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The effect of mineral oil treatment of milk on the vitamin A content of the butterfat

Elise E. Morrell

To the Graduate Council:

I am submitting herewith a thesis written by Elise E. Morrell entitled "The effect of mineral oil treatment of milk on the vitamin A content of the butterfat." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in .

Martha Koelmer, Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

UNIVERSITY OF TENNESSEE

Upon the request of the Committee on Graduate Study the undersigned have examined a thesis entitled

THE EFFECT OF MINERAL OIL TREATMENT OF MILK ON THE VITAMIN A CONTENT OF THE BUTTER FAT.

presented by Elise Morrell

candidate for the degree of Master of Science

_____, and hereby certify that it is worthy of acceptance.

Martha Koelsch
Margaret B. MacDonald
} Examiners

Date 5/8/30

THE EFFECT OF MINERAL OIL TREATMENT OF MILK ON THE VITAMIN A CONTENT OF THE BUTTER FAT

A THESIS

Submitted to the Graduate Committee

of the

University of Tennessee

in

Partial Fulfillment of the Requirements

for the degree of

Master of Science

ELISE EMMA MORRELL

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The Effect of Mineral Oil Treatment on the Vitamin A Content of the Butter Fat.

Throughout the pastures of Tennessee are often found heavy infestations of wild onion. This objectionable condition is evidenced by the frequent occurence of the onion flavor and odor in milk and butter. The Biochemistry Department of the University of Tennessee Agricultural Experiment Station has recently (MacDonald 1927, 1930) devised a practical and efficient method for removing these objectionable features of the milk. The effective principle in the treatment of milk to remove the onion flavor and odor, is washing with mineral oil. The pertinent question as to whether this process is as harmless as it is effective has been studied at the University of Tennessee Agricultural Experiment Station, on raw milk, pastuerized milk, cream and butter fat, in a series of experiments designed to determine any change in the physical and chemical properties of milk so treated. Color tests for vitamin A were included in these experiments.

Dutcher, et al (1927) carried out feeding experiments with rats and found that when varying amounts of butter fat were fed as the sole supplement to a vitamin A-free diet the expected improvement invariably resulted. However when these same amounts of butter fat were fed dissolved in mineral oil in the proportions of 2.5 parts

of mineral oil to 1 part of butter fat, the animals failed to respond. Dutcher states, "It is quite evident from the results obtained that mineral oils possess the property of dissolving the vitamin A from food materials, thereby depriving the body of this vitamin, even though vitamin A be present in excess of body needs." There seems to be a possibility however, that the butter fat may have been excreted along with the mineral oil, in place of merely the vitamin A content of the butter fat, in as much as mineral oil is an excellent solvent for butter fat in the above proportions.

Since the substance which gives the strong odor and flavor of onion to milk is so much more soluble in mineral oil than it is in butter fat, it seemed desirable to determine whether or not the vitamin A present displayed the same variation in solubility. Should this be the case the mineral oil treatment would be decidedly detrimental to the nutritive value of the milk. The following experiments were conducted at the Home Economics Department of the University of Tennessee in cooperation with the Agricultural Experiment Station in an effort to arrive at some definite conclusion on this point.

EXPERIMENTAL

Albino rats and those of the piebald variety were used as experimental animals and were kept in individual cages throughout the duration of the experiment. The cages used were manufactured by the A.B. Hendryx Company, New Haven, Conn., and had false bottoms. This prevented coprophagy. Food and distilled water were supplied to the rats ad libitum.

Methods Employed for Experimentation.

Composition of rations: Two general types of diets were used in these experiments: the customary synthetic ration, and a modification of the McCollum rolled oats ration. Variations of these were produced by irradiation or by changing the constituents. These rations were satisfactory in all respects except that they were devoid of vitamin A and required no supplementing except with those substances whose vitamin A content was being determined.

Table I

Composition of Rations

Foods	Number of Ration				
	2	2A	2E	5	5A
Casein	18[a]	18[a]	18[b]		
Rolled oats				40	40
Gelatin[c]				10	10
Crisco	10	10	10		
Wesson oil				10	10
Yeast[d]	5	5	5		
Agar-agar[e]	2	2	2		
Dextrin[e]	61	61	61	36	36
Salt Mixture[f]	4	4	4	4	4
Ration Irradiated[g]	+	-	+	-	+

a) Washed casein from Eimer and Amend. Extracted 24 hours for seven consecutive days with dilute acetic acid. (10 cc. per gal. distilled water.) Extracted with boiling 80% alcohol three times for periods of one hour each, using a new lot of 80% alcohol each time. Extracted with ether for 40 hours in a Soxhlet type of continuous extractor. Dried for 48 hours in open pans at 200°F.

b) Acetic acid extracted casein, as above. Heated in open pans at 200°F. for three days.

c) "Difco Standardized" Bacto-Gelatine and Bacto-Agar from Digestive Ferments Co. Detroit, Mich.

d) Powdered Yeast Foam Tablets from Northwestern Yeast Co. Chicago, Ill.

e) Argo cornstarch hydrolyzed with dilute citric acid. (10 gm. per gal. distilled water, mixed with 25 lbs. cornstarch.) Autoclaved for 4 hours at 18 lbs. pressure.

f) Osborne and Mendel's salt mixture made from C.P. reagents.

g) The ration was spread out in thin layers (60 gm. per sq. ft.) and was exposed for 30 minutes, with frequent stirring to insure thorough irradiation, at a distance of 30 inches from the quartz tube of an Hanovia Alpine Sun-lamp.

