LACTATION STUDIES IN MICE
I. TECHNIQUE

THESIS FOR THE DEGREE OF M. S.

Kenneth B. DeOme

1934

Lactation
Mice
Tietz

LACTATION STUDIES IN MICE

I. TECHNIQUE

THESIS FOR THE DEGREE OF M. S.

Kenneth B. DeOme

1934

THESIS

LACTATION STUDIES IN MICE

I. TECHNIQUE

Thesis

Submitted to the Faculty of the Michigan State College in Partial Fulfillment of the Requirements for the Degree of Master of Science

by

Kenneth B. DeOme

July, 1934

ACKNOWLEDGMENTS

The author wishes to express his appreciation for the assistance offered by his associates in the course of the experiments described in this paper. To Dr. H.R. Hunt, who suggested the problem and under whose thoughtful supervision it was carried out, special gratitude is due. Mr. Leo Klever has been especially helpful in preparing the apparatus necessary.

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

Part I

INTRODUCTION

The genetic basis of lactation has been investigated extensively for many years. The material available for such studies is largely confined to records gathered by cattle improvement associations. The advanced registry system maintained by the various dairy breed associations, and the dairy breed improvement association records have offered a great mass of data for biometrical studies on the inheritance of milk production. Such competent investigators as John W. Gowen, M. H. Fohrman, W.E. Agar, R. R. Graves, H.D. Goodale, and many others have used the data from these records as a means of attacking the problem.

It must be borne in mind, however, that such breeding records were not compiled from controlled breeding experiments, but were merely performance records of selected animals together with records of their ancestry. This fact offers two very serious objections to their use in genetic investigations-namely, the records represent only a selected portion of the whole cattle population (those whose records for breeding and production make them eligible), and the records are from matings made to produce a certain type of cattle rather then to discover genetic relationships.

In view of the limitations mentioned above, those investigating the inheritance of milk and butterfat production have been confined to the use of the correlation technique.

Much suggestive material has been derived by this method, however, and no attempt to minimize it's value will be made here. For instance, such studies have pointed out that the process of milk production is at least partly hereditary, as would be expected. John W. Gowen, working at the Maine Agricultural Experiment Station, has published a number of analyses which show that daughters of high producing cows tend to be high producers; also, that the daughters of certain bulls make high records, while the daughters of other bulls are usually poor producers, and that there is a higher correlation between the production of full sisters than half sisters, etc. Studies on correlation between production records and body conformation have also been made with the result that production "at the pail" plus a knowledge of ancestry is the only reliable method available at present for selecting high producing animals. R.R. Graves ('26) states, "The evidence seems to point to both parents contributing equally to the inheritance governing the milk and butterfat producing capacity of their daughter", and again that, "when the records of a large number of daughters are compared with the records of their dams, there is a limited correlation, or a tendency for high record daughters to come from high record dams". H.D. Goodale ('27) calculated a sire's breeding index from the observations of Cole, Castle, and Gowen on crosses of breeds differing markedly in production levels. He found that the offspring from such crosses tended to be intermediate between the two parental types, though not exactly half way between the two. W. E. Agar ('26) pointed out that the correlation coefficient between mother and daughter for red poll cattle of Australia was .293 $\pm$ .071 for the total milk production and .235 $\pm$ .060 for total butterfat production. M. H. Fohrman ('26) from a study of the entry and re-entry records of the Guernsey, Jersey, and Ayshire cattle concluded that the production of a cow depends about 60% upon heredity and about 40% upon environment.

Such data, while inclusive, show that heredity plays a large part in the production of milk and butterfat in cattle. They give no idea, however, of the number of factors involved, nor of the mode of inheritance. It is extremely improbable that such information will ever be extracted from the records of breed associations. It is also improbable that any organization will soon undertake the task of carrying on controlled experiments among cattle to determine the exact genetic structure influencing milk production. The excessive cost of maintaining such a project, the small number of offspring produced by a dam, the length of time required to carry out the experiment, and the difficulty of obtaining breeding stock representing the whole cattle population would make such a venture difficult if not impossible.

A search for a method of over-coming these difficulties provided the motive for this study. It has been realized by a number of people that small rapidly breeding laboratory animals might be used to help solve the problem if some adequate method of measuring the quantity of milk given by them could be developed. In human mothers, Holt & Howland ('22) were able to estimate the amount of milk given by weighing the infant before and after nursing. Enzman ('33) adapted this method to mice, obtaining quantitative measurements of milk production by taking the difference between the weight of a litter before and after nursing. By this technique he was able to construct milk curves for albino mice.

With this method developed it seemed possible to use mice as a means of studying the genetics of lactation. It will be the purpose of this paper to describe the technique used in studying lactation in mice, to study the milk production of mice in successive lactations, and to discuss some of the factors influencing the experiment in question.

Part II

THE STANDARD TECHNIQUE

The technique used in measuring the amount of milk given by an individual mouse is a modification of that suggested by Enzman ('33). The difference between the weight of a litter before and after nursing is due to the milk obtained by suckling. Since the mother's milk is the only possible source of food until the eyes of the young mice are open, the above assumption must be correct. A fairly accurate quantitative measurement of the amount of milk given can be obtained, then, if the number of young in the litter is kept large enough to take all of the mother's milk. The technique described below is built about this idea, and has gradually grown through many trials and failures. It may be necessary to modify it still further as new relationships are revealed by future work.

THE CAGE

It is evident that the mother must be kept separated from her young except at such times as measurements are being made. During the early stages of the experiment the mother was left in a regular stock cage and the young were kept in a tin can partially filled with paper shavings. This plan worked quite well but was abandoned since it was thought that the situation would be more nearly normal if the young mice could be kept within sight and hearing of their mother.

Another plan was tried whereby the young mice were left with their mother for 6 hours and then separated automatically from her for the next 6 hours before measurements were made. This was accomplished by placing the mother and her young in a small cage of ½ inch hardware cloth. This small cage was suspended in a glass battery jar by means of a fine wire which was attached to the end of a lever. The lever was fastened by means of a swivel clamp to a heavy ring stand. A 500 c.c. flask was suspended from the end of the lever opposite the cage containing the mice, in such a position that it would nearly counterbalance the weight of the cage and mice. A rubber tube connected the flask on the lever with a flask full of water which was placed about one foot above the lever. A stop-cock was placed in the rubber tube which was fastened to the alarm winding stem of an alarm clock. The alarm clock was set at 2:00 o'clock. When the alarm "went off", the winding stem reversed, turning the stopcock to an open position and permitting the water to flow from the upper flask through the stop-cock into the flask on the end of the lever. The added weight of the water in the flask overbalanced the weight of the small wire cage and mice, thus causing them to be raised up a few inches from the bottom of the battery jar in which the cage was suspended. The young mice would then fall through the ½ inch hardware cloth into the batter jar, but the mother mouse, due to her greater size, was unable to follow them. The young were unable to suckle then until 8:00 o'clock when measurements were made. This plan, though good in theory, was abandoned, due to the unnatural conditions involved.

Small cages made of galvanized iron and hardware cloth were constructed, removing both of the objections mentioned above. One of these cages is shown in Plate I. These cages measured $5\frac{1}{2}$ inches X 8 inches X 9 inches. They were separated into two compartments by a partition of fine hardware cloth. Both compartments were accessible from a door in the front of the cage. A small door, which could be opened and closed from outside the cage, furnished communication between the two compartments. The back compartment was provided with the same paper shavings, food, and water as was used in our regular stock cages. The mother mouse was kept in the back compartment under normal conditions. The front compartment, which was smaller than the back, was provided with enough paper shavings to make a nest for the young mice. No food or water was placed in this compartment. The small cage permitted the young mice to be kept with their mother, separated from her by a fine hardware cloth partition only. Her interest in the young was maintained since she was constantbly aware of their presence. This cage provided a much more natural arrangement than any other device developed to date. In addition it was compact, well ventilated, and minimized the chance of error in handling the young mice. The adult female mouse need not be handled during the feeding period since she soon learns to enter the front compartment when it's entrance door is open.

THE TEMPERATURE

Early in the course of the experiment it was found that the young mice must be kept warm when they were separated from their mother. They were normally warmed by the heat of their mother's body and the structure of the nest built by her. They became very inactive when cold and consequently did not suckle normally. To overcome this difficulty, an incubator was constructed in which the temperature was maintained at 28 degrees to 30 degrees C. The whole cage was placed in the incubator so that the mother was subjected to the same temperature as the young. Under these conditions the young mice were normally active. The exact influence of temperature will be discussed more fully later.

THE SUCKLING PERIODS

Normally , young mice suckle very frequently. No method has been devised as yet to measure the amount of milk obtained by the young under these conditions. An arbitrary time was adopted for the suckling period, based largely on the convenience of the experimenter. It was decided to make measurements at twelve hour intervals. The suckling periods were arranged at 8:00 o'clock A. M. and 8:00 o'clock P. M. At these two periods the young were weighed, allowed to suckle , and weighed again. The date, time of day, time required for suckling, weight before suckling, weight after suckling, the difference between the two weights, and the number of young in the litter were recorded at each measurement.

THE TIME REQUIRED FOR SUCKLING

The young mice were normally hungry enough to suckle immediately when given the opportunity. The older litters will finish suckling within an hour. The younger litters require more time than this. After a certain time the mother will leave her young. This is taken to mean that the young mice have taken all of her milk. However, it is possible that the mother might leave her young for a variety of other reasons. Since the only way that the operator can tell when the young mice have taken all of their mother's milk is by observing the time at which suckling ceases; it was decided to subject this to test.

Female #9 normally suckled her young for about 30 minutes and then left them. If the young had taken all of the milk that female #9 produced during this period,then there should not have been any futher gain in weight even if they had appeared to suckle again within the next hour, since very little more milk should be available. For eleven successive measurements the young were weighed as soon as their mother left them and the amount of milk calculated on that basis. The young were then returned to their mother, who would immediately nestle over them with every appearance of suckling. The young were allowed to remain with her for the remainder of a two hour period, and then weighed. In ten of the eleven trials the young showed a loss in weight during the second "suckling" period,whilein one trial they showed a slight gain. From these trials it seems that the mother leaves her young usually when she has no more milk for them. An arbitrary limit of two hours has been established at which time the young were removed and weighed, whether they appeared to be nursing or not. The time required for suckling depended upon the individual mother and was, roughly, inversely proportional to the age of the young mice.

THE NUMBER OF YOUNG PER LITTER

The number of young per litter was not held constant during the experiment, for it should be large enough to take all of the mother's milk. There is no direct way of telling whena mouse's mammary glands are empty, as in cattle. Some idea can be gained, however, from the rate at which the young mice gain in weight. The normal average daily increase in weight for young mice during the first 14 days after birth, as indicated by Robertson's ('16) work on growth, is about .5 of a gram. This figure represents litters of various sizes and is probably a fair average where the young have all the milk that they need. In other words, a gain of .5 of a gram per day per mouse would indicate that the young had all the milk they could eat. For a lactating female which produced large quantities of milk a gain of .5 of a gram per day would only indicate that the young had more milk available than they could take. Since there is no way of determining the rate of milk production of a mouse other than by the young, it was decided to keep the daily gain in weight much lower than .5 of a gram. This was done by increasing the size of the litter to such a point that the young would gain less than .5 of a gram per day per young mouse. In many cases it was necessary to decrease the number of young in the litter rather than increase it, since the young would lose instead of gain and would finally become emaciated and die. An average of the daily gains for the experiment proves to be about .2 of a gram per mouse per day. This is much less than the average daily gain where an abundance of milk is available. Such a small increment assured that the young were hungry enough to take all of the milk that their mother had and yet gain enough to keep them active. It was thought best to start each female with a large litter so that she would produce the maximun amount of milk as early as possible. Small litters were, therefore, built up to seven as soon as possible after birth. These litters were then increased or decreased as the individual case demanded.

METHOD OF ADDING YOUNG MICE

It is not difficult to add young mice to the already existing litter if a few precautions are taken. It is advisible to add mice of about the same size as those in the litter. A little "pine oil" rubbed on the hands while handling the young to be added will usually cover up the odor of their former nest. The young mice should be introduced after the suckling period so that they have an opportunity to acquire the odor of the nest before the adult female finds them.

THE INITIAL GROWTH PERIOD

Observations of the feeding habits of very young mice shows that they suckle almost continually. It was found, if the young are removed from their mother at birth and returned only at twelve hour intervals for nourishment, that they die or become very emaciated before a week has passed. The second lactation of females #30-#37 failed for this reason as will be indicated later. To overcome this it was decided to allow the young to remain in the nest with their mother for the first four days. This allowed them to gain strength enough to withstand the twelve hour feeding schedule during the course of the experiment. This four day period will be refered to hereafter as"the initial growth period".

FOOD, NESTING MATERIAL, AND WATER

"Fox chow", manufactured by the Purina Milling Company, was used to feed the mothers throughout the experiment. This is the regular stock ration in this rodent colony and has proven to be a very well balanced diet. It is made in small bricketts which could be placed in the back compartment of the experimental cages. Food was always available. Water was furnished from gravity bottles which were thrust through the tops of the cages. Wood shavings were used on the floor of the cages as litter. Paper shavings were found to be the most satisfactory nesting material.

ISOLATION OF THE FEMALES

The females were isolated in the small experimental cages and placed in the incubator at the temperature mentioned above. The small door between the two compartments was left open so that the female mouse would have access to both compartments. She would usually build her nest in the front compartment. By the time the litter was born she had ample time to become thoroughly acclimated to the new situation.

OTHER FACTORS TO BE CONSIDERED

There is no doubt that psychological factors enter into the experiment. The natural temperament of mice, like people, varies. A part of the task of the operator, then, is to know the individual mice with which he is working and to handle them accordingly. Some mice are very tame and do not object to any type of handling while others must be handled with the greatest of care. A number have been found which can not be used even with the most thoughtful attention. Unnecessary noise or quick movements about the cages should be avoided. All of this is a matter of experience and cannot be adequately described. The whole procedure requires endless patience. It was found that some mice are particularly sensitive to the odor from the hands of the operator on the young mice. This can be easily overcome by rubbing a little pine oil on the hands before attempting measurements.

ERRORS DUE TO URINATION AND DEFECATION

An unavoidable error entered into the experiment due to the unination and defecation of the young mice during the suckling period. The mother mouse licked the young thoroughly while nursing, which evidently stimulated urination and defecation. She appeared to eat these waste products in the process of licking the young so that no quantitative estmate of their weight could be obtained. It is evident that the difference between the weight of a litter before and after suckling would be less than the weight of the milk consumed, by a quantity equal to the weight of the waste products. This error would be quite constant, and always in one direction since it would never tendto increase the difference in weights but rather to decrease it. Such an error would not effect the results since only the relative ability of mice to lactate was being studied. It was, therefore, disregarded.

THE ROUTINE OF A LACTATION

It seems advisable to describe in detail the method of carrying out the process outlined above. The female mice to be tested were bred and then isolated in the experimental cages. These cages were then placed in the incubator as described. The small door between the compartments was left open so that the adult mouse could build her nest in the front compartment. The cage was then inspected each day and the date of birth and initial number of young were recorded. The size of the litter was raised to seven if smaller than that number. Twelve hours before the initial growth period was over, the small door between the two compartments was closed so that the mother could not suckle the young. On the fifth day the young were removed from the cage and weighed at the regular time. The gate between the two compartments was opened and the mother would usually enter the front compartment without further persuasion. The gate was then locked shut and the young returned to the front compartment with their mother. As soon as suckling was completed, or at the end of two hours as the case may be, the gate was opened again and the mother driven into the back compartment. The gate was locked shut again to prevent her return. The young were then removed and weighed again. The records were written up at this time, as described above. Any adjustment in the size of the litter, revealed by a study of the gain in weight of the young mice, was made before returning the young. They were then returned to the front compartment until the next suckling period. This process is repeated for each mouse every twelve hours for the duration of the test. The precautions mentioned above must be observed throughout the experiment.

Part III

THE GENERAL CONSIDERATION OF LACTATION

Studies of the rate of milk production have been confined largely to cattle. The author has been unable to find any conclusive quantitative studies on milk production in any other animal, excepting the recent paper by Enzman('33) dealing with albino mice. A number of authors have tried to estimate the relative rate of milk secretion in such animals as rats, mice, and rabits. All of these studies were made in connection with studies of the growth of the young. Robertson ('14) in his growth studies assumed that the differing growth rates of litters of mice of the same size must be due to differences in the amounts of milk given by their mothers. In cattle, Brody, Ragsdale, and Turner ('23) fouend that the curve of the production of milk rose gradually until the thirty-fifth day, onthe average, and then declined gradually until the end of the lactation period. They found a close correlation between the character of the rise of the curve and the curve of monomolecular reaction in chemistry. The period of decline in the curve they found to be similar to the curve expressing the formula of recurrent reactions. Weatherford[(29)] used the method of Golgi, which correlates the cytological picture of the cell with the rate of secretion. He found that in mice the quantity of milk produced increased until the tenth day and then gradually decreased. His work does not indicate what the total length of the lactation period might be. McDowell, Gates, and

McDowell ('29), and Parks ('26) and Enzman and Pincus ('32) have assumed that the rate of milk production in the small rodents could be calculated from the rate of growth of the litters of young mice consumming the milk. The validity of this assumption will be considered later. It will suffice to say here that such a method does not take into consideration the natural differences in the ability of various nursing animals to utilize food. Such differences would be expressed in the rate of growth of individuals on the same diet and under exactly the same conditions.

Turner correlated the general morphology of the whole mammary gland with the rate of milk production. By making whole mounts of the mammary glands of lactating mice he was able to study the number of lobules present in the entire gland. He assumed that the number of lobules present was proportional to the rate of milk secretion. His results agree with those of Weatherford.

Part IV

A TEST OF THE STANDARD TECHNIQUE

Enzman ('33) was able to obtain quantitative measurements of the milk of albino mice. His results were obtained by the use of a technique much like that described above. His method differed from mine in that he allowed the young mice to eat solid food as soon as they would. Thus, the young were weaned during the third week. His records were taken over a period of twenty-one days. During the third week it was necessary to correct his gross measurements for the amount of solid matter eaten by the young mice. Such a procedure introduces unnecessary complications, which the author belives are overcome in the technique described above. The type of curve obtained by Enzman agrees quite well, for the 14 days, in which mice receive only milk, with those to be presented in the following discussion.

The problem to be discussed in the remainder of this paper must be clear at the outset. It is the desire of the author to devise a technique that is reasonably accurate and then to test that technique on a group of mice. It's application over a number of successive lactation of the same mice should demonstrate the reliability of the method. Gowen has shown that the amount of milk produced by a cow increases until she is about eight years old, or the average. Similar variations would be expected in the successive lactations of mice, so that it would be unreasonable to expect a mouse to produce the same quantity of milk in her first, second, third, and fourth lactation. However, if a group of females are considered, the same order of yields should be expected over successive lactations, providing that the method used regulates the environmental factors closely enough. But the conditions effecting the lactation of mice are not well known, so that we could not expect too much from any technique until all of the environmental factors can be controlled.

Therefore, if the conditions of the external environment are carefully controlled, and the data collected by the use of the technique mentioned should place the members of a group of mice in the same relative order with reference to the amount of milk produced, then the technique used would be proven sufficiently accurate. Data will be presented in the following pages to show that our method meets these requirements.

No attempt was made in this experiment to measure the maximum milk production of mice. Our object was to determine production under the specific conditions of the investigation. The external environmental factors were kept as constant as possible throughout the trials.

No breeding experiments of a genetic nature have been carried out to date. It is hoped that the invention of a well proven technique will make it possible to discover the genetic mechanism of lactation in mice.

Part V
THE ANIMALS USED

The animals used in the course of the experiment are fully described in Table # I. The bulk of the data to be presented was obtained from the twenty animals listed. Some additional remarks will be made on the first lactations of other animals not indicated in the table. These particular mice were selected on the basis of their availability at the beginning of the experiment, with no preconceived idea of their preformance. Each animal was ear marked so that it could always be identified.

Females #1-#12 inclusive were treated as one group, females #30-#37 as a second group. Measurements were made on all of the members of a group at the same time, so that the conditions of the experiment would be as nearly alike as possible.

Part VI

A RESUME' OF THE EXPERIMENT

The first, second, and fourth lactations of group #1-#12 were carried out strictly according to the standard technique. During the third lactation of these females a temporary incubator was constructed by enclosing a section of the shelves with a sheet of heavy canvas. Two large electric light blubs were placed in this enclosure to supply the necessary heat. While the third lactation was still in progress, the outside temperature became high enough to permit the opening of the doors and windows of the colony house during the day. No thermostatic control was provided for the temporary incubator and the temperature fluctuated within wide limits. This arrangement provided extra space but proved to be a poor substitute since it was subject to great variations in temperature. The results obtained during the third lactation will be considered later.

The first and fourth lactations of females #30-#37 were carried out as required by the standard technique. During the second lactation an attempt was made to shorten the initial growth period to two days, with the result that the young mice failed to suckle properly. The second lactation failed completely from this reason. The third lactation of group #30-#37 was carried out at room temperature instead of 28^{o}-30^{o}C., which proved to be too low and resulted in failure again.

Since only the relative ability of mice to produce milk was being studied, no attempt was made to obtain maximun milk production. Special feeds were not used since the conditions were kept as nearly normal as possible.

Part VII

LACTATION IN MICE

Tables #2, #3, #4, and #10 represent the records of females #2-#12 inclusive for the first, second, third, and fourth lactations. Each entry represents the sum of two measurements, one taken at eight o'clock in the morning and the other at eight o'clock in the evening. No corrections of any kind have been applied to the figures. In a few instances measurements were not obtained for reasons beyond the control of the operator. Such omissions in the records were estimated by taking the average of the four adjacent measurements from the record book. This is accepted as good practice in the keeping of dairy records.

The length of the individual records is quite variable. It was originally planned to continue the measurements over a period of only fourteen days. The reason for this decision will be considered later. In practice, however, the measurements were continued as long as the cages were not needed for the next series of mice. The result of this practice was the accumulation of a number of complete lactation records.

No records were obtained for females #4, #5, #6, #7, #11, and #12 during the first lactation. Litters were born to these females, but they did not suckle normally. No clue can be gained from the records for these failures since the females in question did not lactate. Females #4, #7, #11, and #12 were only three months old when introduced into the experiment. Experience has shown that it is often impossible to use animals of this age because of their excitable nature.

