracies.

It is of interest to note that the "corn solubles" agar showed a higher number of colonies than any other media used, andthat the other three media gave differing results. That the use of different

media always results in a different bacterial count has been known ever since the time of Koch. This data simply emphasizes the point that bacteria grow on agar only if they happen to be certain kinds which find the suitable media. This data helps to explain the varying divergence between the plate and microscopic counts.

In Plate 12, the distribution of bacteria per microscope field demonstrates very well the unevenness with which bacteria are spread out in the Breed method of making films. This unevenness of the distribution of bacterial cells is no doubt due to two causes. It is well known that the different kinds of bacteria are characterized by forming large, medium, or small sized groups. Also all who have had experience in spreading films of milk on glass slides know that milk refuses to be spread evenly.

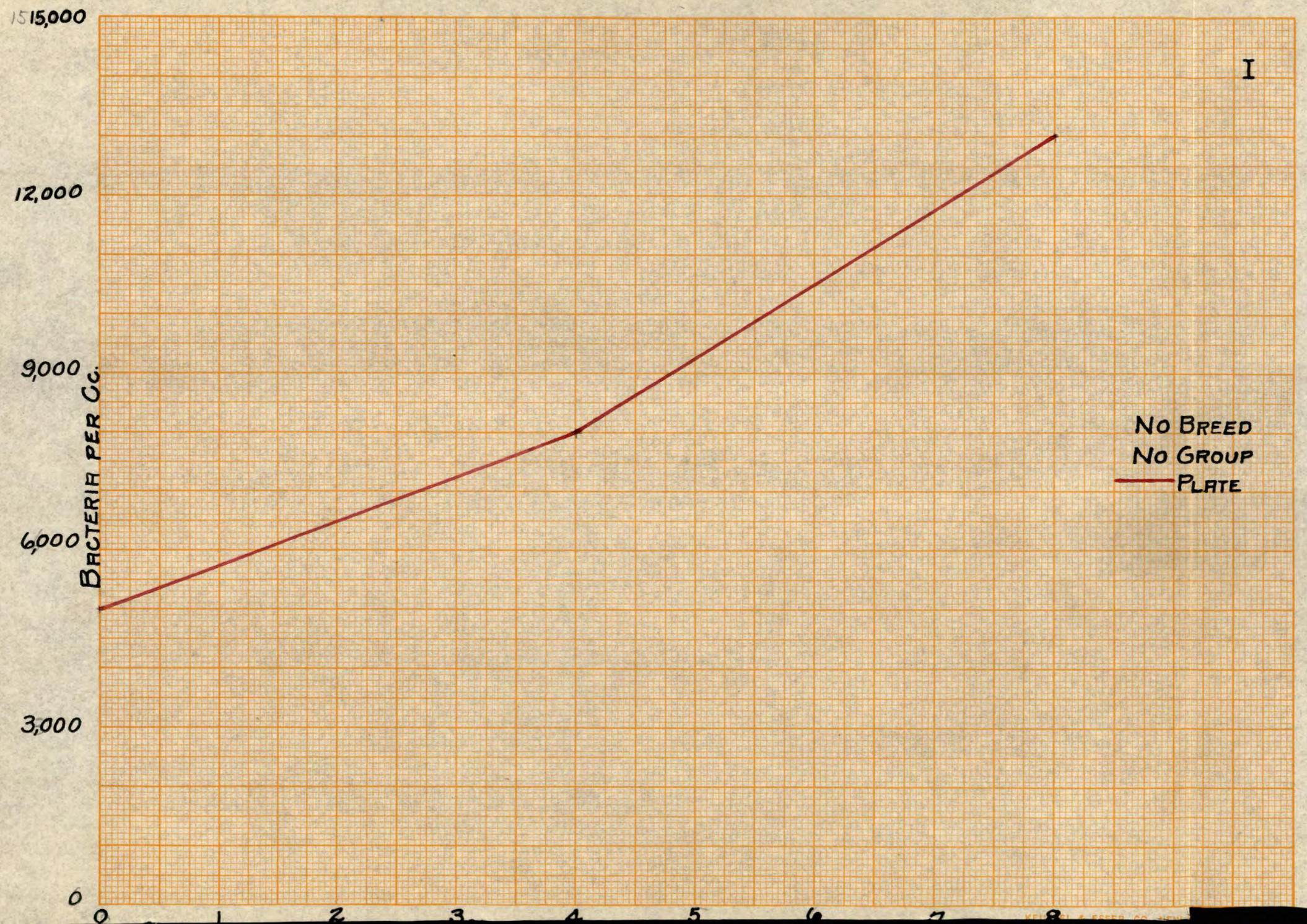
CONCLUSIONS.

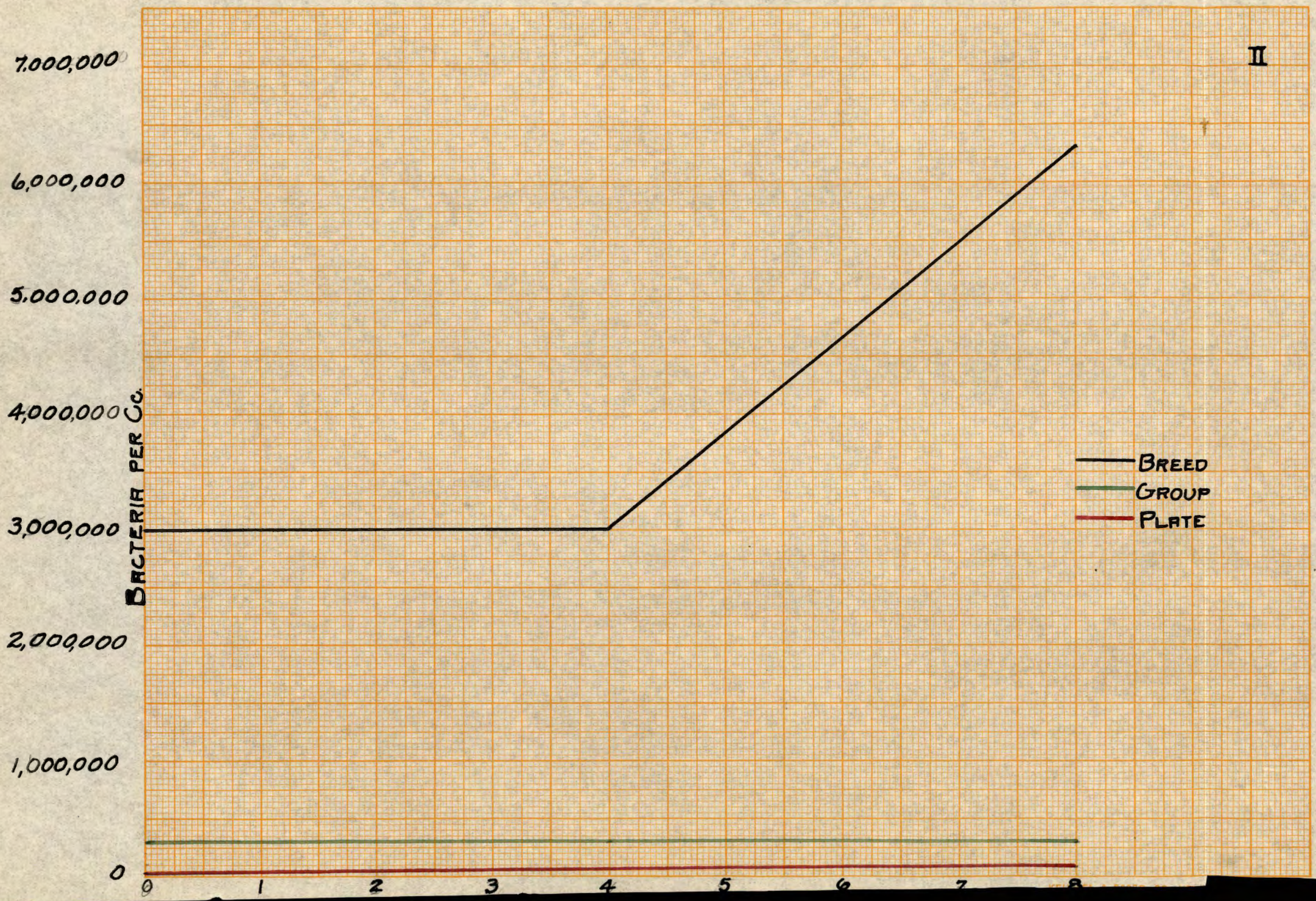
I. There is a fairly constant relation between the plate count and the Breed count of groups.

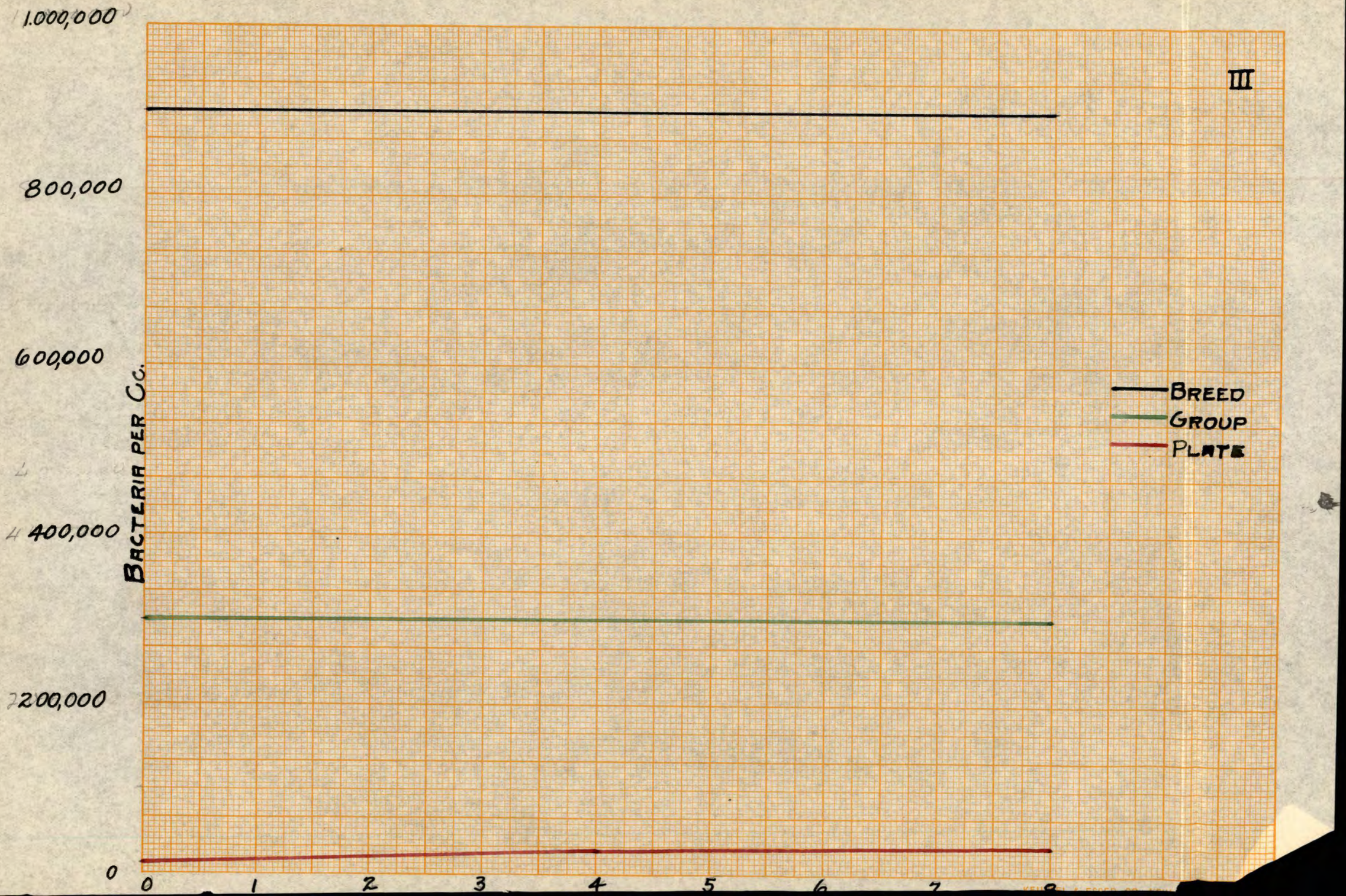
II. The relation between the plate count and the Breed count varies with the age of the milk and the kind of bacteria which become dominant in it.

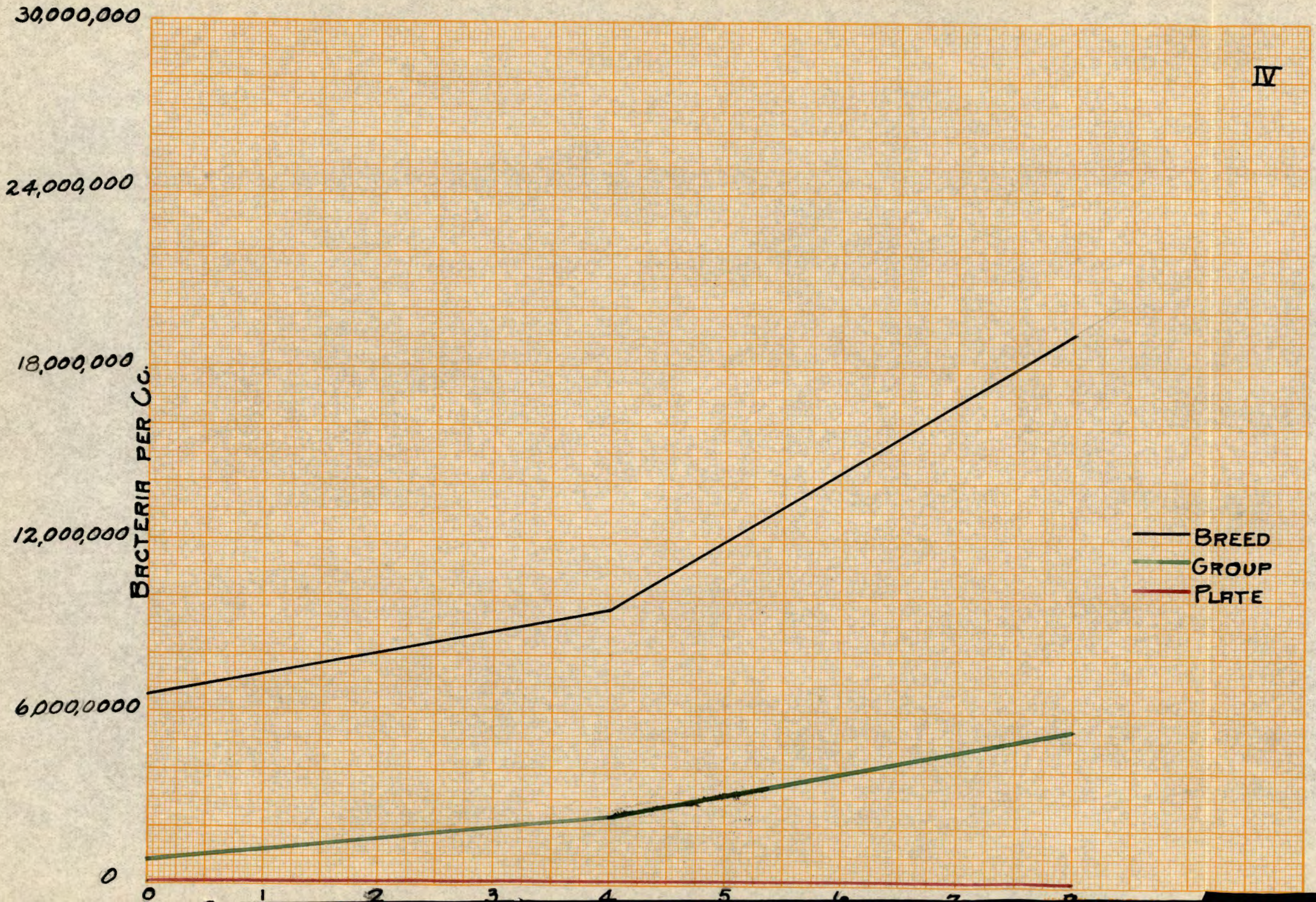
III. In general the greatest divergence was found between the plate count and the total Breed count on samples of milk of high bacterial content.

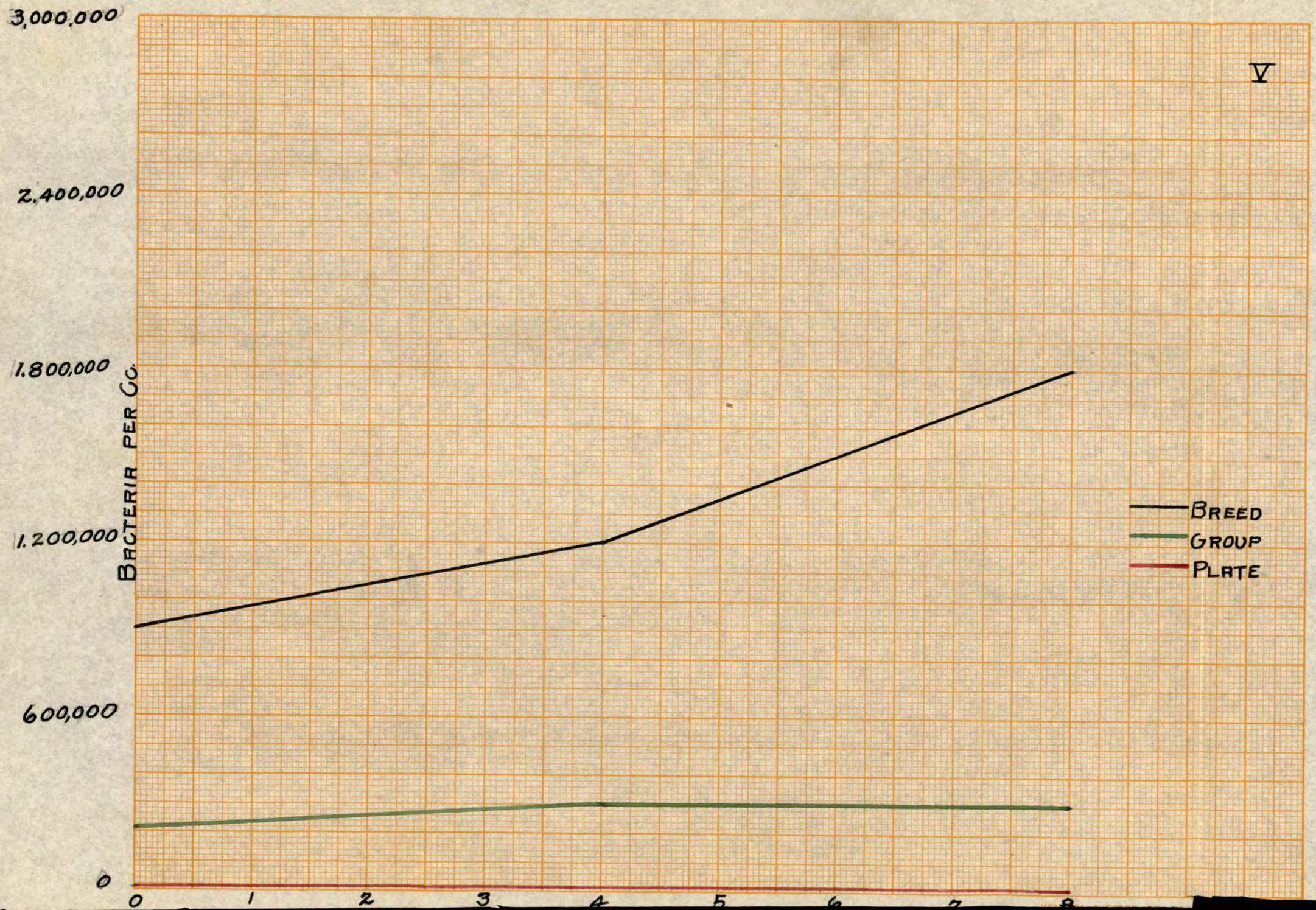
IV. While the individual count is the most reliable index of the total bacteria in milk, however it is not feasible to attempt to establish a constant factor between the individual count and the plate count.