Determination of fore-period and test period: The rats were weaned at 21-29 days and placed on one of the vitamin A-free diets. Their weight at that time was not less than 28 gm. and not more than 50 gm. The fore-period was determined after the manner of Sherman and Munsell (1925). When the weight of the rat remained approximately stationary for about one week the fore-period ended and the test period was begun immediately. If xeropthalmia developed before the animal's weight had become stationary its reserve of vitamin A was considered exhausted and the test period was begun.

Butter fat supplements: When the fore-period was ended the rat was given daily supplements of butter fat. The amounts given daily varied from 40 mgm. to 140 mgm. and were of two types, one (designated as #1) from milk which had received no treatment other than that incident to butter making and the other (#2) from a portion of the same lot of milk which had been washed with mineral oil to remove the onion flavor and odor. The butter fat was placed in the cage on an inverted crucible lid and was usually eaten immediately. The supplements were measured from pipettes made to deliver drops of butter fat weighing 20 mgm.

Criteria used in analysis of results: 1. A test animal was considered cured of vitamin A deficiency when the eyes were completely healed, when there was no external

evidence of lung infection and when the rate of gain and general appearance were that of a normal animal. At this point the animal was killed and autopsied.

2. Evans (1928) points out as one sign of vitamin A deficiency the continuous presence of cornified cells in the vaginal smear. It was of interest to watch for this symptom in following the oestrus cycle of rats on the experiment. The method used in making smears and also the system of recording the findings of these examinations, were devised by Helen T. Parsons, University of Wisconsin.

A few drops of physiological salt solution in a medicine dropper were introduced into the vagina of the rat, and immediately withdrawn by releasing the pressure on the bulb of the medicine dropper. In the case of young animals whose vagina had just opened, or in which the oestrus cycle had not yet been definitely established, this proceedure frequently had to be repeated.

One drop of the material thus obtained, placed on a microscope slide is sufficient for examination. Rats quickly become accustomed to this routine and with a little practice can easily be handled throughout the whole proceedure by one person. In laboratory practice the records are kept in colors; each of the three different types of cells found being designated by a different color. The examinations for each day are recorded in small squares

with the dates. The proportion of each type of cell found is indicated by the varying number of dots of the respective colors placed in a given square. A square containing dots of one color only would indicate that only one type of cell was present in the material from the vagina for that date. Chart 15 gives the results of these examinations on representative animals. In this chart different symbols have been substituted for the colors used in the original records.

Vaginal smears were made in a group of animals for periods of 3 to 4 consecutive days each at weekly intervals before and after supplementary feeding, and in some of the negative controls. In Chart 15 a lapse of more than seven days indicates that the same stage as found in the preceding examination had been maintained throughout this period. When the continuous presence of cornified cells in smears made from the negative controls had been observed for three consecutive weeks, the examinations were discontinued.

3. Hemoglobin determinations were made according to the method of Newcomer (1919). The glass color standard used was one supplied by Bausch and Lomb. The instrument used was a new Duboscq colorimeter. Blood samples were obtained by puncture of the caudal vein. The blood of the negative controls became very dark and samples

were hard to take on account of the rapidity with which the blood coagulated. Because of this difficulty determinations on this group were finally discontinued. Table IV gives the results of hemoglobin determinations on a group of animals.

RESULTS

Each rat on the experiment was given two numbers, the first used as a group designation to indicate the type of ration fed. This number was sometimes accompanied by a letter to show certain variations of the ration used. The second number was that of the individual rat on the indicated ration. The letter following the numbers and the sex designation show from what litter the rat was taken. 2E-5♂J and 2E-6♀I are rats number 5 and 6, on ration 2E and are from different litters. 5A-4♀H and 5-13♂H are rats from the same litter but on different variations of ration 5.

For complete data on types of rations fed, see Table I. Table III gives full information on significance of the letters indicating the litter to which a given animal belonged.