Female #1 died during the first lactation. Her records are not included. Female #4 died in the interval between the second and third lactations, therefore her record is missing from the third and fourth lactations. Females #11 and #12 died during the interval between the third and fourth lactations and their records are not included in the fourth lactation.

Figures #1-#10 represent graphically the production of ten females whose records are complete. Figure #11 shows graphically the daily mean production for all animals in each lactation. It must be remembered that measurements were not begun until the fifth day, so that the first day indicated on the graphs is the fifth day after partuition.

A study of figure #11 will show that the average production starts on the fifth day with from one to two grams of milk. The rate of production rises rapidly until the maximum is reached between the sixteenth and nineteenth days. A maximum average daily production of 3.896 grams was reached on the sixteenth day of the first lactation. From this peak production the curve slope gradually downward to the end of lactation between the thirty-ninth and forth-fourth days.

The production curves of individual mice show considerable variation from the mean. The variations in the production from day to day are very striking. The general shape of the production curve, the time at which the peak production is reached, the duration of lactation, and the total amount of milk produced, also show considerable variation.

While these daily variations are very striking, it is interesting to note that there are similarities between the successive records of certain mice. Complete lactation records are available for three females for two successive lactations. Figures #8 and #9 show the records of the second and third lactations of female #11. The general shapes of the two curves are much alike. In the second lactation the peak was reached on the twenty-first day, which was two days later than the peak for the whole population in the second lactation. In the third lactation the peak was reached on the seventeenth day, which was one day later than the mean peak for the third lactation. Lactation ceased on the fortieth day for the second lactation and on the forty-third day for the third lactation. The total amounts of milk were 91.99 grams for the second lactation and 89.63 grams for the third lactation.

The production curves of female #8 for the second and third lactations, figures #5 and #6, show no similarity as to general shape and duration of lactation. The time at which the peak production was reached was in each case one day later than the mean of the group. The total amounts of milk produced were 45.59 grams for the second lactation and 43.26 grams for the third lactation. The records of the second and third lactations of female #5 do not show such similarities.

Figure #2 showing the third lactation of female #6, and figure #10 showing the second lactation of female #12 are of interest, since the general shape of the curves and the durations of lactation are so very different. The curve for female #6 covers a period of 37 days but at no time exceeds the mean of series #2-#12 females in their third lactation by more than .54grams. The curve for female #12 is for only 24 days but exceeds the mean of females #2-#12 for their second lactation by 2.36 grams at the highest point. The total amounts of milk produced by the two females are very nearly equal, that of female #6 being 57.21 grams and that of female #12 57.51 grams.

The production curve of female #10 for the second lactation, figure #7, is of the same general shape as that of the mean for that lactation.

The complete production curves are of interest since they show the entire course of the process of lactation. Young mice used in the experiment were kept dependent on their mother's milk for nourishment so that she would produce milk as long as she was able. The extreme variability of that part of the production curve after the twentieth day is to be expected since neither natural nor artifical selection has acted upon it. The duration of lactation in mice would be of the same nature since selection has acted only upon that part of the curve during which the young normally suckle.

A study of the complete production curves will show that there are a number of marked depressions in the curve of each animal. These points of depression tend to recur at rather regular intervals. The rhythmical character of the curve suggests that cyclic influences might effect the production of milk. The nature of such forces will be discussed later.

Considering it's size, the mouse shows a remarkable ability to produce milk. Female #11 gave 6.36 grams of milk in one day. At the age of three months she weighed 31.61 grams.

A daily production of 6.36 grams represents 20.1% of her body weight. An inspection of the records will show that daily records of more than four grams are very frequently obtained. Enzman records a few daily records of more than 7.00 grams. This is not surprising when one considers that the weight of the suckling mice usually exceeds that of the mother before the nursing period is over.

The records presented agree very well with those given by Enzman ('33). His records are, as a whole, slightly higher. He made measurements at six hour intervals, which might account for the increase in production. Observations on the suckling habits of young mice indicate that they nurse very frequently. It is possible that the total amount of milk produced by a mouse lactating under normal conditions would be much higher than that indicated by the records by Enzman and ourselves. This is known to be the case in cattle, and milkings are made more than twice a day when the highest possible amounts of milk are desired. In this experiment it was not thought necessary to make measurements more often since only the relative ability of mice to lactate was to be studied.

From the records shown, it seems that the amount of milk produced by mice is a measurable quantity. This being so, there is no reason why mice can not be used in a genetic study of lactation. The greatest difficulty lies in understanding and controlling the external and internal environmental influences affecting the process of lactation.

Part VIII

A STUDY OF SUCCESSIVE LACTATIONS

THE CHOICE OF COMPARABLE SAMPLES

The length of time required to obtain complete lactation records for a large number of animals over a number of lactations, has made it advisable to consider the reliability of comparable lactation samples. The seven day yield, properly timed and supervised, has been used with much success in cattle. It seemed reasonable to adopt some similar scheme here.

The first fourteen days of measurement, that is, from the 5th-18th days after partuition were selected as a sample period for the following reasons:

1. During this period the young mice are normally dependent upon their mother's milk for nourishment. This period would, then, be free from any complications due to feeding on solid food while still nursing.

2. This sample covers the time of rise in production. The peak is reached between the sixteenth and nineteenth days.

3. This period is a fair sample of the whole lactation. If the total milk production of the animals, whose complete lactation records are shown in Figures #1 to #10 are arranged in order of decreasing quantities of milk produced, this order will correspond exactly to the order of their fourteenth day samples, with the exception of female #4. This is shown in Table # XIV.

COMPARISON OF FOURTEEN DAY SAMPLES FOR SUCCESSIVE LACTATIONS

A test of the adequacy of the standard technique (previously described) to measure genetic differences between animals, would be to determine whether under constant environmental conditions a group of females maintained their relative positions in successive lactations. Data will be presented to show that the method describe in the section on technique actually reveals these inherent differences.

A number of the animals used failed during one or more of the lactations. As a result, the records of only five animals are complete for four successive lactations. The data for these five are shown in Table # VI, where they are arranged in the order of decreasing quantities of milk produced during each lactation.

The order of the animals in Table # VI is constant for the first, second, and fourth lactations with the exception of the position of females #3 and #10, which have changed places from the first to second lactation. These three lactations were carried out under the exact conditions of the standard technique. The order in the third lactation is altered considerable. The possible reasons for this change of order will be discussed under the heading of " The Effect of Temperature on the Experiment". It should be said here, however, that the third lactation was not carried out strictly under the conditions specified in the standard technique. Even under the altered situation in the temporary incubator, the changed order of the five animals is explainable on the basis of temperature variations within the incubator.

A study of the five females whose records are complete for four successive lactations shows that these mice produced their greatest quantities of milk during their second lactation. The table below shows the mean daily production of each of these five mice for four successive lactations. The data is taken from the fourteen day samples of their records.

Lactation	♀#2	♀#3	♀#8	♀#9	♀#10
First	1.930	2.012	1.528	3.407	3.257
Second	2.102	3.038	1.848	3.664	2.706
Third	2.105	1.214	1.126	1.408	3.088
Fourth	1.662	2.432	1.406	2.901	2.103

This table shows that mice #2, #3, #8, and #9 produced their greatest quantities ofmilk during the second lactation. Mouse #10 produced the greatest amount during the first lactation.

The rate of milk production varies in mice in successive lactations. While the number of animals considered is too small to be conclusive, it appears that the greatest quantity of milk is produced in the second lactation. Gowen has shown that cows produce their greatest quantity of milk at 8.25 years of age,on the average.

Figures #12 -#21 show graphically the fourteen day samples of females #2-#12 for their first, second, third, and fourth lactations. Several of the records are missing for the reasons indicated in Table # I and in the text. The shapes of the curves produced on successive lactations by a mouse are quite different when only the fourteen day samples are considered. The daily variations are very great but no variations of a cyclical nature are observed. It is evident from these curves that the daily fluctuations are not due to pecularities

in the individual mouse which occur in the same fashion in successive lactations. It is interesting to note, in spite of the extreme variations, that the total quantity of milk produced during the fourteen day sampling period places the mice in the same order over successive lactations. This is shown in Table #VI.

The females of the #30-#37 series show similar results. The first lactation of these animals is shown in Table # XI. The second lactation failed due to the reduced initial growth period. The third lactation failed because the temperature of the room was too low. The fourth lactation included only females #30 and #32. The others having died or failed to breed. The 14 day samples of females #30 and #32 are shown below for the first and fourth lactations, which were carried out under comparable conditions.

Female	First Lactation			Fourth Lactation	
	Total	Mean		Total	Mean
#30	27.82	1.987	(grams)	26.51	1.893
#32	23.17	1.655	(grams)	24.56	1.754

This table shows, even after the failure of the second and third lactations, that an animal will preform in same manner in successive lactations if the conditions of the standard technique are followed. The agreement between the first and fourth lactations is very close.

The data presented above, though limited, shows that the technique described controlls the external environmental conditions well enough to permit constant preformance over successive lactations.

Part IX

RESULTS OBTAINED FROM MICE #30-#37

Table # XI shows the records of females #30-#37 inclusive during their first lactation. The history of these animals is shown in Table #I. They were subjected to the usual conditions of the experiment during the first lactation with very satisfactory results.

The temperature was maintained at 28°-30° C. in the incubator and the initial growth period was maintained at four days.

During their second lactation the initial growth period was reduced to two days instead of the customary four day period. All other conditions were kept the same as in the first lactation. As a result of the reduced initial growth period, the young mice became very emaciated and did not suckle well. The results obtained were very unreliable and measurements were attempted for only 7 days.

During the third lactation of females #30-#37 the conditions of the experiment were maintained as usual, except that the experimental cages were kept at room temperature. The temperature of the rodent colony is thermostatically maintained at about 74° F. (23½° C.). Some small variation may take place due to the opening of doors and windows. As a result of the reduced temperature the young mice became emaciated and failed to suckle properly.

Unfortunately, only the records of females #30 and #32 were available for the fourth lactation. The other members having died or failed to breed as indicated in Table # I.

The fourth lactation was carried out under the standard conditions with an initial growth period of 4 days and a temperature of 28°-30° C. The records of females #30 and #32 are given in Table # XII. They compare very favorable with performance in the first lactation as is hown in Table # VI.

The data obtained from females #30-#37 are of special value since they show that the standard conditions described on page 5 must be closely adhered to, if reliable results are to be obtained. They prove that the technique is fundamentally sound and that a mouse will preform in essentially the same way over successive lactations if the conditions of the experiment are kept uniform as described under "Technique".

Part X

THE EFFECT OF TEMPERATURE ON THE EXPERIMENT

The conditions of the standard technique, described in the section above, specify a temperature of 28°-30° C. for nursing mice. During the course of the work, deviations from this standard have taken place. The results obtained under these variations in temperature are very interesting since they point out the necessity of maintaining the conditions as specified.

The third lactation of females #30-#37, inclusive, was carried out at room temperature which is maintained automatically at about 74° F. (23½° C.). Some slight variation in the room temperature doubtless took place. It was pointed out in the preceding section that the third lactation of females #30-#37 failed. Since mice in the regular stock cages at room temperature lactate sufficiently to raise normal litters, it could hardly be assumed that the reduction in temperature effected the lactating females. The young mice, however, were kept away from their mothers for eleven hours at a time so that they receive no heat from her body. They felt cold when handled and their movements were very sluggish, so that no doubt this sluggishness prevented their nursing properly when the mother was introduced. The young are not protected much by hair until about the tenth day.

The third lactation of females #2-#12 was carried out in an improvised incubator which provided more adequate space. The necessity of <u>accurate</u> control of temperature was not appreciated fully until this lactation was finished.

This lactation was begun about April tenth and continued until May twenty-fifth. During this time the weather became warm enough so that the steam could be turned off and the windows left open part of the time. The temperature of the room was then affected by the outside temperature. The incubator was heated by a large electric light bulb which worked very well when the room temperature was constant, but which offered no means of compensating for variations in the room temperature. The exact range of temperature variation in the improvised incubator is not known since the lowest temperatures came during the night. Observation during the day showed variations from 23° C. to 32° C.

Table # IV and # V show the results obtained from the third lactation of females #2-#12. Table # VI shows that females #2 and #10 maintained a production similar to the first and second laction, while females #9, #3, and #8 showed marked decreases in production. The cages of females #10 and #2 were very close to the electric bulb which was used to heat the temporary incubator. This doubtless explains their failure to show decreases in production. Females #9, #3, and #8, whose cages were some distance from the source of heat, show decreases. It is interesting to notice, however, that the females showing decreases in production, fall into the same order in Table # VI as in the first and second lactations, but are lower in the series. Female #10 produced more than female #2 in the first and second lactations. This is also true of the third lactation, although their positions with reference to the other animals is changed.

It may be concluded that temperature is an important factor in the technique described. Deviations from the standard temperature of 28°-30° C. have been shown to produce fluctations in the amount of milk obtained. It is probable that the reduced temperature effects the suckling mice rather than the lactating females by chilling them until they do not nurse properly.

Part XI

THE DAILY GAIN IN WEIGHT OF THE YOUNG MICE AS AN INDICATION OF THE RATE OF MILK PRODUCTION

A number of investigators, MacDowell, Gates and MacDowell ('29), and Enzman and Pincus ('32) have assumed that the daily gain in weight of the young mice might be taken as an indication of the amount of milk produced by their mother. The studies refered to above were not made directly upon the lactation of mice.

If such a relationship existed, there should be a constant relationship between the total gain in weight of the young mice and the total amount of milk consumed. This would be true only if the total number of days considered were the same in each case and if the number of young in the litters considered were constant.
Or expressed mathematically--

$$\frac{\text{The total gain in weight of the young mice}}{\text{The total amount of milk consumed}}$$

would equal a constant (k) if the assumptions were correct. The available records are tabulated in Table # XIII. Only litters in which seven young were carried through 14 consecutive measurements are included. The weight of the young at the first weighing varied but little.

The value (k) varies within wide limits, showing that no constant relationship exists between the gain in weight of the young and the amount of milk consumed.
This variation is suggestive of a difference in the ability of young mice to turn food consumed into tissue.

The records of females #12 and #10 show this clearly. Female #12 produced 37.21 grams of milk in 14 days. Her 7 young gained 13.01 grams. Female #10 produced 37.91 grams of milk in 14 days and her young gained only 9.77 grams.

Part XII

THE EFFECT OF OESTRUS UPON LACTATION

Figures #1-#10 represent the record of one complete lactation for each of ten females. These curves show depressions which tended to recur at more or less regular intervals after the peak production was reached. Figure #4 illustrates this point particularly well. The peak was reached on the sixteenth day, which was followed by a gradual decline until the nineteenth day. A sharp drop in production took place on the twentieth and twenty-first days, followed by a sharp rise. Another depression appeared on the thirtieth and thirty-first days, and on the thirty-ninth days. Lactation stopped on the forty-second day. The interval between these recurring periods of depression was about ten days, which suggests that a cyclic influence may be the cause.

Figure #9 shows similar recurring depressions on the twenty-second, thirty-third, and fourtieth days. The regular low points are not so clearly shown in the remaining figures though similar tendencies can be made out in most cases.

In each of these ten figures a low point is reached at some time between the twentieth and thirtieth days, which is followed and frequently preceded by other similar depressions.

Except in the case of Figure #2, the highest point in a curve is followed by a sharp decline within a very few days.

The cyclic nature of these depressions suggests that they might be correlated with the oestrous cycle. Allen ('22) studied the oestrous cycle of normal mice and found that the stages of the cycle could be detected by daily microscoöpical examinations of the vaginal contents. Parks ('26) applied Allen's method to lactating mice. He found, when the young were weaned normally, that oestrus recurred "at about the third week", but if lactation was continued by giving the female a second litter, oestrus reappeared from the twenty-fifth to the fortieth days. McClandish ('26) showed that the quantity of milk produced by cattle decreases during the period of heat.

Allen made daily vaginal smears which he stained with eosin and hemotoxylin. Briefly his observations revealed the following periods in oestrus.

Di-oestrus:-Epithelium (chiefly nucleated) and leucoytes are present during this stage. The vaginal smear tends to be stringy.

Pro-oestrus:-Lightly staining nucleated epithelial cells predominate the picture. During this stage the vaginal contents are serous in nature.

Oestrus:-Non-nucleated, eosinophilous, cornified cells constitute the smears during this stage. As this period begins, the vaginal epithelium may be dry; subsequently the contents of the vagina become granular.

Meta-oestrus:- The oestrus period is followed by a large infiltration of leucocytes which gradually removes the cornified elements. During this stage the vaginal content becomes first pasty and the milky.

During the fourth lactation of females #2-#12 daily vaginal smears were made and stained with eosin and hematoxylin. Plate # II shows the typical di-oestrous smear with its scattered polymorphonuclear leucocytes, moderate quantities of mucus, and epithelial cells in various stages of degeneration. A pro-oestrous smear is presented in Plate # III which shows the absence of polymorphonuclear leucocytes, and numerous lightly staining epithelial cells. The typical oestrous smear is shown in Plate # IV containing nothing but cornified, heavily eosinophil, non-nucleated cells. Plate # V is from a meta-oestrous smear. In this case polymorphonuclear leucocytes are very numerous in the later stages but scarce immediately following oestrus, while the epithelial cells during the early stages are almost entirely cornified, non-nucleated, and eosinophil.

Plates #IV , #VII, #VIII, #VI, #IX, and #X show the first oestrus smears of females #8, #3, #5, #2, #9, and #10 repectively. Figure #21 shows the milk production curves and Table #X the records of these mice during their fourth lactation. In Figure #21 the days of oestrus are marked by small circles. Oestrus occured in these animals from the twenty-fourth to the thirty-first days. In each case the oestrus followed shortly after a high point in the curve, and occured at a low point. These figures agree very well with those given by Parkes, and according to him, oestrus should recurr again within seven days, on the average.

The evidence presented here suggests that after the twenty-fourth days, the recurring oestrus is correlated with the periodic depressions in the milk curve. This may explain the cyclic variations seen in Figures #I-#X.

Many other daily variations are present which cannot be explained now, unless they are normal for mice.

The figures given above were obtained from a study of 250 stained vaginal smears, from females #2, #3, #5, #8, #9, and#10 and from a number of unmated females.

It is interesting to note in the cases of females #9, #5, and #3, that the decline in the quantity of milk produced preceded the oestrous period. The forces which cause decreased milk secretion, then, must be separate from those causing oestrus, for otherwise the two events would always occur simultaneously. It is probable that both oestrus and the decreased mammary activity are the results of a series of events involving the anterior pituitary gland and the ovary.

Part XIII

DISCUSSION

The purpose of this work was to develop a technique for measuring the milk production of mice and then to apply that technique to a number of animals over successive lactations. The procedure described in the section on "Technique" outlines the method used.

The application of the standard technique has produced the results discussed above. It is regretable that the number of animals used was not larger but the time and equipment necessary to carry out the experiment limits the number of animals that it is possible to handle. The method described must be followed closely. Two measurements must be made <u>every</u> day at the prescribed hours if success is to be attained.

The results of Enzman's ('33) experiments are comparable to those presented above, only in a limited way. He was studying the amount of milk given by a mouse during a normal lactation when the young were permitted to eat solid food as soon as possible. He did not attempt to study the complete nor the successive lactations of mice. The curves presented by him are, when smoothed, of the first order. The production of milk rises gradually to the tenth day on the average and then declines to zero at about the twentieth day.

The results of the technique described above show a somewhat different type of curve, since the whole lactation is considered. The production of milk rises rather sharply to the 16th or the 19th day and then declines slowly to zero at some point between the twenty-seventh and fortieth days, depending upon the persistency of lactation. The peak production is reached somewhat later in this experiment than in Enzman's work. The reason for this difference is not evident. The young mice would need the greatest amount of milk at about the 13th to the 20th days when their eyes are opening and before they take enough solid food to satisfy their needs. The results obtained from these experiments show that the peak production is reached during that period.

The daily variations in milk production are very great in the results described above and by Enzman. Other than oestrus, no reason can be given for these variations at present. It may be that such variations are normal events in the lactation of mice, or they may be due to some environmental factors which are not properly controlled by the standard technique.

Enzman presents several very smooth curves for females suckling only four mice. It is possible, however, that these curves represent the maximum rate at which the young mice consume milk rather than the rate at which the female could produce it. Since the average size of litter is between 7 and 8 young mice, it is possible that the females producing these smooth curves were capable of secreting more milk than four young mice could consume. The curves in this case would represent the rate of milk consumption of four young when an abundance of milk was available. This consideration emphasizes the necessity of regulating the size of litter as is described in the section on "Technique".

The use of 14 day samples for comparing the production of mice is equally applicable to Enzman's data, since his curves are of the first order. Such a method of sampling makes it unnecessary to carry animals through the complete cycle of lactation to determine their relative worth. The method of sampling is surprisingly accurate and very convenient.

It may be desirable at some future date to study the persistency of lactation in mice. The technique described will make such a study possible. Interesting variations in persistency will be noticed in the complete production records presented in Figures #I-#X. Among these ten animals the persistency of lactation varies from 27 to 43 days, with as average of 36.9 days for the group.

The mean daily production among these mice, as estimated from the 14 day test, ranged from .665 grams (female #30, first lactation) to 3.664 grams (female #9, second lactation).

Similar variations can be seen in the duration of lactation, the time at which peak production is reached, and the general shape of the curve produced. If the technique is sufficiently accurate these variations may be looked upon as individual differences. Consideration of the Figures #I-#X would tend to substantiate this view, as mentioned before.

The available data shows that the greatest quantity of milk is produced during the second lactation.

The recurrence of oestrus after pártuition has been shown to exert a cyclic influence upon the quantity of milk produced. It is doubtful whether oestrus itself effects the mammary gland in such a way as to cause a decrease in milk secretion, but rather both events are the result of a series of influences involving the anterior pituitary gland, and the ovary. This view is substantiated by the records of females #9, #5, and #3 as shown in Figure #XXI. In these cases the decline in milk production preceded the actual occurance of oestrus by several days showing that the force which caused the decrease in milk preceded that which caused oestrus.

The technique described and tested, should be sufficiently reliable to permit the use of mice in future studies on lactation. The application of this technique may make it possible to establish a situation among mice similar in every desirable feature to that in cattle. The rapidity with which mice reproduce, the large size of litters, the ease with which they can be raised and studied, should make such a type of experiment exceedingly helpful in studying the complicated phenomena of lactation.

Finally, the author hopes to be able , by the use of the information presented, to work out the genetics of lactation. Much information concerning the physiology of lactation and reproduction will have to be gathered before this study can be completed. The only aim of this paper was to develop and study a means by which this future work may be accomplished.