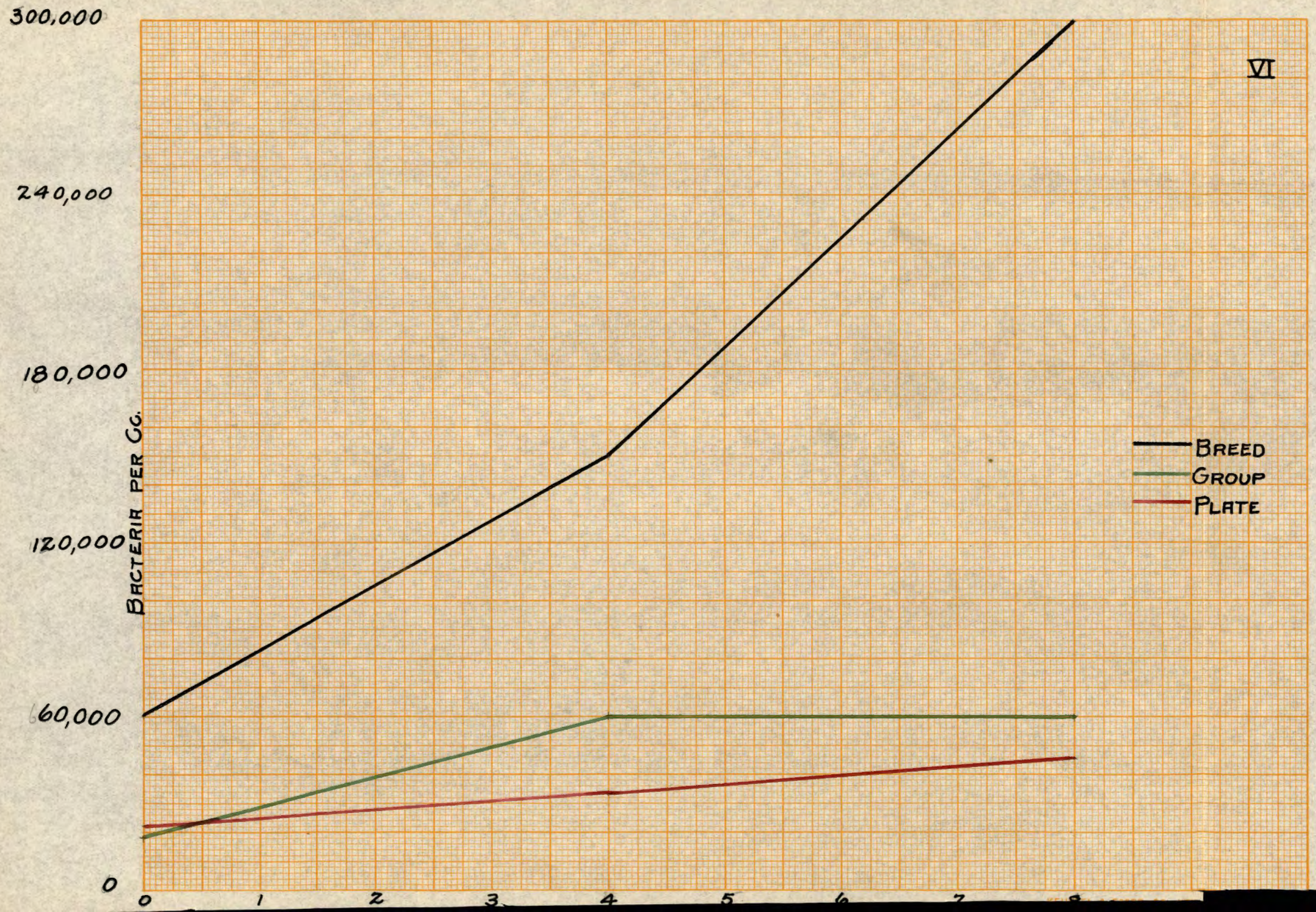

15,000
12,000
9,000
6,000
3,000
0
BACTERIA PER Cc.
0
1
2
3
4
5
6
7
8
I
NO BREED
NO GROUP
PLATE

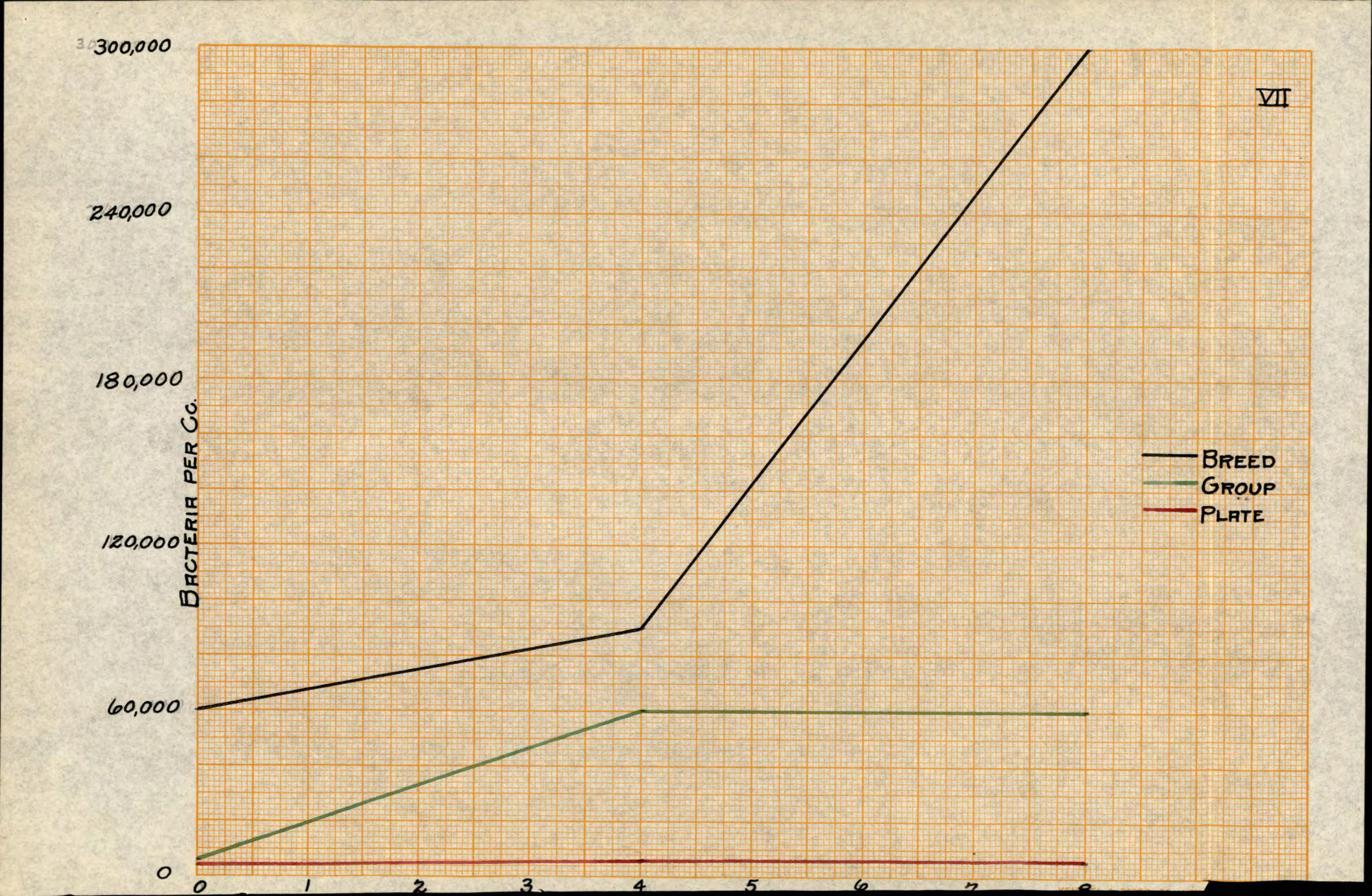

II
7,000,000
6,000,000
5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
0
BACTERIA PER Cc.
0
1
2
3
4
5
6
7
8
BREED
GROUP
PLATE

