The University of Oregon

STUDIES ON THE FAT SOLUBLE A CONTENT OF EGGS.

II. ON PERFECTING A DIET WITH PARTICULAR REFERENCE TO THE VITAMIN A CONTENT OF LACTOSE, CRISCO, AND FRESH SPRING AND WINTER EGGS.

Department of Physiology

By

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STUDIES ON THE FAT SOLUBLE A CONTENT OF EGGS.

II. ON PERFECTING A DIET WITH PARTICULAR REFERENCE TO THE VITAMIN A CONTENT OF LACTOSE, CRISCO, AND FRESH SPRING AND WINTER EGGS.

In some early work in which we were using a Vitamin A deficient diet, we experienced considerable difficulty which we attributed to variations in the Vitamin A factor in our basal diet. The results that we obtained were rather unsatisfactory in the following respects:

1. No specific opthalmia occurred.
2. Cessation of growth was in all cases obtained but loss in weight followed in only a few instances.
3. It was necessary to run the experiment for a much longer period of time than would have been necessary with a more highly purified diet.
4. Death due to the deficiency was greatly delayed.

Because of these objections we felt that there was ample reason to believe that Vitamin A was present in amounts sufficient to prevent the classical signs of a Vitamin A deficiency and that it had gained access through some ingredient or ingredients of the diet which we had been led to believe was practically devoid of this factor.

This diet, which we shall call Diet A, consists of the following: Protein (purified casein) 18; Carbohydrates 51 (Lactose 15 and Rice starch 36); Crisco (fat) 20; salt 6 and agar agar 5. Yeast and orange juice were added daily to the drinking water in sufficient quantities to insure an adequate amount of Vitamins B and C. In no instance was the orange juice over 10 c.c. nor the yeast over 3 grams. To the above diet was added varying amounts of the ether soluble portion of egg yolk as a carrier of Vitamin A.

It will be seen that the above diet supplies all the factors that an adequate diet demands. Examining the diet for the purpose of detecting the possible presence of Vitamin A, we can look with suspicion on all the items except agar and salt. Coming as it does from milk in which there is a marked amount of Vitamin A, casein is at once suspected of having carried down with it some of this Vitamin. In fact Drummond (1) states specifically that commercial caseinogen contains relatively large amounts of Vitamin A. For that reason we carefully purified it by thrice dissolving it in alkali and precipitating with acid. This proceed-

1. Drummond. Biochem.Jour. Vol.14,1920 p.661.

ure was followed by an alcohol and an ether extraction after which the casein was subjected to a warm air drying for several hours. Even with this proceedure it is conceivable that there would still remain minimal amounts of Vitamin A.

Lactose is also open to criticism for McCollum and Davis (2) in some work they were conducting with lactose found that commercial preparations contained adequate amounts of Vitamin B when fed at a 20% level and they strongly suspected it also of containing some Vitamin A. As we shall later show, diets in which we reduced the lactose to one-third its original amount, showed a Vitamin A deficiency much sooner and in a more marked degree.

In regard to rice starch, Drummond (3) states that it is practically devoid of Vitamin A.

Crisco was the only fat added to the diet aside from the fat (egg yolk) to be tested. Crisco is a commercial preparation of cotton seed oil which had been partially hydrogenated. The makers refer to it as a hydrostearâleine. For two reasons Crisco could be considered as being fairly free of Vitamin A. First because cotton seed oil is fairly inactive in

2. McCollum and Davis. Jour.Biol.Chem.Vol.23, 1915, p.231
3. Drummond. Biochem.Jour.Vol.14,1920,p661.

this respect and secondly because the process of preparation would tend to destroy any Vitamin A present. Hess(4) used it in as large quantities as 17% with no reported difficulties. In Diet A, it was used in 20% quantities. As mentioned before, we were not satisfied with the results and in looking over the diet first suspected Crisco of containing more Vitamin A than we had at first believed. To test this out, we made up the following four diets:

	Diet I	Diet 2	Diet 3	Diet 4
Casein	18	18	18	18
Lactose	15	15	15	15
Rice Starch	56	51	51	36
Agar Agar	5	5	5	5
Salt	6	6	6	6
Crisco	0	5	2	20
Egg Yolk	0	0	3	0
Orange Juice	q.s.	q.s.	q.s.	q.s.
Yeast	q.s.	q.s.	q.s.	q.s.

The growth curves for these series are shown in Chart I. It will be seen here that the growth curves for Crisco at a 20% level are much better than where the growth stimulant was contained in only a tenth of this amount. This we have attributed to their being some Vitamin A present. In fact Daniels and Laughlin (5) found by feeding cotton seed oil at a level of 28% they were able to get adequate growth with repro-

4. Hess. Jour.Biol.Chem.Vol.47,1921,p.395.
5. Daniels and Laughlin.Jour.Biol.Chem.Vol.42, 1920,p.360.

duction of young. They found, however, that at a 21% level these results were not obtained. While at a 20% level we got results indicative of a Vitamin A deficiency, we do not feel that these results are decisive enough to warrant definite statements concerning the Vitamin A content of the fats we were testing.

In order to investigate the statement made above concerning the presence of Vitamin A in lactose, we made up a diet as follows:

	Low Lactose Diet	Diet A
Casein	18	18
Starch	60	36
Lactose	5	15
Crisco	5	20
Agar Agar	8	5
Salt	4	6

The results are graphically shown in Chart II. It will be seen that the growth curves not only attained a less height but that the decline in growth occurred earlier and with greater abruptness. It would seem therefore that our earlier results with Diet A were fogged to a great extent by the presence of Vitamin A contained in Crisco and in Lactose fed at 20% and 15% levels respectively.

Osborne and Mendel(6) showed that diets containing as high as 42.5 parts of yeast failed to protect rats against a Vitamin A deficiency. Drummond and

6. Osborne and Mendel. Jour.Biol.Chem. Vol.45, 1921,p.277.

Coward(7) found that only .08% of yeast was ether soluble and that only one-half of this was true fat. In a diet containing 5% yeast they considered they were maintaining a fat deficiency.

It has long been considered that orange juice is a rich source for Vitamin C, but it had not been thought, until recently, that it provided appreciable quantities of Vitamins A and B. In 1916, Funk and McCallum(8) stated that orange juice had neither growth promoting nor growth sustaining properties. In 1920, Drummond(9) stated that orange juice was devoid of Vitamin A.