Part XIV

CONCLUSIONS

1. A technique has been presented by means of which the entire milk production of mice can be determined.

2. By means of this technique several mice have been carried through four complete lactations.

3. It has been shown that a group of mice will produce milk in the same relative quantities in successive lactations if the conditions of the external environment are controlled according to the standard technique.

4. Ten complete lactation curves are presented which show that lactation may be continued as long as 43 days. The persistency of lactation varies from 27 to 43 days with an average of 36.9 days for the group of animals.

5. The fourteen day sample has been developed and proved to be a fair sample of the entire lactation.

6. Mice produce the greatest quantity during the second lactations.

7. The recurrence of oestrus after partuition has been shown to correlate with rhythmical depressions in the lactation curve.

8. Oestrus appeared between the twenty-fourth and the thirty-first days.

9. In three cases the decline in milk secretion preceded oestrus by several days so that the influence, causing the depression in the curve must be separate from that causing oestrus.

BIBLIOGRAPHY

Agar. W.E., 1926 Heredity and milk production. An analysis of the inheritance of milk and butterfat production in red poll cattle. Victoria (Australia) Dept. of Agric. Jour. 24, pages 1-9.

Allen, Edgar, 1922 The oestrous cycla in the mouse. Am. Jour. of Anat. 30:297-321.

Brody, S., Ragsdale, A.C., and Turner, C.W., 1924 The relation between the initial rise and the subsequent decline of milk secretion following partuition. Jour. of Gen. Phys. 6:541-545.

Enzman, E.V., 1933 The milk production curves of albino mice. Anat. Record 65:345-358.

Enzman, E.V. and Pincus, G., 1932 The effect on lactating mice of injecting an extract of the urine of pregnancy. Am. Jour. of Phys. 103:30.

Fohrman, M.H., 1926 Official records as material for studying inheritance of milk and butterfat production. Jour. of Dairy Science 9:286-292.

Gates, W.H., 1925 Litter size, birth weight, and early growth rate of mice (Mus musculus).Anat. Record 29:183-193.

Goodale, H.D., 1927 A sires breed index with special reference to milk production. Am. Naturalist, 61:539-544.

Gowen, John W., 1924 Milk secretion. The Williams and Wilkins company, Baltimore.

Gowen, John W., 1926 Studied in milk secretion XVIII. Transmitting qualities of guernsey sires for milk yield, butterfat percentage, and butterfat. Maine Agric. Exp. Station Bulletin # 329, page 1-48.

Graves, R.R., 1926 Transmitting ability of 23 Holstein-Friesian sires.U.S. Dept. of Agric. Bulletin 1372.

Holt and Howland, 1922 Diseases of Infancy and Childbirth. (quoted from Enzman)

MacDowell, E.C.,Gates, W.H., and MacDowell, C.G., 1929 The influences of the quantity of nutrition upon the growth of the suckling mouse. Jour. of Gen. Phys. 13:529-545.

McClandish, Andrew C., 1926 The influences of the period of heat on milk production. Jour. of Dairy Sci. 9(1):65-67.

Parks, A.S., 1926 Observations on the oestrous cycle of the albino mouse. Proceeding of the Royal Society of London, series B, 100:151-199.

Robertson, T.B., 1916 Growth studies on mice. Jour. of Bio. Chem. 24:347-383.

Robertson, T.B., 1917 Growth studies on mice. Jour. of Bio. Chem. 31:567-514.

Turner, C.W., 1932 The mammary glands. page 544. Sex and internal secretion. Williams and Wilkins Co.

Weatherford, H.L., 1929 A cytological study of the mammary gland: Golgi apparatus, trophospongium and other cytoplasmic canaliculi, mitochondra. Am. Jour. of Anat. 44:199-282.

Part XVI

Table # I

Animals used in the experiment

Female	Strain	Age at begin. of exp.	Wt. in grams	Remarks
#1	F_1 from P_1 cross of	5 mo.	29.12	died during first lactation
#2	dark eyed black	5 mo.	28.78	
#3	agouti ♀ X	5 mo.	28.13	
#4	chinchilla ♂	3 mo.	27.14	died between second and third lactation
#5	"	5 mo.	30.38	
#6	"	5 mo.	30.40	
#7	"	3 mo.	32.02	
#8	"	3 mo.	30.40	
#9	"	3 mo.	29.15	
#10	"	3 mo.	29.91	
#11	"	3 mo.	31.61	died between second and third lactation
#12	"	3 mo.	30.23	Developed tumor. killed between second and third lactation
#30	dark eyed white	4 mo.	34.10	
#31	"	4 mo.	28.20	failed to breed for fourth lactation
#32	"	4 mo.	28.43	
#33	dark eyed black	3 mo.	25.37	failed to breed for fourth lactation.
#34	dark eyed black agouti	3 mo.	28.92	failed to breed for 2 nd& 3rd lactation.
#35	pink eyed dilute brown	5 mo.	29.17	developed infection in ear. Killed during fourth lactation.
#36	dark eyed white	4 mo.	27.18	died during third lactation.
#37	"	4 mo.	29.13	died during third lactation.

Table # II

Daily production records of females # 2-#12 for the first lactation.

Days	♀#2	♀#3	♀#4	♀#5	♀#6	♀#7
5	.46	.78	F.*	F.*	F.*	F.*
6	.68	.29				
7	.78	.29				
8	1.07	1.15				
9	1.79	1.18				
10	1.24	1.68				
11	1.00	1.82				
12	1.52	2.10				
13	1.05	1.29				
14	2.82	3.90				
15	3.48	3.10				
16	3.76	3.84				
17	3.65	3.25				
18	3.73	3.51				
19	2.96	2.92				
20	3.27	3.23				
21						
22						
23						
24						
25						
Total	33.26	34.28				
Mean	2.08	2.14				

* See Table # I

Table #II Continued

Daily production records of females #2-#12 for the first lactation.

Days	♀#8	♀#9	♀#10	♀#11	♀#12	Mean
5	.59	1.31	2.28	F.*	F.*	1.084
6	.08	1.33	2.30			.936
7	.46	2.04	2.68			1.250
8	.54	2.26	2.73			1.550
9	.80	4.48	3.08			2.338
10	1.15	2.78	3.64			2.078
11	1.55	3.04	3.86			2.264
12	1.65	4.63	2.99			2.578
13	2.16	4.69	3.85			2.608
14	2.34	4.57	3.08			3.342
15	2.48	3.95	3.45			3.292
16	2.62	4.64	4.62			3.896
17	2.41	4.26	3.90			3.494
18	2.56	4.36	4.14			3.660
19	3.23	4.53	3.05			3.332
20	3.44	5.41	1.82			3.434
21	3.16	2.64	.57			2.123
22	1.85	1.86				1.855
23	2.22	3.68				2.950
24						
25						
Total	35.30	66.82	52.06			
Mean	1.85	3.51	3.06			

* See Table#I

Table #III

Daily production records of females #2-#12 for the second lactation.

Days	♀#2	♀#3	♀#4	♀#5	♀#6	♀#7
5	1.64	1.46	2.39	1.80	2.08	1.42
6	.48	.99	1.17	.99	1.42	1.23
7	.81	1.43	2.17	.97	.72	1.55
8	1.68	2.31	2.14	1.01	2.52	1.04
9	.37	2.83	2.57	.66	2.82	.81
10	1.23	3.97	4.41	.56	2.59	1.55
11	2.78	4.59	4.07	.26	2.92	.81
12	2.90	2.95	2.83	.14	2.86	4.27
13	2.72	3.46	2.86	.40	2.15	3.71
14	2.84	3.81	.98	.62	2.20	3.94
15	3.14	4.22	2.51	1.86	3.14	3.19
16	3.36	4.29	2.66	1.96	3.38	2.26
17	2.90	3.98	2.83	.67	2.28	.48
18	2.80	2.26	2.14	1.53	2.72	.00
19	2.22	1.83	2.52	2.74	3.00	
20	.75		2.50	3.13	3.93	
21	3.10		1.98	2.66	1.77	
22	2.18		1.75	.51		
23	3.07		.56	1.88		
24	1.88		1.59	1.82		

Table #III Continued

Daily production records of females #2-#12 for the second lactation.

Days	♀#2	♀#3	♀#4	♀#5	♀#6	♀#7
25	2.60		1.47	2.49		
26	1.11		1.86	2.40		
27	1.29		2.31	1.45		
28	1.77		.48	.90		
29	2.15		.13	.79		
30	1.45		.51	.42		
31	2.32		.41	.73		
32	.57		.52	.98		
33	.01		.41	.68		
34	.00		.00	.34		
35			.00	.00		
36						
37						
38						
39						
40						
Total	56.12	44.38	53.77	37.25	44.50	26.26
Mean	1.870	2.958	1.734	1.266	2.617	1.875

Table #III Continued

Daily production records of females #2-#12 for the second lactation.

Days	♀#8	♀#9	♀#10	♀#11	♀#12	Mean
5	2.39	2.52	2.23	1.77	1.81	1.955
6	.86	1.56	1.19	1.90	1.62	1.219
7	1.43	2.03	1.69	2.88	.47	1.468
8	2.85	2.35	1.43	2.39	1.40	1.919
9	2.74	2.52	2.02	3.48	1.04	1.896
10	2.53	2.71	2.58	4.05	2.61	2.526
11	1.91	4.85	3.64	3.58	2.67	2.916
12	1.21	5.56	3.73	4.48	3.05	3.089
13	1.43	4.92	2.56	3.41	2.90	2.774
14	.54	4.31	2.57	4.72	3.47	2.727
15	1.97	4.13	2.38	3.48	2.98	3.000
16	1.35	3.86	2.18	3.91	3.96	3.015
17	1.65	5.56	4.37	3.61	5.09	3.036
18	3.02	4.43	5.34	2.24	4.14	2.783
19	3.17	3.40	3.93	4.35	5.66	3.288
20	4.11	1.17	4.41	4.75	3.21	3.105
21	3.51		4.13	5.95	.62	2.965
22	3.63		4.07	3.60	2.51	2.607
23	2.38		2.93	2.02	2.83	2.238
24	1.56		.96	3.52	3.58	2.130

Table #III Continued

Daily production records of females #2-#12 for the second lactation.

Days	♀#8	♀#9	♀#10	♀#11	♀#12	Mean
25	.00		2.57	2.75	1.56	1.920
26	.35		2.69	3.18	.33	1.702
27	.00		2.99	2.90	.00	1.562
28	.00		3.35	3.55	.00	1.434
29	.00		2.60	2.03		1.283
30			2.58	2.28		1.548
31			1.14	1.30		1.180
32			1.10	.74		.782
33			.55	.67		.465
34			.45	1.28		.416
35			.17	.78		.237
36			.00	.30		.15
37				.03		.03
38				.03		.03
39				.08		.08
40				.00		.00
Total	45.59	55.88	78.53	91.99	57.51	
Mean	1.823	3.492	2.454	2.555	2.396	

Table # IV

Daily production records of females #2-#12 for the third lactation.

Days	♀#2	♀#3	♀#4	♀#5	♀#6	♀#7
5	1.41	.22	F.*	.70	.08	2.31
6	1.47	.70		.26	.69	2.84
7	1.32	1.05		1.04	1.23	1.93
8	1.08	1.64		.20	1.18	2.03
9	1.83	1.07		.87	1.13	2.62
10	2.72	1.64		2.15	1.52	1.80
11	2.94	1.38		2.33	1.85	1.69
12	2.16	1.27		1.92	2.74	1.97
13	1.58	.82		2.71	3.64	2.39
14	1.71	1.41		2.74	3.45	4.36
15	2.72	1.69		1.99	2.95	.40
16	1.64	1.35		3.99	3.38	3.00
17	4.13	1.49		3.42	2.28	2.93
18	1.86	1.27		3.34	2.21	2.97
19	1.85	.80		3.19	2.88	2.82
20	2.24	1.99		.76	2.51	2.98
21	1.56	1.99		.19	2.65	2.06
22	2.95	2.44		.61	1.60	1.96
23	.86	2.27		1.35	2.00	1.14
24	1.75	2.36		1.87	1.74	1.84
25	1.50	2.28		1.86	1.99	3.34

*See Table # I

Table # IV Continued

Dily production records of females #2-#12 for the third lactation.

Days	♀#2	♀#3	♀#4	♀#5	♀#6	♀#7
26	1.44	1.59	F.*	1.66	.41	3.47
27	2.08	1.85		2.22	1.55	2.90
28	2.83	.63		1.88	1.53	3.10
29	4.03	1.07		1.30	1.75	2.58
30	3.59	1.27		.56	2.16	2.77
31	3.75	1.77		.27	.06	
32	4.27	2.39		.80	.45	
33	4.13	3.01		.82	.76	
34		2.78		.92	.45	
35		4.20		1.30	1.55	
36		2.33		1.36	1.65	
37		1.83		1.73	.84	
38		2.71		1.41	.67	
39				.60	.18	
40				.28	.00	
41				.09		
42				.00		
Total	67.40	58.57		54.69	57.21	64.20
Mean	2.355	1.723		1.397	1.634	2.469

* See Table # I

Table # IV Continued

Daily production records of females #2-#12 for the third lactation.

Days	♀#8	♀#9	♀#10	♀#11	♀#12	Mean
5	.79	2.66	.26	.30	.67	.940
6	.00	1.13	1.19	1.33	.37	.998
7	.34	.73	2.57	2.41	.69	1.331
8	.24	1.51	2.99	1.11	.94	1.291
9	.67	.51	3.22	2.52	.68	1.512
10	.44	1.13	4.25	2.50	2.12	2.027
11	.76	.81	4.28	2.79	2.43	2.126
12	.51	2.35	3.30	3.48	.91	2.061
13	.63	1.05	2.99	4.95	2.22	2.298
14	1.10	.79	3.73	4.27	2.35	2.591
15	1.65	1.65	3.42	3.90	.54	2.091
16	1.96	2.25	3.74	3.41	1.84	2.656
17	3.07	2.11	3.81	5.27	2.55	3.106
18	3.52	1.03	3.50	6.36	2.87	2.893
19	1.62	1.82	2.89	5.25	3.56	2.668
20	2.70	.10	1.97	5.46	4.13	2.484
21	1.51	2.51	1.56	2.92	2.47	1.942
22	1.35	3.11	.26	1.03	4.43	1.974
23	1.42	2.67	.05	3.05	1.77	1.658
24	2.40	3.75	.44	2.64	2.83	2.162
25	2.33	2.40	.86	2.31	2.88	2.175

Table # IV Continued

Daily production records of females #2-#12 for the third lactation.

Days	♀#8	♀#9	♀#10	♀#11	♀#12	Mean
26	1.34	1.87	1.01	2.32	3.41	1.852
27	1.32	3.72	1.57	2.67	3.88	2.376
28	1.17	1.82	1.97	2.69	4.20	2.182
29	1.22	1.23	1.73	2.55	4.32	2.178
30	.91	.85	1.47	2.26	2.99	1.883
31	1.61	1.12	1.09	1.41	2.55	1.363
32	1.22	.98	1.00	1.50	1.97	1.458
33	1.16	1.27	1.14	.00	3.34	1.563
34	.86	1.70	1.03	1.00		.874
35	.94	2.12	1.43	1.48		1.302
36	.95	2.51	1.37	1.03		1.120
37	.75	3.96	1.24	1.09		1.144
38	.38	1.82	1.34	.95		.928
39	.28	2.07	.27	.59		.399
40	.00	2.47	.38	.17		.330
41	.00		.94	.18		.121
42	.14			.21		.035
43	.00			.00		.000
Total	43.26	65.58	70.26	89.36	69.91	
Mean	1.136	1.821	1.898	2.350	1.834	

Table # V

Table showing the Mean Daily Production of All Mice for the First, Second, Third, and Fourth Lactation.

Days	1st.Lact.	2nd.Lact.	3rd.Lact	4th.Lact.
5	1.084	1.955	.940	1.582
6	.936	1.219	.998	1.875
7	1.250	1.468	1.331	1.810
8	1.550	1.919	1.292	1.988
9	2.338	1.896	1.512	2.083
10	2.078	2.526	2.027	2.081
11	2.264	2.916	2.126	2.035
12	2.578	3.089	2.061	2.151
13	2.608	2.774	2.298	2.460
14	3.342	2.727	2.591	2.085
15	3.292	3.000	2.091	2.543
16	3.896	3.015	2.656	2.916
17	3.494	3.036	3.106	2.533
18	3.660	2.783	2.893	3.088
19	3.332	3.288	2.668	
20	3.434	3.105	2.489	
21	2.123	2.965	1.942	
22	1.855	2.607	1.974	
23		2.238	1.658	
		2.130	2.162	

Table # V Continued

Table showing the Mean Daily Production of All Mice for the First, Second, Third, and Fourth Lactations.

Days	1st.Lact.	2nd.Lact.	3rd.Lact.	4th.Lact.
25		1.920	2.175	
26		1.702	1.852	
27		1.562	2.376	
28		1.434	2.182	
29		1.283	2.178	
30		1.548	1.883	
31		1.180	1.363	
32		.782	1.458	
33		.465	1.563	
34		.416	.874	
35		.237	1.302	
36		.150	1.120	
37		.030	1.144	
38		.030	.928	
39		.080	.399	
40		.000	.330	
41			.121	
42			.035	
43			.000	
44				

Table # VI

Mean Daily production of those females of the #2-#12 series whose records are complete for four successive lactations, arranged in order of decreasing milk production. Fourteen day samples are considered in each lactation.

First Lact.		Second Lact.		Third Lact.		Fourth Lact.	
♀#	Mean	♀#	Mean	♀#	Mean	♀#	Mean
9	3.407	9	3.664	10	3.088	9	2.901
10	3.257	3	3.038	2	2.105	3	2.432
3	2.012	10	2.706	9	1.408	10	2.103
2	1.930	2	2.102	3	1.214	2	1.662
8	1.528	8	1.848	8	1.126	8	1.406

Daily mean production of females #30 and #32 for the first and fourth lactations.

First Lact.		Second Lact.	Third Lact.	Fourth Lact.	
♀#	Mean			♀#	Mean
30	1.987	F.*	F.*	30	1.893
32	1.655			32	1.754

* See Text-page 30

Table # VII

Daily production records of females #2-#12 for the first lactation, 5-18 days inclusive.

Days	♀#2	♀#3	♀#4	♀#5	♀#6	♀#7
5	.46	.78	F.*	F.*	F.*	F.*
6	.68	.29				
7	.78	.29				
8	1.07	1.15				
9	1.79	1.18				
10	1.24	1.58				
11	1.00	1.82				
12	1.52	2.10				
13	1.05	1.29				
14	2.82	3.90				
15	3.48	3.10				
16	3.76	3.84				
17	3.65	3.25				
18	3.73	3.51				
Total	27.03	28.18				
Mean	1.930	2.012				

* See Table # I

Table # VII Continued

Daily production records of females #2-#12 for the first lactation, 5-18 days inclusive.

Days	♀#8	♀#9	♀#10	♀#11	♀#12	Mean
5	.59	1.31	2.28	F.*	F.*	1.084
6	.08	1.33	2.30			.936
7	.46	2.04	2.68			1.250
8	.54	2.26	2.73			1.550
9	.80	4.48	3.08			2.338
10	1.15	2.78	3.64			2.078
11	1.55	3.04	3.86			2.264
12	1.65	4.63	2.99			2.578
13	2.16	4.69	3.85			2.608
14	2.34	4.57	3.08			3.342
15	2.48	3.95	3.45			3.292
16	2.62	4.64	4.62			3.896
17	2.41	4.26	3.90			3.494
18	2.56	4.36	4.14			3.660
Total	21.39	48.70	46.60			
Mean	1.528	3.407	3.257			

* See Table # I

Table # VIII

Daily production records of females #2-#12 for the second lactation, 5-18 days inclusive.

Days	♀#2	♀#3	♀#4	♀#5	♀#6	♀#7
5	1.64	1.46	2.39	1.80	2.08	1.42
6	.48	.99	1.17	.99	1.42	1.23
7	.81	1.43	2.17	.97	.72	1.55
8	1.68	2.31	2.14	1.01	2.52	1.04
9	.37	2.83	2.57	.66	2.82	.81
10	1.23	3.97	3.41	.56	2.59	1.55
11	2.78	4.59	4.07	.26	2.92	.81
12	2.90	2.95	2.83	.14	2.86	4.27
13	2.72	3.46	2.86	.40	2.15	3.71
14	2.84	3.81	.98	.62	2.20	3.94
15	3.14	4.22	2.51	1.86	3.14	3.19
16	3.36	4.29	2.66	1.96	3.38	2.26
17	2.94	3.98	2.83	.67	3.28	.48
18	2.80	2.26	2.14	1.53	2.72	.00
Total	29.68	42.55	34.77	13.43	34.80	26.25
Mean	2.120	3.038	2.482	.958	2.284	1.875

Table # VIII

Daily production records of females #2-#12 for the second lactation, 5-18 days inclusive.

Days	♀#2	♀#3	♀#4	♀#5	♀#6	♀#7
5	1.64	1.46	2.39	1.80	2.08	1.42
6	.48	.99	1.17	.99	1.42	1.23
7	.81	1.43	2.17	.97	.72	1.55
8	1.68	2.31	2.14	1.01	2.52	1.04
9	.37	2.83	2.57	.66	2.82	.81
10	1.23	3.97	3.41	.56	2.59	1.55
11	2.78	4.59	4.07	.26	2.92	.81
12	2.90	2.95	2.83	.14	2.86	4.27
13	2.72	3.46	2.86	.40	2.15	3.71
14	2.84	3.81	.98	.62	2.20	3.94
15	3.14	4.22	2.51	1.86	3.14	3.19
16	3.36	4.29	2.66	1.96	3.38	2.26
17	2.94	3.98	2.83	.67	3.28	.48
18	2.80	2.26	2.14	1.53	2.72	.00
Total	29.68	42.55	34.77	13.43	34.80	26.25
Mean	2.120	3.038	2.482	.958	2.284	1.875

Table # VIII Continued

Daily production records of females #2-#12 for the second lactation, 5-18 days inclusive.