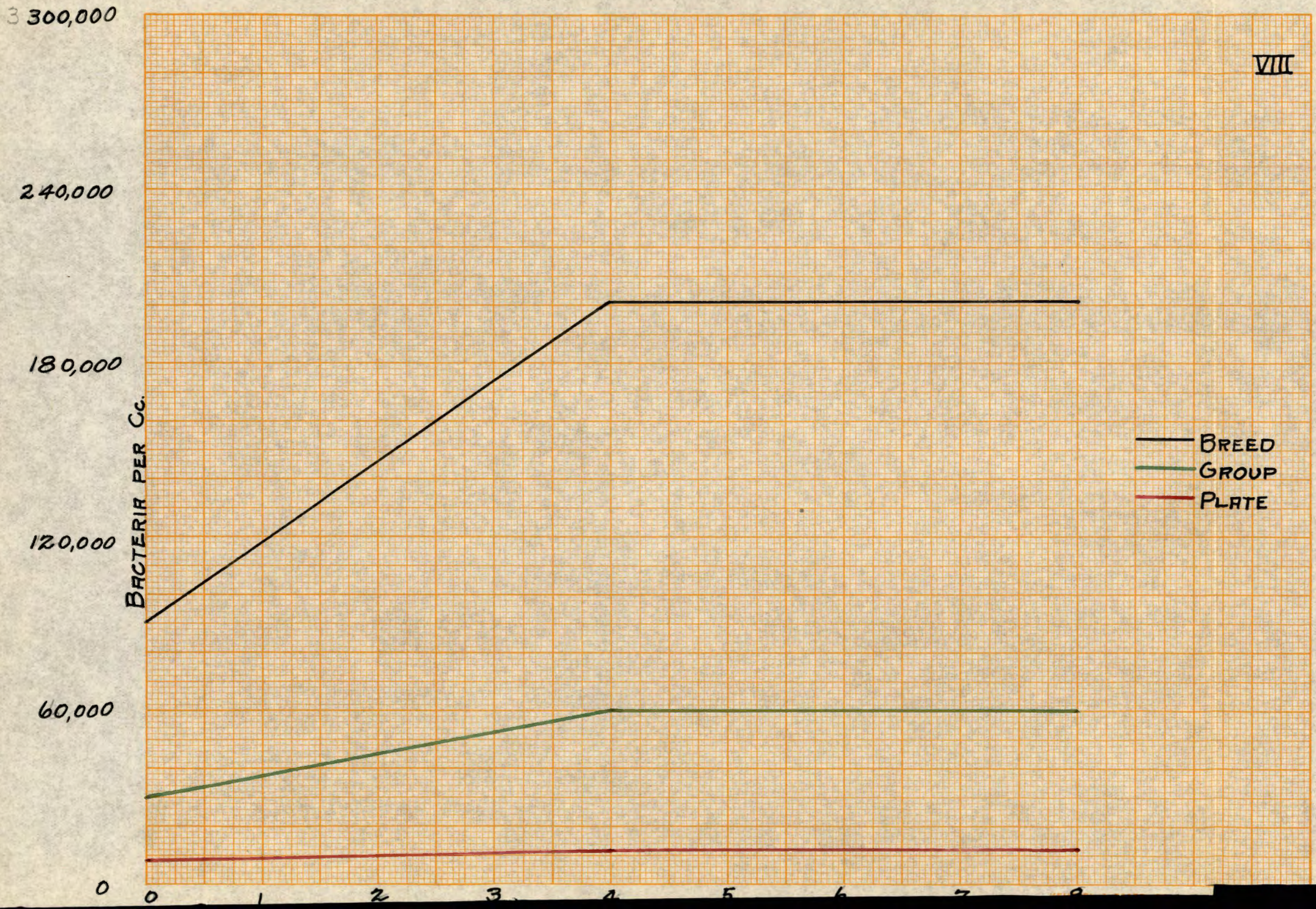

III
1.000,000
800,000
600,000
400,000
200,000
0
BACTERIA PER CC.
0
1
2
3
4
5
6
7
8
BREED
GROUP
PLATE

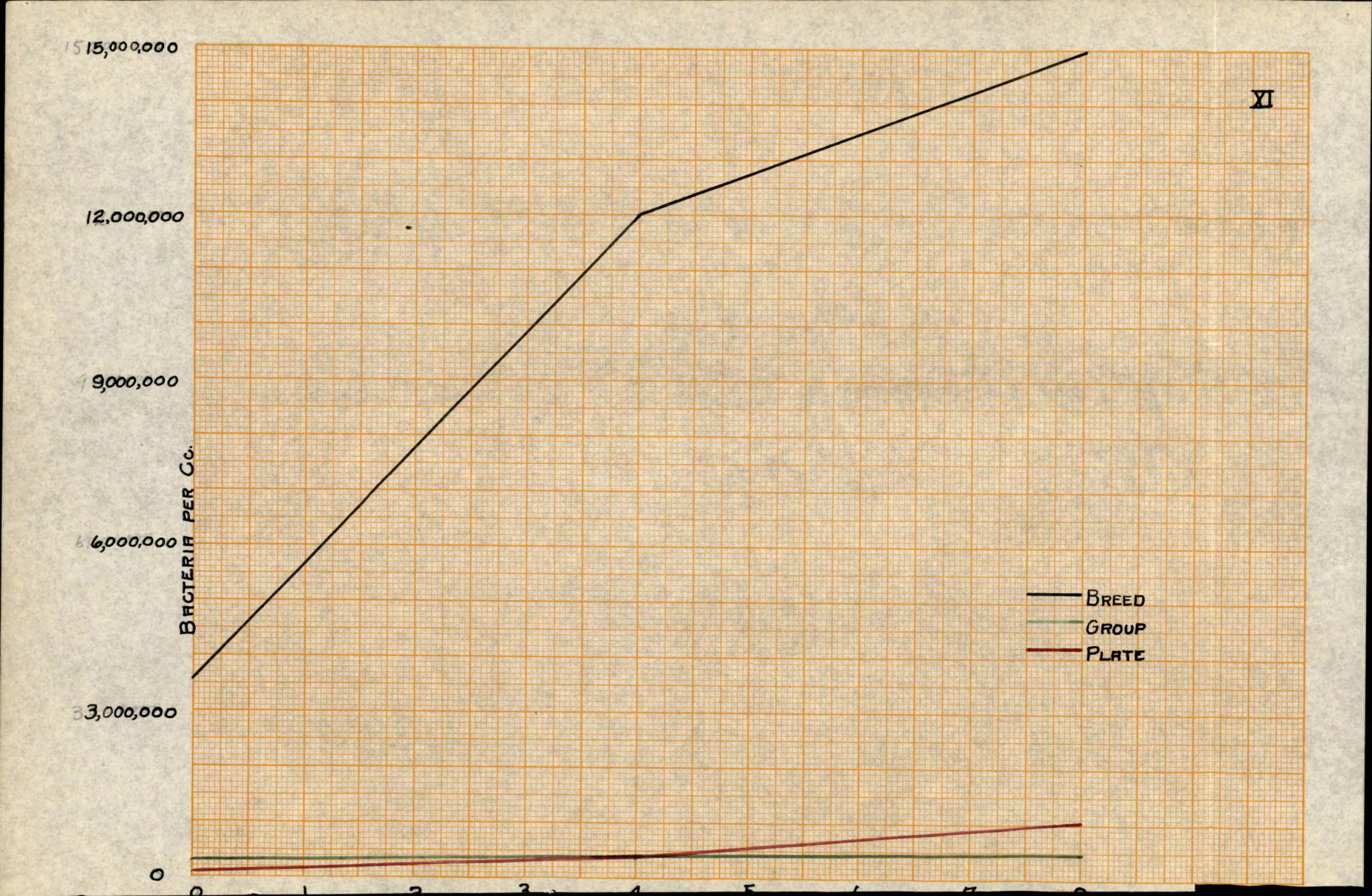

IV
30,000,000
24,000,000
18,000,000
12,000,000
6,000,0000
0
BACTERIA PER C.C.
0
1
2
3
4
5
6
7
8
BREED
GROUP
PLATE

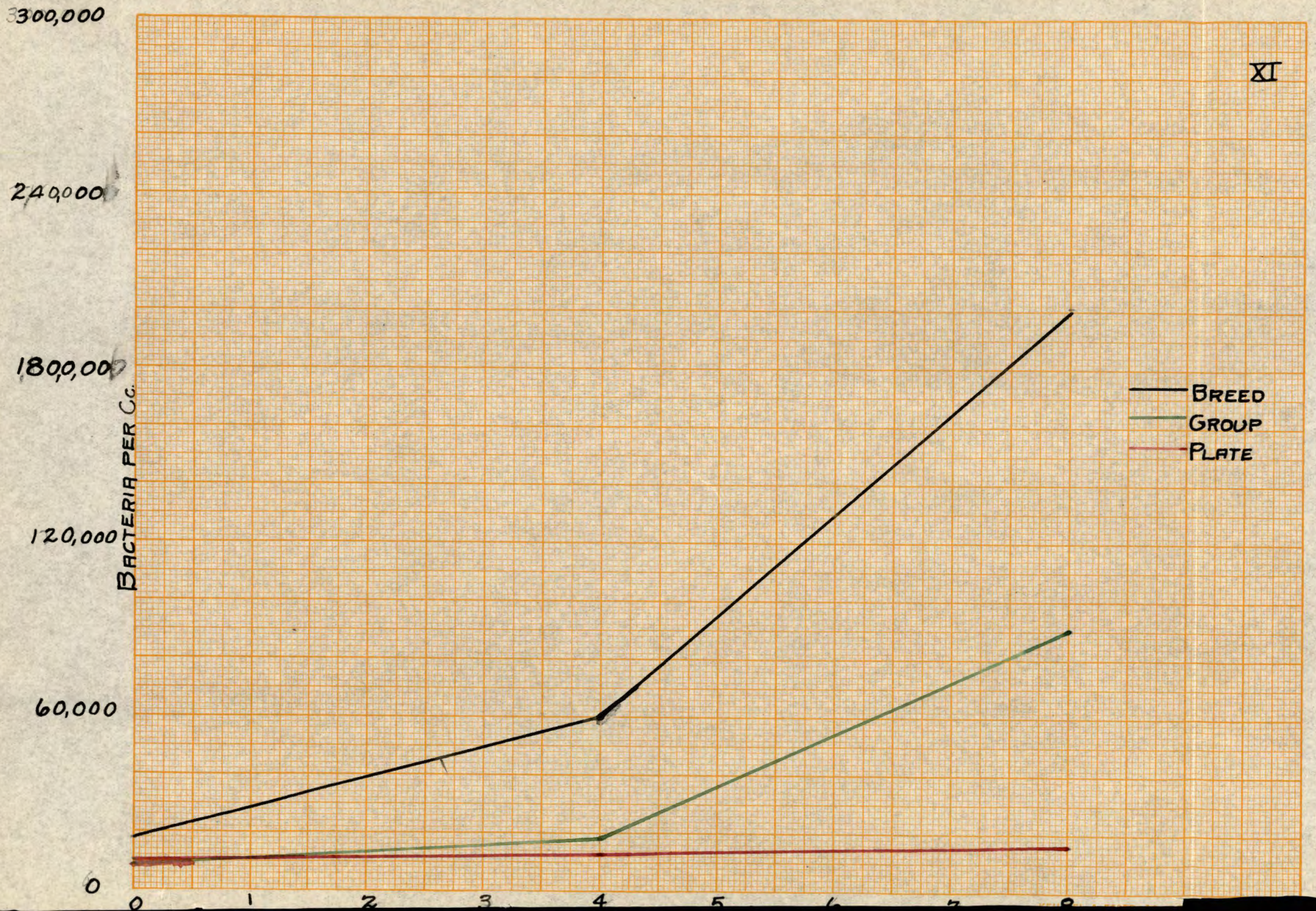

V
3,000,000
2,400,000
1,800,000
1,200,000
600,000
0
BACTERIA PER CC.
0
1
2
3
4
5
6
7
8
BREED
GROUP
PLATE

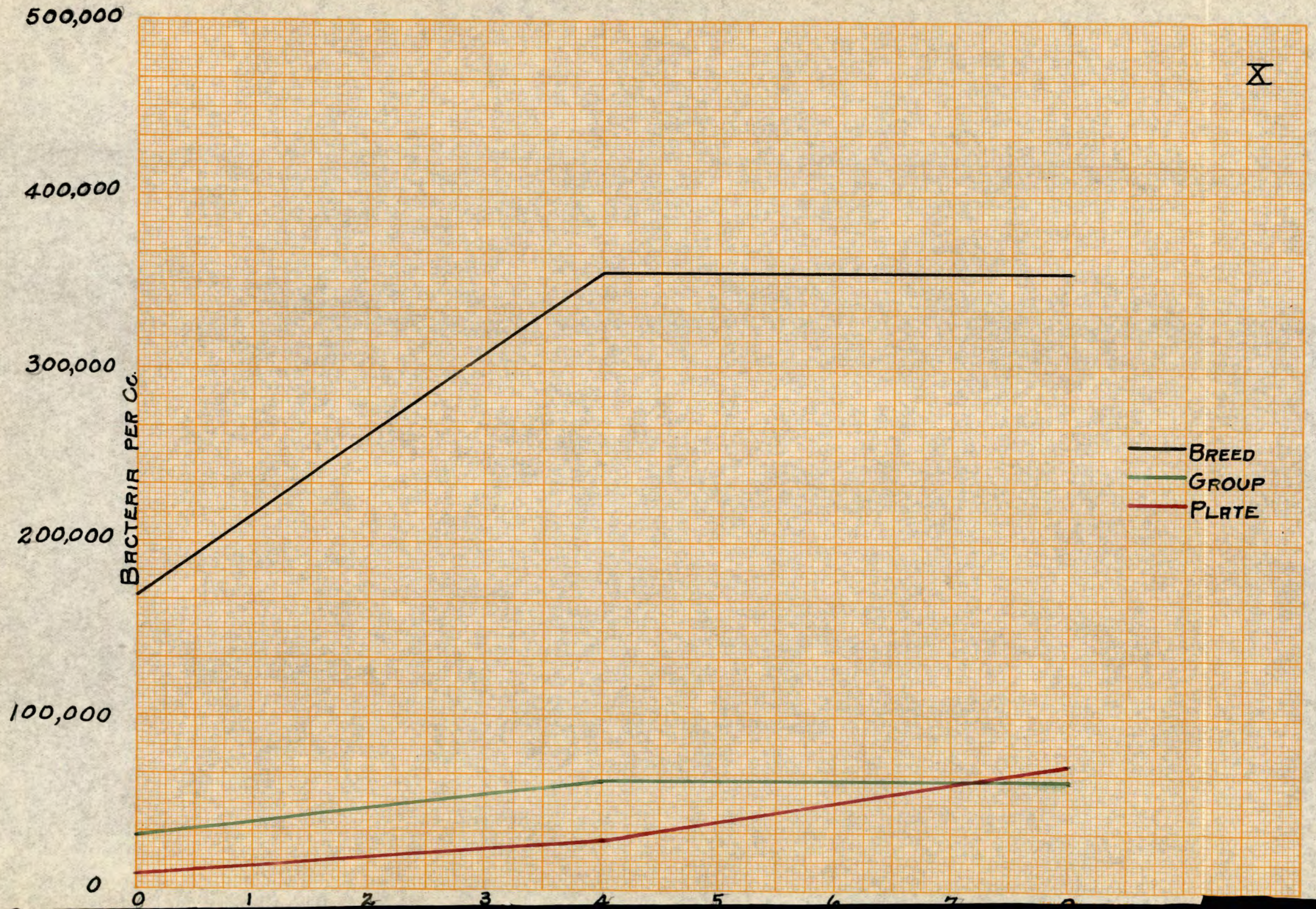

VI
300,000
240,000
180,000
120,000
60,000
0
BACTERIA PER CC.
0
1
2
3
4
5
6
7
8
BREED
GROUP
PLATE

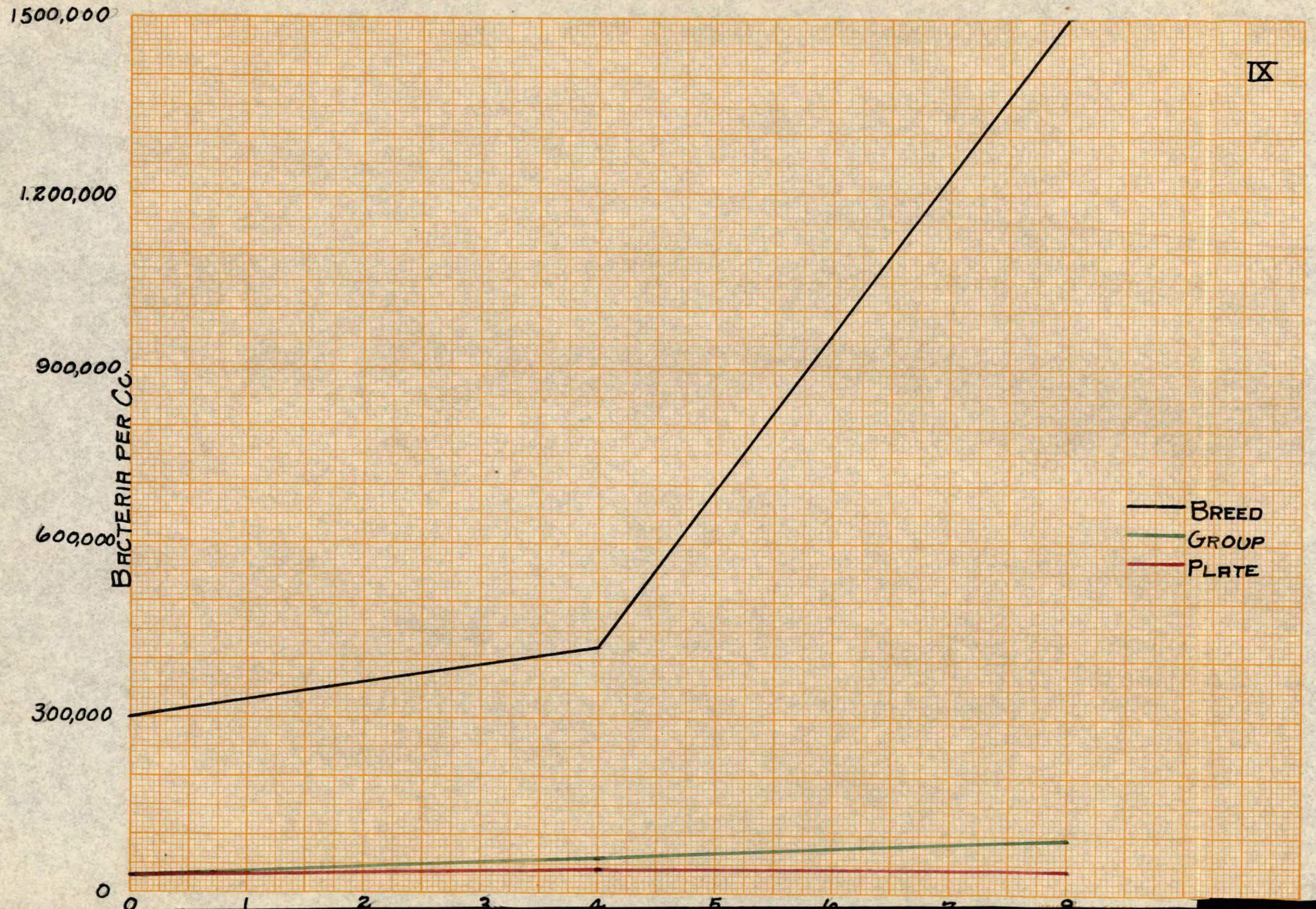

VII
300,000
240,000
180,000
120,000
60,000
0
BACTERIA PER CC.
0
1
2
3
4
5
6
7
8
BREED
GROUP
PLATE

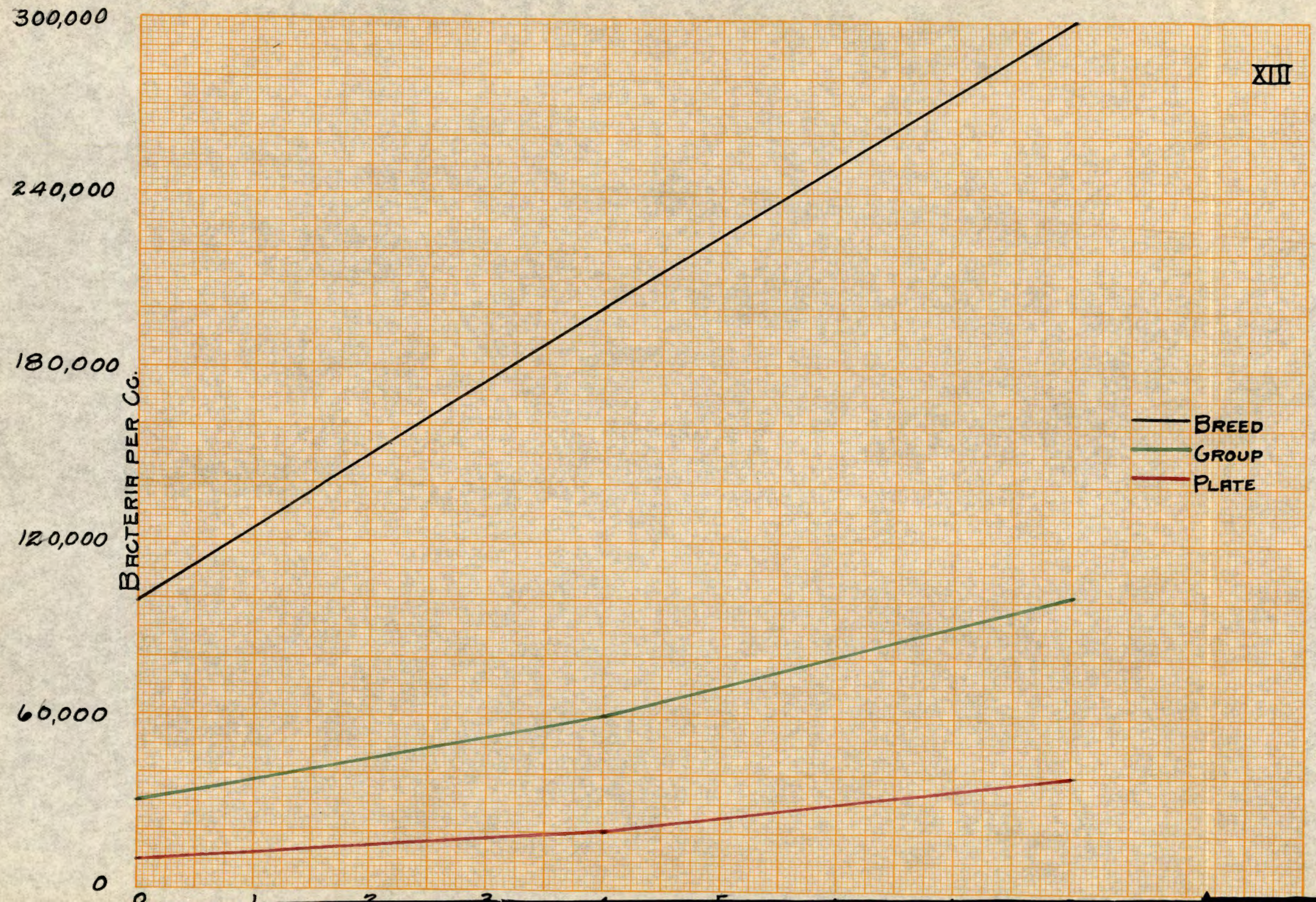

300,000
240,000
180,000
120,000
60,000
0
BACTERIA PER Cc.
0
1
2
3
4
5
6
7
8
VIII
BREED
GROUP
PLATE

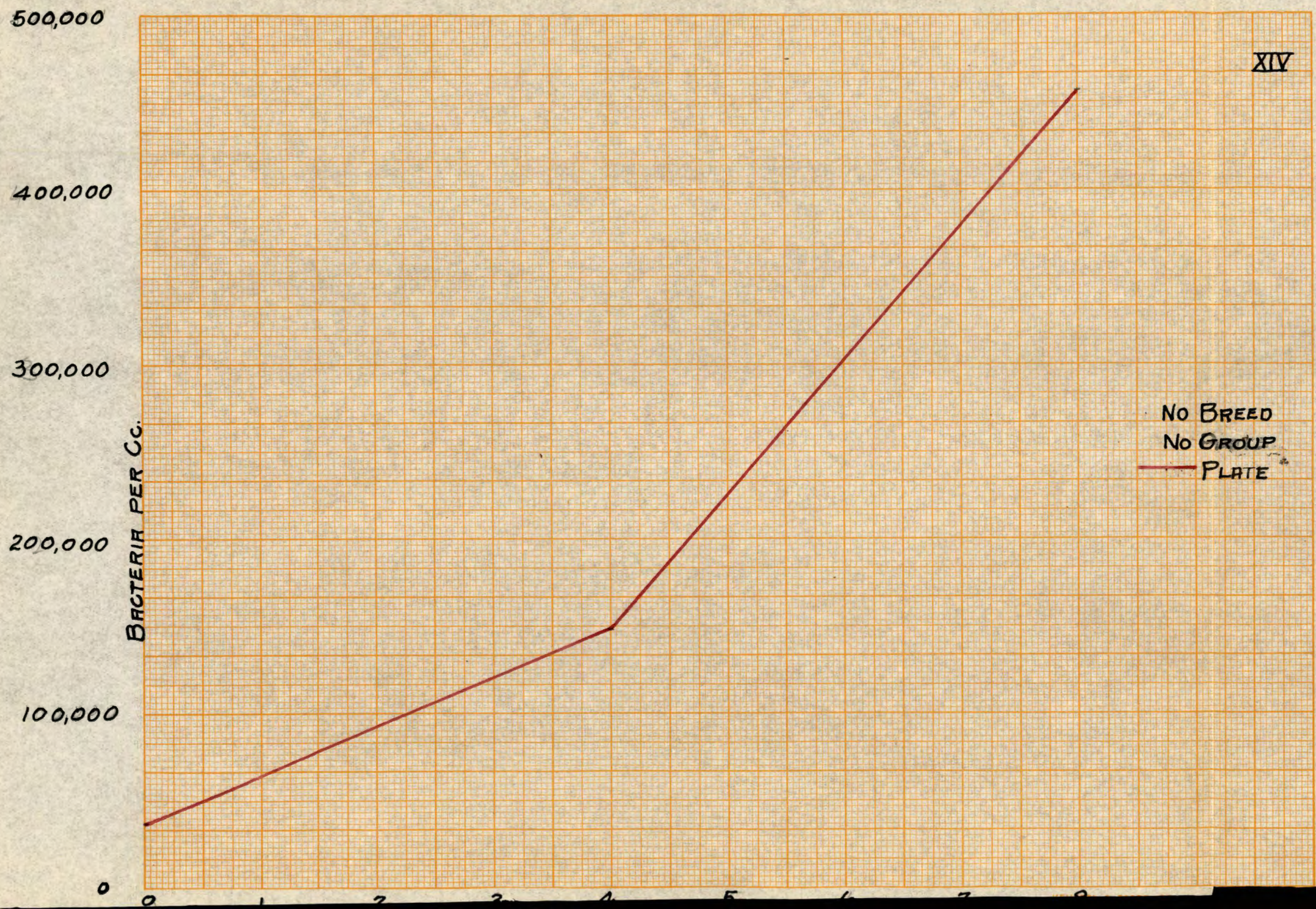