In spite of these assertions, various workers noted that whenever orange juice was included in their diets, signs of Vitamin A deficiency were not as pronounced. For instance, Hess(10) noticed that keratitis developed with less frequency. Drummond (11) considered Vitamin C as an augmentor of the action of Vitamin A and thus sought to explain the discepepancies arising from including orange juice in the diet. We found in our work that the only eye lesions obtained were limited to a slight redness of the lids. In

7. Drummond and Coward.Lancet,Oct.1,1921,p.698.
8. Funk and McCallum,Jour.Biol.Chem.Vol.27,1916, p.51.
9. Drummond.Biochem.Jour.Vol.14,1920,p.661
10. Hess.Jour.Biol.Chem.Vol.47,1921,p.395.
11. Drummond.Biochem.Jour.Vol.13,1919,p.78.

only one rat in all the animals we have run on Vitamin A deficient diets have we succeeded in obtaining keratomalacia. This was unilateral and preceeded the death of the animal by only two days.

In 1920, Byfield and Daniels(12) and Osborne and Mendel(13) showed that orange juice had Vitamin B present. The latter stated that it was present to the same extent as the same volume of fresh cows milk. In 1921, Cooper(14) reported that orange peel contained Vitamin A. These two findings threw considerable doubt on the vitamin content of oranges. Because of our own results and the above indications we considered it advisable to not only carefully peel the oranges (or lemons) but to add the expressed juice in amounts barely sufficient to supply the Vitamin C requirement. Davey(15) showed that the minimum was 1.5 c.c. daily. To avoid the possibility of similar complications in respect to Vitamin B, yeast was added in the minimum amount of .2 grams as stated by Osborne and Mendel(16). This reduces to a minimum

12. Byfield and Daniels.Amer.Jour.Dis. Child. Vol.19,1920,p.349.
13. Osborne and Mendel. Jour.Biol.Chem.Vol.42, 1920,p.465.
14. Cooper.Proc.Soc.Exp.Biol.and Med. Vol.18, 1921,p.243.
15. Davey. Biochem.Jour.Vol.15,1921,p.695.
16. Osborne and Mendel,Jour1Biol1Chem.Vol.32, 1917,p.309.

any synergistic action the vitamins may have for each other. The yeast was placed in water in such proportions that 1 c.c. of the suspension was equivalent to .2 grams of the dried yeast.

Osborne and Mendel in the work above referred to, in stating that orange juice contained as much Vitamin A as similar amounts of fresh cows milk, added that it took 10 c.c. of the juice to insure a normal rate of growth and prevent opthalmia and that 5 c.c. merely prevented the onset of eye symptoms. They stated that .1 gram of butter fat accomplished the same results as the 10 c.c. of orange juice. In using 1.5 c.c. of orange juice, we felt that we were keeping well within the limits of any complications arising from its use (or the use of lemon juice).

Drummond(17) showed that in all probability Vitamin A is a photosynthetic product associated with the action of sunlight on chlorophyl. If this is true,it could be assumed that all plants showing the presence of chlorophyl at some stage of their growth would have some Vitamin A present.

The evidence of Cooper, the inference drawn from Drummond's work, and the conflicting results obtained by others as well as ourselves whenever orange juice

17. Drummond.Biochem.Jour.Vol.15,1921,p.530.

was used led us to suspect strongly that orange juice possessed Vitamin A. We therefore changed to lemon juice with practically no change in our results. In the meantime a series of experiments were instituted to determine the Vitamin A value of lemon juice. These will be reported later. During the preparation of a preceeding report, we received a communication from Mendel(18) stating that orange juice contains Vitamin A. Our evidence will not only substantiate this but add further knowledge concerning citrus juices as carriers of Vitamin A.

In summarizing the above with regard to any possible presence of Vitamin A in the items of our diet, w we can make the following statements:

1. Casein, though carefully purified, probably retains traces of Vitamin A.
2. Lactose contains Vitamin A but at a 5% level would not seriously effect the results.
3. Rice starch is practically devoid of Vitamin A.
4. Crisco contains Vitamin A but at a 5% level would not seriously impair the diet.
5. Yeast is practically devoid of Vitamin A.
6. Orange juice contains Vitamin A but an addendum of 1.5 c.c. per rat per day would not seriously impair a diet in this respect. The same can probably be said of

18. Private communication. Later appeared in Proc. Soc.Exp.Biol. and Med.Vol.19,1922,p.187.

lemon juice.

Although a diet may conform to the above limitations, it is still subject to the following criticisms;

1. Why include lactose in a diet when it is known to contain Vitamin A.and another carbohydrate is already present?
2. Why include Crisco(fat) in the diet when it also prepresents Vitamin A and is not an indispensible item since fat can be synthsized by the organism?
3. Why include Vitamin C in the diet when a rat, so far as we know, is not particularly susceptible to scurvy?

We shall attempt to answer these questions in the following manner. Eggs and milk are the only natural foodstuffs elaborated by nature for the special purpose of nourishing the young. The best artificial diet can be only a close approximation of these. Grossly, they are made up as follows:

	Protein	Carbohydrate	Fat	Salts
Egg yolk	15.7	0.	33.1	1.
Milk	3.3	5.(lactose)	4.	.7

It will thus be seen that nature has perfected a diet for the young in which protein, carbohydrate (including lactose) fats and salts are well represented. Vitamins are in the milk according as the mother receives them in her food. The young rat, in contradistinction to the young bird, before birth derives very little nourishment from the egg. Any storage of foods, such as is necessary for birds, is obviated in the rat by its placental attachment to the maternal organism. After birth the maternal milk nourishes the young until they are able to care for themselves.

Lactose is therefore added to the diet to make it conform more completely to the natural diet. There are still other reasons why fats should be added. It is true that Drummond(19) showed that rats could show practically normal growth on an artificial diet that was entirely free of fats but he also mentions that these same rats showed a more marked lowering of vitality than those whose diet contained fat. Until the action of fats and lipoids in immunity reactions is much better understood, we do not deem it wise to increase the chances for experimental errors by probable decreases in vitality above referred to. Another reason why fats should be included in the diet--and probably the most practical reason--is for the sake of an accurate balance for the control diet. How can the control diet and the test diet be declared similar when one contains no fat and the other contains the fat which is being tested as the carrier of VitaminA ? For example,

19. Drummond. Lancet, Oct.1,1921,p.699.

the control diet has 5% Crisco and the test diet 2% Crisco and 3% egg yolk in it. Both have 5% fat--the former practically Vitamin A free and the latter containing it. Had the former had no Crisco and had the latter contained egg yolk only, the two would not have been fat-balanced. If one could separate Vitamin A from the fats without altering its potency, it would be possible to test it in diets containing no fats. But, so far at least, Drummond is the only one who has reported the ability to do this. His objections to a fat free diet have already been mentioned.