Chart 1 Negative Controls

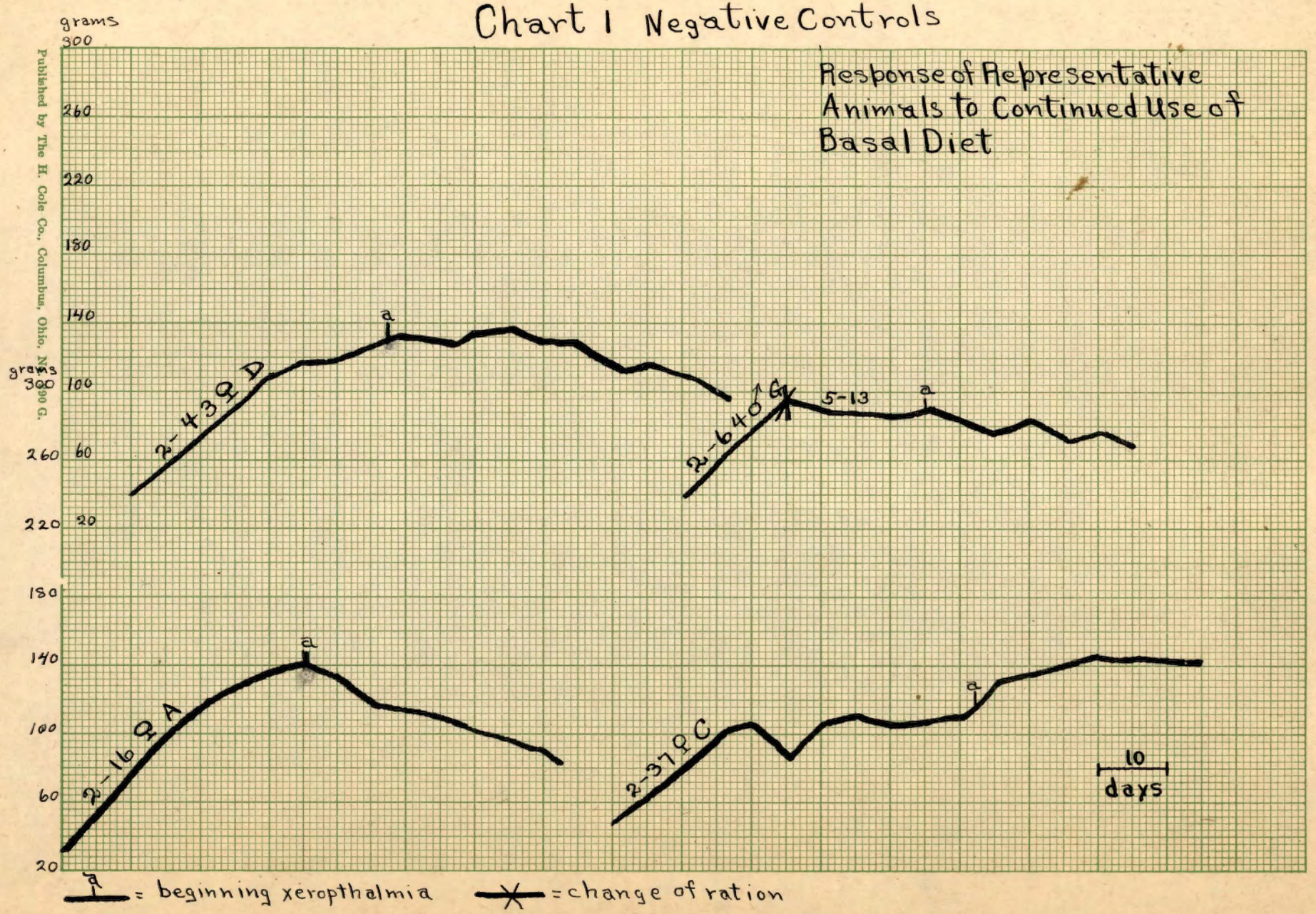

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Chart 2 Negative Controls

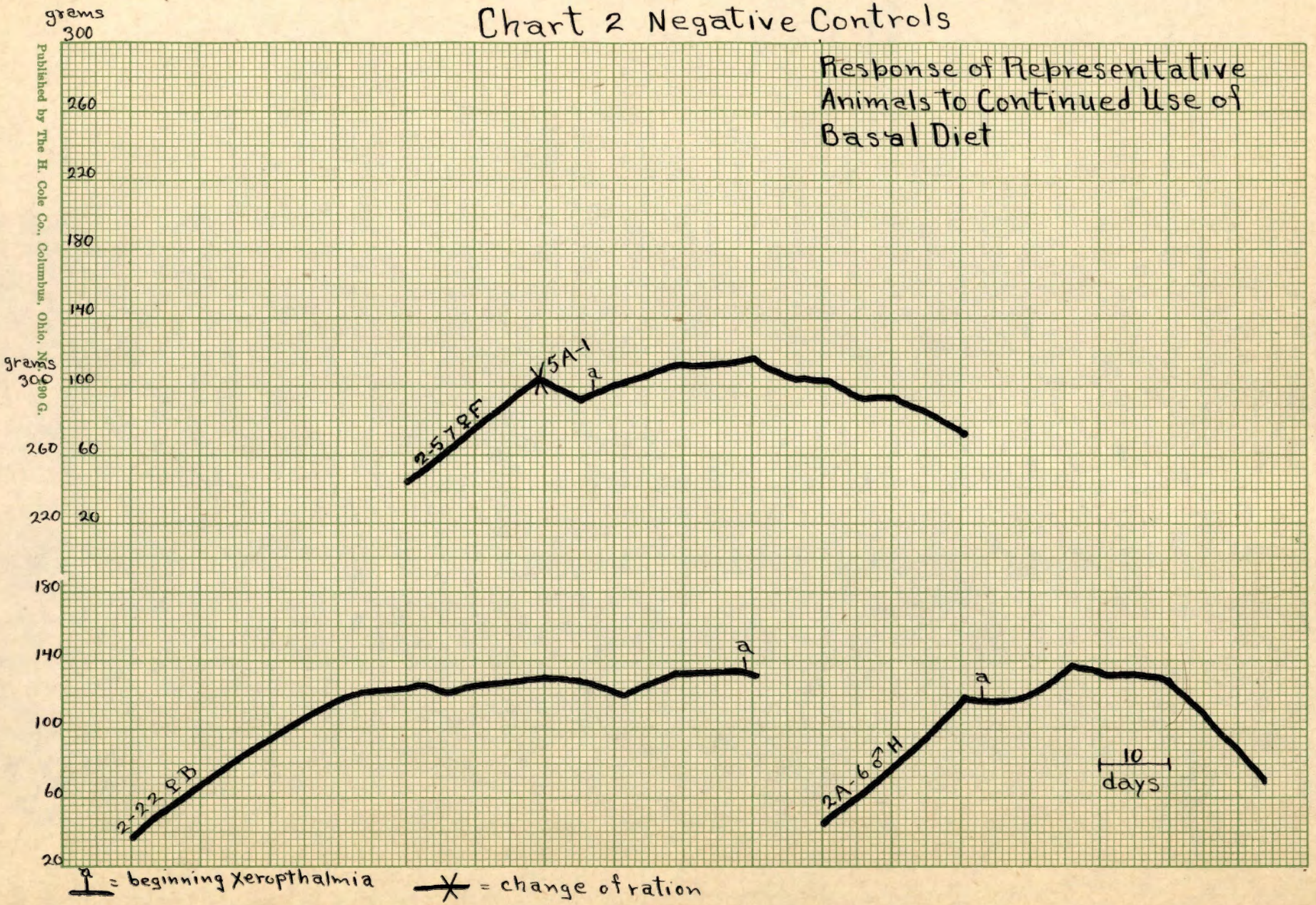