XI
15,000,000
12,000,000
9,000,000
6,000,000
3,000,000
0
BACTERIA PER Cc.
BREED
GROUP
PLATE


300,000
240,000
180,000
120,000
60,000
0
BACTERIA PER C.C.
0
1
2
3
4
5
6
7
8
XI
BREED
GROUP
PLATE

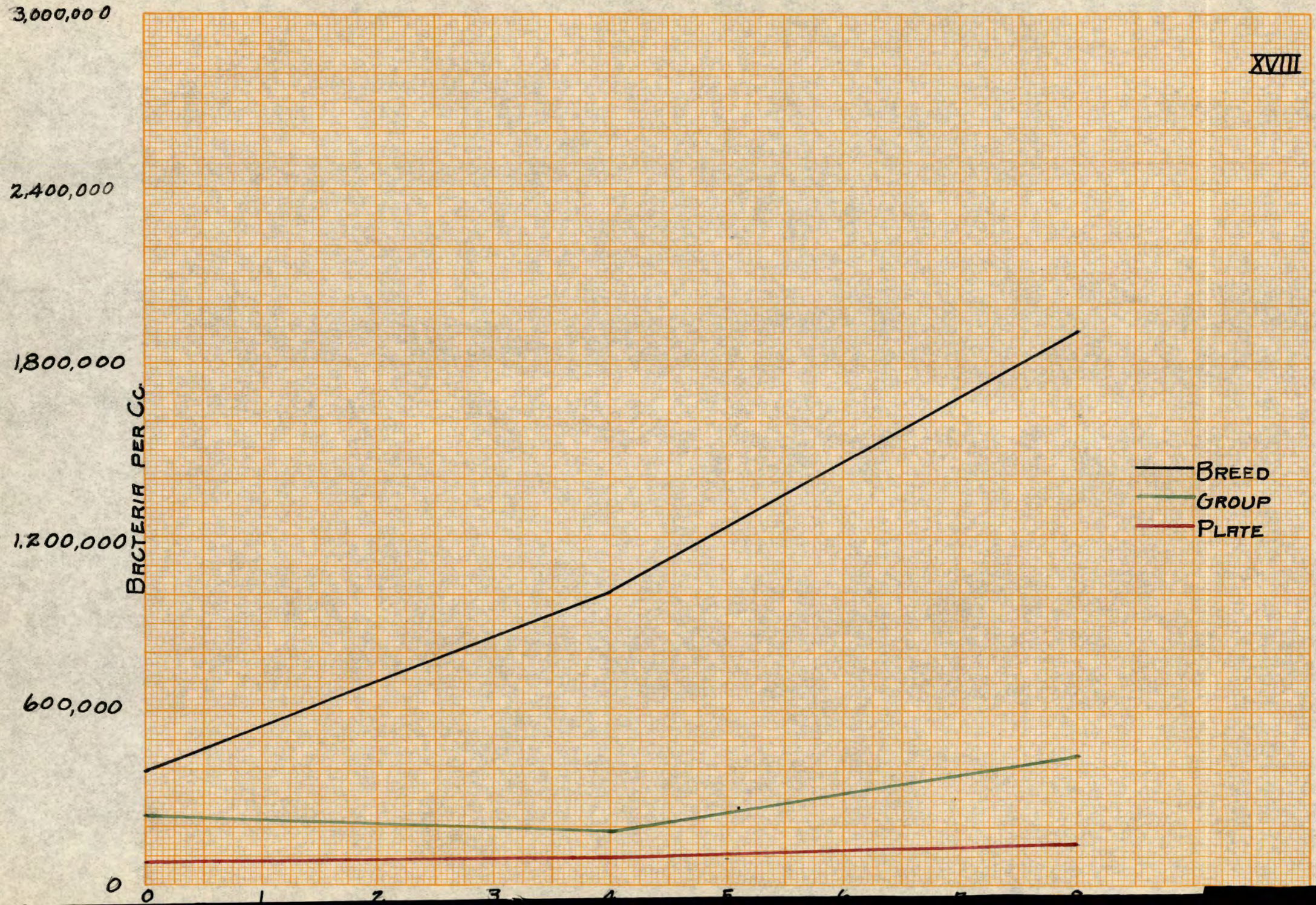

500,000
400,000
300,000
200,000
100,000
0
BACTERIA PER Cc.
0
1
2
3
4
5
6
7
8
X
BREED
GROUP
PLATE

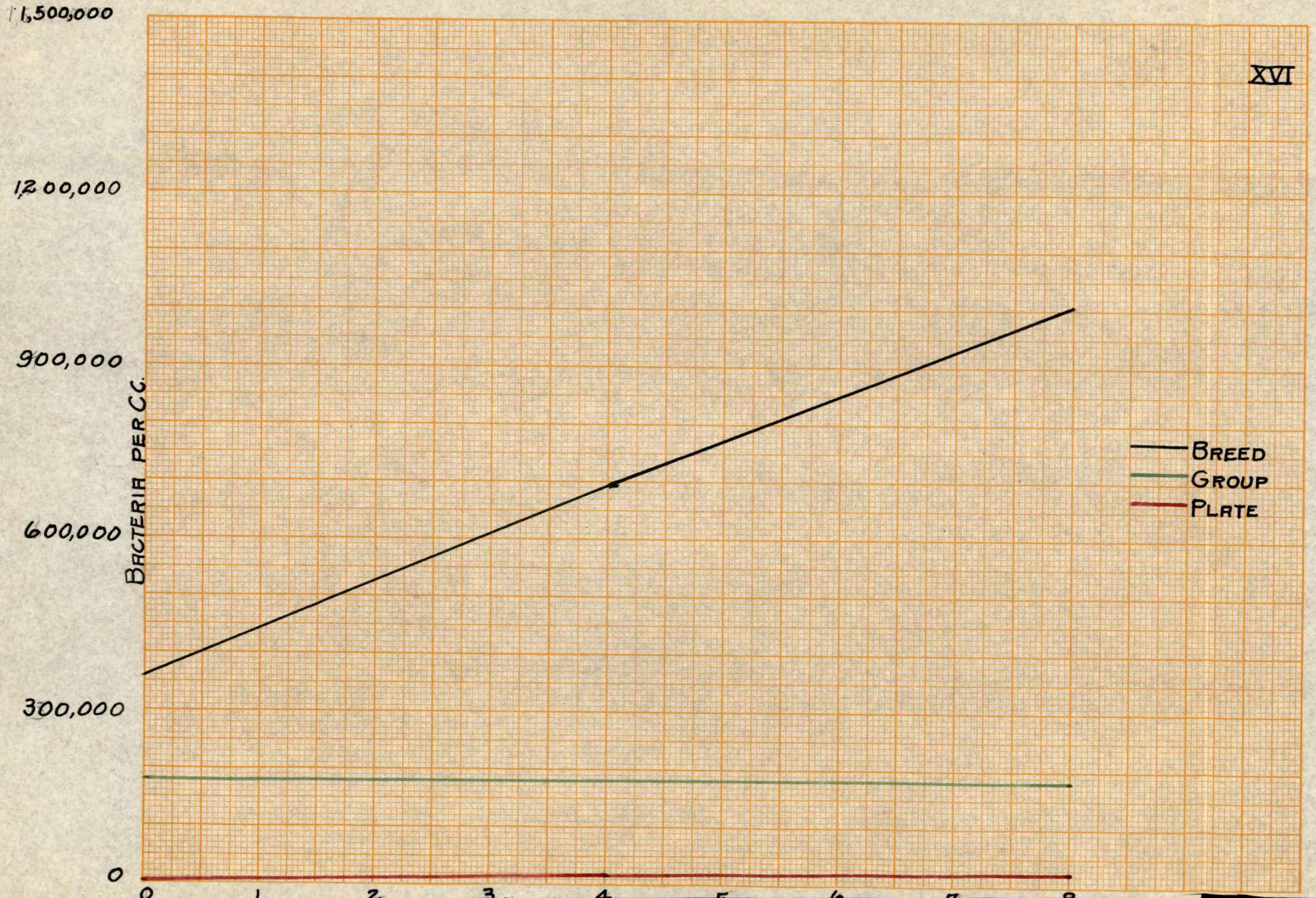

IX
1500,000
1.200,000
900,000
600,000
300,000
0
BACTERIA PER CC.
0
1
2
3
4
5
6
7
8
BREED
GROUP
PLATE

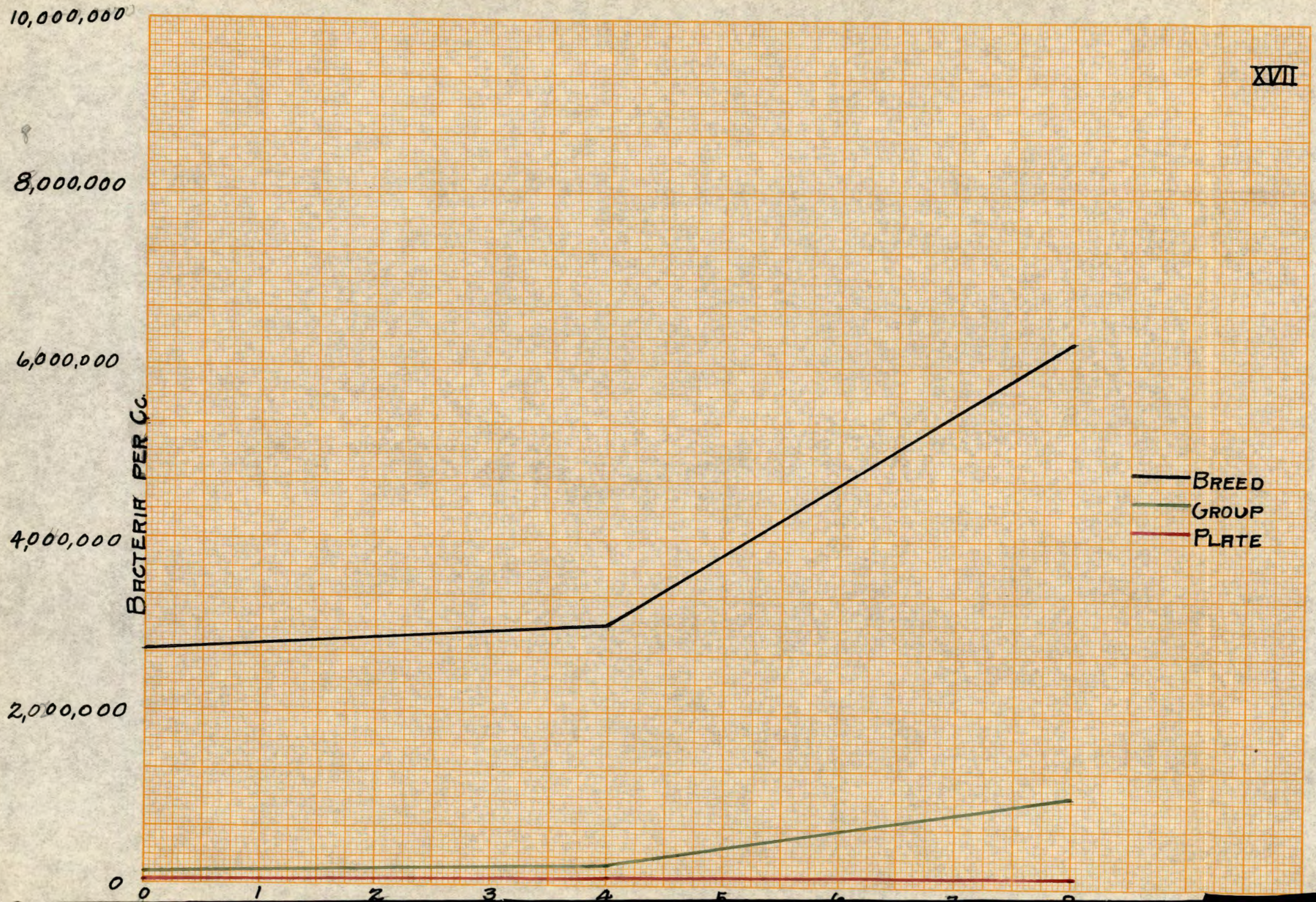

XIII
300,000
240,000
180,000
120,000
60,000
0
BACTERIA PER CC.
BREED
GROUP
PLATE

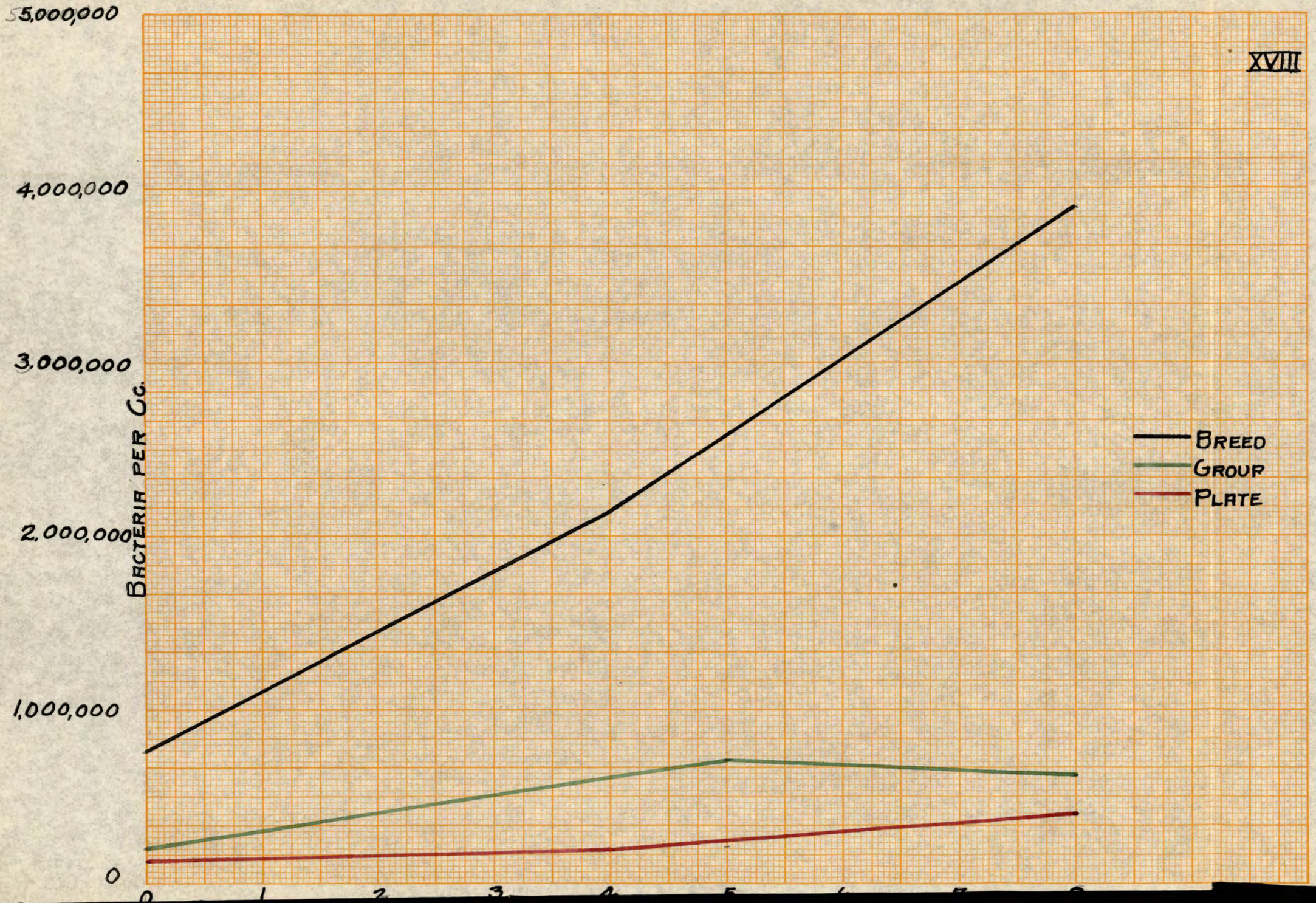

XIV
500,000
400,000
300,000
200,000
100,000
0
BACTERIA PER CC.
NO BREED
NO GROUP
PLATE

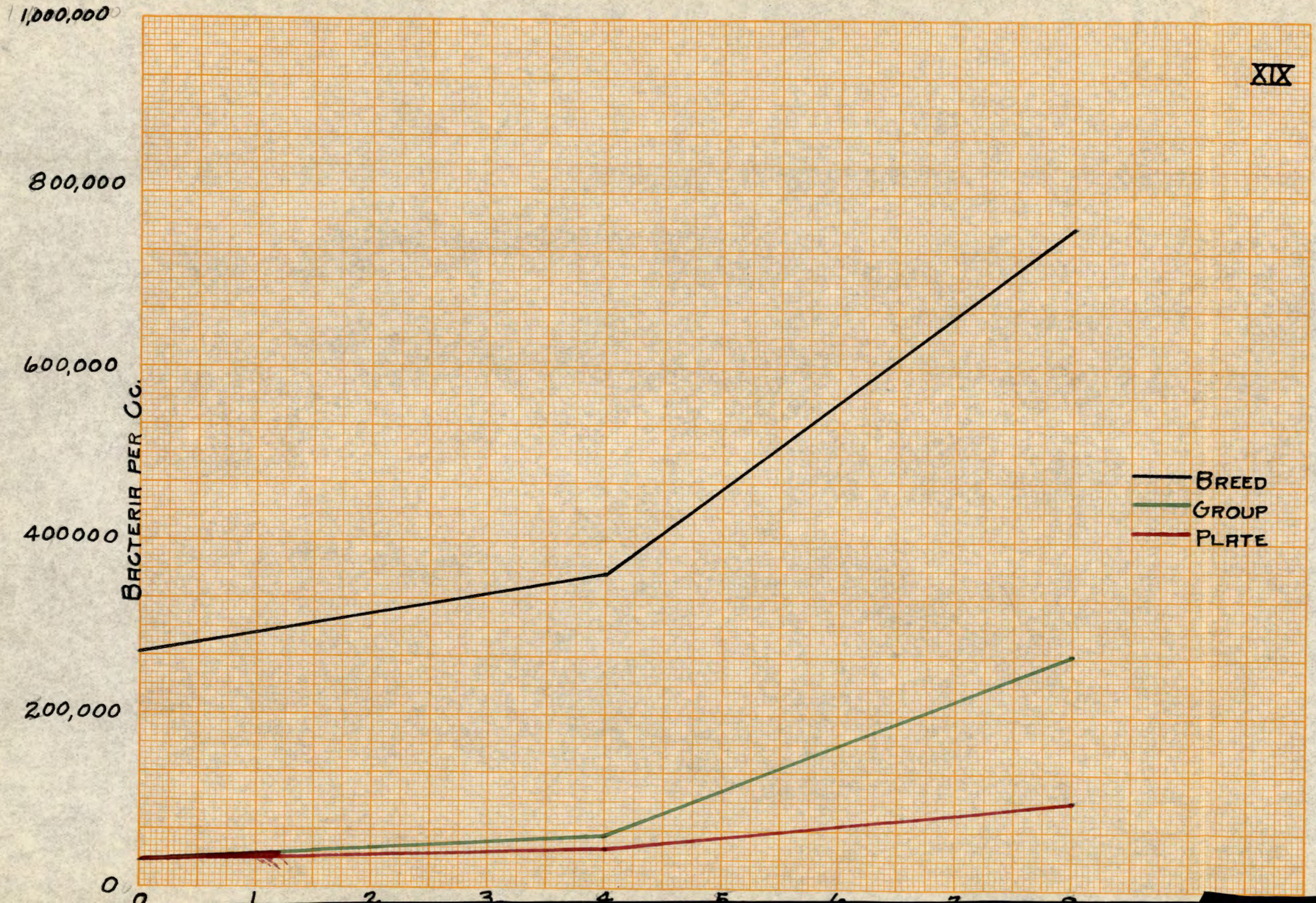
XVIII

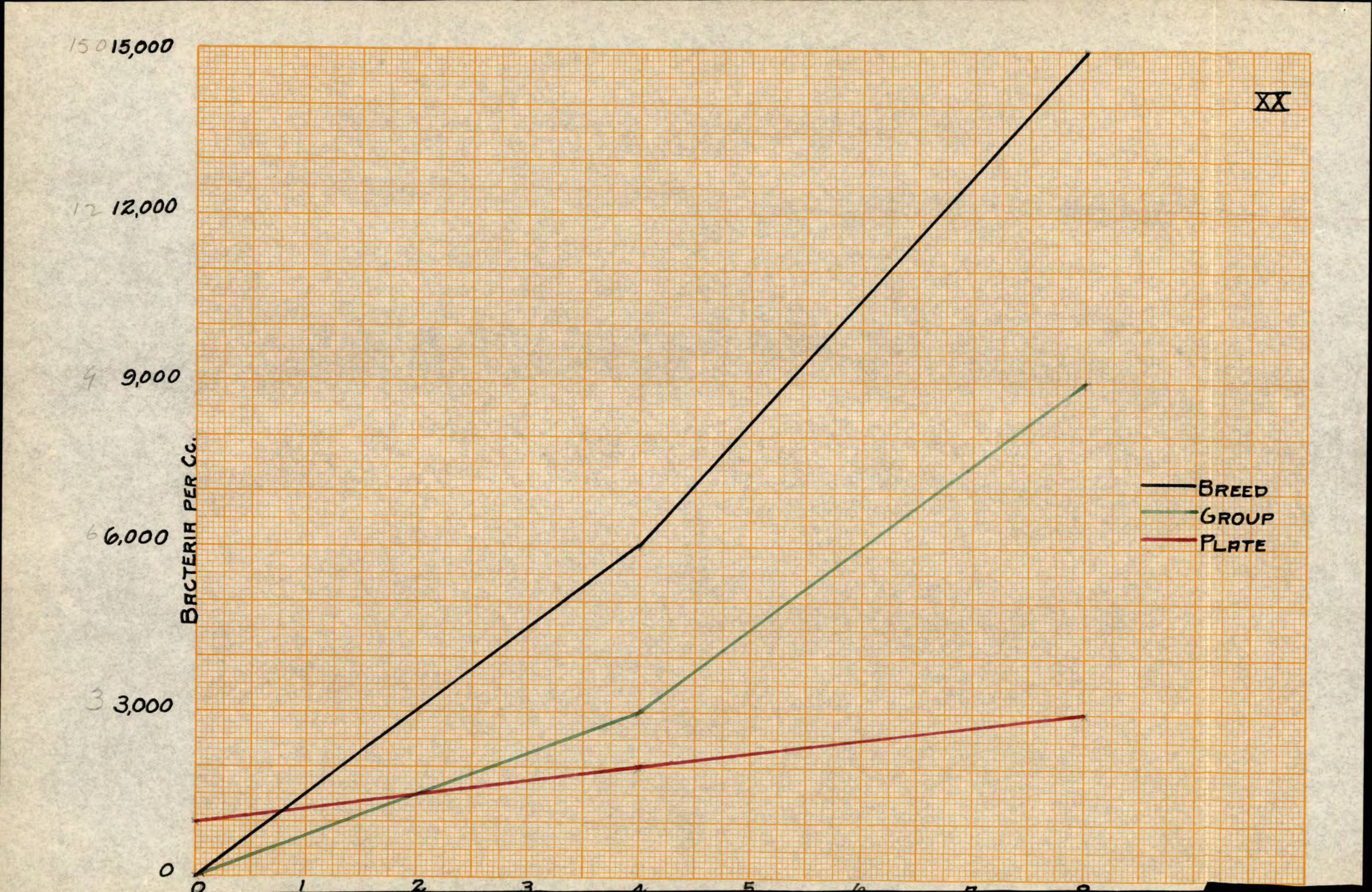

3,000,000
2,400,000
1,800,000
1,200,000
600,000
0
Bacteria per Cc.
0
Breed
Group
Plate