It has been assumed that because rats did not easily manifest signs of scurvy, it was not necessary to include Vitamin C in an artificial diet. McCollum, Simmonds, Shipley and Park(20) stated that rats synthesize Vitamin C. The inference is that because of this fact it is unnecessary to add such an item to d diet. On the other had Harden and Zilva(21) claim that rats thrive better when they have the antiscorbutic vitamin. Drummond(22) states that optimal experimental conditions are only attained when Vitamin C is present as well as the other factors.

Although rats may not show marked lesions of scur-

20. McCollum, Simmonds, Shipley and Park. Jour. Biol. Chem. Vol. 47, 1921, p. 507.
21. Harden and Zilva. Biochem. Jour. Vol. 12, 1918, p. 408.
22. Drummond. Biochem. Jour. Vol. 13, 1919, p. 77.

vy, it would seem advisable, inasmuch as the bone lesions of rickets and of scurvy are easily confused, to include the antiscorbutic factor in the diet and thus rule out any possibility of objections from a theoretical nature or any opportunity for the fogging of results from some factor at present neither thoroughly understood nor even suspected.

The three above objections can therefore be met by the following arguments in brief:

1. In feeding young animals on an artificial diet, that diet should conform in all respects as nearly as possible to the natural diet that nature has already furnished for their nourishment. The omission of any item by the plea that it is unnecessary or that the organism is able to synthesize it, is adding, under test conditions, handicaps not existent under a normal condition whose influence on the outcome of the experiment is unknown.
2. There is evidently some relationship between fats and immunity. Animals deprived of fats or having their diets so restricted that not only is fat storage impossible but those organic substances generally present in fats are absent, present such a marked lowering of resistance to infections and disease processes, that no other conclusion is possible. A-

side from the raising the resistance to infections, fat should be present in both test and control diets for the sake of an equal balance.

From the above considerations, then, we modified our diet A in the following manner:

	Diet A	Diet B	Diet C
Casein	18	18	18
Rice starch	36	51	60
Lactose	15	10	5
Crisco	20	10	5
Agar agar	5	5	8
Salt	6	6	4
Yeast	q.s.	q.s.	.8 gm.daily
Orange juice	q.s.(peeled lemon)	q.s.	1.5 c.c.daily

In Diet C, it will be observed that the principal changes are an addition to the amount of rice starch; a cutting of the lactose to one-third the original amount; a similar reduction in the fat to one-fourth the original amount; a slight increase in the roughage and a decrease in the salt. Another change is the limitation of yeast to the minimal amount and the substitution of lemon for orange juice. In the preparation of the juice, each lemon was carefully peeled and the juice expressed from the pulp and filtered. We felt that these minimal amounts, while on the border line and founded on the basis of a diet different from ours, would be sufficiently augmented by Vitamins B and C in the other items of the diet to obviate any difficulties arising from a possible shortage of them.

The arbitrary use of a minimum quantity of any vitamin carrier by various experimentors working with different diets is extremely objectionable because of unavoidable variations in the amount of vitamin present in their basal diets. This may be due to (1) the kinds of food used; (2) the method of their preparation; (3) variations in the percentages used in the diet; (4) variations due to the season in which the foods were grown; (5) variations due to methods of handling, exposure to sunlight and other factors such as the form in which the substance is used.

Take, for example, a discussion of the minimal amount of yeast necessary for satisfactory growth. This may be expressed in absolute quantities or in terms of a percentage of the diet. The diets used will be as numerous as the experimentors working with them. Drummond(23) used 5% yeast extract; Osborne and Mendel(24) used .4 gm. of the extract daily and .2 gm. of dried yeast which was equivalent to 2%; Seidel(25) used .5 to 1 c.c. of the clear filtrate of autolyzed yeast daily; Funk(26) used 3% as a minumum. It is thus seen how difficult it is to state arbitrarily what the minimum requirement of a certain vitamin is. At the best

23. Drummond.Lancet,Oct.1,1920,p.698.
24. Osborne and Mendel.Jour.Biol.Chem.Vol.32,1917, p.309.
25. Seidell.Jour.Biol.Chem.Vol.29,1917,p.145.
26. Funk.Jour.Biol.Chem.Vol.27,1916,p.1.

it is only an approximation and can apply specifically only to thediet chosen and the technique used. Uniformity cannot be attained in this respect unless the ingredients of a diet, their preparation,and their proportions are standardized.

There still remains bo be discussed under the subject of diet, the preparation of the egg yolk. The yolks of the eggs were carefully separated from the whites and then thoroughly shaken up with ether. That portion soluble in ether was then poured off and evaporated. It was evaporated in two ways: in sunlight and in a hot water bath. Two sets of experiments were run to test out the efficacy of the Vitamin A contained in the two samples thus prepared. As Chart III shows, the egg yolk subjected to sunlight did not possess as much growth stimulating properties as that prepared in the hot water bath. This can be explained on the basis of some work by others who found that Vitamin A is destroyed by oxidation. This varies with the amount of surface exposed, the duration of the exposure and the temperature at which exposure occurs. We, accordingly, subjected our ether solution to evaporation with a minimum of surface exposed for as short a time as possible at a temperature approximating 65' C. With this proceedure, 500 c.c. of ether solution was evaporated in 20 minutes. Hopkins(27) showed that butter fat heated to 120' for one hour and aerated lost very little of its Vitamin A potency as compared with samples treated in this same manner but for longer periods of time.

The vitamin content of milk has been shown to vary with the season and with the diet fed the cow. The Vitamin A content of fat deposits in the body has been found to have the same variations and in addition has been shown to vary with the tissues examined. It might be supposed therefore that the Vitamin A content of eggs would also vary with the season and with the diet of the hens laying them. The former we have confirmed as the growth curves in Chart IV show. Here it is seen that fresh November eggs did not promote as good growth as did fresh eggs gathered in the spring. Communications(28) we have received indicate that variations due to diet are also true.