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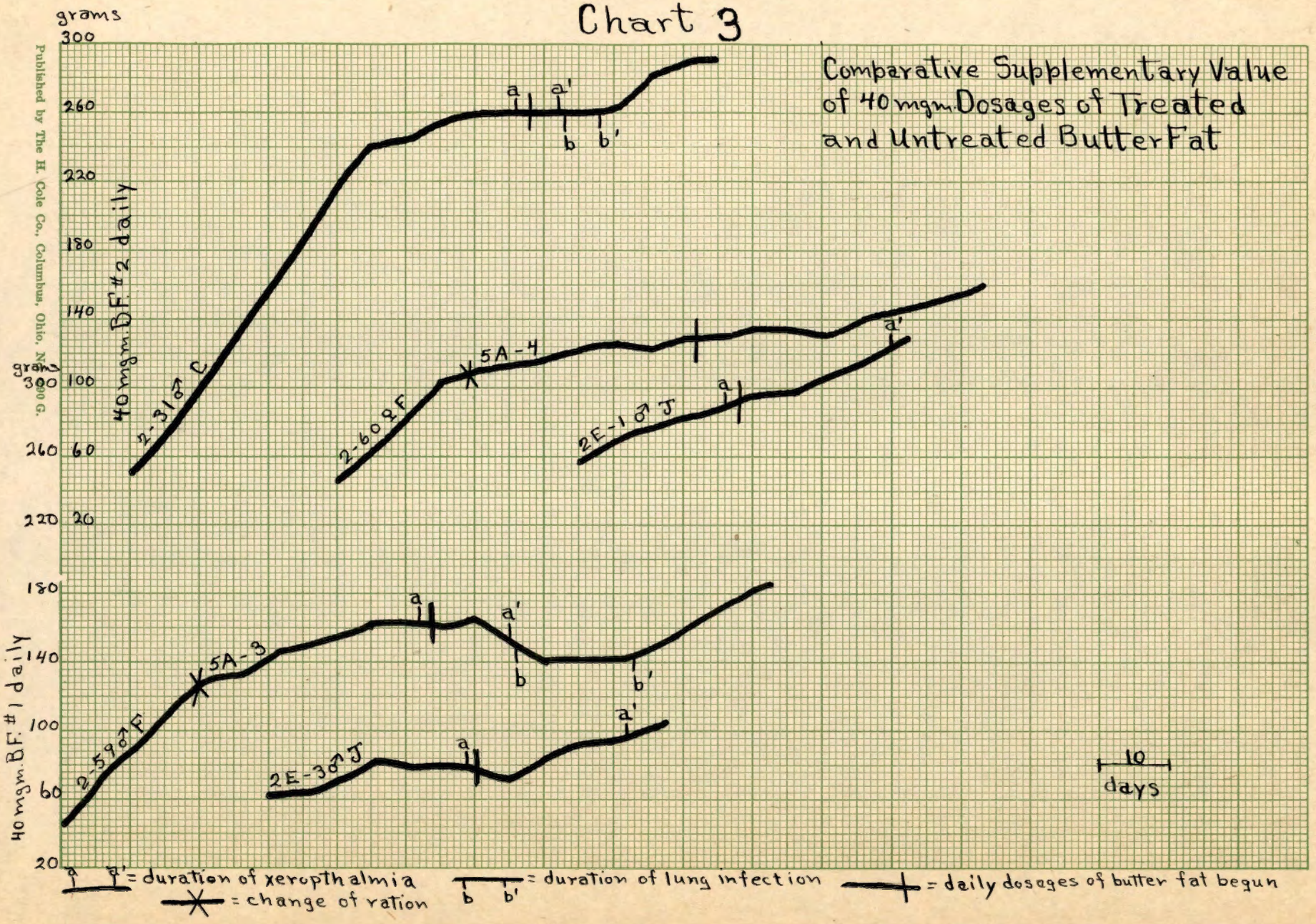
Chart 3



Comparative Supplementary Value of 40 mgm. Dosages of Treated and Untreated Butter Fat