XVI
1,500,000
1,200,000
900,000
600,000
300,000
0
BACTERIA PER C.C.
0
1
2
3
4
5
6
7
8
BREED
GROUP
PLATE


XVII
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0
BACTERIA PER Cc.
0
1
2
3
4
5
6
7
8
BREED
GROUP
PLATE

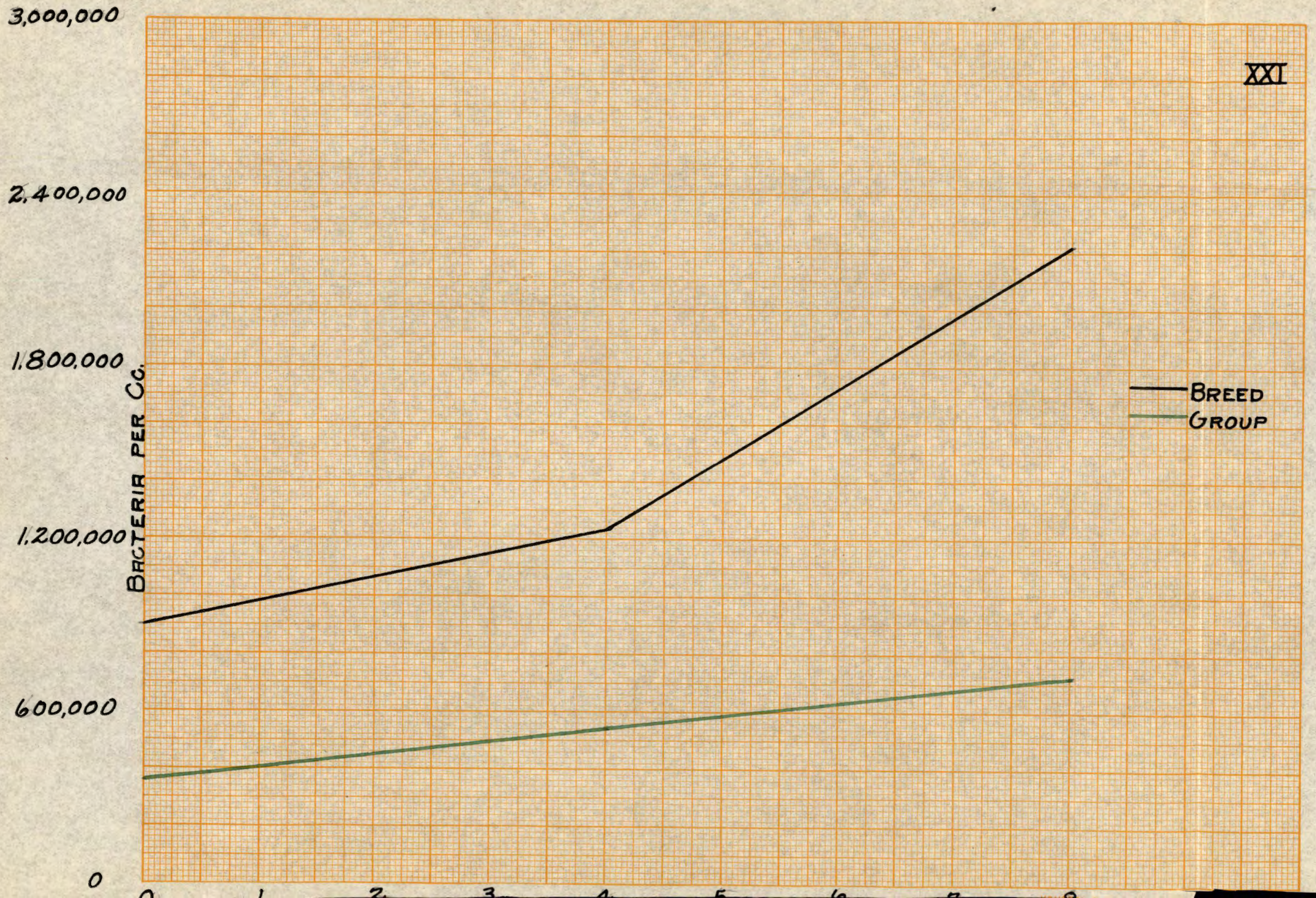

5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
0
Bacteria per Cc.
XVIII
Breed
Group
Plate

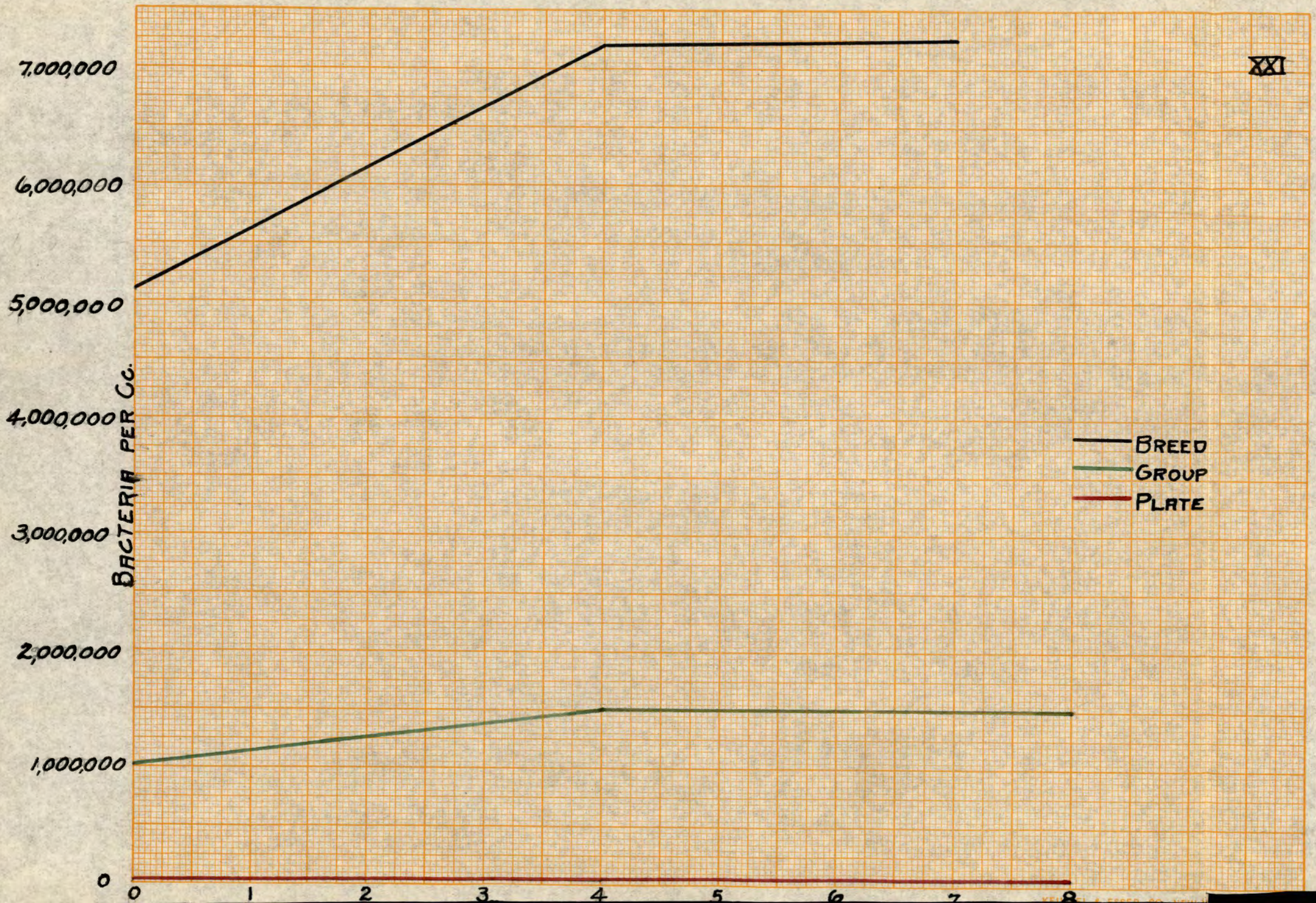

XIX
1,000,000
800,000
600,000
400000
200,000
0
BACTERIA PER CC.
BREED
GROUP
PLATE

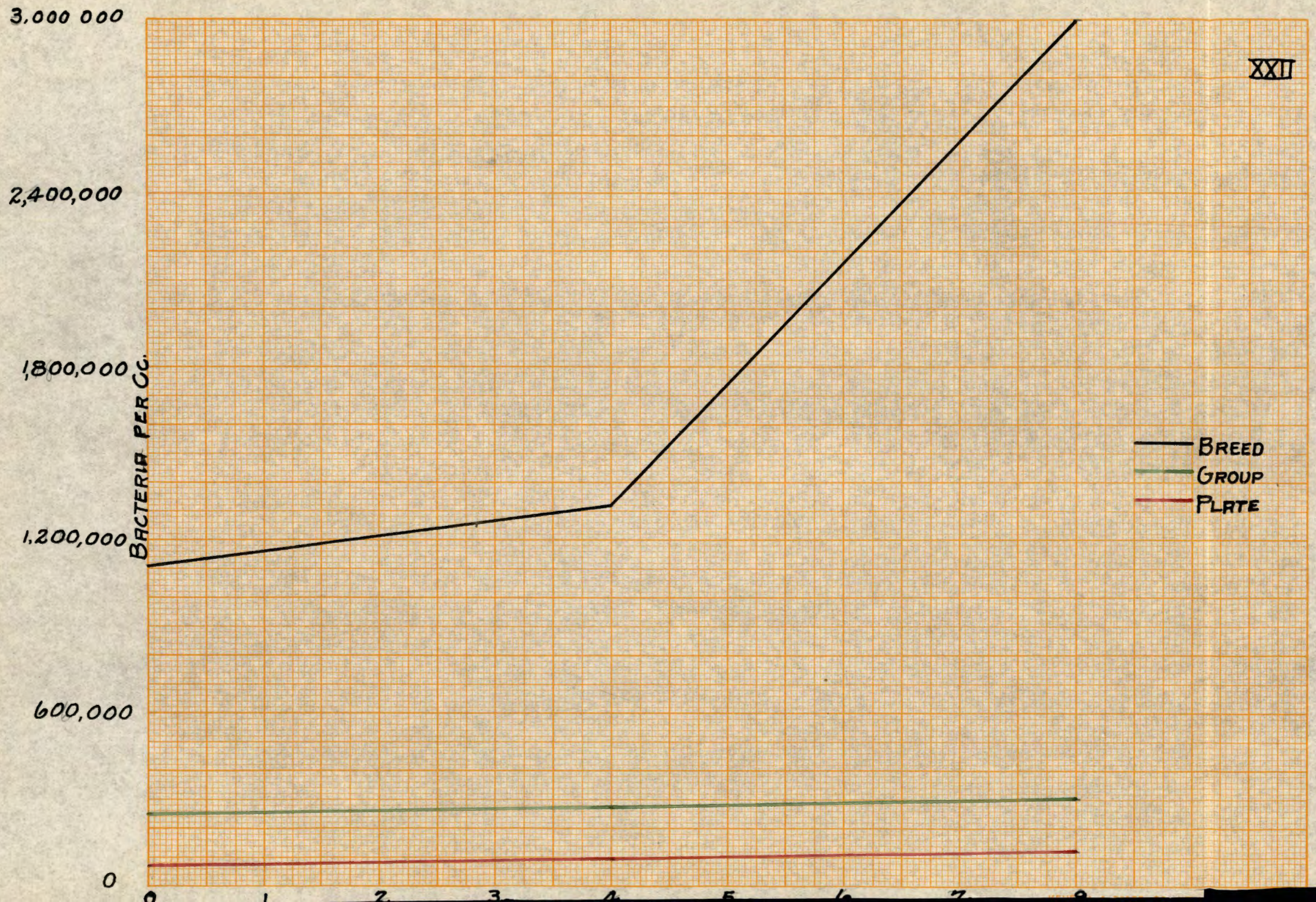

15,000
12,000
9,000
6,000
3,000
0
BACTERIA PER CC.
0
1
2
3
4
5
6
7
8
XX
BREED
GROUP
PLATE


XXVII
3,000,000
2,400,000
1,800,000
1,200,000
600,000
0
Bacteria per Cc.
0
1
2
3
4
5
6
7
8
Breed
Group
Plate