Days	♀#8	♀#9	♀#10	♀#11	♀#12	Mean
5	2.39	2.52	2.23	1.77	1.81	1.955
6	.86	1.56	1.19	1.90	1.62	1.219
7	1.43	2.03	1.69	2.88	.47	1.468
8	2.84	2.35	1.43	2.39	1.40	1.919
9	2.74	2.52	2.02	3.48	1.04	1.896
10	2.53	2.71	2.58	4.05	2.61	2.526
11	1.91	4.85	3.64	3.58	2.67	2.916
12	1.21	5.56	3.73	4.48	3.05	3.089
13	1.43	4.92	2.56	3.41	2.90	2.774
14	.54	4.31	2.57	4.72	3.47	2.727
15	1.97	4.13	2.38	3.48	2.98	3.000
16	1.35	3.86	2.18	3.91	3.96	3.015
17	1.65	5.56	4.37	3.61	5.09	3.132
18	3.02	4.43	5.34	2.24	4.14	2.783
Total	25.88	51.31	37.91	45.90	37.21	
Mean	1.848	3.664	2.706	3.278	2.800	

Table # IX

Daily production records of females #2-#12 for the third lactation, 5-18 days inclusive.

Days	♀#2	♀#3	♀#4	♀#5	♀#6	♀#7
5	1.41	.22	F.*	.70	.08	2.31
6	1.47	.70		.26	.69	2.84
7	1.32	1.05		1.04	1.23	1.93
8	1.08	1.64		.20	1.18	2.03
9	1.83	1.07		.87	1.13	2.62
10	2.72	1.64		2.15	1.52	1.80
11	2.94	1.38		2.33	1.85	1.69
12	2.16	1.27		1.92	2.74	1.97
13	1.58	.82		2.71	3.64	2.39
14	1.71	1.41		2.74	3.45	4.36
15	2.72	1.69		1.99	2.95	.40
16	1.64	1.35		3.99	3.38	3.00
17	4.13	1.49		3.42	2.28	2.93
18	1.86	1.27		3.34	2.21	2.97
Total	29.47	17.00		27.66	28.33	32.24
Mean	2.105	1.214		1.974	2.022	2.302

* See Table # I

Table # IX Continued

Daily production records of females #2-#12 for the third lactation, 5-18 days inclusive.

Days	♀#8	♀#9	♀#10	♀#11	♀#12	Mean
5	.79	2.66	.26	.30	.67	.940
6	.00	1.13	1.19	1.33	.37	.998
7	.34	.73	2.57	2.41	.69	1.331
8	.24	1.51	2.99	1.11	.94	1.291
9	.67	.51	3.22	2.52	.68	1.512
10	.44	1.13	4.25	2.50	2.12	2.027
11	.76	.81	4.28	2.79	2.43	2.126
12	.51	2.35	3.30	3.48	.91	2.061
13	.63	1.05	2.99	4.95	2.22	2.298
14	1.10	.79	3.73	4.27	2.35	2.591
15	1.65	1.65	3.42	3.90	.54	2.091
16	1.96	2.25	3.74	3.41	1.84	2.656
17	3.07	2.11	3.81	5.27	2.55	3.106
18	3.52	1.03	3.50	6.36	2.87	2.893
Total	15.78	19.71	43.25	43.60	21.38	
Mean	1.126	1.408	3.088	3.082	1.522	

Table # X

Daily production records of females #2-#12 for the fourth lactation, 5-18 days inclusive.

Days	♀#2	♀#3	♀#4	♀#5	♀#6	♀#7
5	1.28	1.23	F.*	3.04	F.*	F.*
6	.77	1.97		1.62		
7	1.04	2.06		2.06		
8	1.34	2.18		1.95		
9	1.53	2.19		2.22		
10	2.08	1.80		2.37		
11	1.82	2.48		1.86		
12	2.26	2.04		3.11		
13	1.82	2.40		3.82		
14	2.08	2.33		2.02		
15	1.38	2.51		3.79		
16	2.35	3.43		4.32		
17	2.30	3.10		1.16		
18	3.25	4.34		1.35		
Total	25.27	34.06		34.69		
Mean	1.662	2.432		2.470		

* See Table # I

Table # X Continued

Daily production records of females #2-#12 for the fourth lactation, 5-18 days inclusive.

Days	♀#8	♀#9	♀#10	♀#11	♀#12	Mean
5	.44	2.33	1.18	F.*	F.*	1.582
6	.67	1.54	1.58			1.875
7	.83	2.88	1.99			1.810
8	1.46	2.35	2.65			1.988
9	1.31	2.91	1.89			2.083
10	1.06	2.99	2.22			2.081
11	1.16	3.39	1.50			2.035
12	1.04	3.13	1.33			2.151
13	1.51	2.86	2.35			2.460
14	1.46	1.89	2.73			2.085
15	2.13	4.15	1.30			2.543
16	2.35	2.11	2.94			2.916
17	2.19	4.03	2.42			2.533
18	2.08	4.06	3.45			3.088
Total	19.69	40.62	29.63			
Mean	1.406	2.901	2.116			

* See Table # I

Table # XI

Daily production records of females #30-#37 for the first lactation, 5-18 days inclusive.

Days	♀#30	♀#31	♀#32	♀#33	♀#34	♀#35	♀#36	♀#37
5	2.07	2.77	.61	2.49	.52	.55	.40	.53
6	2.84	2.36	1.90	2.98	.51	.68	.42	.21
7	2.33	3.72	1.70	2.63	.71	.89	.59	.42
8	1.43	3.32	1.20	3.20	.18	1.40	.57	.26
9	2.06	2.15	.70	2.49	1.42	1.52	.84	.31
10	1.31	2.94	.43	2.97	.21	2.31	.55	.38
11	.99	2.21	2.51	3.18	.51	.90	1.30	.09
12	.86	2.19	2.25	2.70	.29	2.31	1.03	.40
13	2.76	1.23	2.02	3.01	.24	2.06	.76	.82
14	3.47	2.37	1.54	2.63	1.35	.89	2.27	.83
15	2.11	.79	2.09	2.10	.72	1.78	2.11	2.00
16	1.37	1.38	1.29	3.24	1.27	2.70	2.56	2.27
17	2.01	2.51	2.31	1.95	.59	1.15	3.04	1.72
18	2.21	2.38	2.62	2.64	.80	2.24	2.54	1.92
Total	27.82	32.32	23.17	38.21	9.32	21.38	18.95	12.16
Mean	1.987	2.308	1.655	2.729	.665	1.521	1.353	.868

Table # XII

Daily production records of females #30 and #32 for the fourth lactation, 5-18 days inclusive.

Days	♀#30	♀#32
5	1.25	1.19
6	.79	1.21
7	1.38	1.49
8	1.55	1.30
9	2.09	1.41
10	1.90	1.10
11	2.06	1.88
12	1.61	1.75
13	2.53	2.48
14	2.19	1.88
15	2.09	1.94
16	3.18	2.66
17	2.30	2.35
18	1.59	1.86
Total	26.51	24.56
Mean	1.893	1.754

Table # XIII

Relationship between the amount ofmmilk consumed and the gain in weight ofyoung mice. Only litters of 7 young over a period of 14 days are included.

Female (mother)	Wt. of litter at first measurement. (in grams)	Total gain in wt. of young litter. (in grams)	Total amount of milk consumed. (in grams)	Value of K.
#2	19.97	6.04	29.68	.20
#3	18.76	16.93	42.55	.39
#4	18.31	10.59	34.77	.30
#5	17.65	7.29	13.43	.54
#6	19.36	9.00	34.80	.28
#8	17.31	7.33	25.88	.24
#9	17.61	20.81	51.31	.40
#10	17.55	9.77	37.91	.26
#11	19.11	15.17	45.90	.33
#12	17.18	13.01	37.21	.35

Table # XIV

Females with complete lactation records arranged in order of decreasing milk production on the basis of the total milk produced and their fourteen day samples.

Complete Lactation			Fourteen Day Samples		
Female	Lactation	Total amount of milk in grams.	Female	Lactation	Total amount of milk in grams.
#11	2	91.99	#11	2	45.90
#11	3	89.36	#11	3	43.60
#10	2	78.53	#10	2	37.91
#12	2	57.51	#12	2	37.21
#6	3	57.21	#4	2	34.77
#5	3	54.69	#6	3	28.33
#4	2	53.77	#5	3	27.66
#8	2	45.57	#8	2	25.88
#8	3	43.26	#8	3	15.78
#5	2	37.25	#5	2	13.43

Part XVII

Figure # I

Curve representing the complete milk production record of mouse #4 for the second lactation.

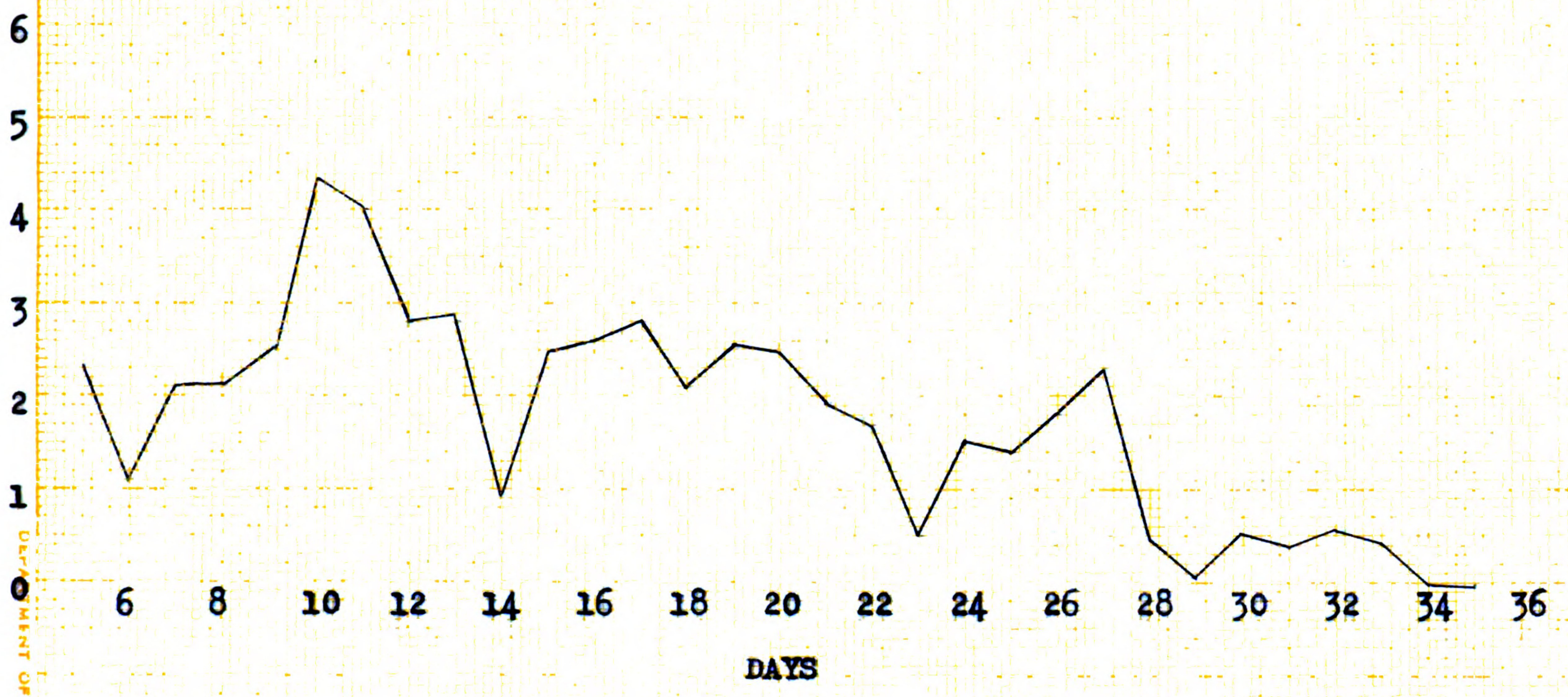


Figure # II

Curve representing the complete milk production record of mouse #6 for the third lactation.

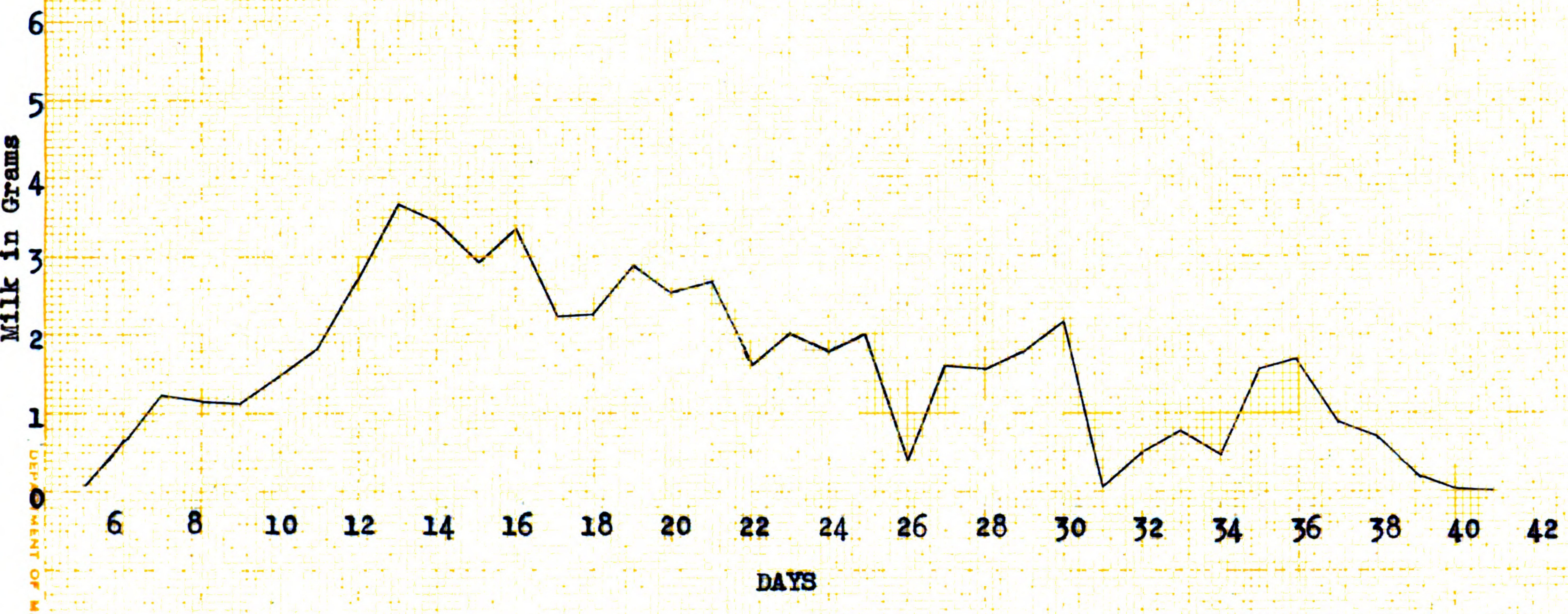


Figure # III

Curve representing the complete milk production record of mouse #5 for the second lactation.

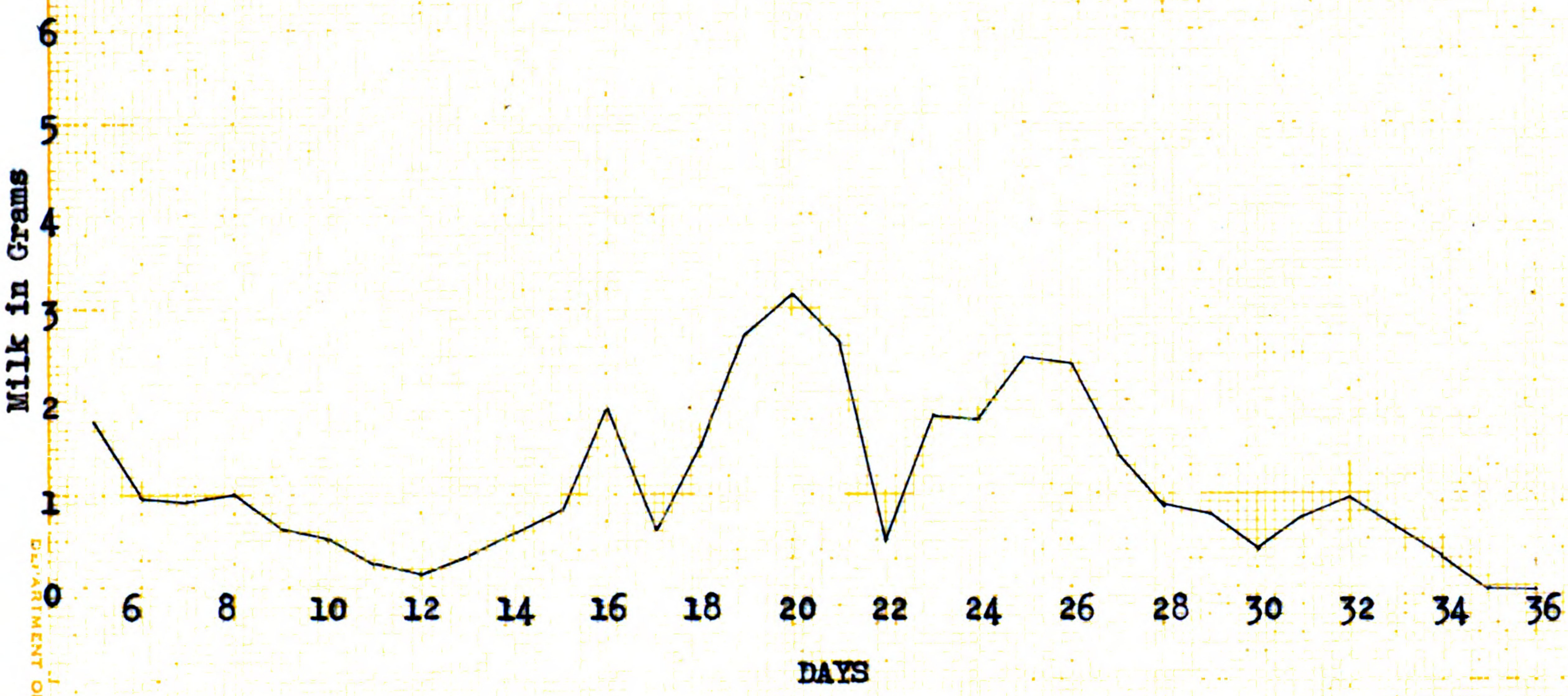


Figure # IV

Curve representing the complete milk production record of mouse #5 for the third lactation.

Figure # V

Curve representing the complete milk production record of mouse #8 for the second lactation.

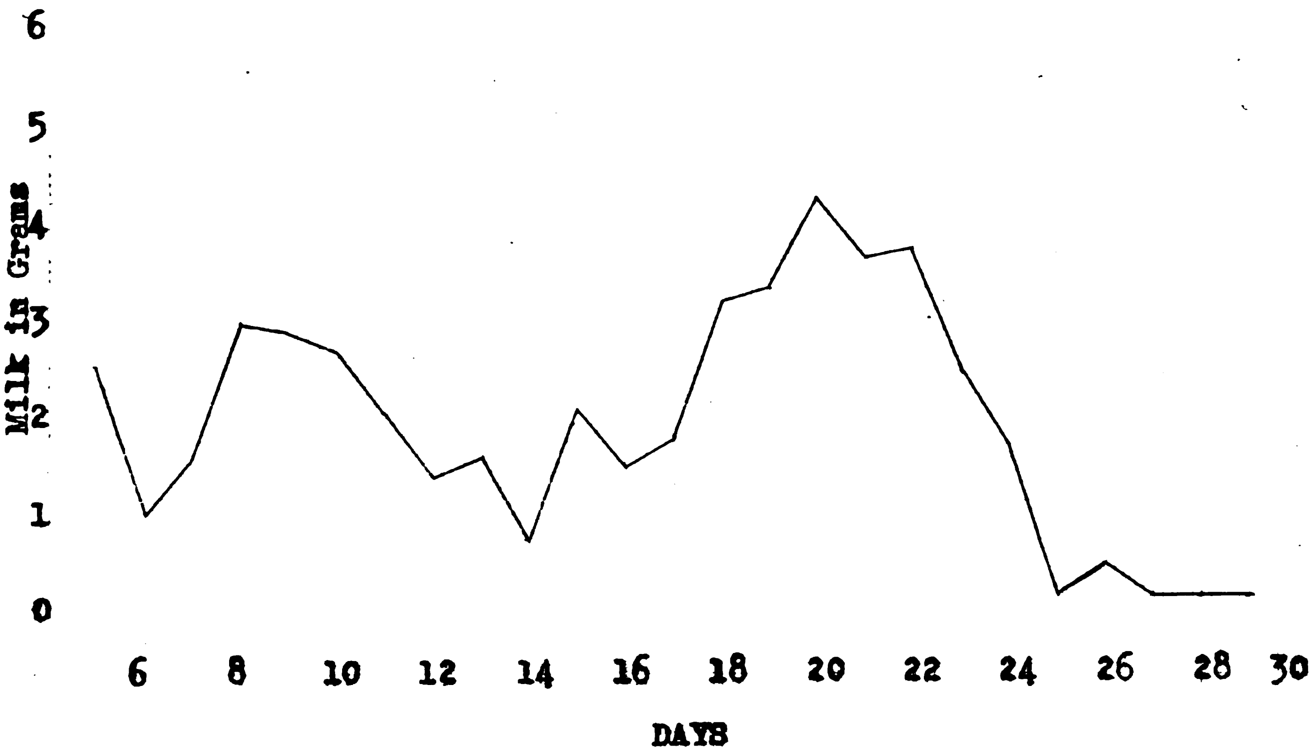


Figure # VI

Curve representing the complete milk production record of mouse #8 for the third lactation.