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Chart 4

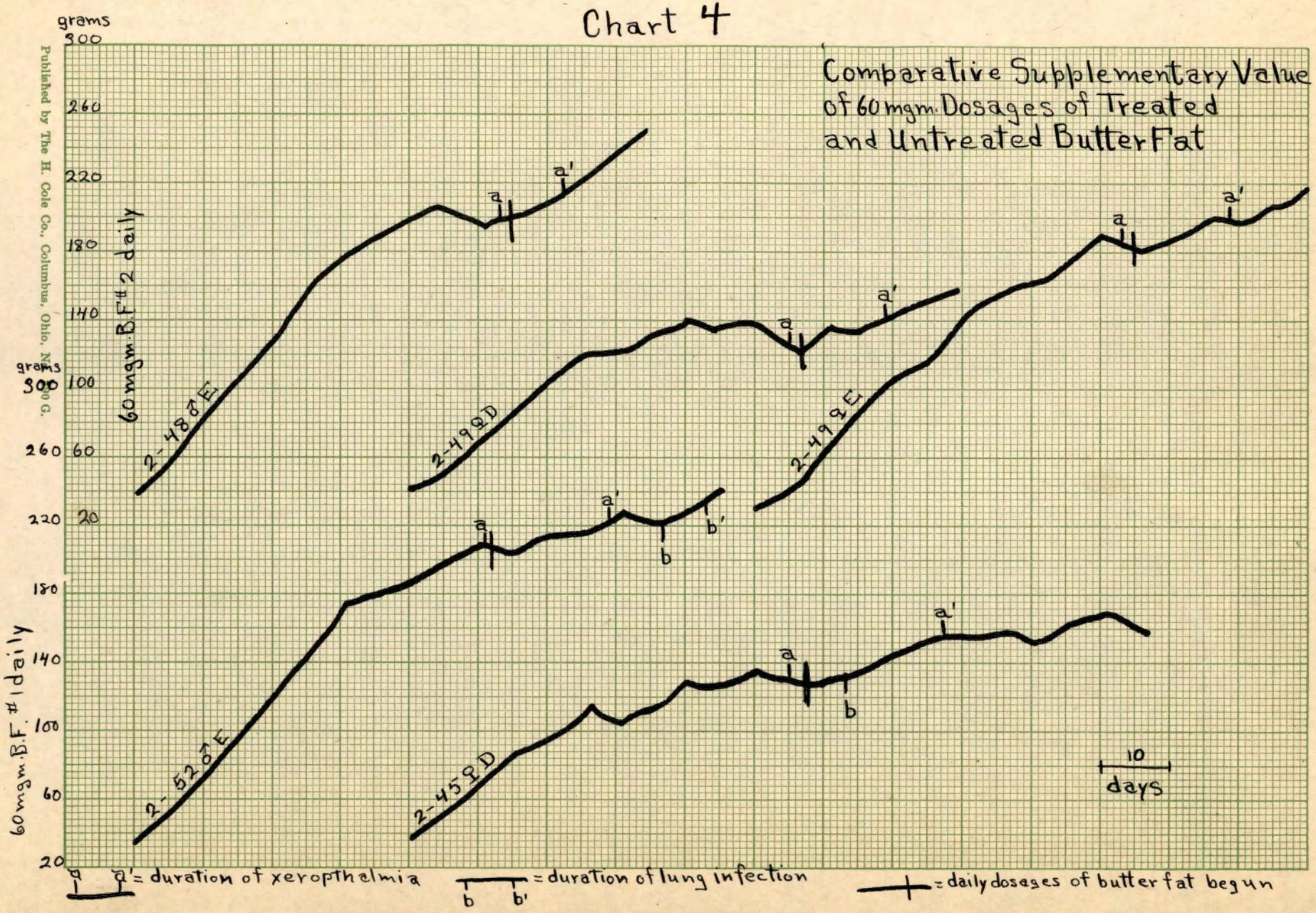


Chart 5

Comparative Supplementary Value of 60 mgm. Dosages of Treated and Untreated Butter Fat.