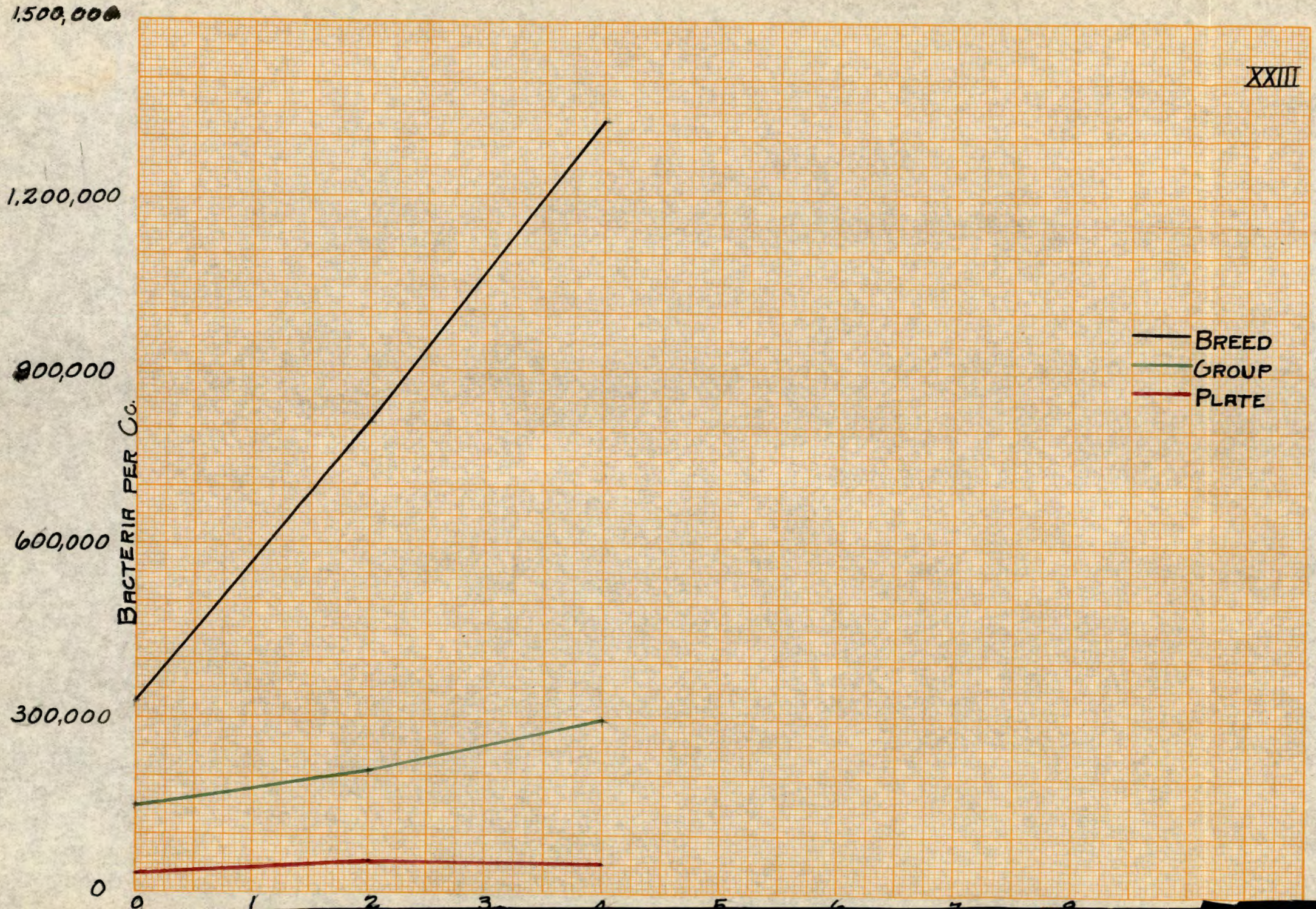
XXI

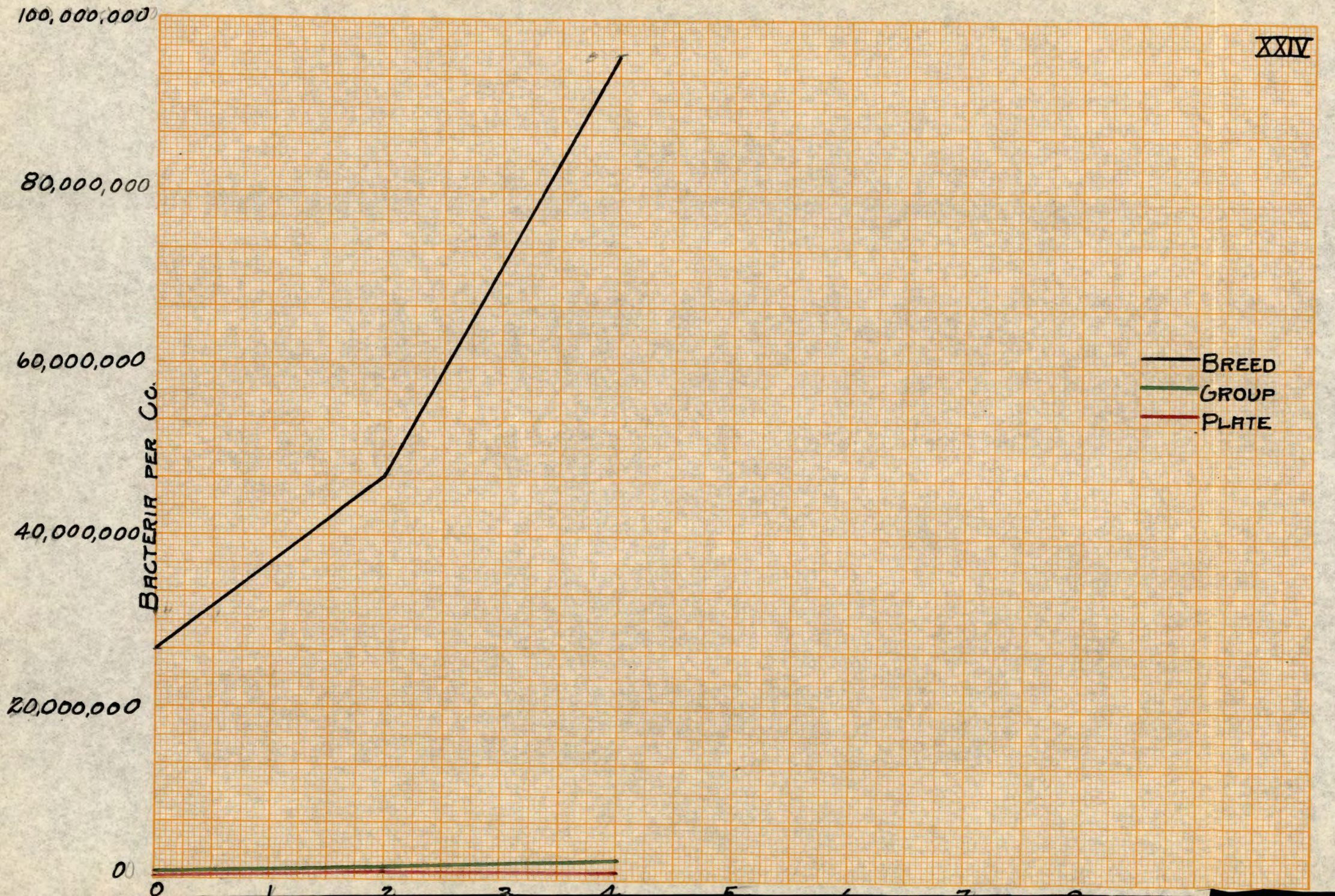

3,000,000
2,400,000
1,800,000
1,200,000
600,000
0
Bacteria per Cc.
Breed
Group

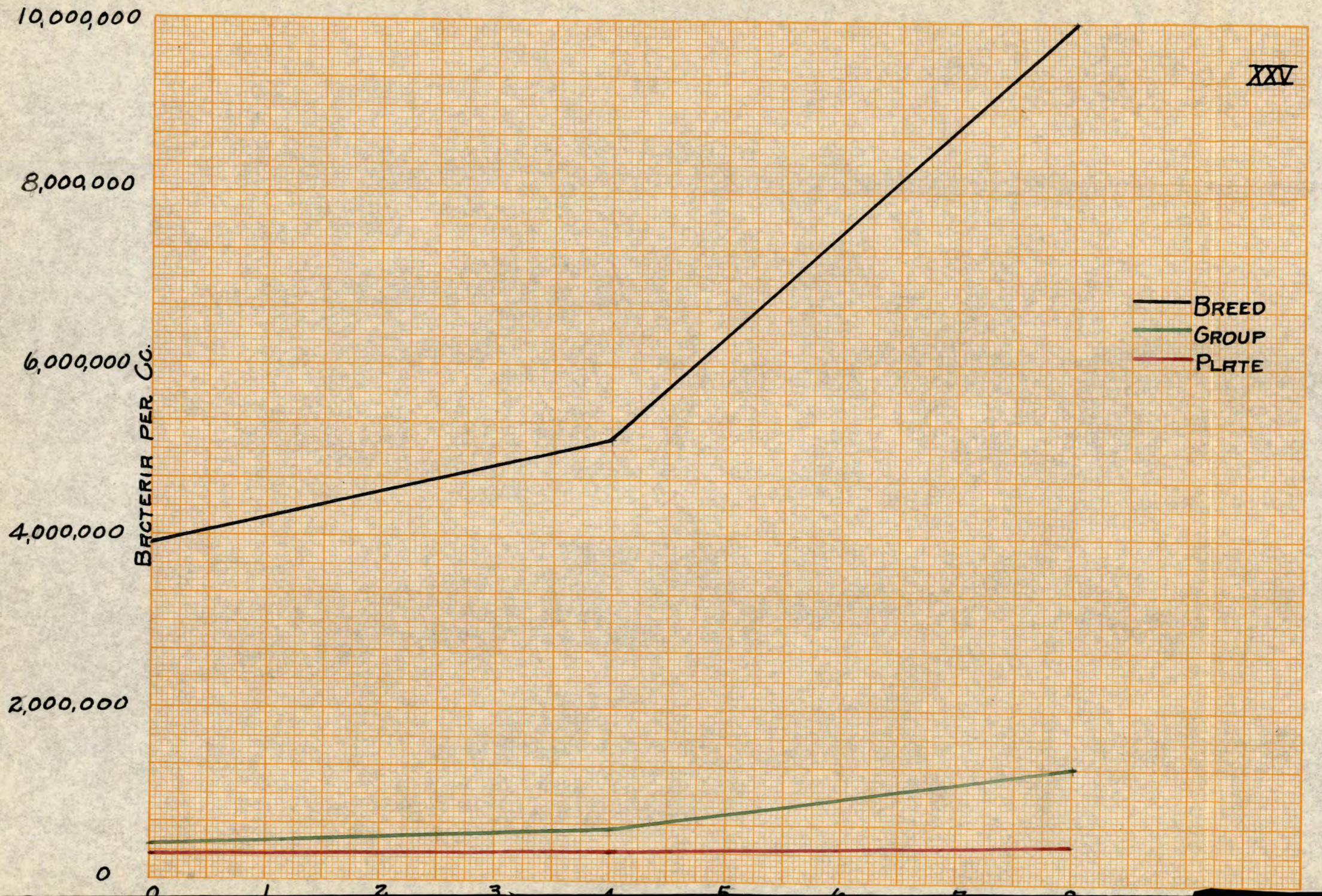

XXI
7,000,000
6,000,000
5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
0
BACTERIA PER Cc.
0
1
2
3
4
5
6
7
8
BREED
GROUP
PLATE


XXII
3,000 000
2,400,000
1,800,000
1,200,000
600,000
0
BACTERIA PER CC.
BREED
GROUP
PLATE

XXIII

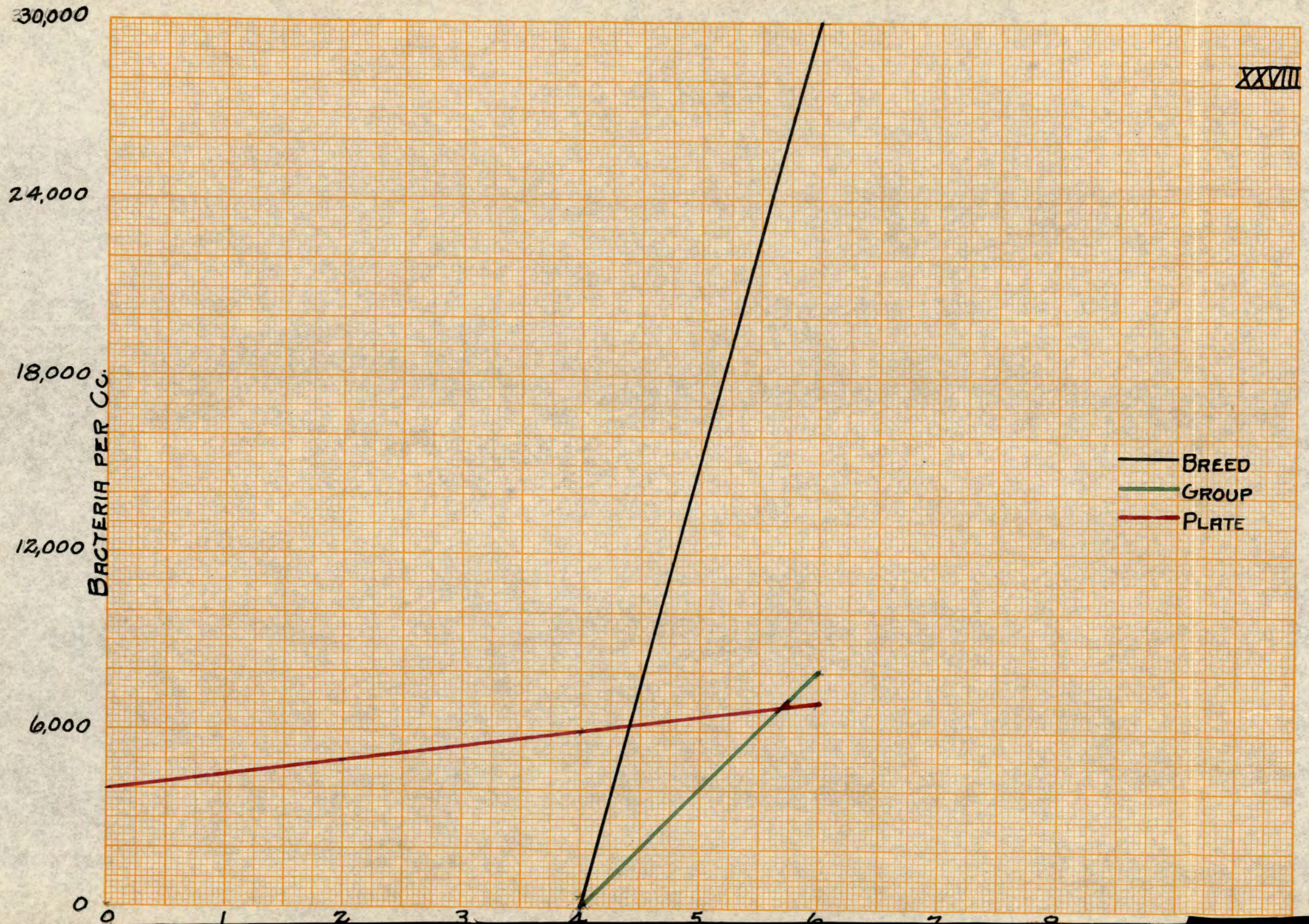

1,500,000
1,200,000
900,000
600,000
300,000
0
Bacteria per Cc.
0
1
2
3
4
5
6
7
8
Breed
Group
Plate

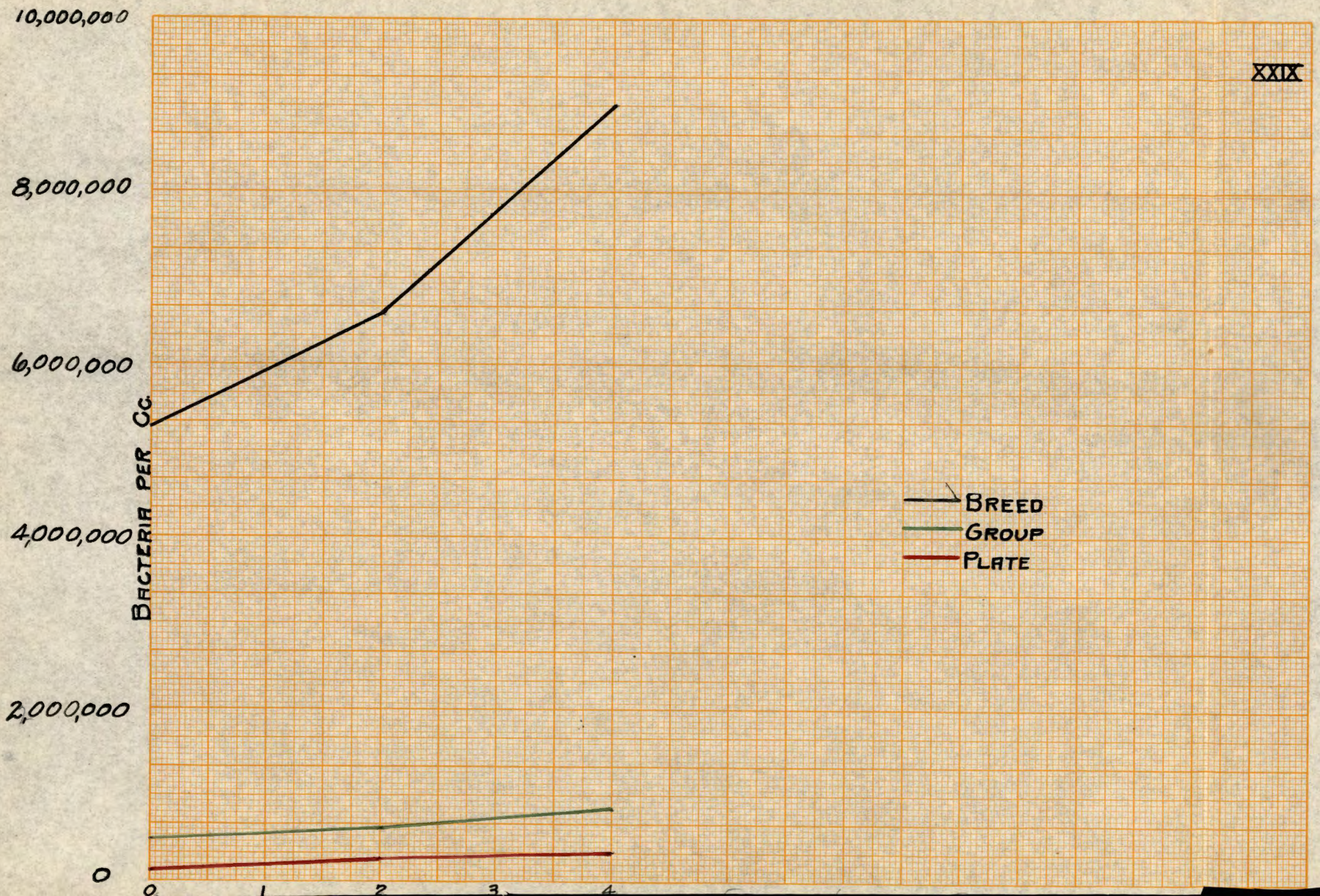

XXIV
100,000,000
80,000,000
60,000,000
40,000,000
20,000,000
0
BACTERIA PER CC.
0
BREED
GROUP
PLATE

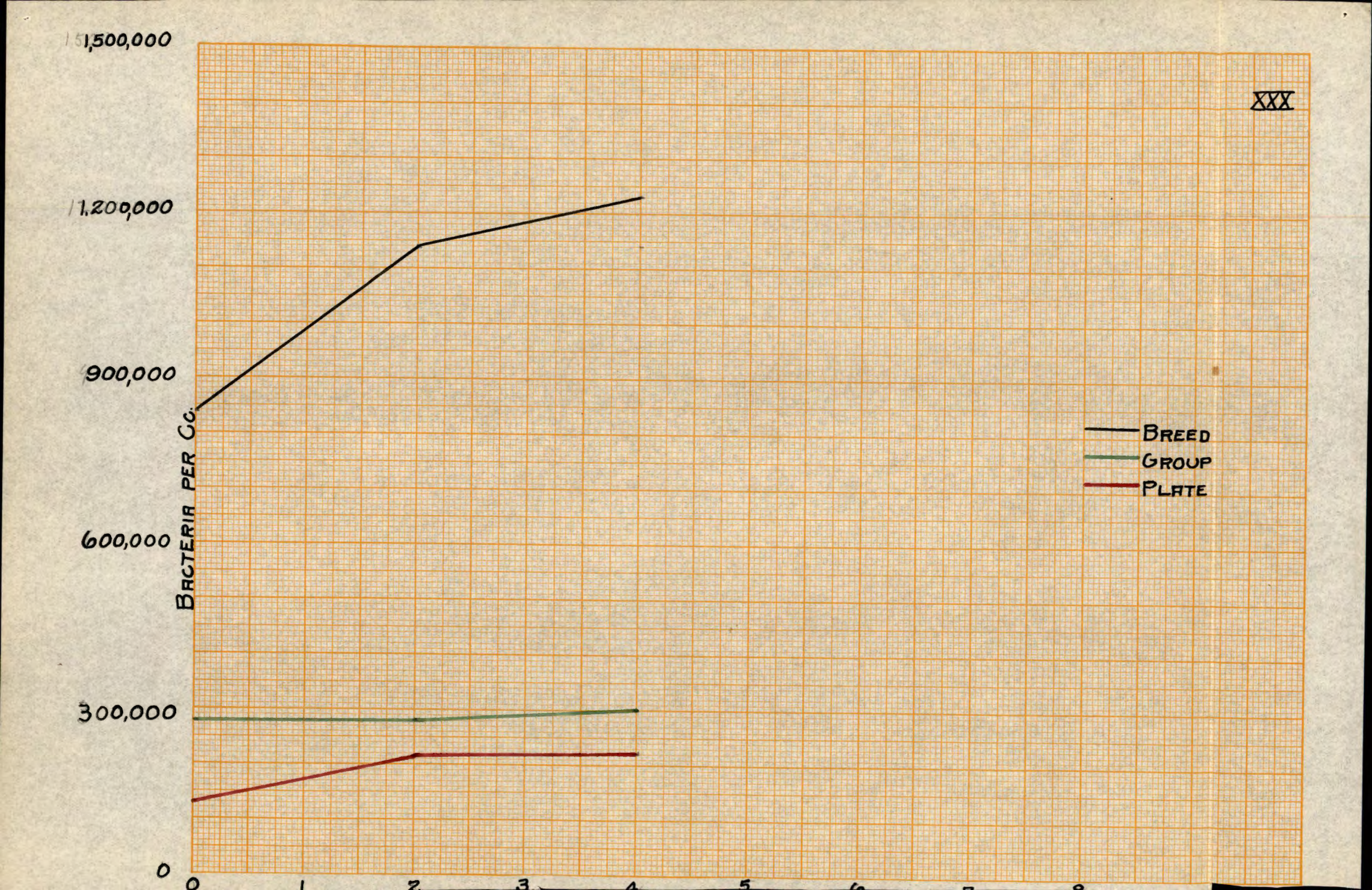

XXV
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0
BACTERIA PER C.C.
BREED
GROUP
PLATE