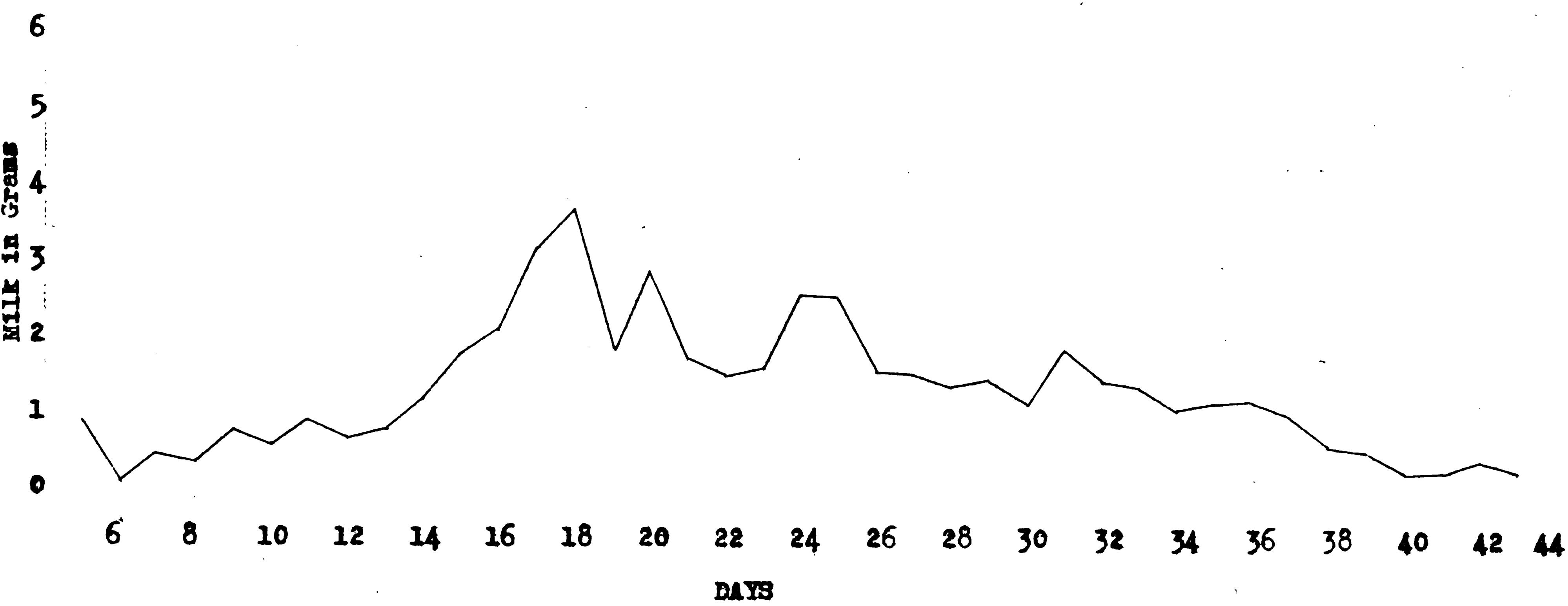


Figure # VI

Curve representing the complete milk production record of mouse #8 for the third lactation.

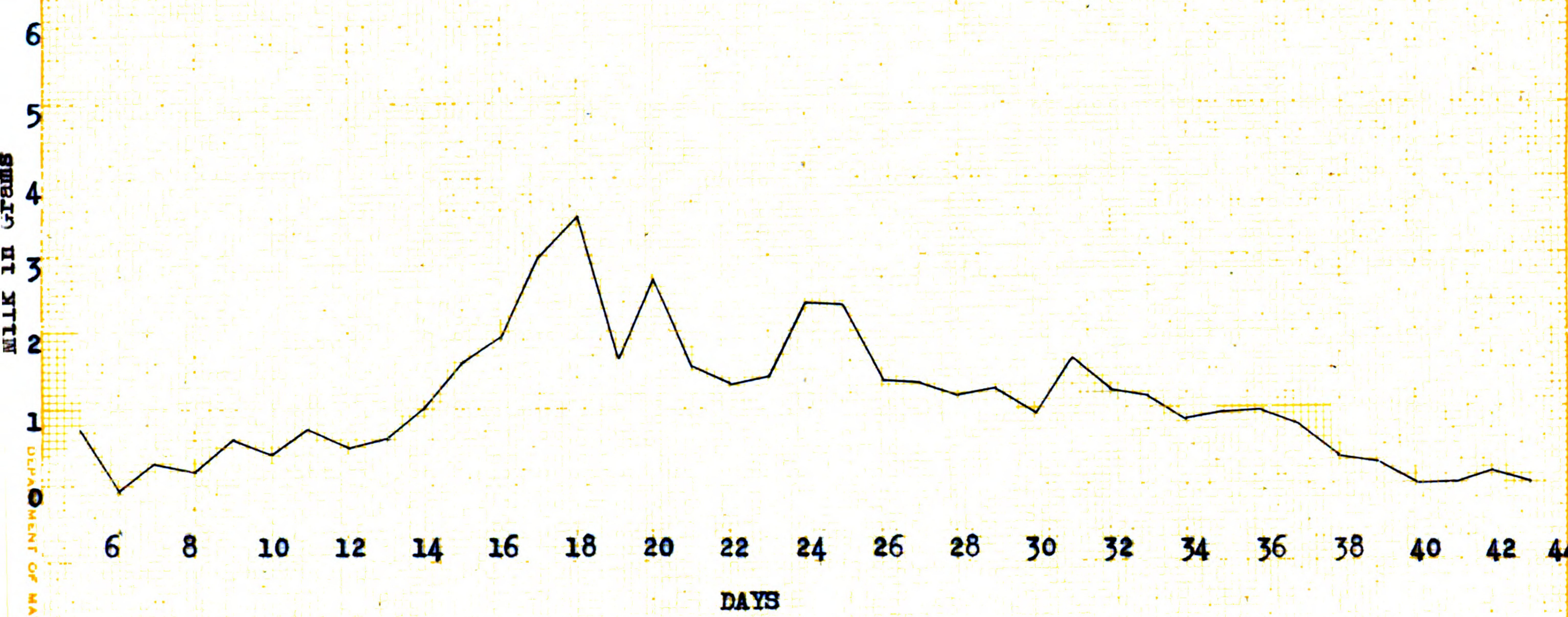


Figure # VII

Curve representing the complete milk production record of mouse #10 for the second lactation.

Figure # VIII

Curve representing the complete milk production record of mouse #11 for the second lactation.

Figure # IX

Curve representing the complete milk production record of mouse #11 for the third lactation.

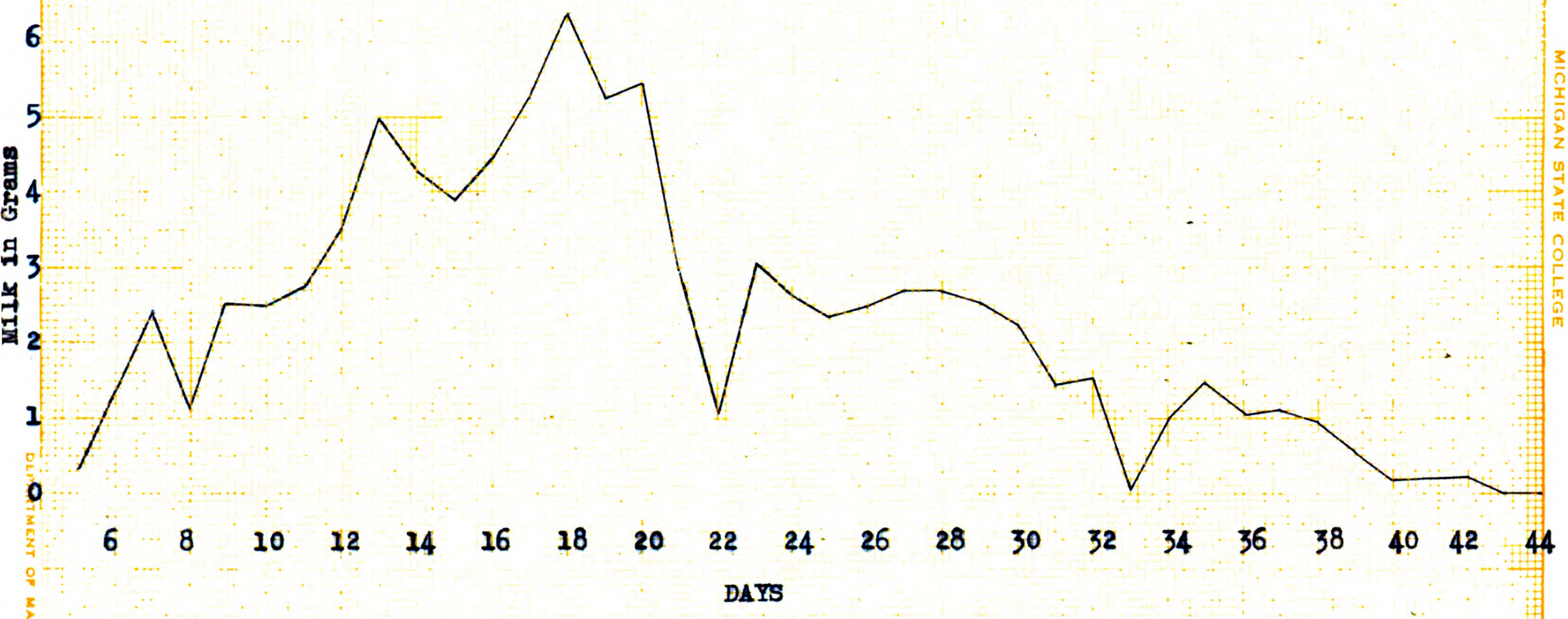


Figure # IX

Curve representing the complete milk production record of mouse #11 for the third lactation.

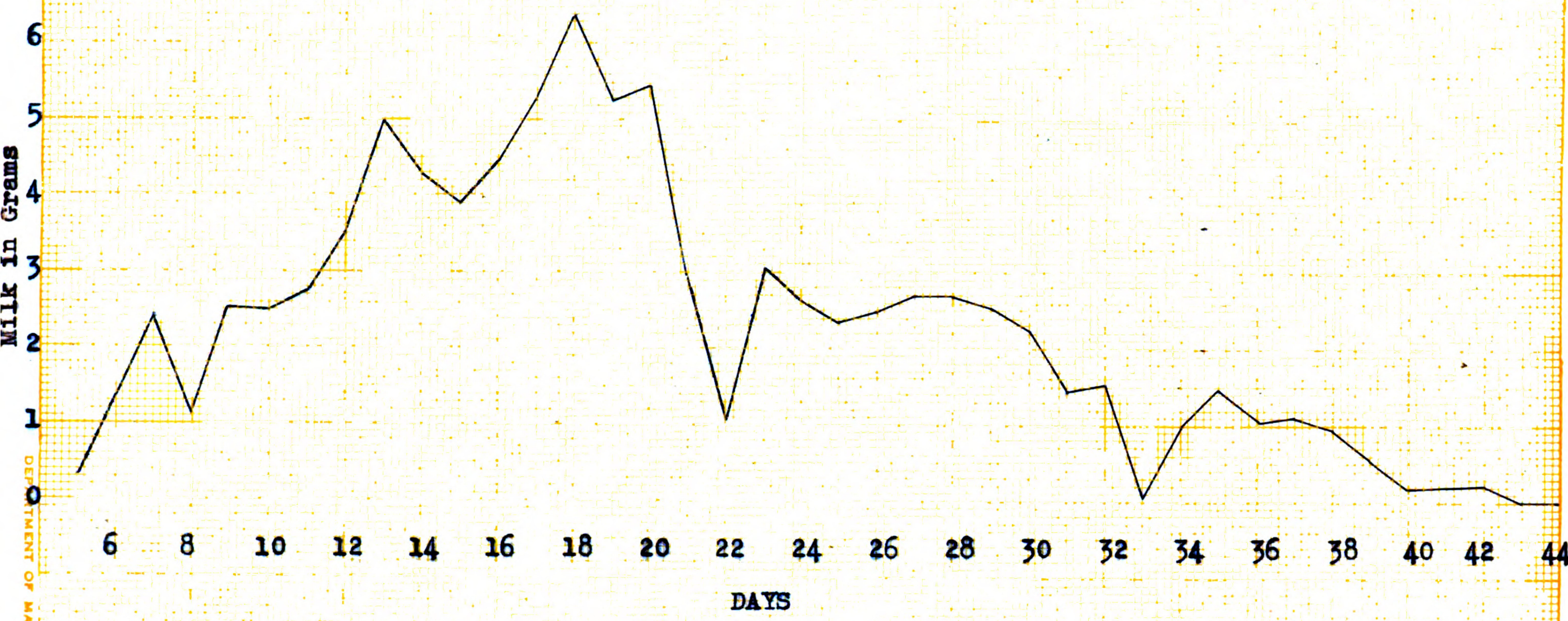


Figure # IX

Curve representing the complete milk production record of mouse #11 for the third lactation.

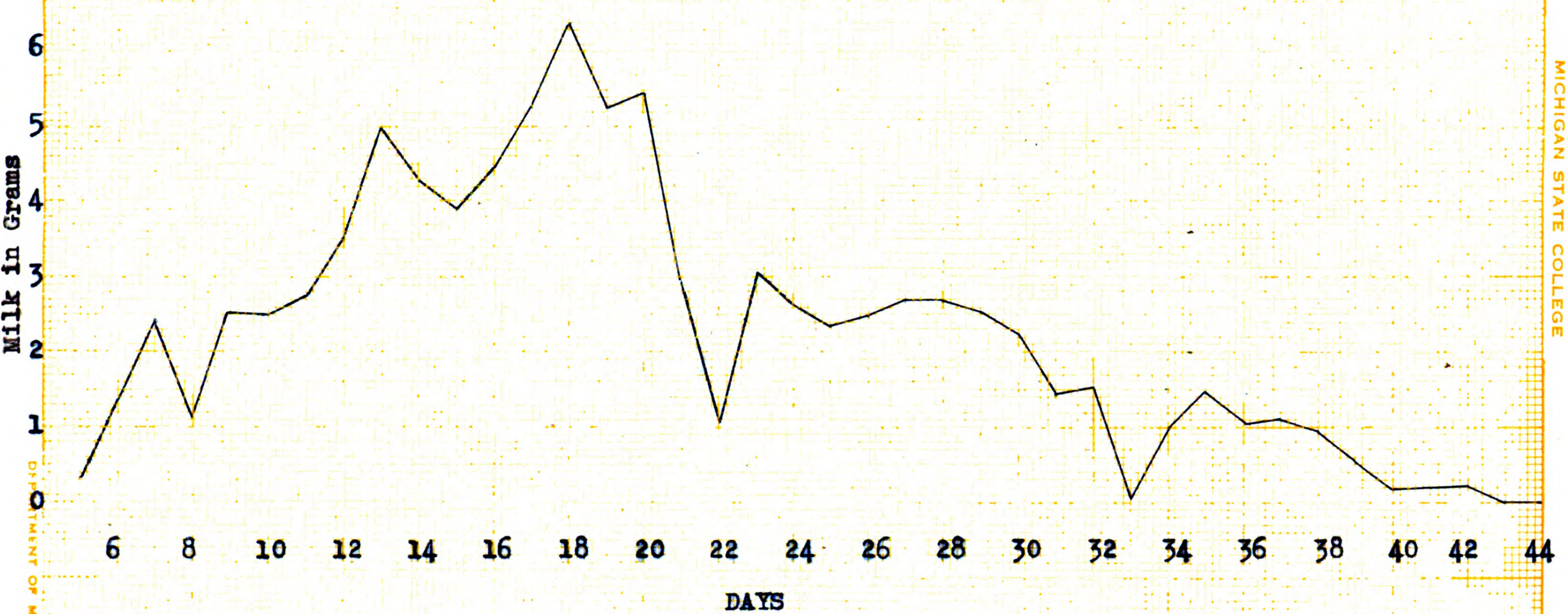


Figure # X

Curve representing the complete milk production record of mouse # 12 for the second lactation.

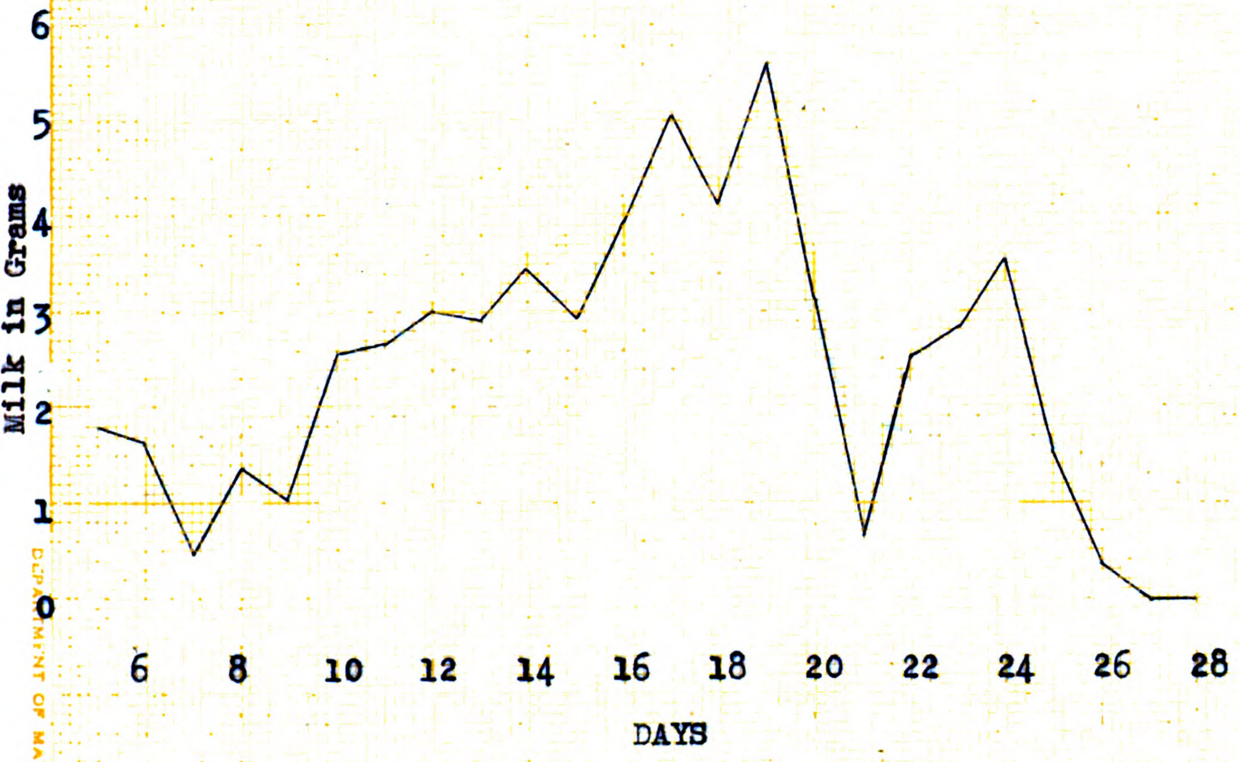


Figure # XI

Curves representing the mean daily production of females #2-#12 for the first, second, third, and fourth lactations.

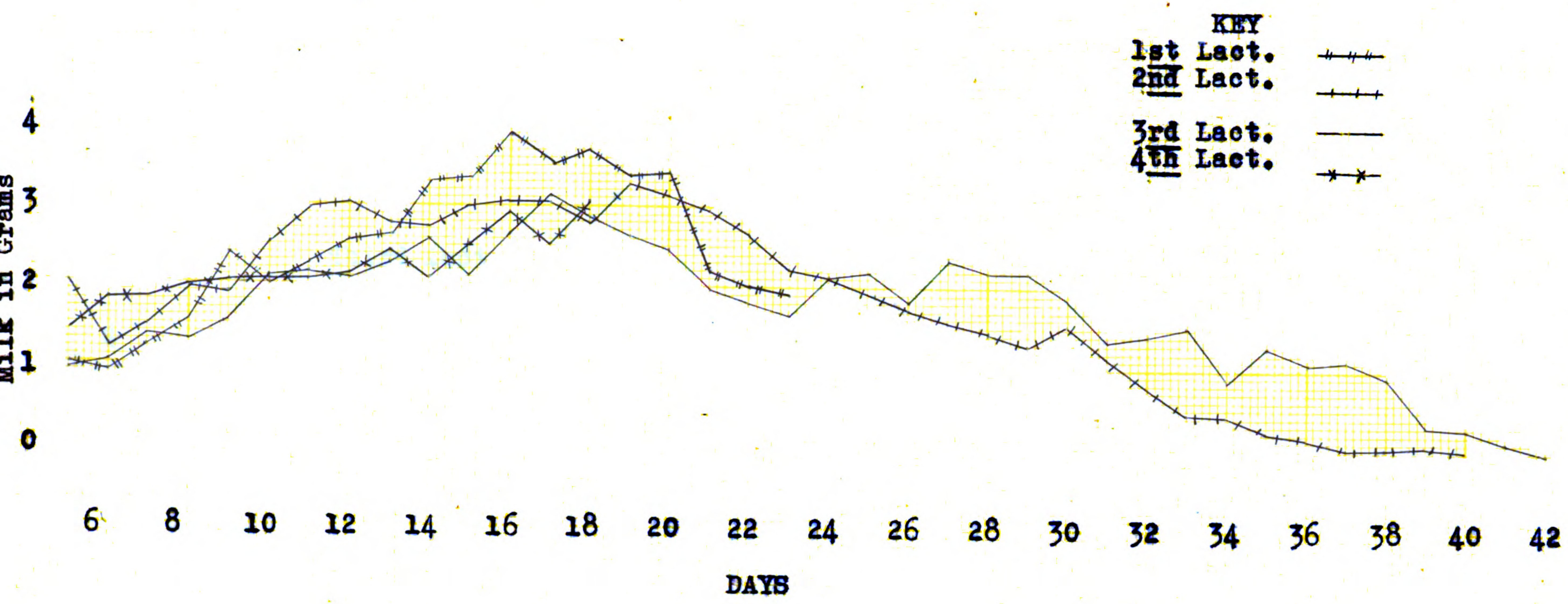


Figure # XII

Curves representing the mean daily milk production of the fourteen day samples of mice #2-#12.