In the feeding experiments which we have conducted during the past two years, we have observed that the rate of growth of rats is proportional to the amount of Vitamin A contained in their diets; that the Vitamin A requirements of a diet constantly lessen as the animal becomes older and that young animals, be-

27. Hopkins.Biochem.Jour.Vol.14,1920,p.725
28. Private communication.

cause of the excess of Vitamin A stored in their tissues (which they acquired from their mother) are able to coast for a short time, showing no effects of a deficient diet.

Chart V illustrates the variations in the rate of growth dependent upon the Vitamin A content of the food. Chart VI contains constructed curves following the curve of expectation of growth and showing the relationship between age and Vitamin A requirement. These charts illustrate graphically why it is that Vitamin A is considered necessary to both growth and maintenance. A young animal attains growth by being able to add new cells to its body tissues. If the elements required in this cellular formation are not present, cell production ceases. If a certain amount of the necessary elements are present, cell replacement will occur and an animal will maintain itself at a certain level. If these elements are lacking or insufficient, cell production not only ceases but cell replacement cannot occur and the body regresses--loses weight and the animal dies. A corollary of this is that any tissue whose function is the constant production--over and above simple growth and maintenance requirements--of cellular elements will be the first tissue to suffer from the general deficiency. An example of such tissues are the gonads and mammae. It is on such a basis that we have conducted a series of experiments on the effects of Vitamin A deficiency on reproduction. This work will be reported in a subsequent paper.

In early life metabolism is at its height. Not only are tissues being maintained but new cellular elements are being rapidly added which constantly increases the burden of maintenance. Ultimately the organism attains its growth--the increase in cells stops--that limit placed by Nature has been reached and simple cellular maintenance is now all that is required. Still later in life, the balance is upset and cell destruction occurs with greater rapidity than cell replacement. Through this entire cycle, it can be seen that the Vitamin A requirement is large at first, dwindles as maturity approaches, maintains a fair constant for a time and finally dwindles again as the animal ages and dies.

The life cycle of a rat is comparitively short. The period of gestation is three weeks. A rat is weaned in three weeks. At three months of age they can bear young. One or two months later they have practically reached adult weight. From this time on they remain fairly constant until retrogressive changes set in. It will therefore be seen that any experiments on Vitamin A requirements must be conducted before the animal reaches maturity--that is, during the first four or five months of its life.

The question now arises, how early should an experiment be started. Most workers start their rats on feeding experiments when the animals average a weight of 40 grams--laying more stress on their initial weight than on their age. Generally speaking, this is good practise because of the variations in vigor of animals. Some rats weigh 40 grams at 30 days of age while others may not attain that weight until they are 50 days old. It is self evident that the latter are poor experimental animals because of the poor growth stimulus manifested. On the other hand, the former animals might give indications just the contrary. These considerations, though real, do not present practical difficulties for the reason that most labratory workers are raising select stock animals whose vitality and vigor are average and contant.

However, it was with the idea of getting some actual data on this condition that we ran a series of three experiments as follows: Group A 44 days old; Group B 35 days old and Group C 21 days old. Group A averaged 42 grams in weight; Group B, 32 and Group C, 33. All were put on Diet B in which the l lemon juice was limited to 3½ c.c. per day and the yeast to .5 gram. 60 days later, Diet C was substituted for Diet B. Chart VII shows the resulting growth curves.

As stated before, Diet C is our improved Vitamin A deficient diet. To this was added 1.5 c.c. of lemon juice and 1 c.c. of yeast suspension. It will be seen that Group A showed very little gain from the outset and that their deaths occurred much earlier than those of the other groups. Group B showed some primary retardation but, following that, manifested fairly good growth for a time and then declined rapidly to the point of death. Group C showed good ga gain from the beginning, ran their weights to nearly 100 grams and then rapidly declined to death. The results can be tabulated as follows:

Group-	Age-	Initial weight-	Days for gain-	Greatest weight-	Days for loss-	Time
A	44	42	60	97	20	80
B	35	32	40	55	30	70
C	21	33	40	60	10	50

For clearness of results, decisiveness and the lack of vitiating complications, Group C offers the most ideal conditions. The poor growth manifested by Group C can probably be explained on the basis of the radical change in their diet, that is, from breast milk to test diet before their digestive system had become well enough established in function to handle this shock without detriment to the animal's general metabolic processes. We conclude therefore that test animals must not only be of average vigorous stock but that their initial ages should closely approximate 44 days and their weights 42 grams.

If an experiment is run within the limits we have specified above, it will be seen that it cannot last much over a period of 120 days. It is imperative therefore that a basal diet be of sufficient purity in regard to the factor tested to produce decisive results within that time. This we believe we have accomplished in our Diet C.

Summary.

In establishing a deficient Vitamin A diet that will give decisive results within experimental time limits, we have shown that

1. Lactose at a 15% level and Crisco at a 20% level contain too much Vitamin A for an ideal diet.
2. In all probability orange juice contains Vitamin A.
3. The action of sunlight on food exposed to the air is destructive to its Vitamin A content.
4. Winter eggs are of lower Vitamin A content than spring eggs.
5. The optimum time for starting rats on a feeding experiment is when they are between the ages of

35 and 45 days and weigh between 40 and 50 grams.

6. Our Diet C will produce a Vitamin A deficiency within experimental time limits and at the same time conform to all the requirements of an adequate diet.

Chart I.