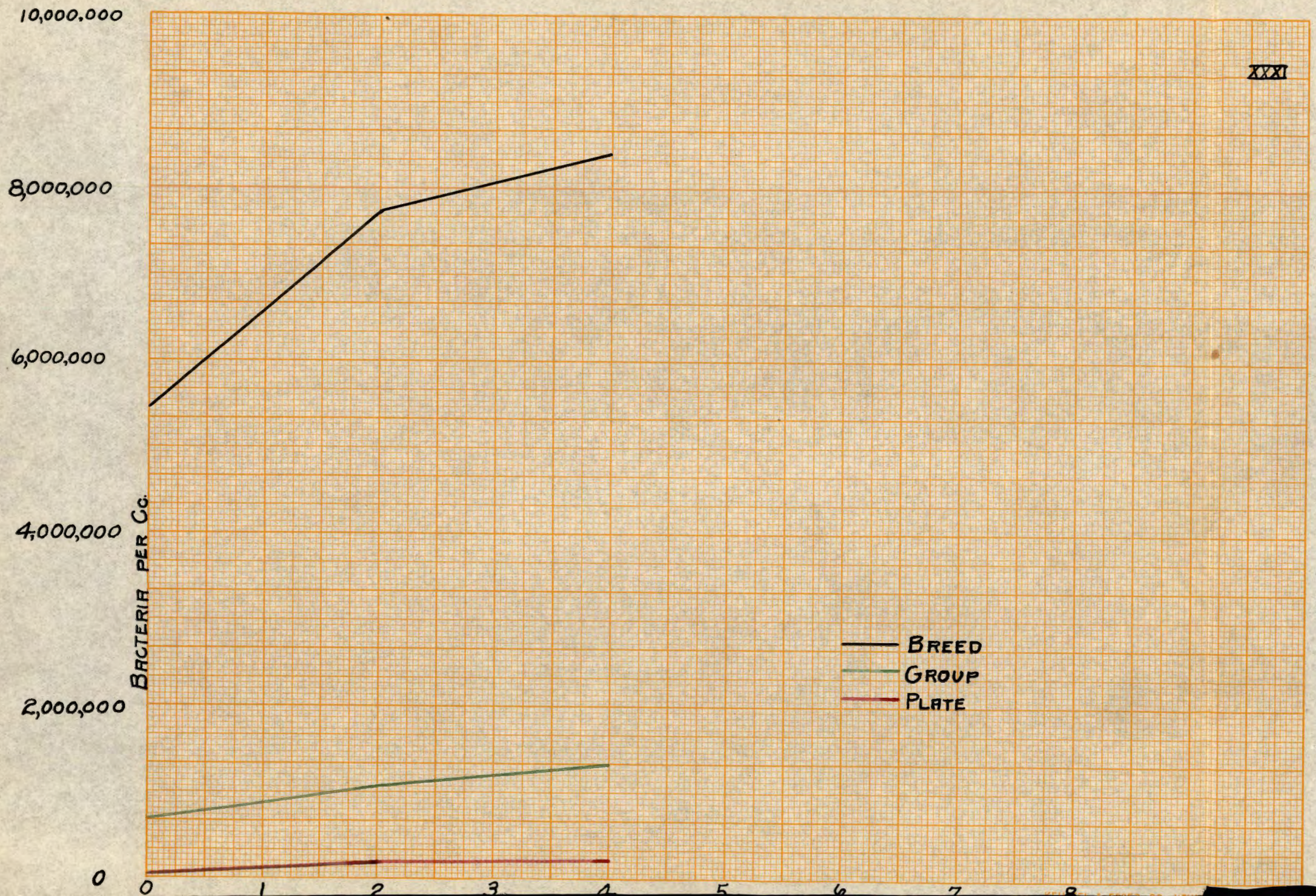

XXVI
3,000,000
2,400,000
1,800,000
1,200,000
600,000
0
BACTERIA PER CC.
BREED
GROUP

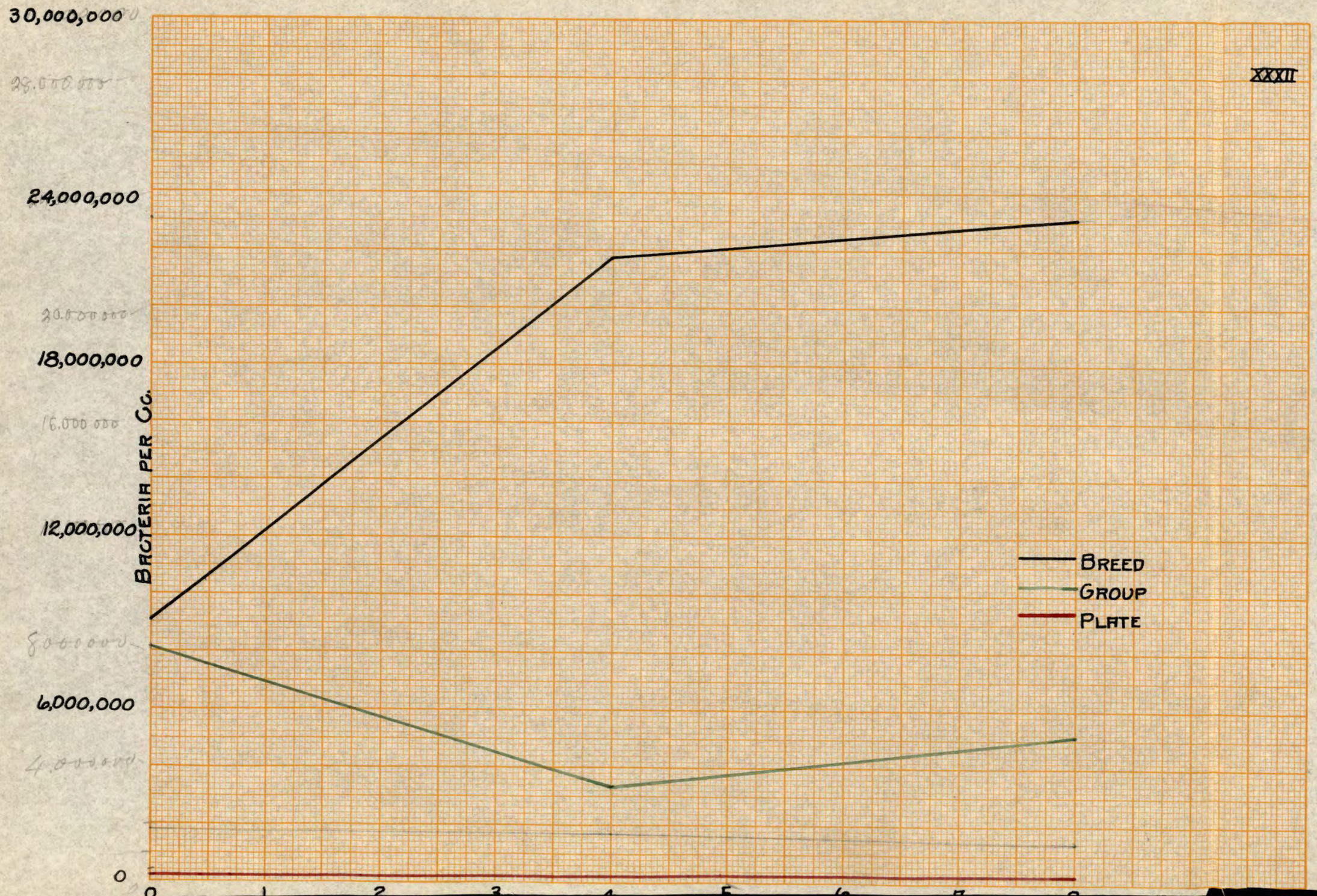
XXVIII

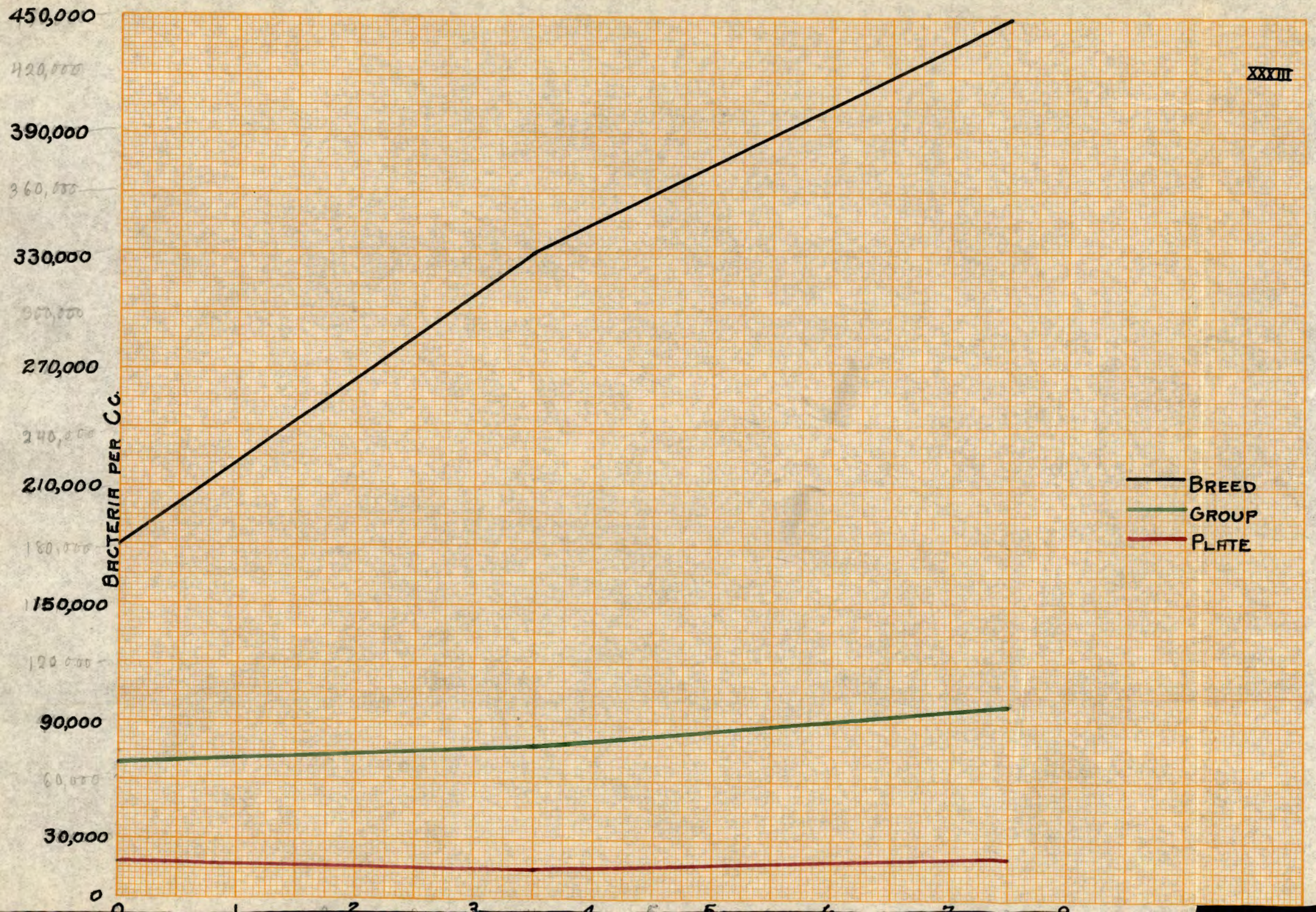

30,000
24,000
18,000
12,000
6,000
0
BACTERIA PER CC.
0
1
2
3
4
5
6
7
8
BREED
GROUP
PLATE

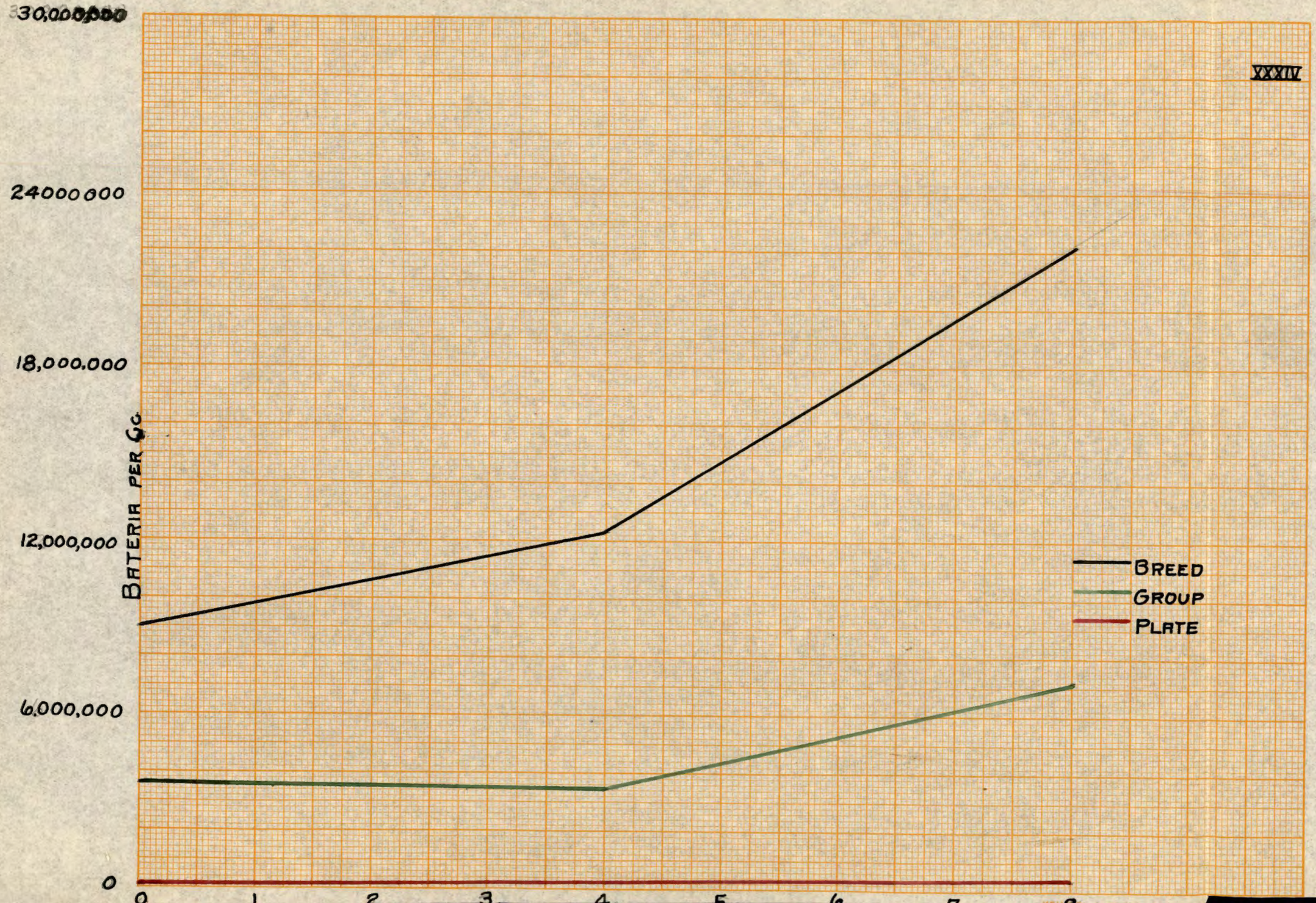

10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0
Bacteria per Cc.
0
1
2
3
4
Breed
Group
Plate

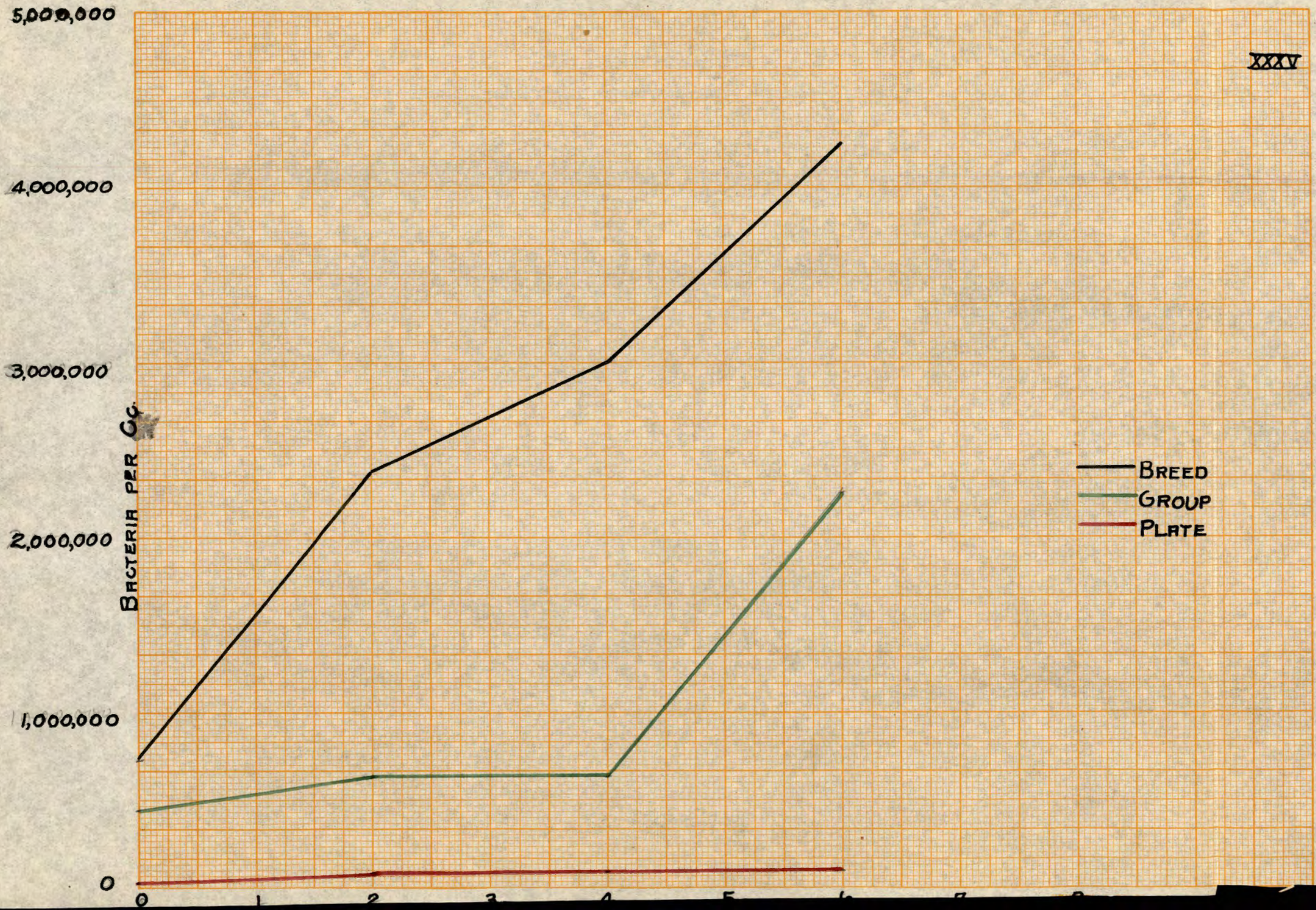

XXX
1,500,000
1,200,000
900,000
600,000
300,000
0
Bacteria per Cc.
Breed
Group
Plate
0
1
2
3
4
5
6
7
8