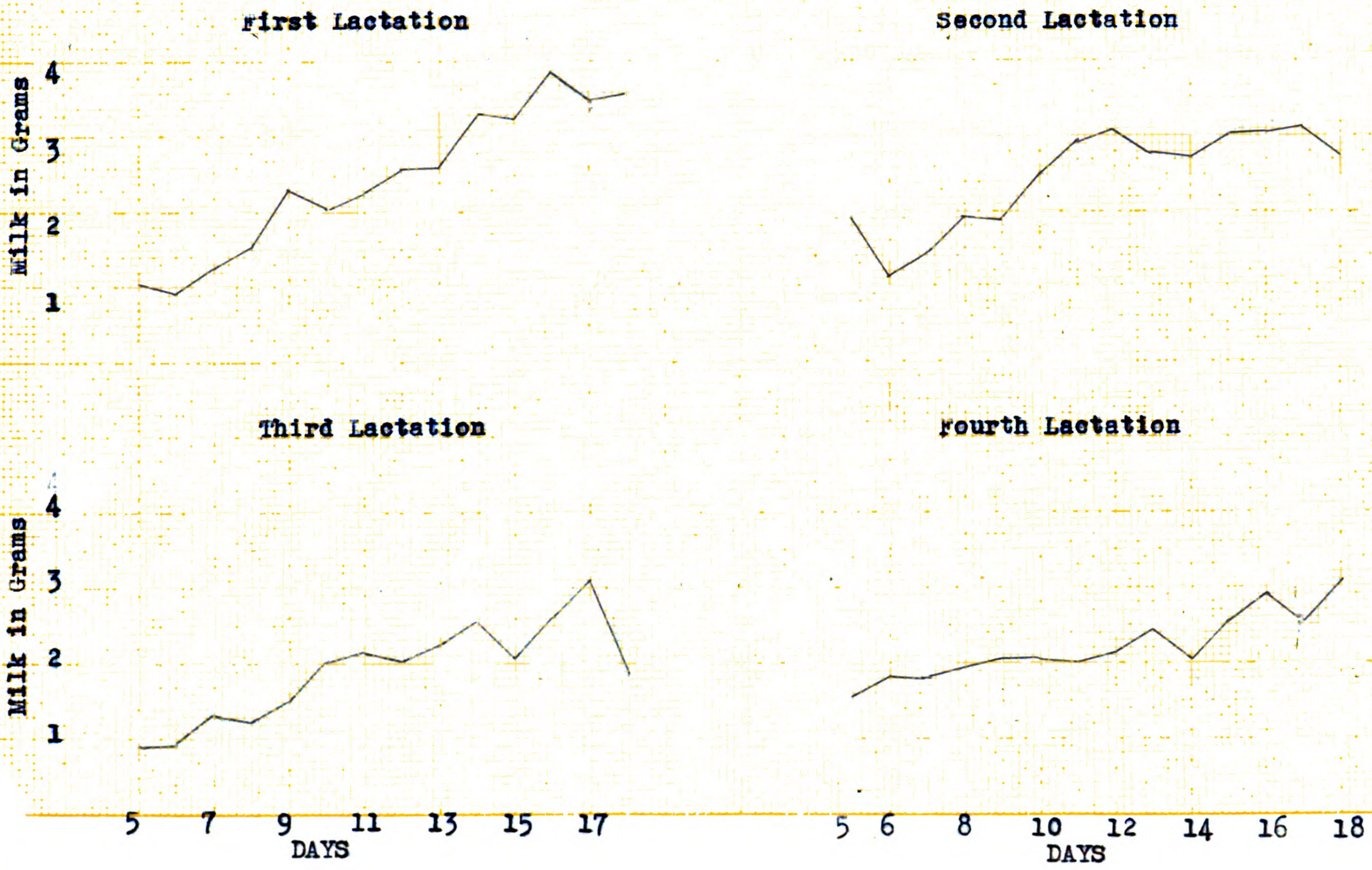


Figure # XIII

Curves representing the fourteen day samples of mouse #2.

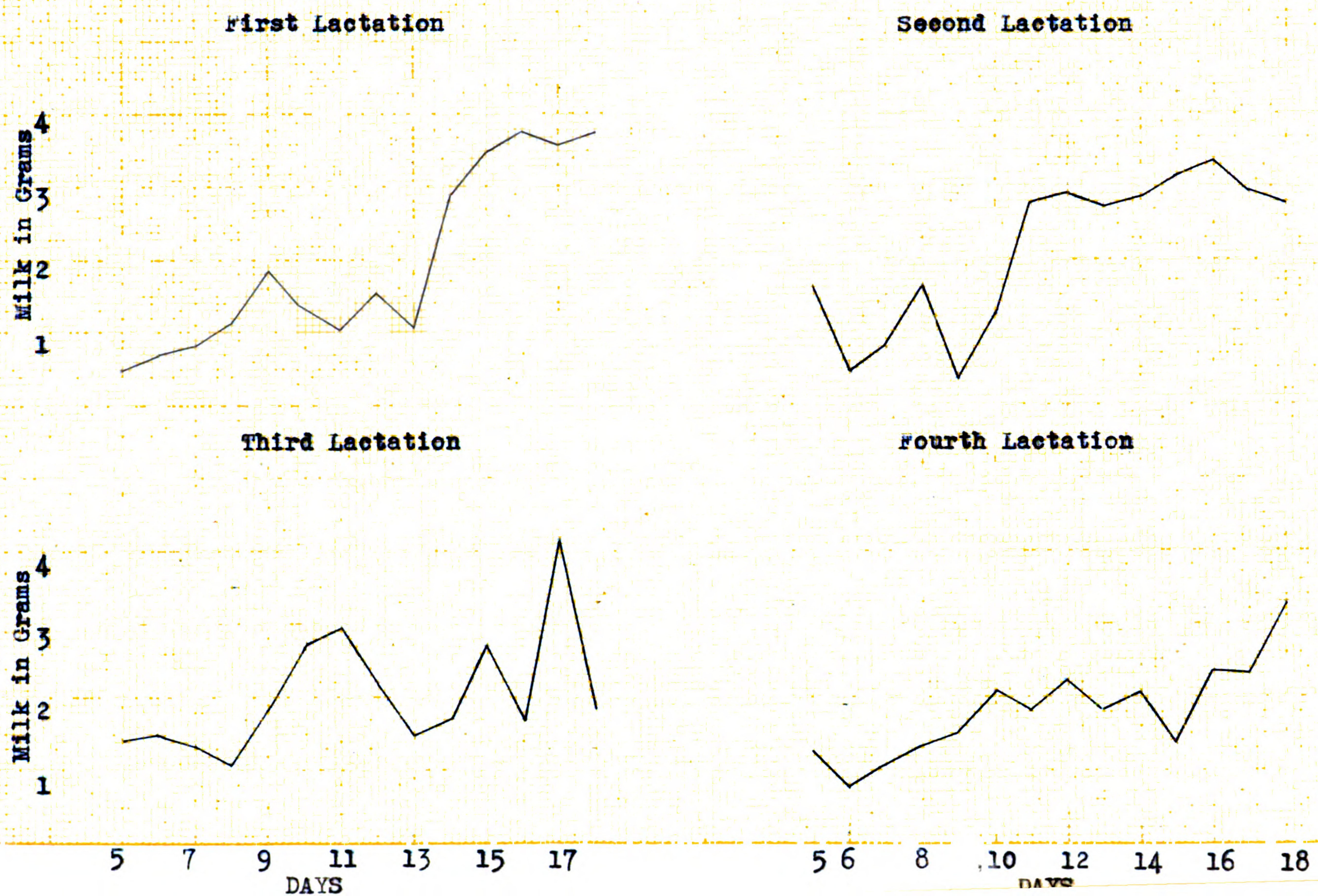


Figure # XIV

Curves representing the fourteen day samples of mouse #3.

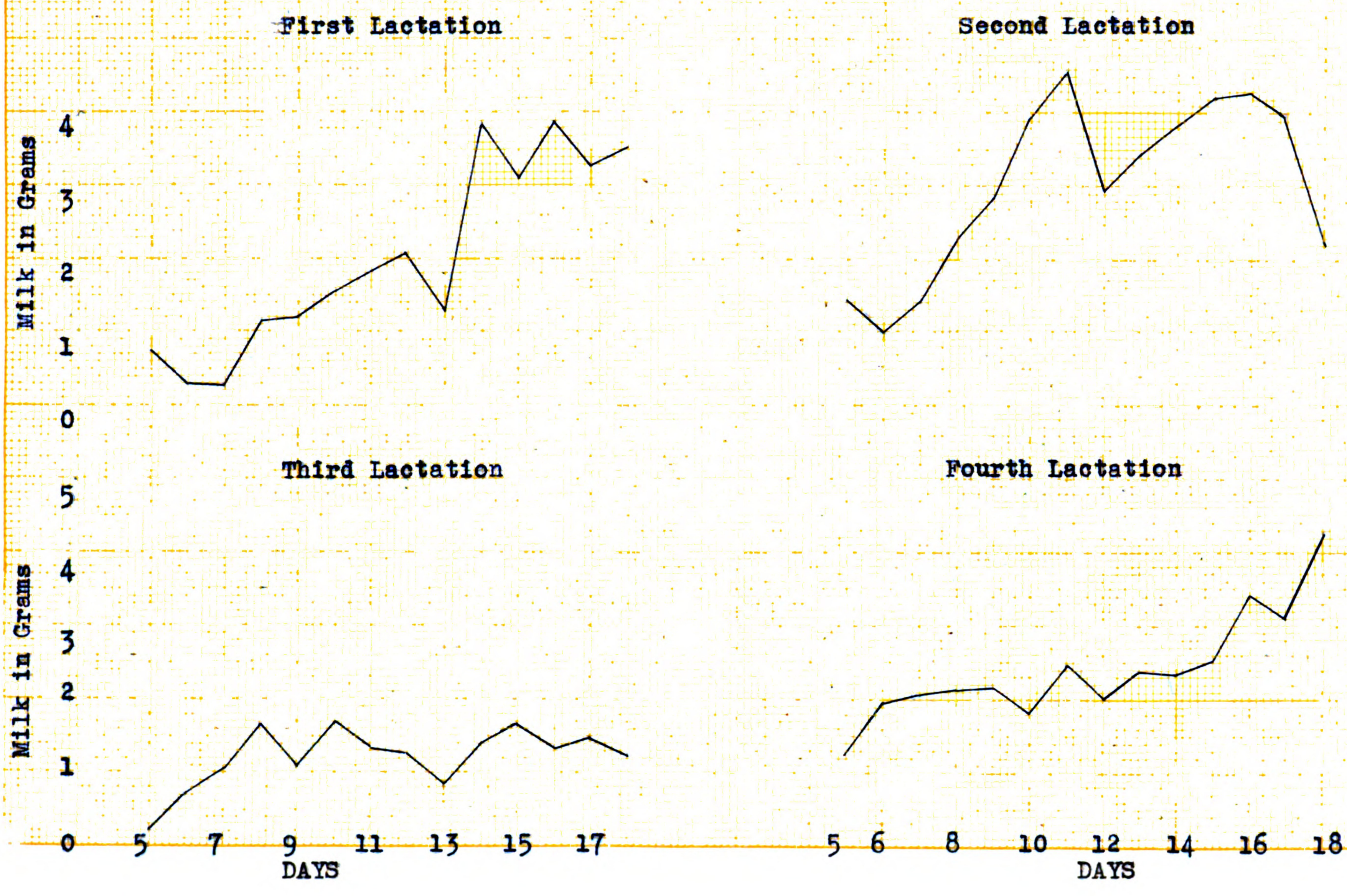


Figure # XV

Curves representing the fourteen day samples of mice #4 and # 5.

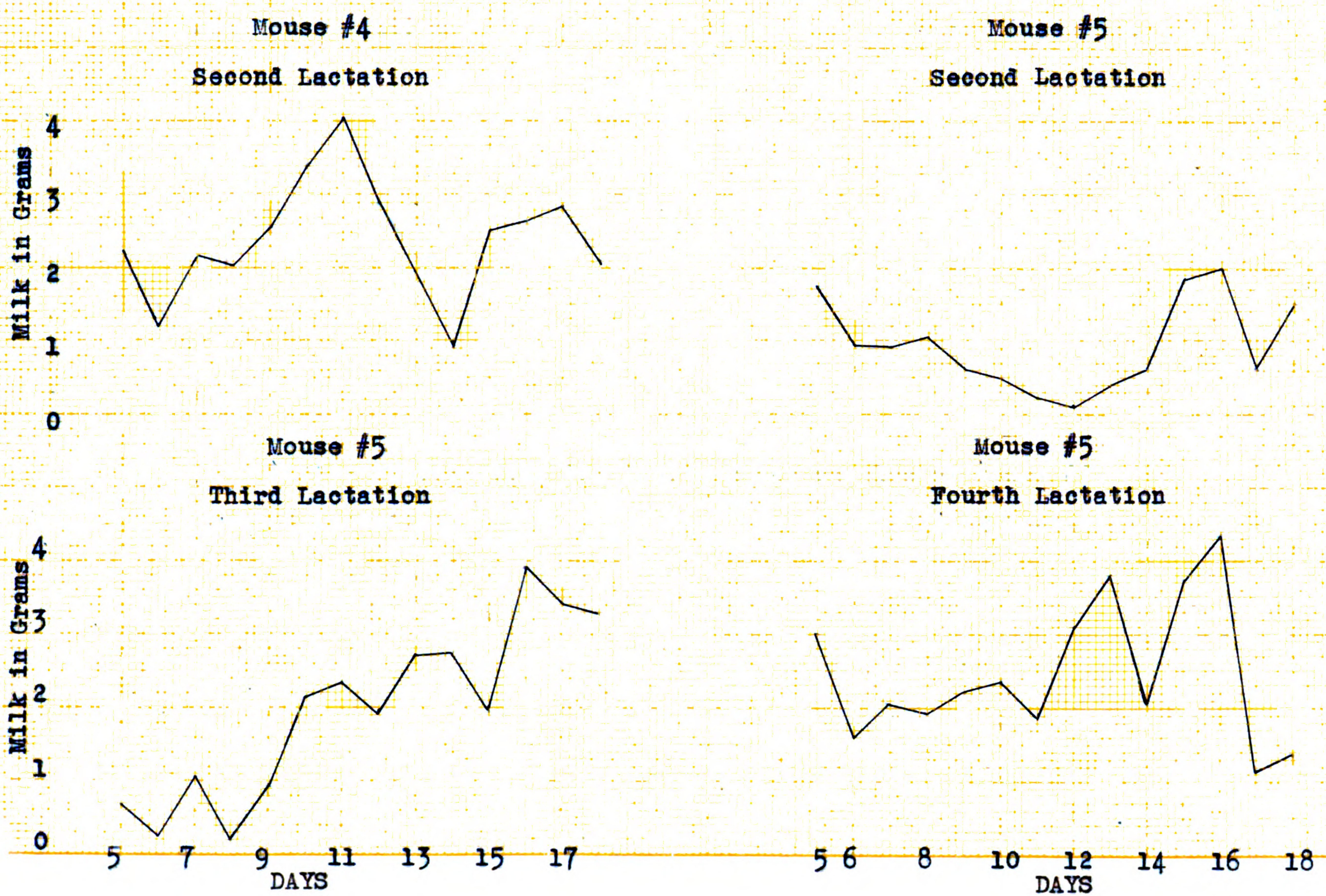


Figure # XVI

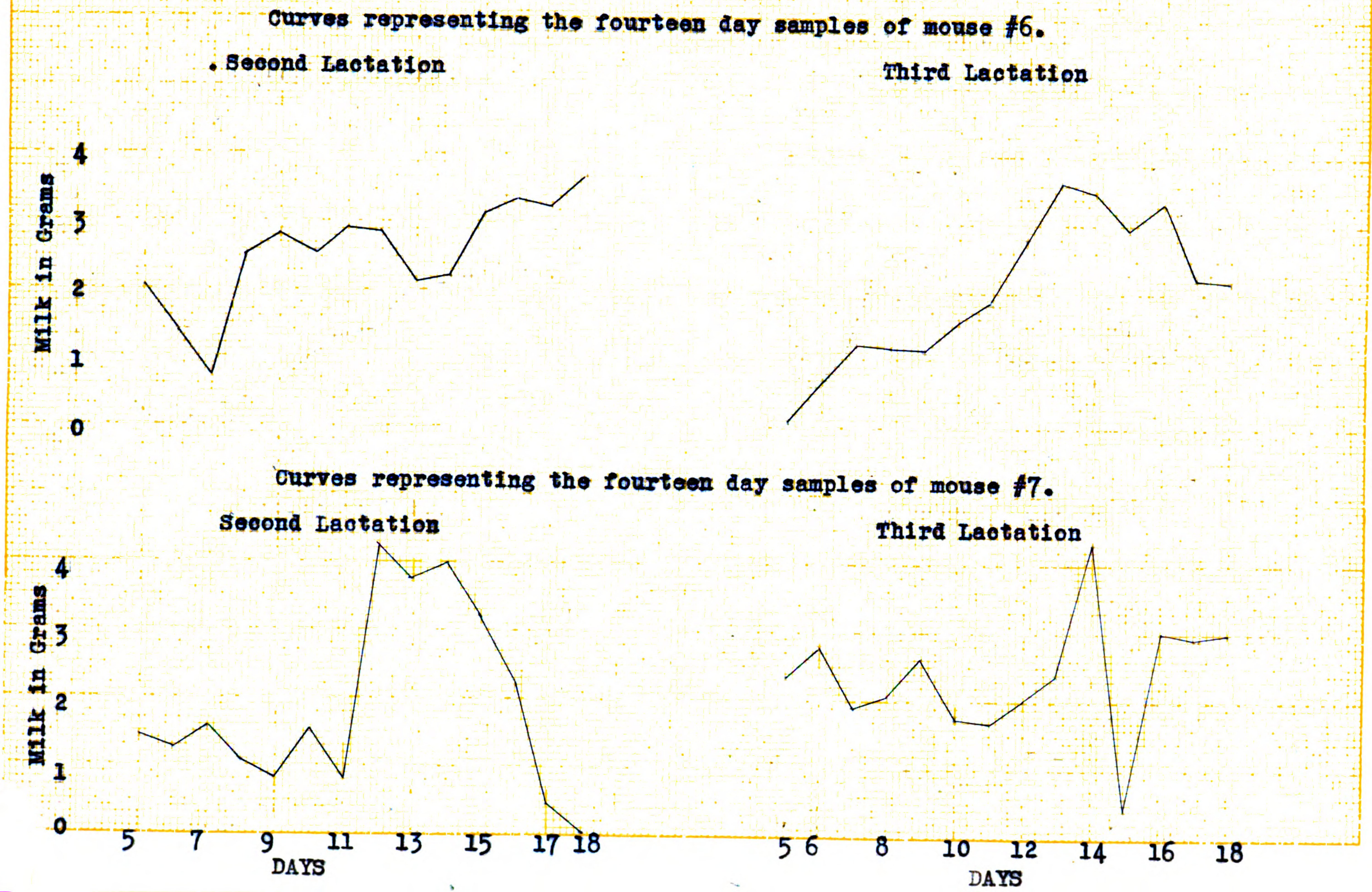
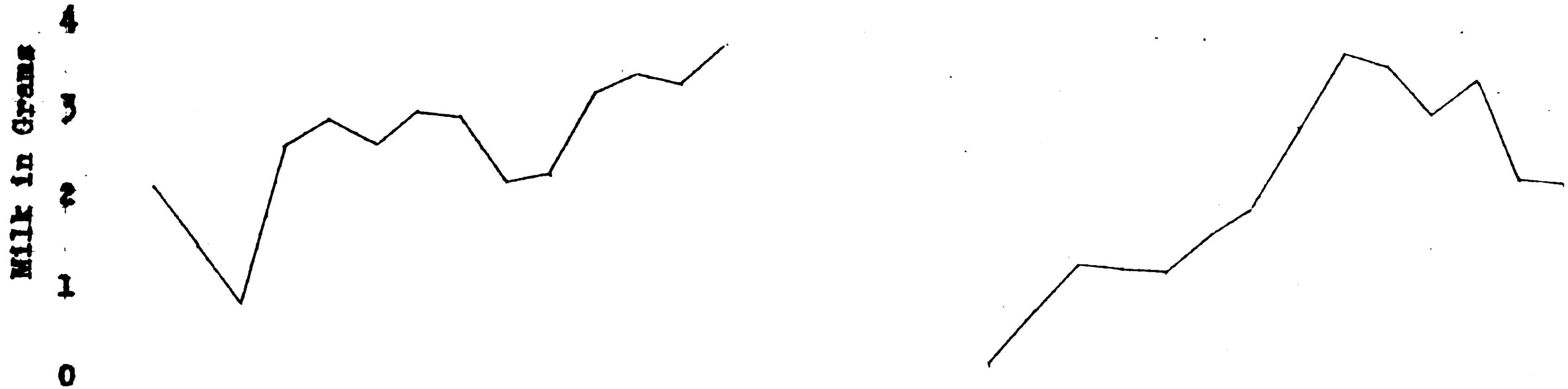

Curves representing the fourteen day samples of mouse #6.
Second Lactation
Third Lactation
Milk in Grams
4
3
2
1
0
Curves representing the fourteen day samples of mouse #7.
Second Lactation
Third Lactation
Milk in Grams
4
3
2
1
0
5
7
9
11
13
15
17
18
DAYS
5
6
8
10
12
14
16
18
DAYS

Figure # XVI

Curves representing the fourteen day samples of mouse #6.

. Second Lactation | Third Lactation



Curves representing the fourteen day samples of mouse #7.

Second Lactation | Third Lactation

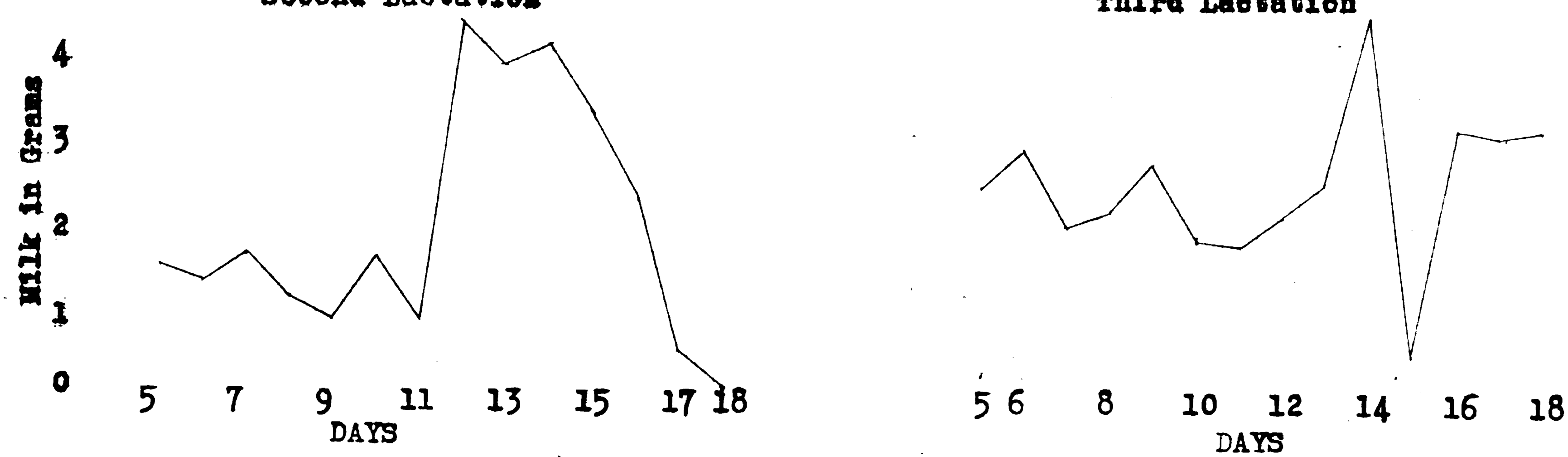


Figure # XVII

Curves representing the fourteen day samples of mouse #8.