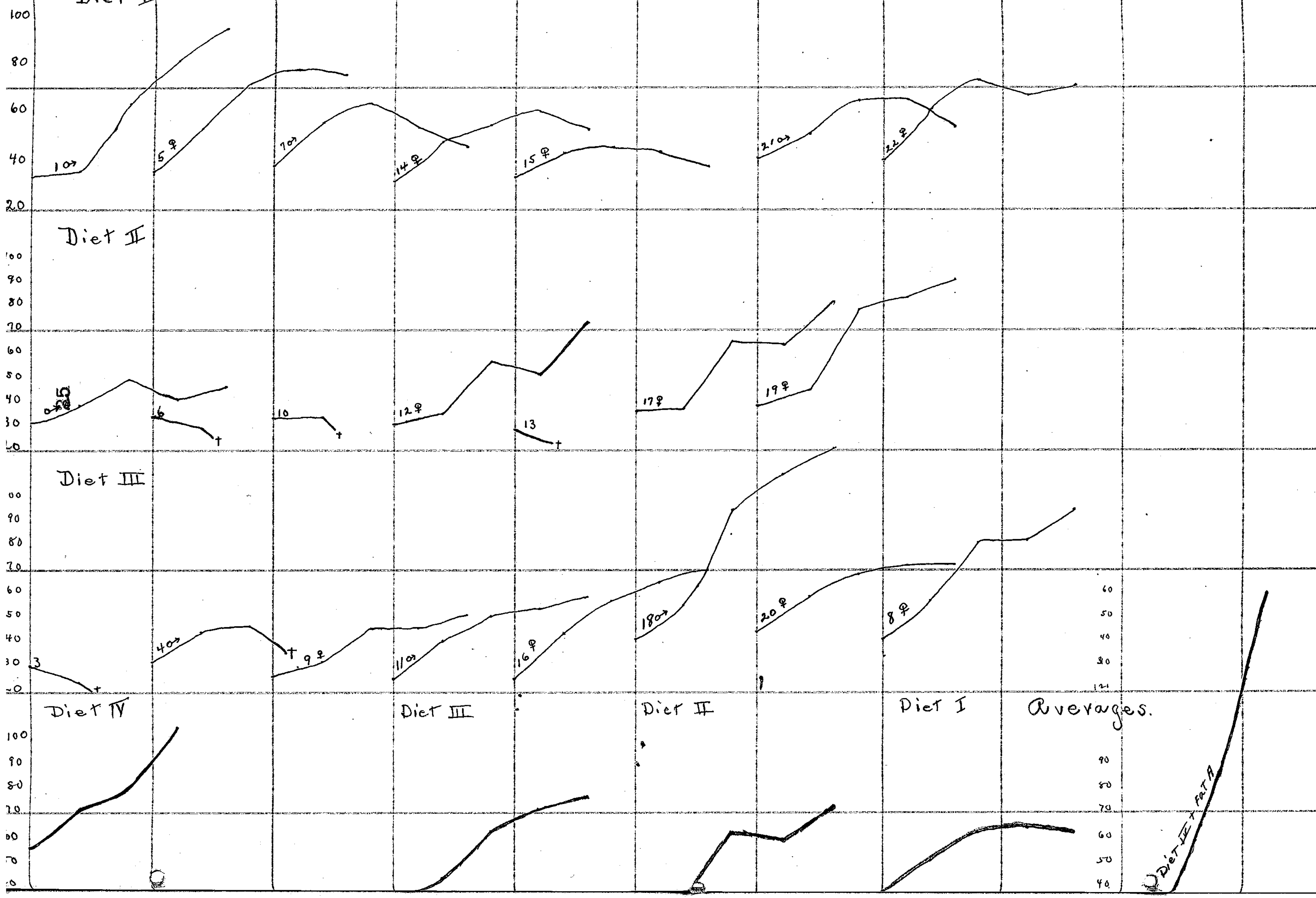
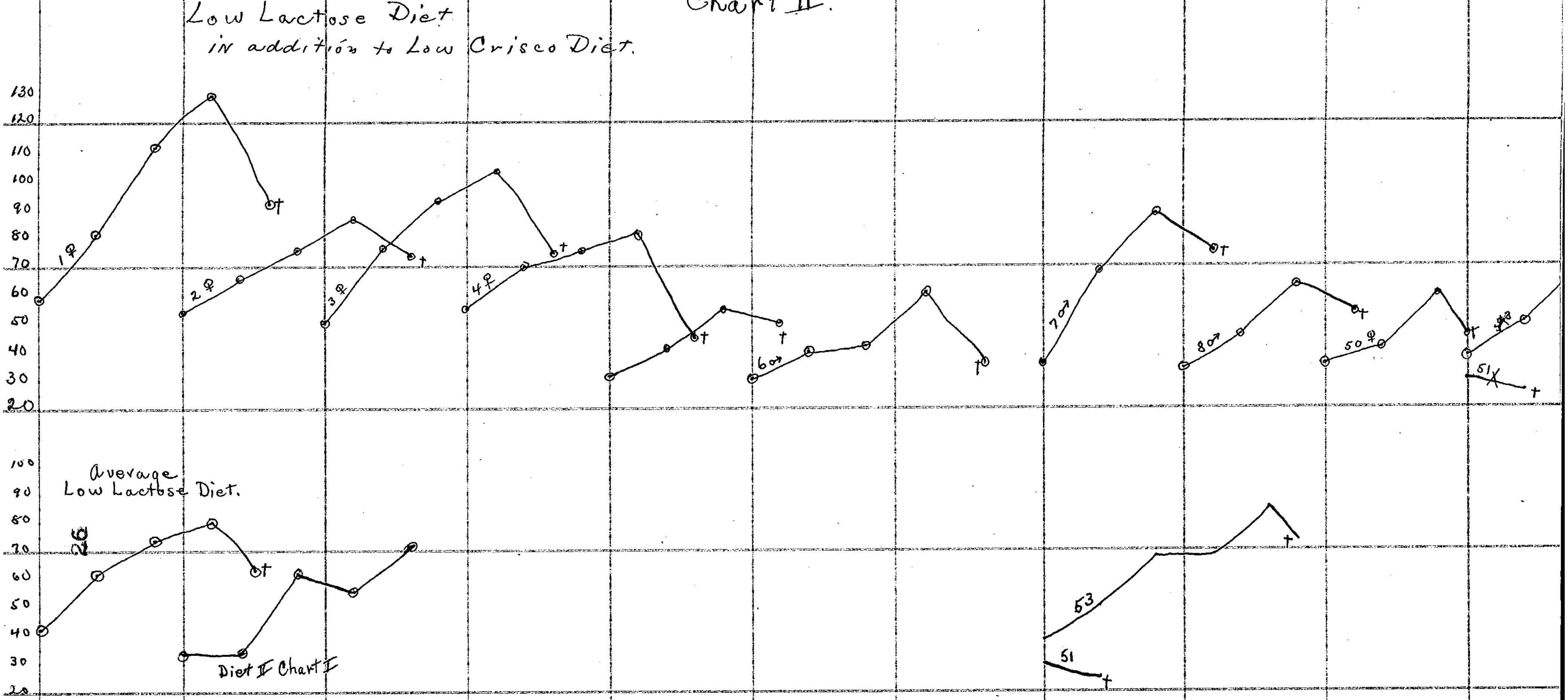

Diet I
Diet II
Diet III
Diet IV
Diet III
Diet II
Diet I
Averages.
Diet IV + Fat A

Chart II.