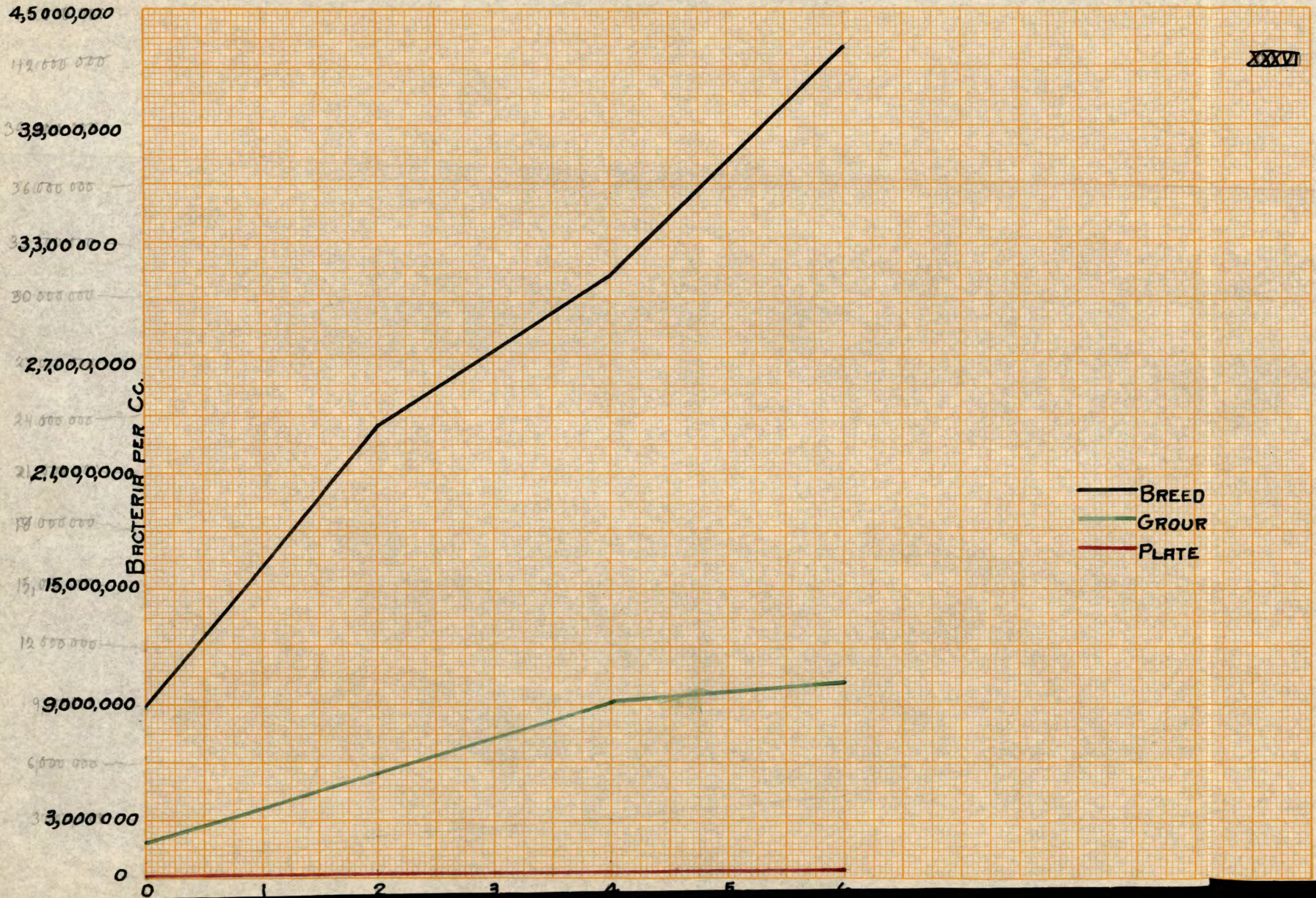

10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0
Bacteria per Cc.
0
1
2
3
4
5
6
7
8
Breed
Group
Plate

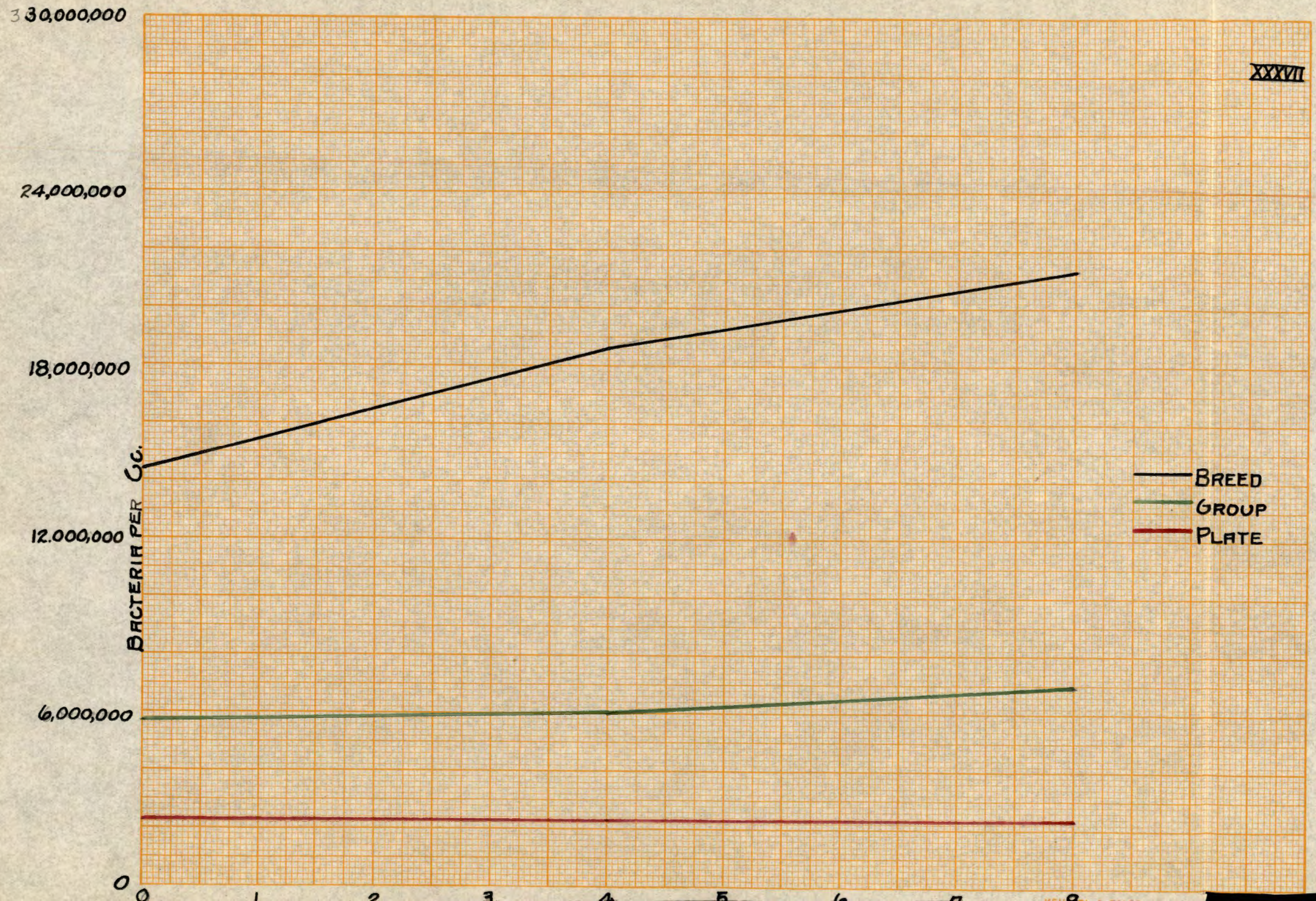

30,000,000
24,000,000
18,000,000
12,000,000
6,000,000
0
Bacteria per Cc.
Breed
Group
Plate

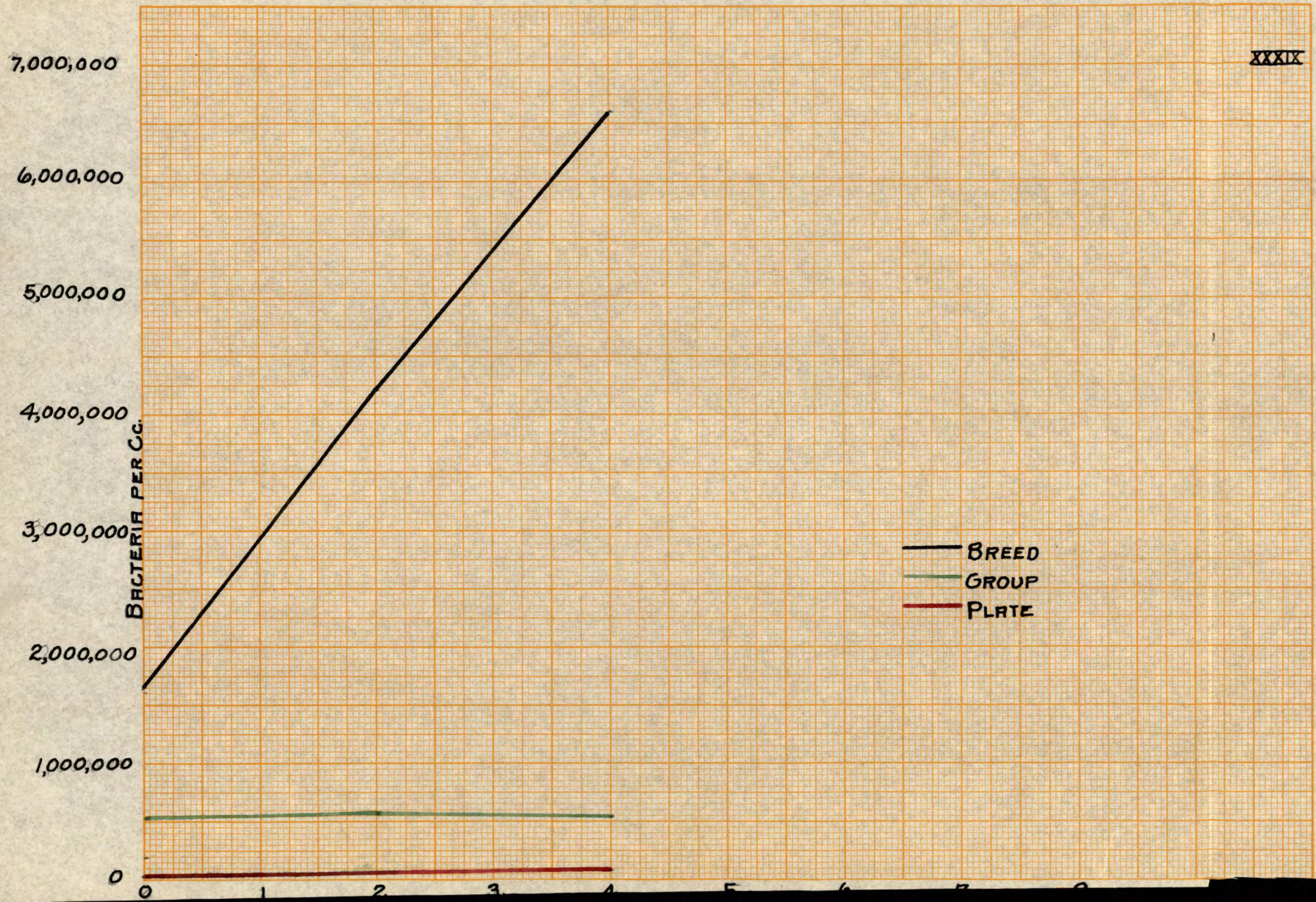

XXXIII
450,000
390,000
330,000
270,000
210,000
150,000
90,000
30,000
0
BACTERIA PER C.C.
BREED
GROUP
PLATE

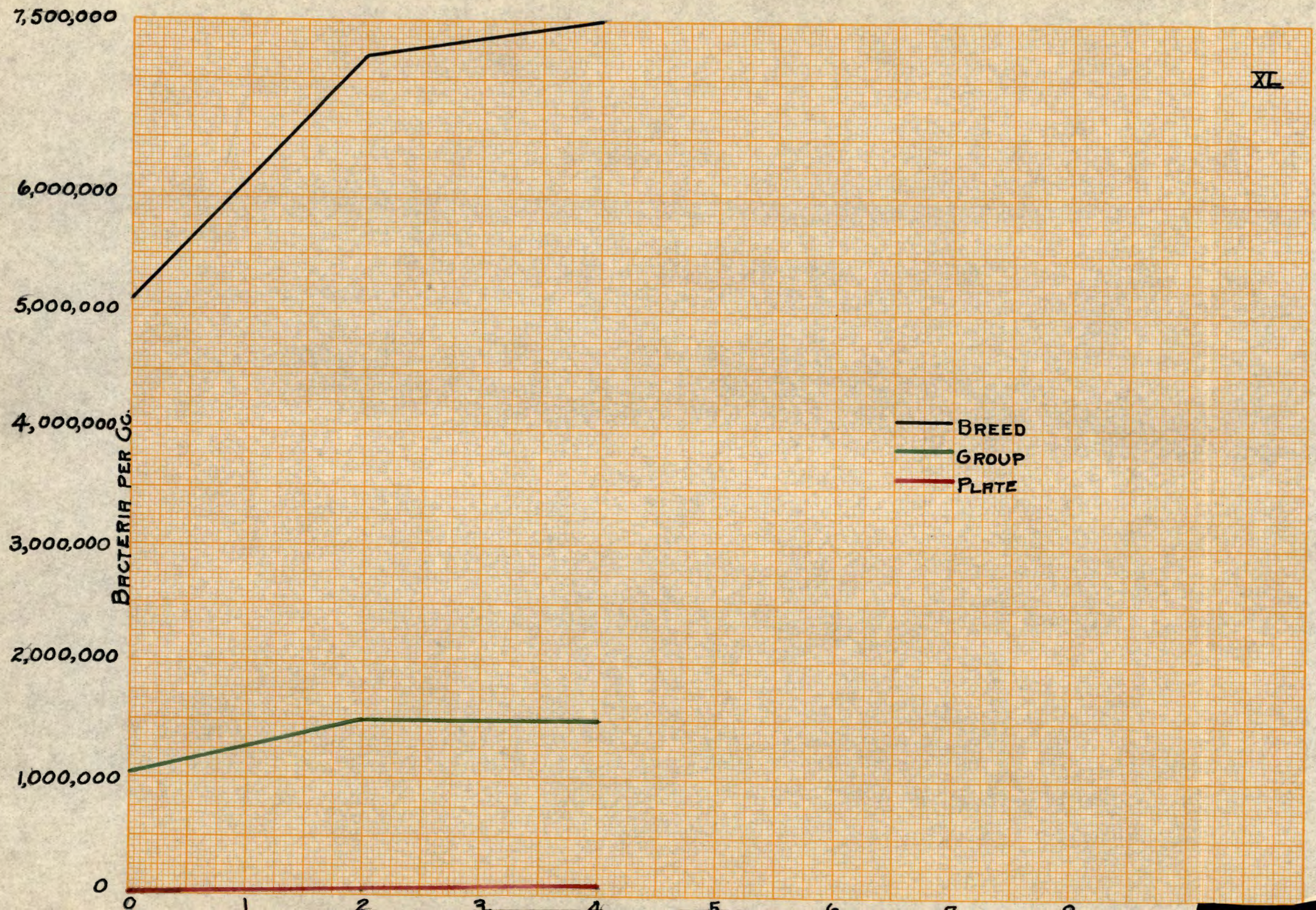

30,000,000
24000000
18,000,000
12,000,000
6,000,000
0
BATERIA PER Gc.
BREED
GROUP
PLATE


XXXV
5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
0
Bacteria per Cc.
0
1
2
3
4
5
6
Breed
Group
Plate


4,5000,000
3,9,000,000
3,3,00000
2,7,00,0,000
2,1,00,0,000
15,000,000
9,000,000
3,000000
0
Bacteria per Cc.
0
1
2
3
4
5
6
Breed
Grour
Plate


XXXVII
30,000,000
24,000,000
18,000,000
12.000,000
6,000,000
0
BACTERIA PER CC.
BREED
GROUP
PLATE


7,000,000
6,000,000
5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
0
BACTERIA PER CC.
0
1
2
3
4
BREED
GROUP
PLATE


7,500,000
6,000,000
5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
0
BACTERIA PER CC.
0
1
2
3
4
5
6
7
8
XL
BREED
GROUP
PLATE

BIBLIOGRAPHY .

(1) Baker, J.C., Brew,J.D., and Conn, H.J.,

1919. The Relation of Lactic Acid Production and Bacterial growth in the Souring of Milk. Technical Bulletin no. 74 experiment Station, Geneva, New York.

(2) Breed, Robert S., and Brew, James D.,

1917. The Control of Bacteria in Market Milk by Direct Microscopic Examination. Experiment Station, Geneva,New York. Bulletin no.443.

(3) Breed, Robert S., and Bright, John W.,

1921. Leakage from the Vacuum Pipe line into the Pail as a Sourse of Contamination of Milk. Experiment Station Geneva New York Bulletin no. 448.

(4) Breed Robert S. and Brew , James D.

1916. Counting Bacteria by Means of the Microscope. Experiment Station , Geneva, New York. Bulletin no. 49.

(5) Breed, Robert S., Ruhle, G.L.A., and Smith, George A.,

1917. Mliking Machines; As a Source of Bacteria in Milk; Methods of Maintaining in a Bacteria Free Condition. Experiment Station, Geneva New York. Bulletin no 450

(6) Breed Robert S., and Stocking W.A.,

1920. The Accuracy of bacterial counts from Milk Samples. Experiment Ststion , Geneva New York. Technical Bulletin no.75.

(7) Brew, James D.,

1914. AComparisonof the microscopical method and the Plate Method of Counting Bacteria in Milk. Experiment Station, Geneva New York. Bulletin no. 373.

(8) Brew, James D.,

1914. A Comparison of the Microscopical Method and the Plate Method of Counting Bacteria in Milk. Experiment Station, Geneva New York. Bulletin no. 439.

(9) Conn, H.W., and Conn, H.J., Text.

The Study of Microorganisms and their Relation to Human Welfare.

(10) Kennedy, Ila,

1924. The Importance of Cell Grouping in the Direct Microscopic Counting of Bacteria in Market Milk. Abstracts of Bacteriology, Volume VIII. no.1 pp.15 and 16.

(11) Robertson, A.H.,

1921. The Relation Between Bacterial Counts from Milk as Obtained by Mocroscopic and Plate Methods. Experiment Station Geneva New York. Technical Bulletin no. 86.

(12) Whiting, William A.,

1923. The Relation Between the Clumps of Bacteria in market Milk and the Flora of Dairy Utensils. Experiment Station Geneva New York. Technical Bulletin, no. 73.