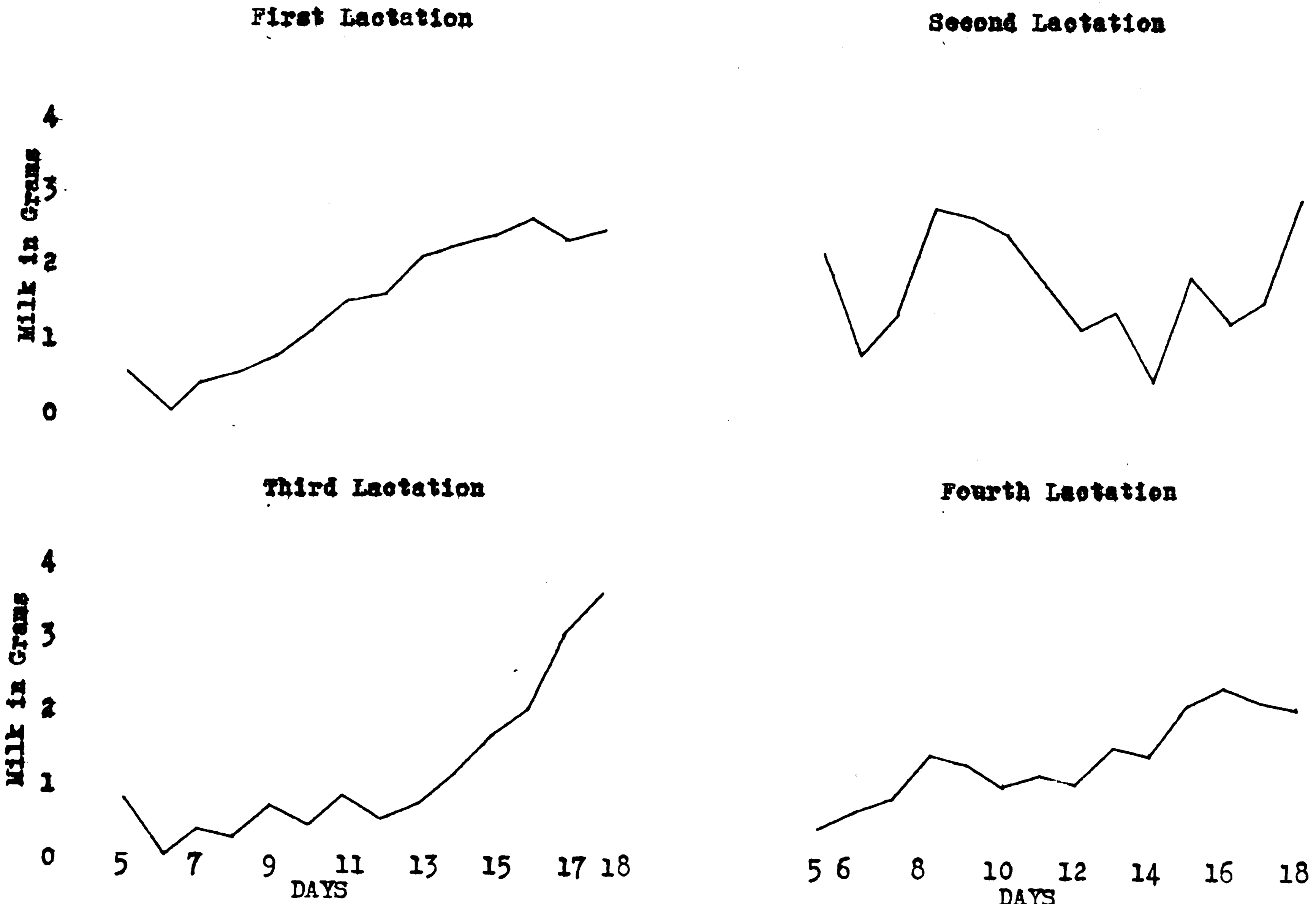


Figure # XVIII

Curves representing the fourteen day samples of mouse #9.

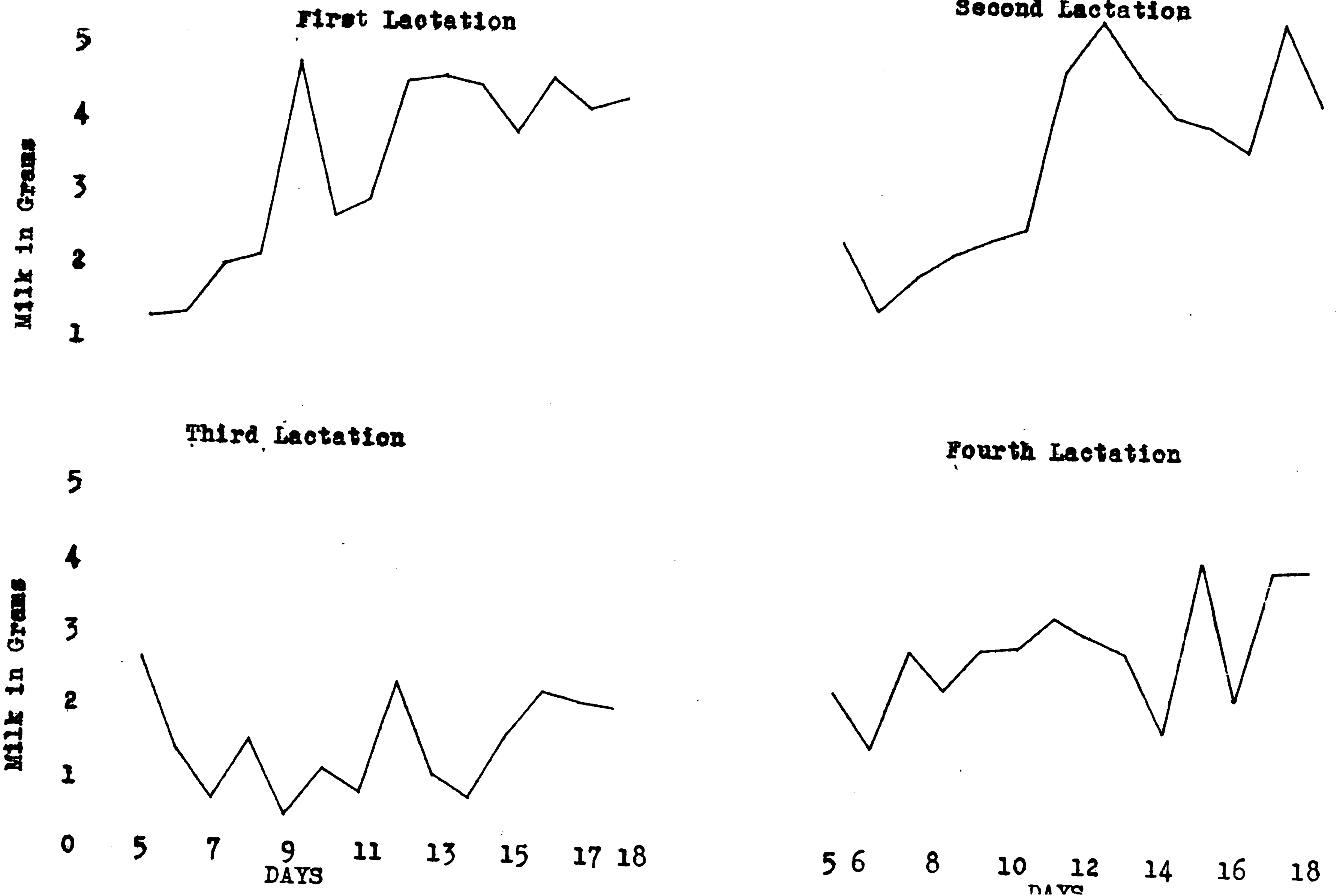


Figure # XIX

Curves representing the fourteen day samples of mouse #10.

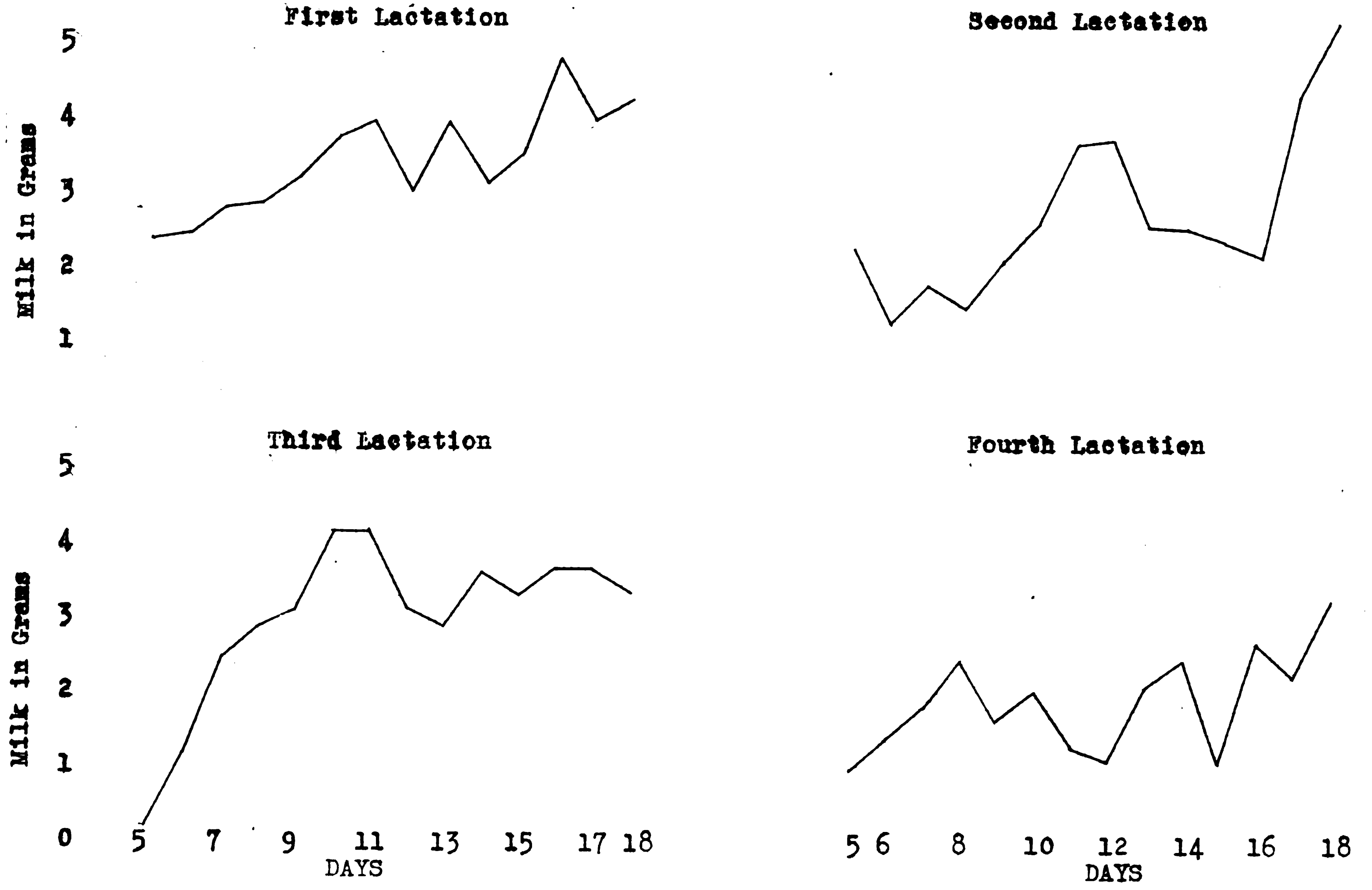


Figure # XX

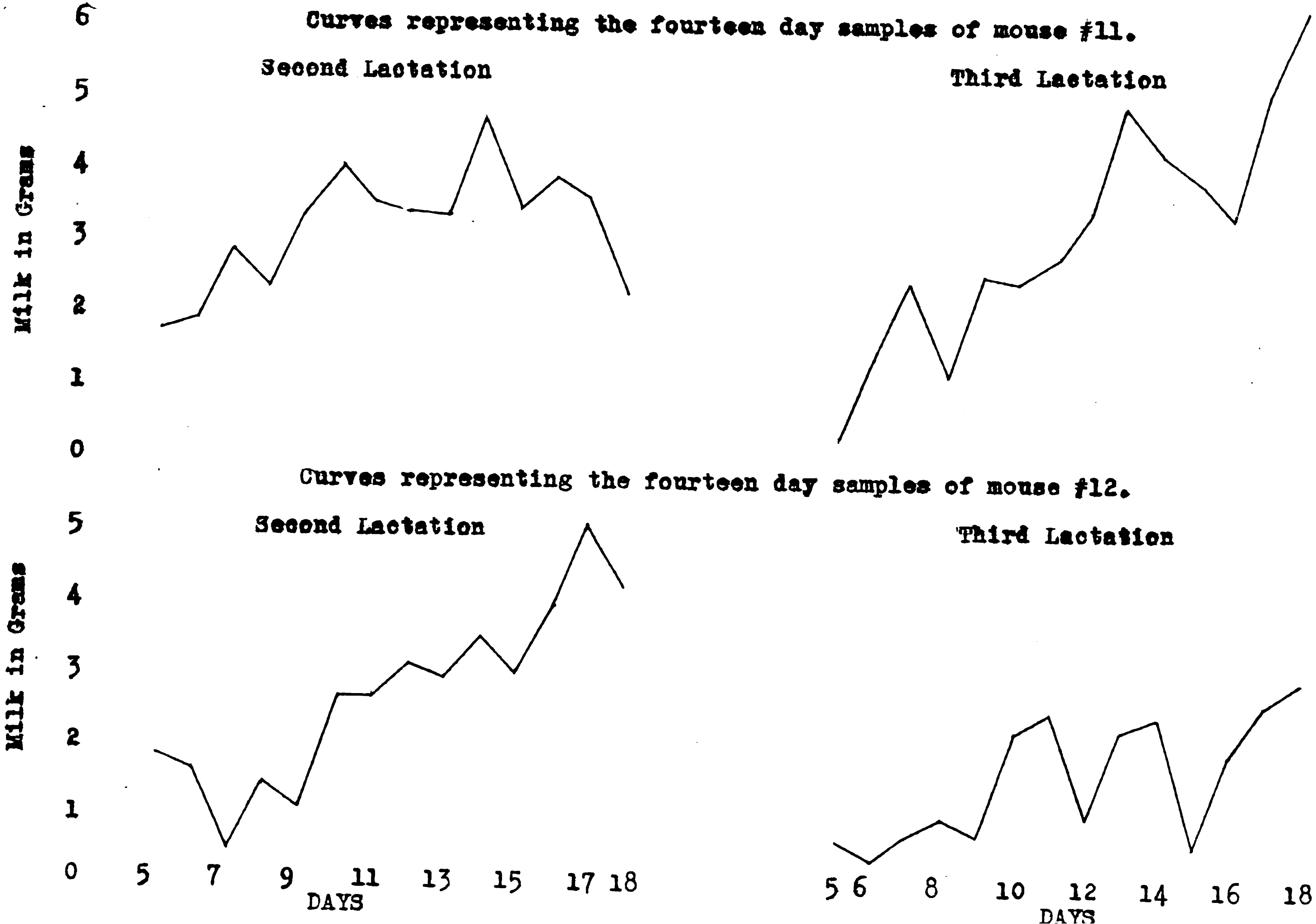

Curves representing the fourteen day samples of mouse #11.
Second Lactation
Third Lactation
Milk in Grams
0
1
2
3
4
5
6
Curves representing the fourteen day samples of mouse #12.
Second Lactation
Third Lactation
Milk in Grams
0
1
2
3
4
5
5 7 9 11 13 15 17 18
DAYS
5 6 8 10 12 14 16 18
DAYS

Figure # XXI

Curves representing the fourth lactation

of females #10, #3, #8, #5, #9, and #2.

Circles (ø) indicate the presence of oestrus.

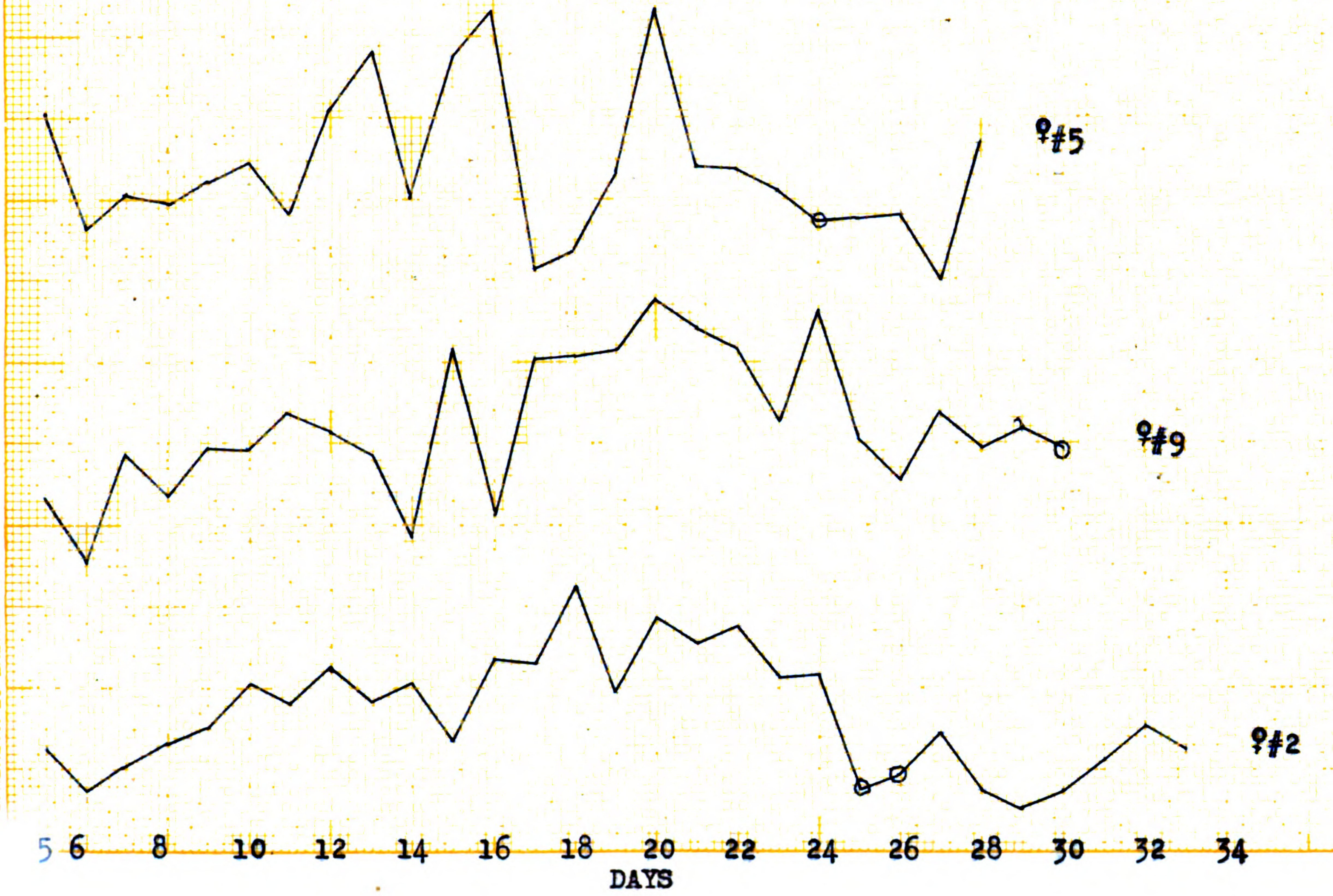


Figure # XXI

Curves representing the fourth lactation of females #10, #3, #8, #5, #9, and #2. Circles (o) indicate the presence of oestrus.