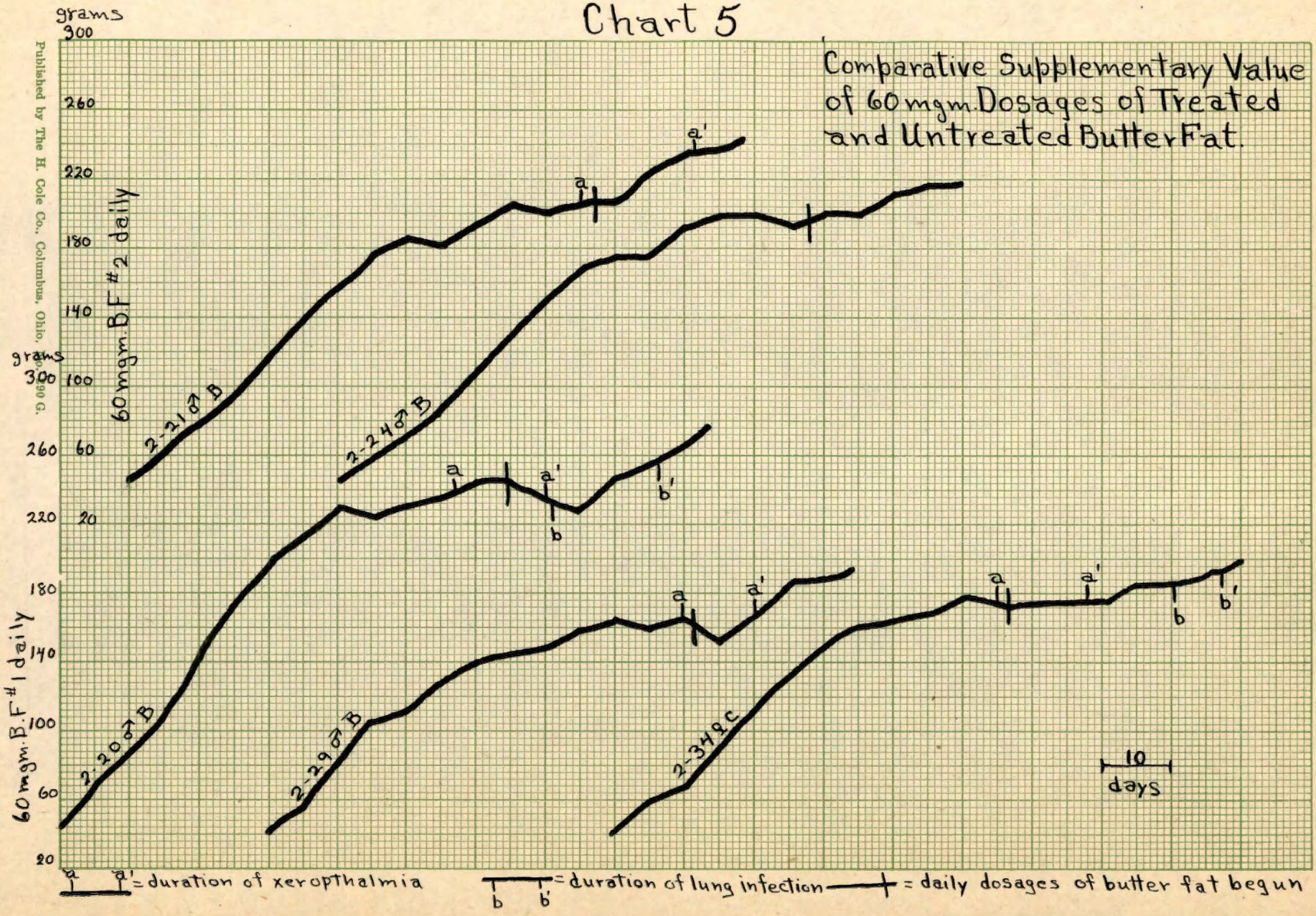
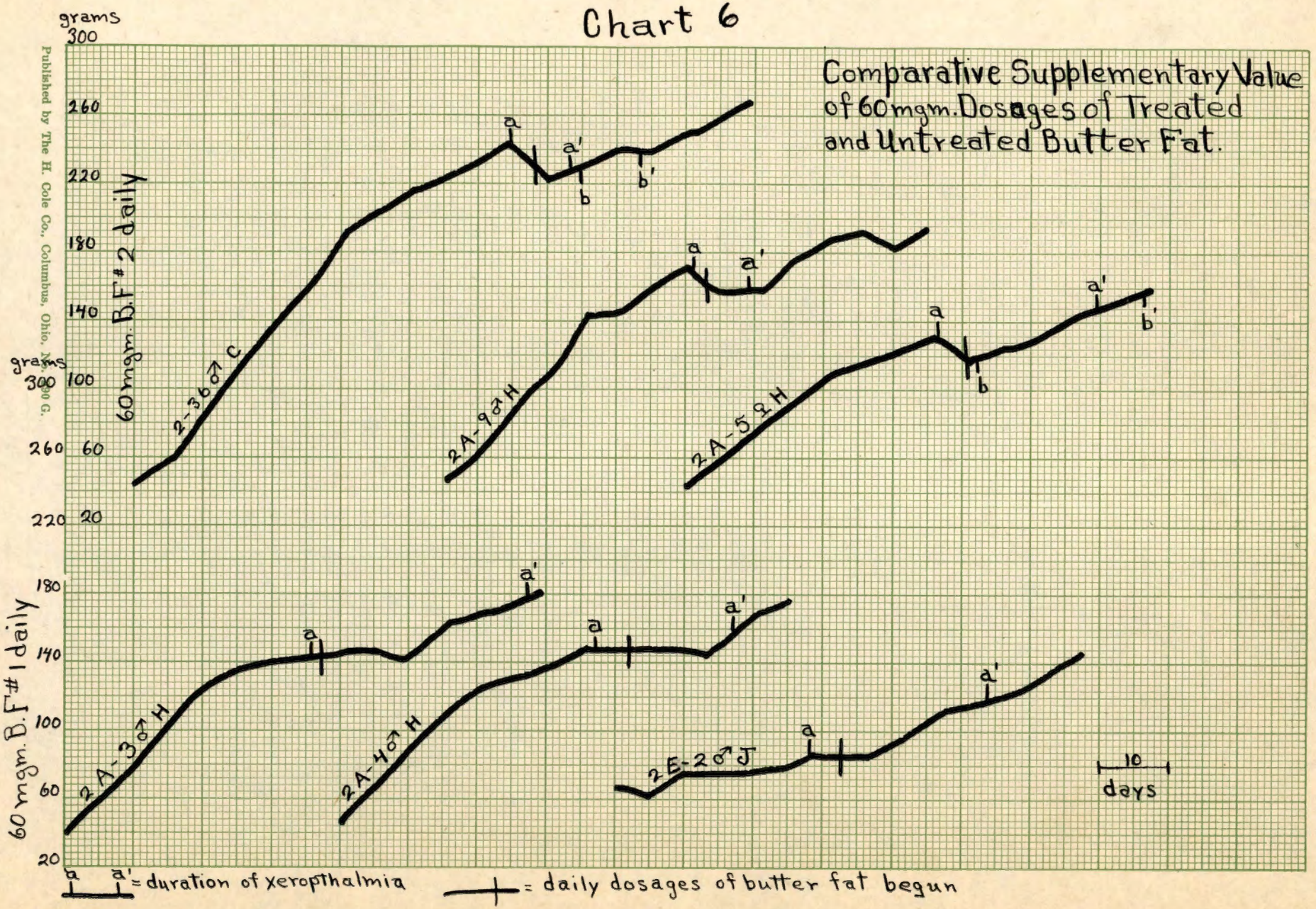


Chart 6

Comparative Supplementary Value of 60 mgm. Dosages of Treated and Untreated Butter Fat.