Low Lactose Diet
in addition to Low Crisco Diet.
130
120
110
100
90
80
70
60
50
40
30
20
1♀
2♀
3♀
4♀
6♂
7♂
8♂
50♀
51
Average
Low Lactose Diet.
26
Diet II Chart I
53
51

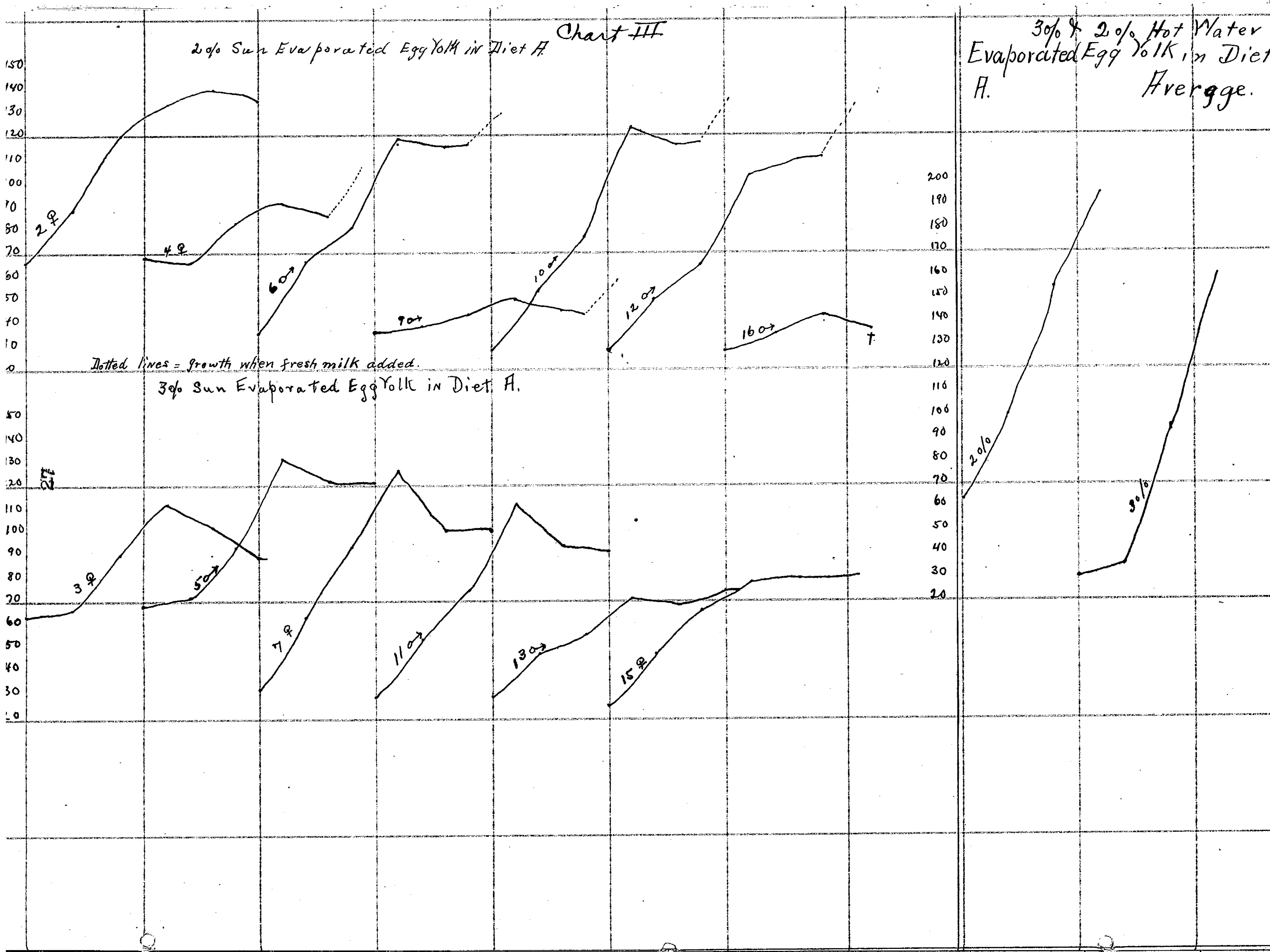
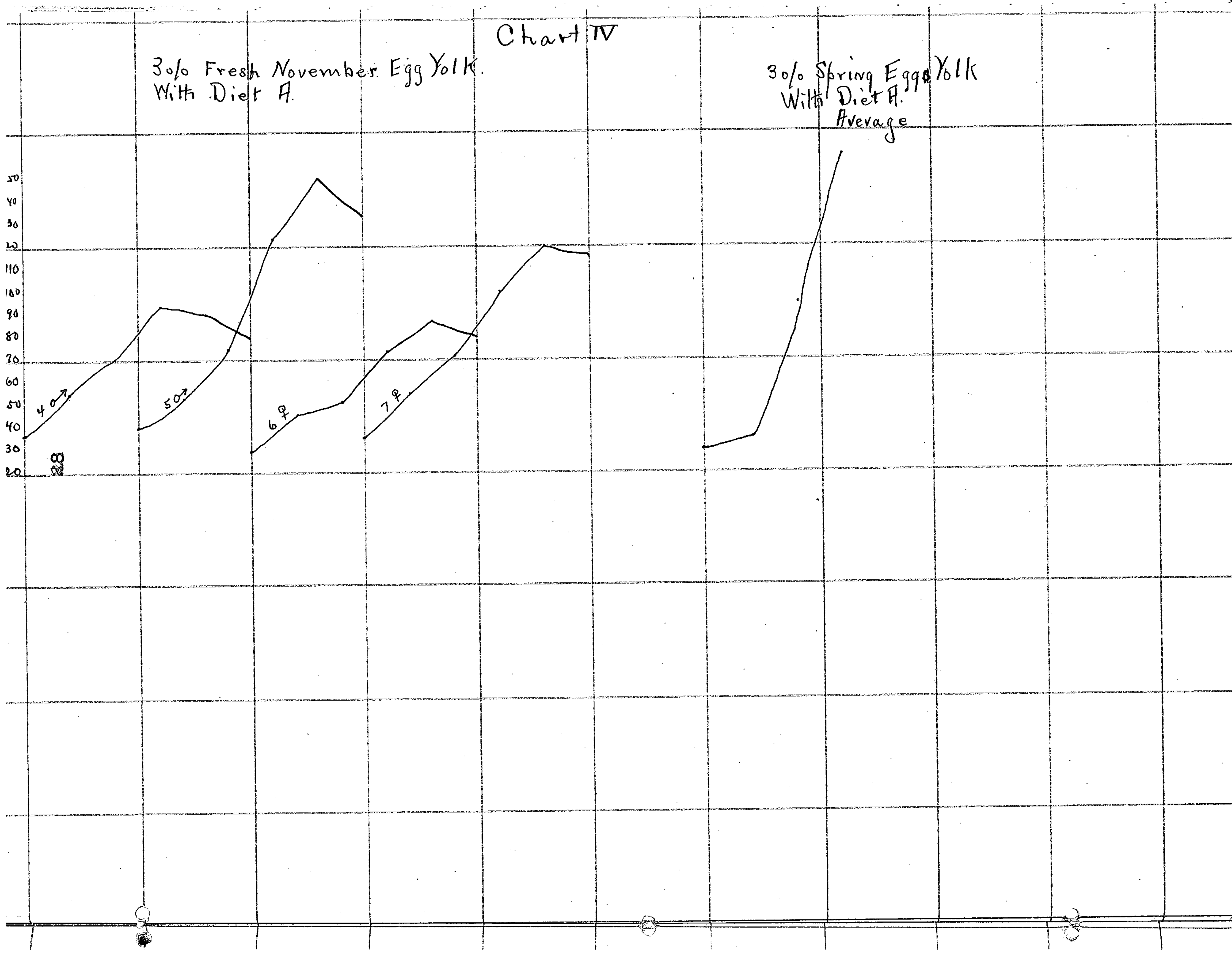