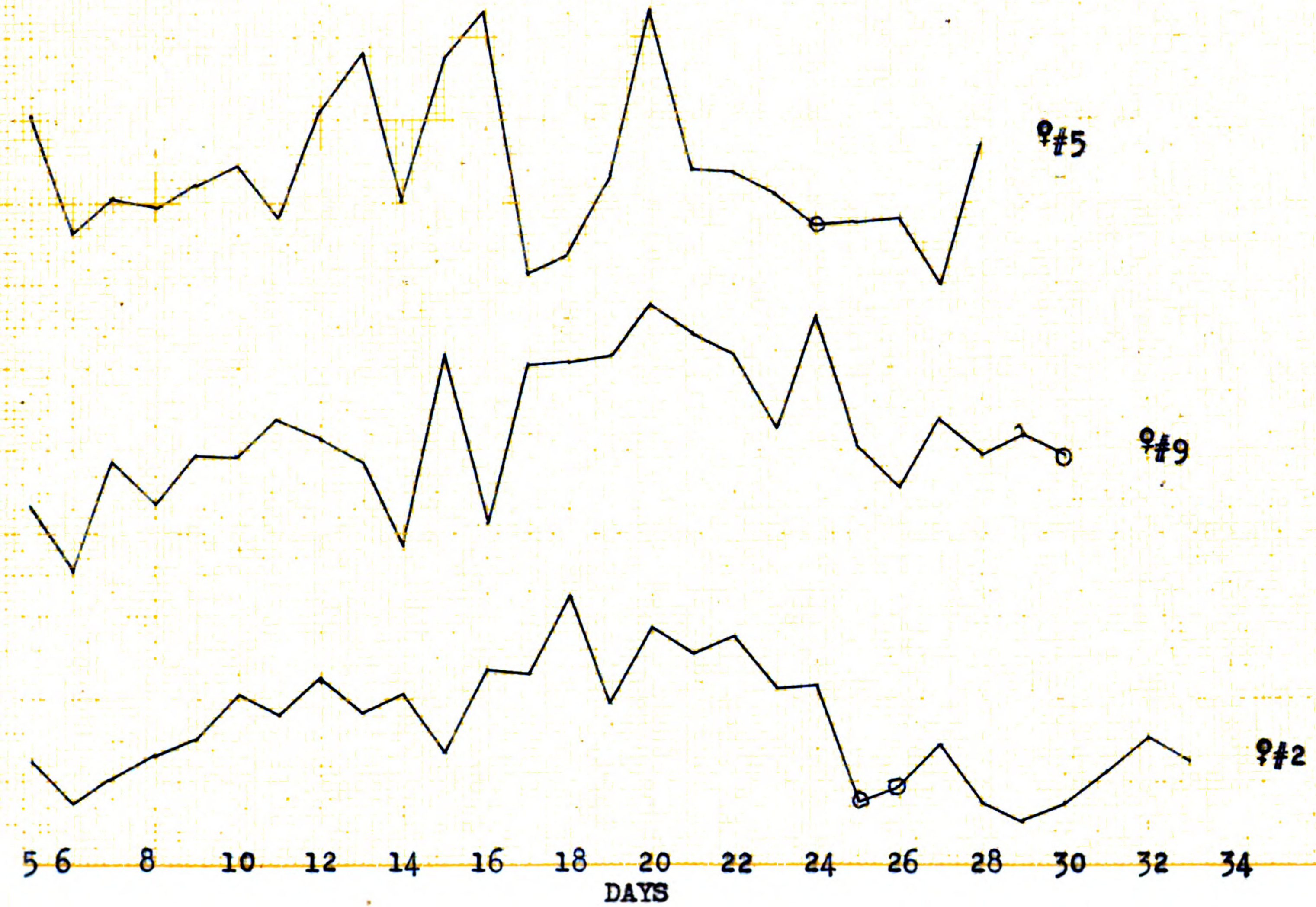
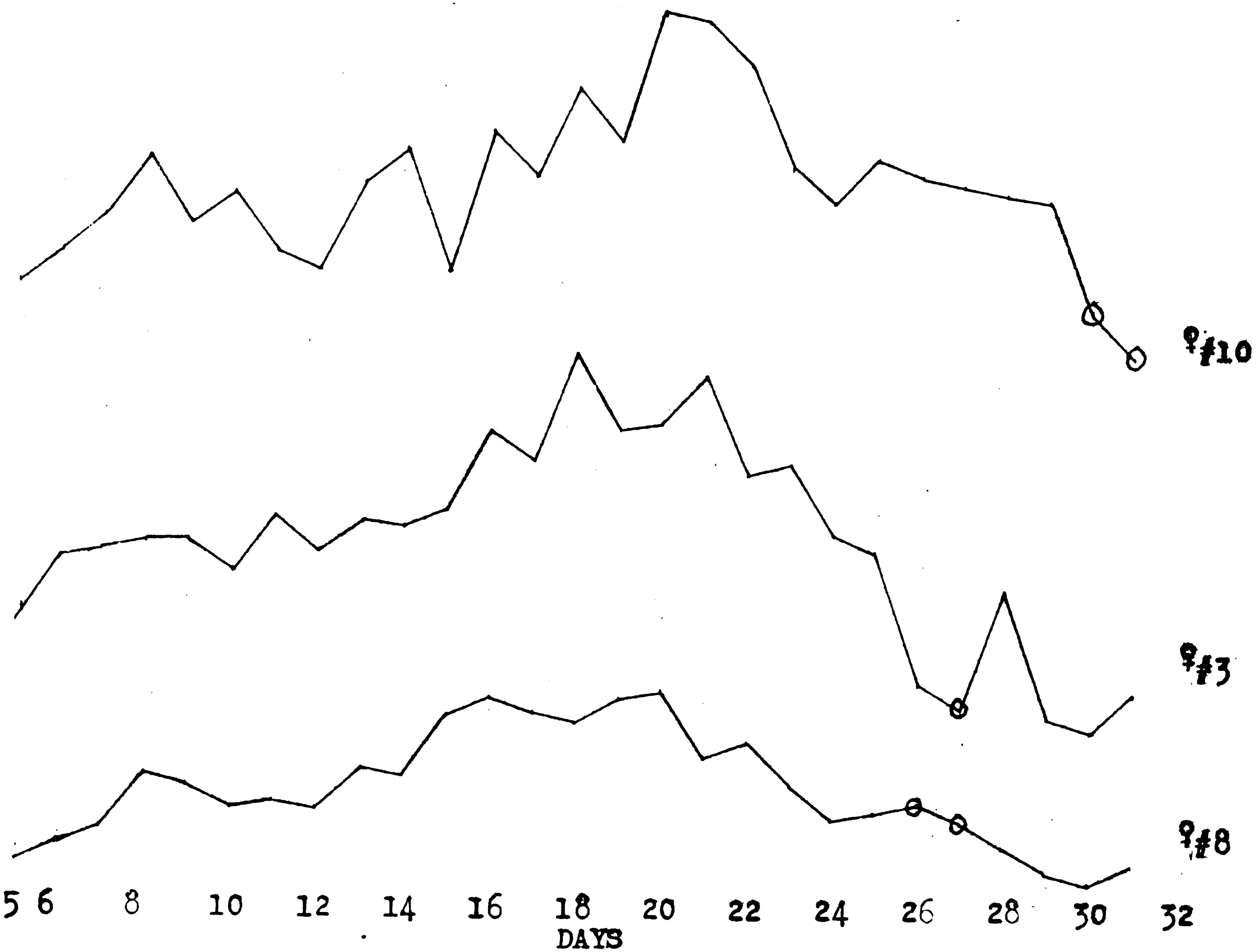


Figure # XXI Continued

Curves representin the fourth lactation

of females #10, #3. #8, #5, #9, and #2.

Circles (ø) indicate the presence of oestrus.



Part XVIII

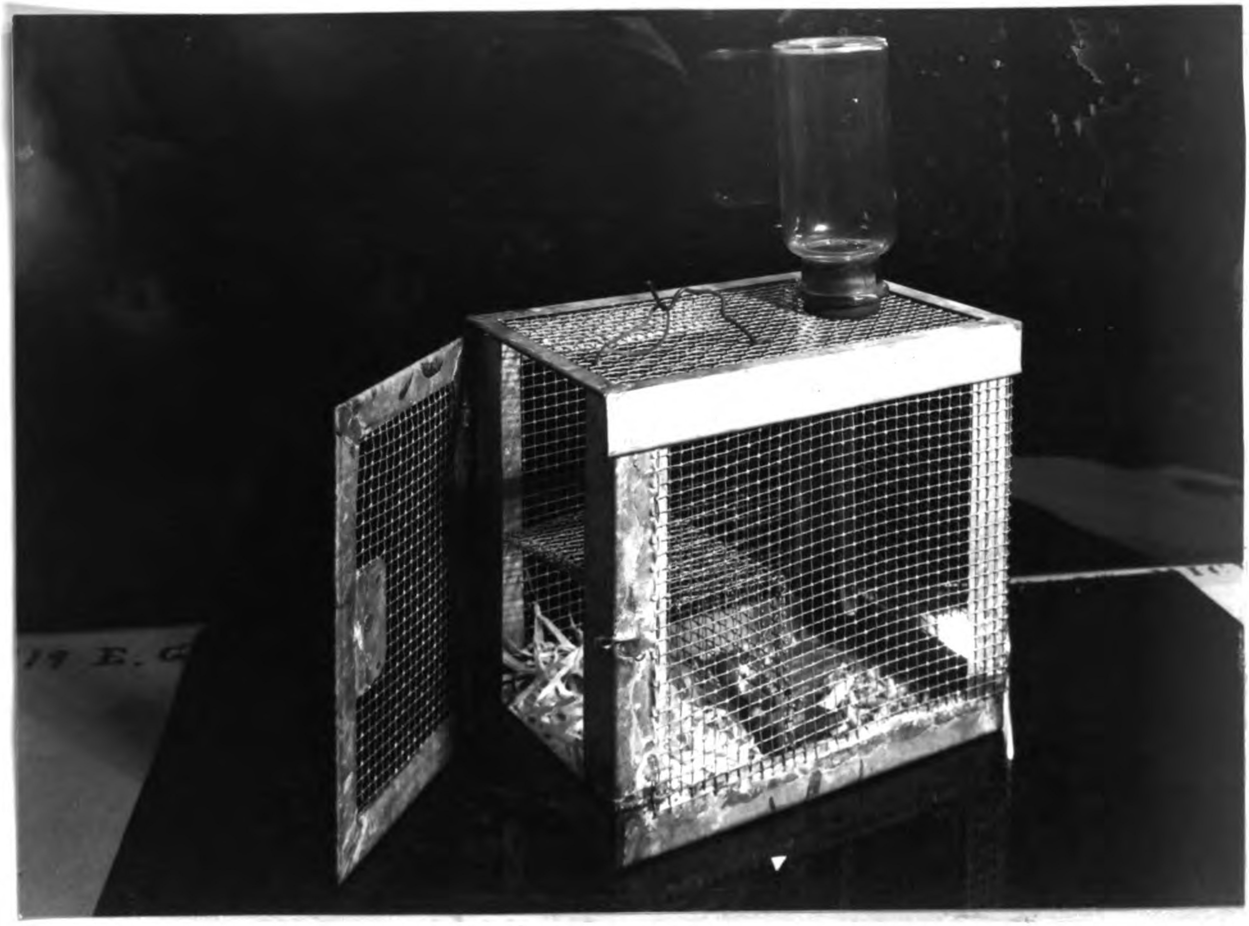


PLATE # I

The experimentsl cage is described on page 5.
It's dimensions are 5½ inches X8 inches X9 inches.

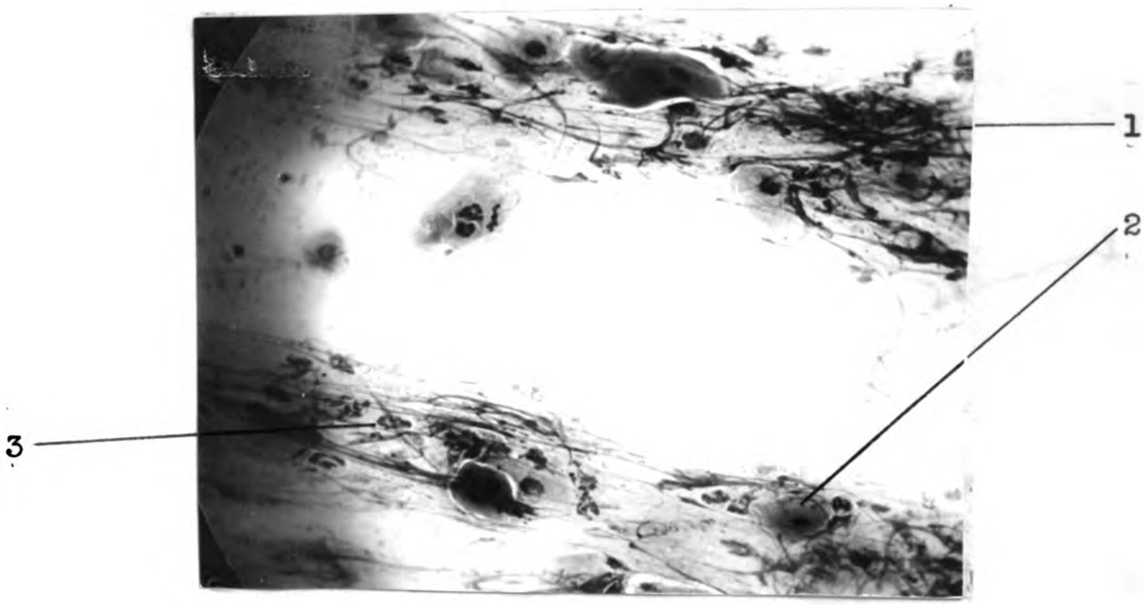


PLATE # II

Photomicrograph of a di-oestrous smear of female #8 on June 27, 1934, stained with eosin and hematoxylin. X 360.

1. Strands of coagulated mucus.
2. Epithelial cells in various stages of degeneration.
3. Polymorphnuclear leucocytes.

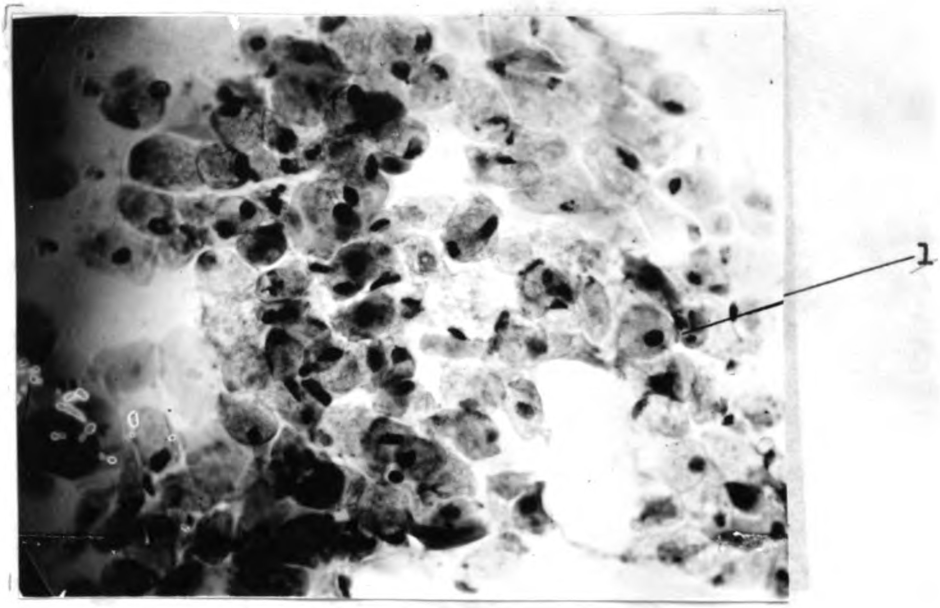


PLATE # III

Photomicrograph of a pro-oestrous smear of females #8 on July 14, 1934, stained with eosin and hematoxylin. X 360.

1. Lightly stained epithelial cells with small compact nuclei.

1

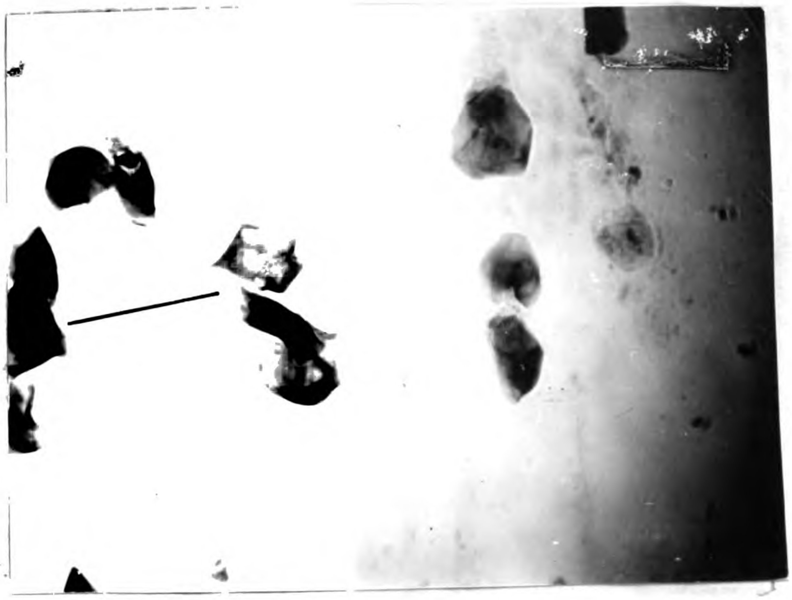


PLATE # IV

Photomicrograph of an oestrous smear of female #8 on July 15, 1934, stained with eosin and hematoxylin. X 360.

1. Cornified, eosinophil, non-nucleated epithelial cells.

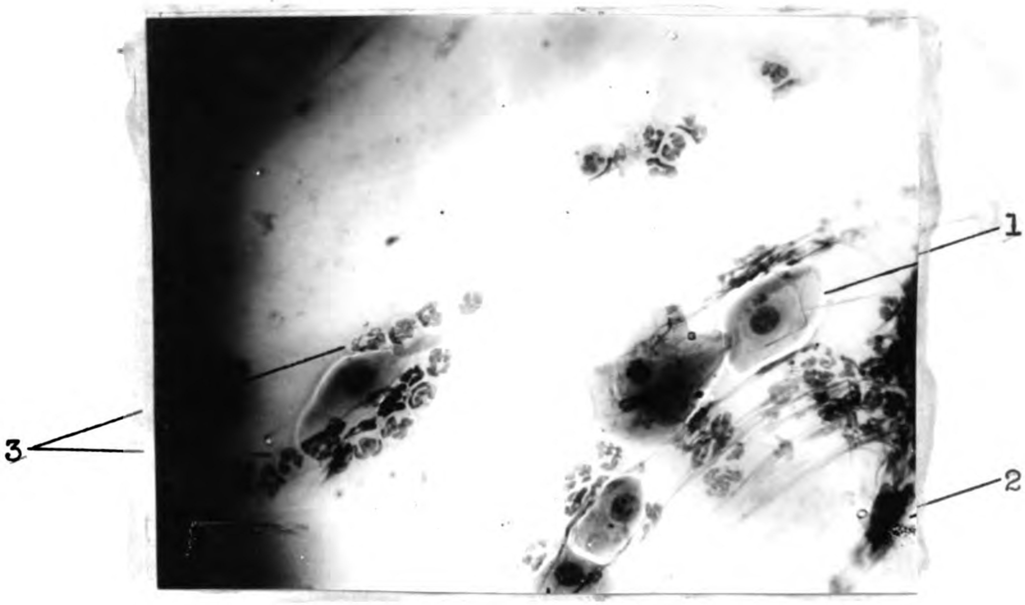


PLATE # V

Photomicrograph of a meta-oestrous smear of female #8 on July 17, 1934, stained with eosin and hematoxylin. X 360.

1. Typical nucleated epithelial cell.

2. Mass of mucus.

3. Polymorphonuclear leucoytes.

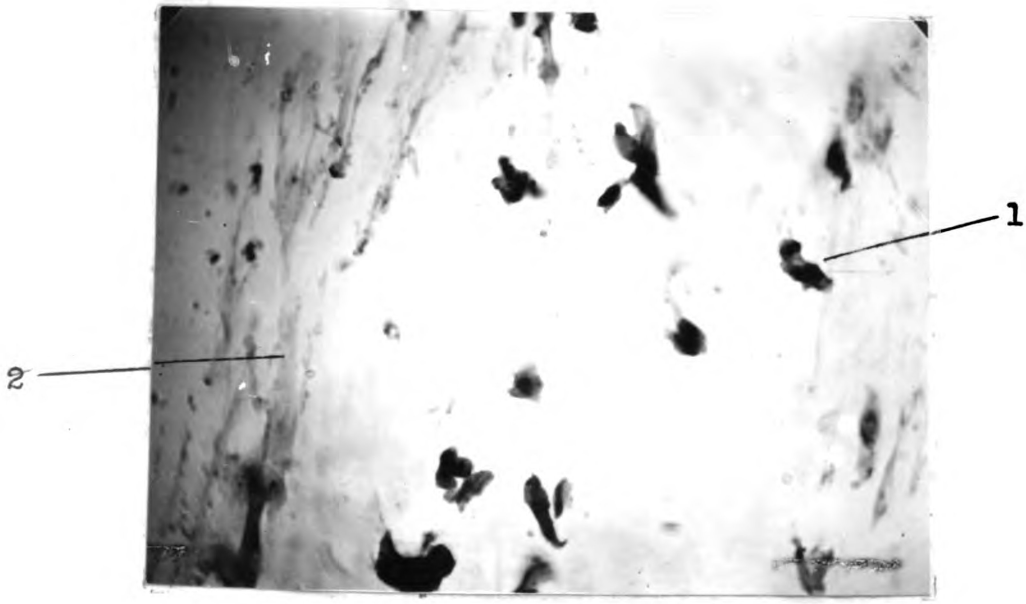


PLATE # VI

Photomicrograph of an oestrous smear of female #2 on July 13, 1934, stained with eosin and hematoxylin. X 360.

1. Cornified, eosinophil, non-nucleated epithelial cells.
2. products of cell decomposition.

1

PLATE # VII

Photomicrograph of an oestrous smear of female #3 on July 20, 1934, stained with eosin and hematoxylin. X 360.

1. Cornified, eosinophil, non-nucleated epithelial cells.

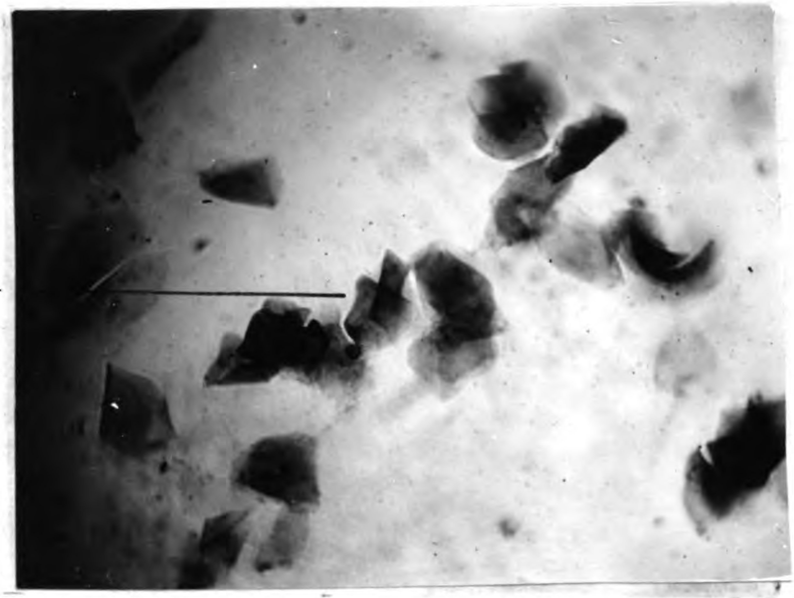


PLATE # VIII

Photomicrograph of an oestrous smear of female #5 on July 16, 1934, stained with eosin and hematoxylin. X 360.

1. Cornified, eosinophil, non-nucleated epithelial cells.

PLATE # IX

Photomicrograph of an oestrous smear of female #9 on July 16, 1934, stained with eosin and hematoxylin. X 360.

1. Cornified, eosinophil, non-nucleated epithelial cell.
2. Typical nucleated epithelial cell indicating the later stages of oestrus.
3. Products of cell degeneration.

PLATE # X

Photomicrograph of an oestrous smear of females #10 on July 20, 1934, stained with eosin and hematoxylin. X 360.

1. Cornified, eosinophil, non-nucleated epithelial cells.
2. Products of cell degeneration.