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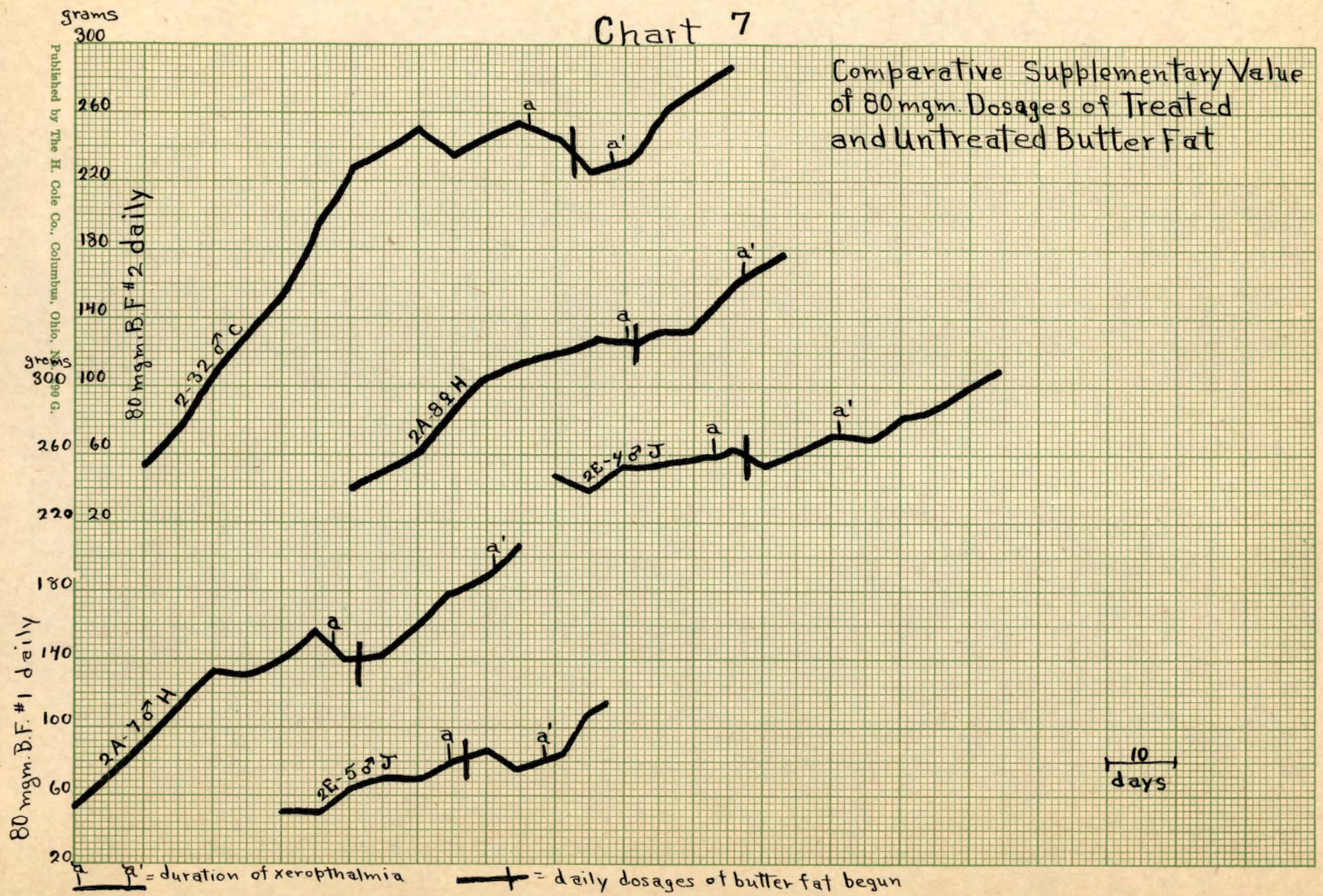
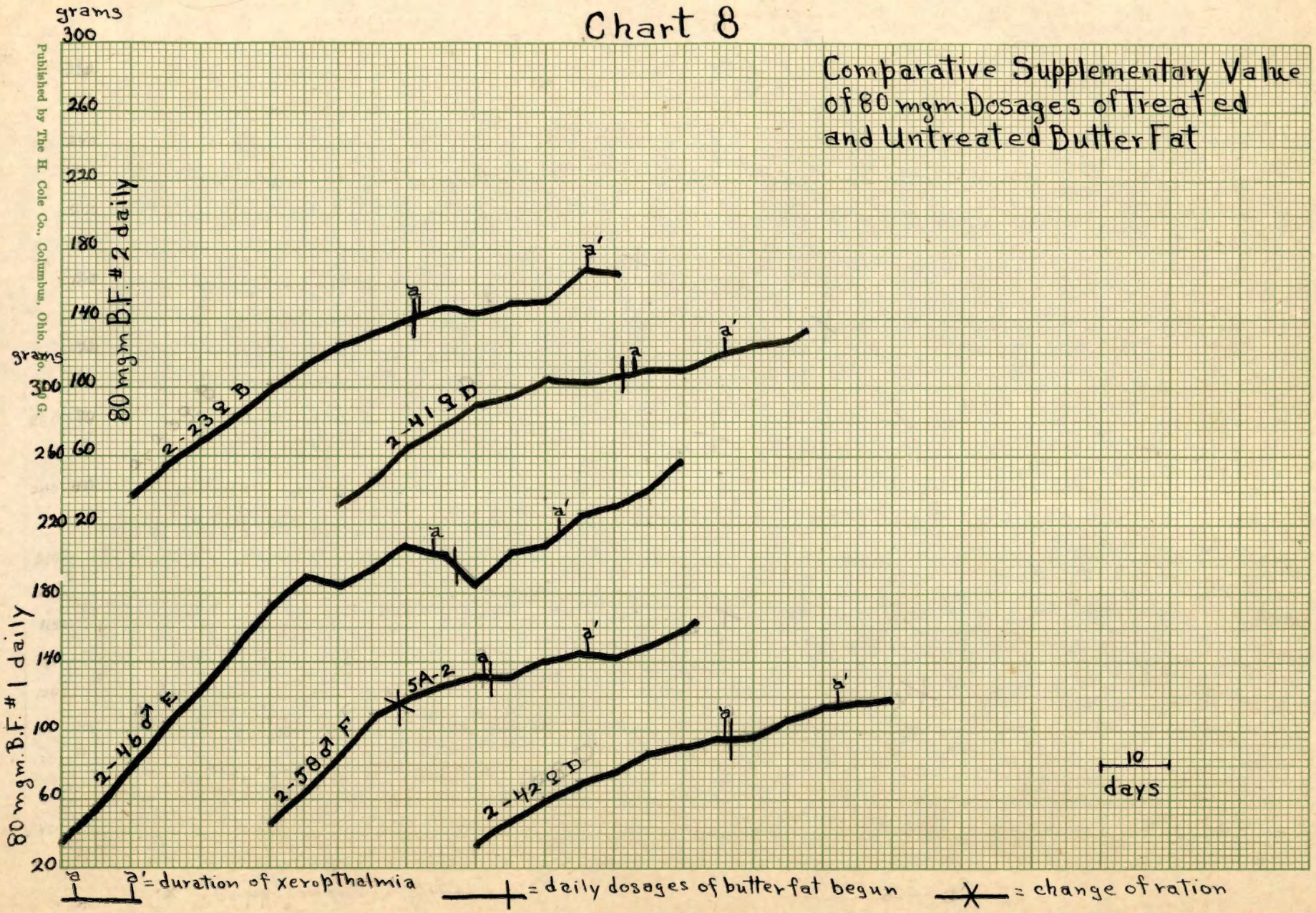