Chart III
2% Sun Evaporated Egg Yolk in Diet A
Dotted lines = growth when fresh milk added.
3% Sun Evaporated Egg Yolk in Diet A.
3% & 2% Hot Water Evaporated Egg Yolk, in Diet A. Average.
2♀
4♀
6♂
9♂
10♂
12♂
16♂
†
3♀
5♂
7♀
11♂
13♂
15♀
20%
30%

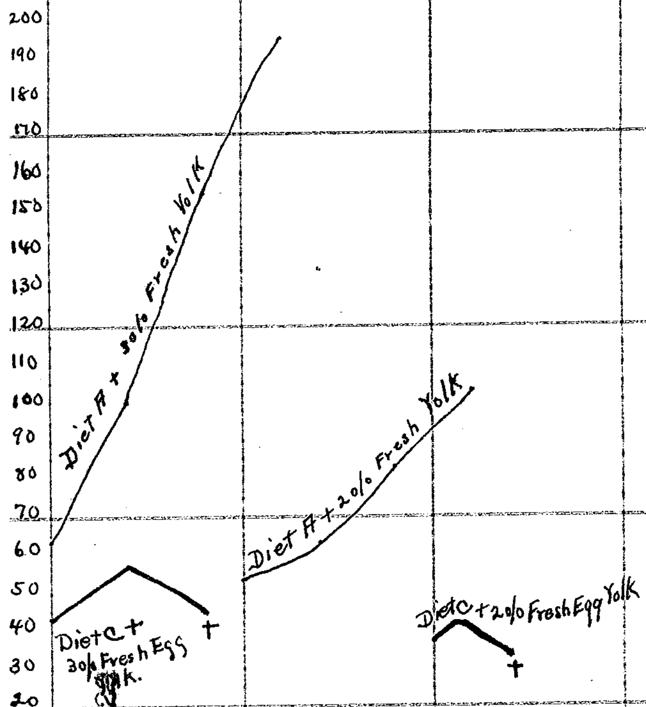
27

Chart IV

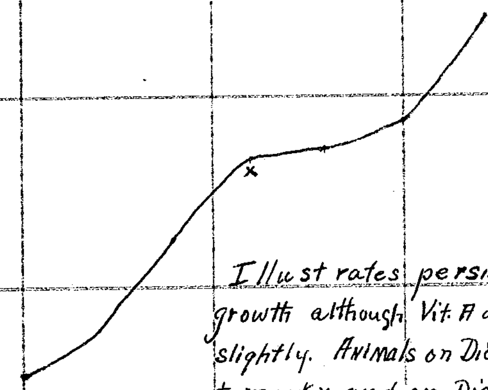


3% Fresh November Egg Yolk.
With Diet A.
3% Spring Egg Yolk
With Diet A.
Average
4 ♂
5 ♂
6 ♀
7 ♀
20
30
40
50
60
70
80
90
100
110
120
130
140
150

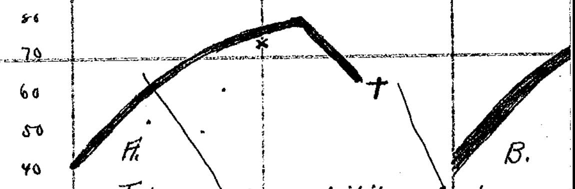
Chart V



Illustrates that rate of growth is proportional to amount of Vitamine in diet.

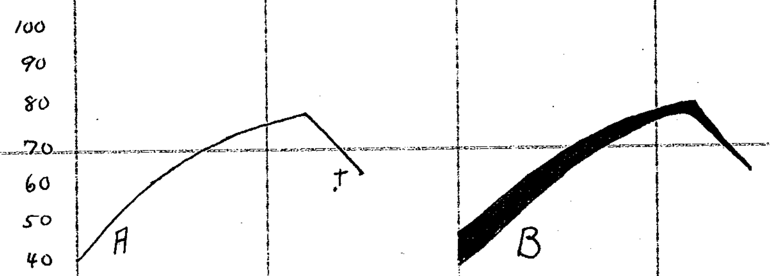


Illustrates persistence of growth although Vit. A decreased slightly. Animals on Diet B up to mark x and on Diet C there after.
Composite for 8 rats.



A. Illustrates ability of vigorous rats to coast for a time, showi[ng] signs of A deficiency

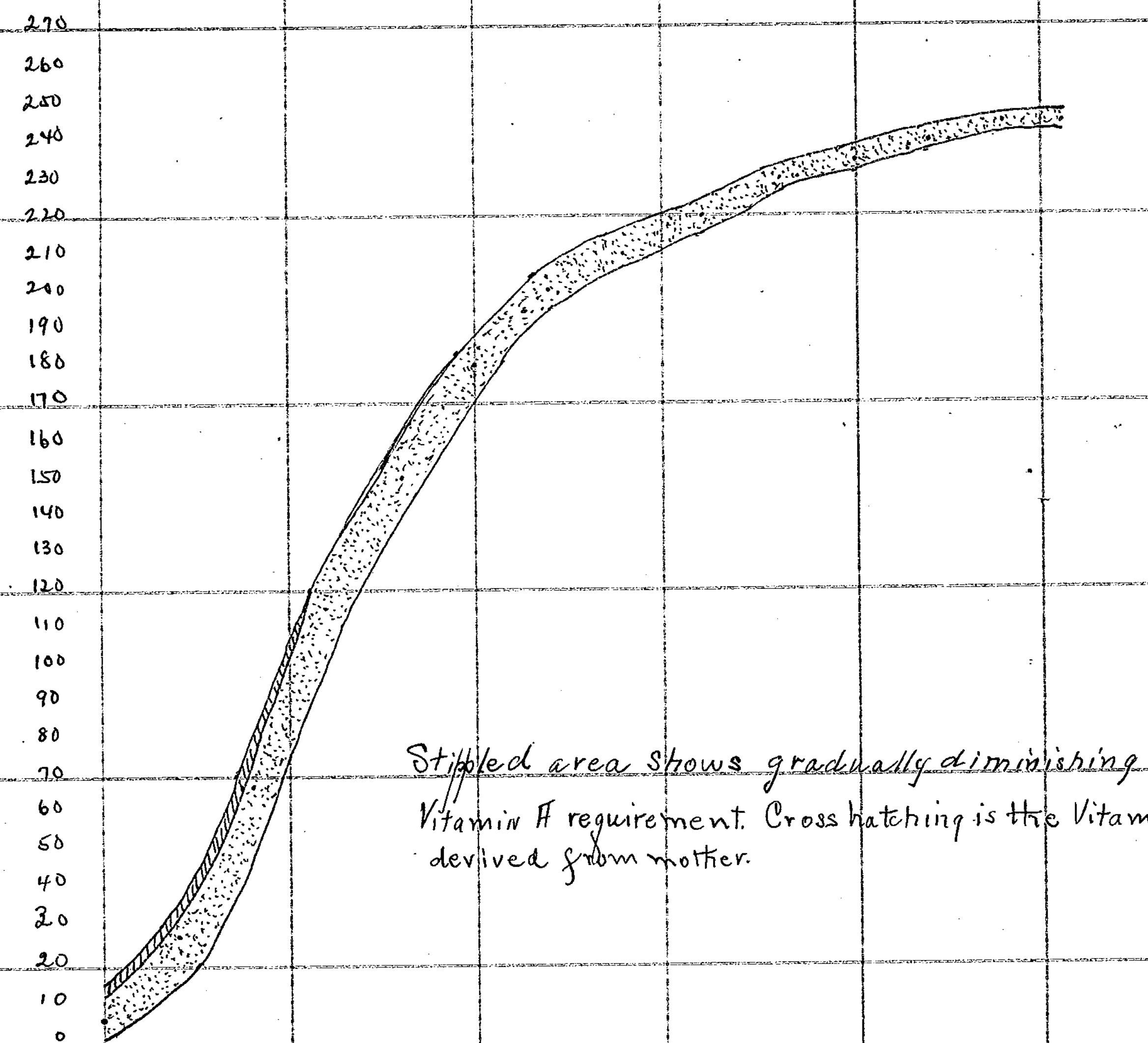
B. Same Curve showing quan[titative] relationship of Vitamine A derived from m[other]



A. Illustrates the ability of vigorous young rats to coast for a time, showing no signs of an "A" deficient diet.

B. The same curve showing the quantitative relationship of Vitamin A derived from the mother.

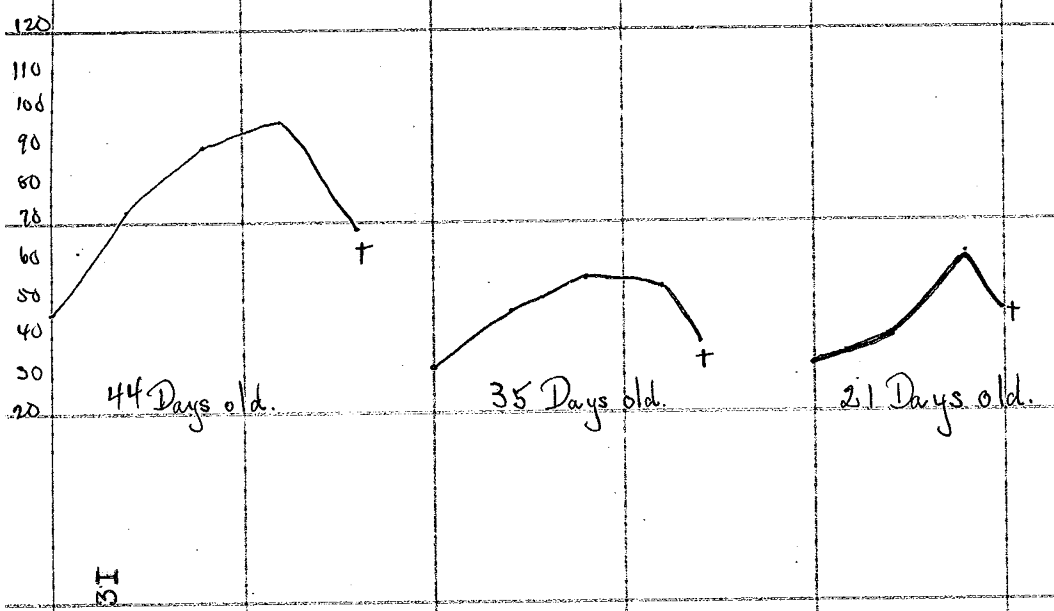
Chart VI



Curve of Expectation of growth of average between Male and Female Rat according to Donaldson.

Arranged to show quantitative Vitamin A relationship.

Chart VII


120
110
100
90
80
70
60
50
40
30
20
44 Days old.
35 Days old.
21 Days old.
3I