Chart 8

Comparative Supplementary Value of 80 mgm. Dosages of Treated and Untreated Butter Fat



a a' = duration of xeropthalmia

| = daily dosages of butterfat begun

X = change of ration

Chart 9

Comparative Supplementary Value of 80 mgm. Dosages of Treated and Untreated Butter Fat

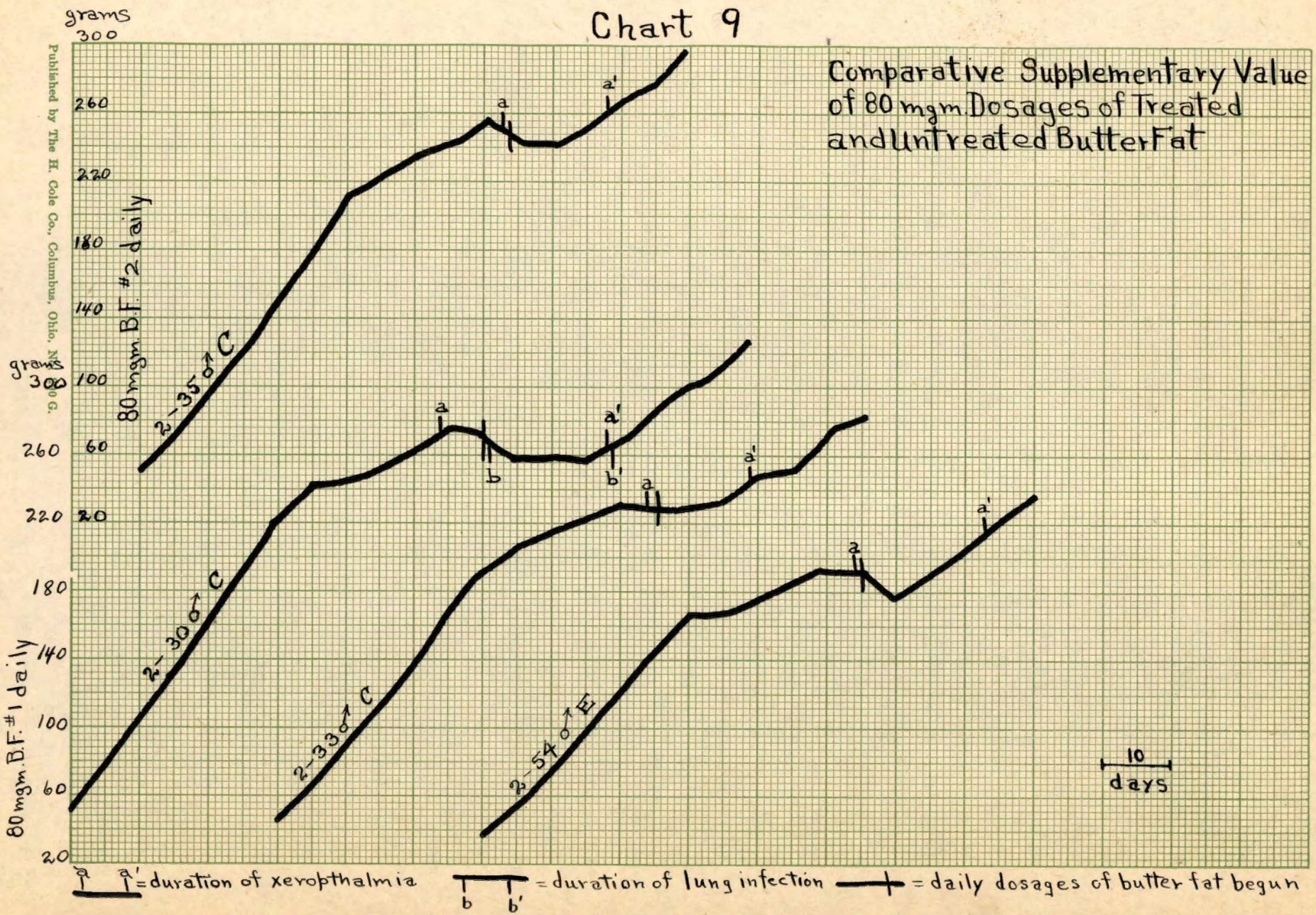


Chart 10

Comparative Supplementary Value of 100 mgm Dosages of Treated and Untreated Butter Fat

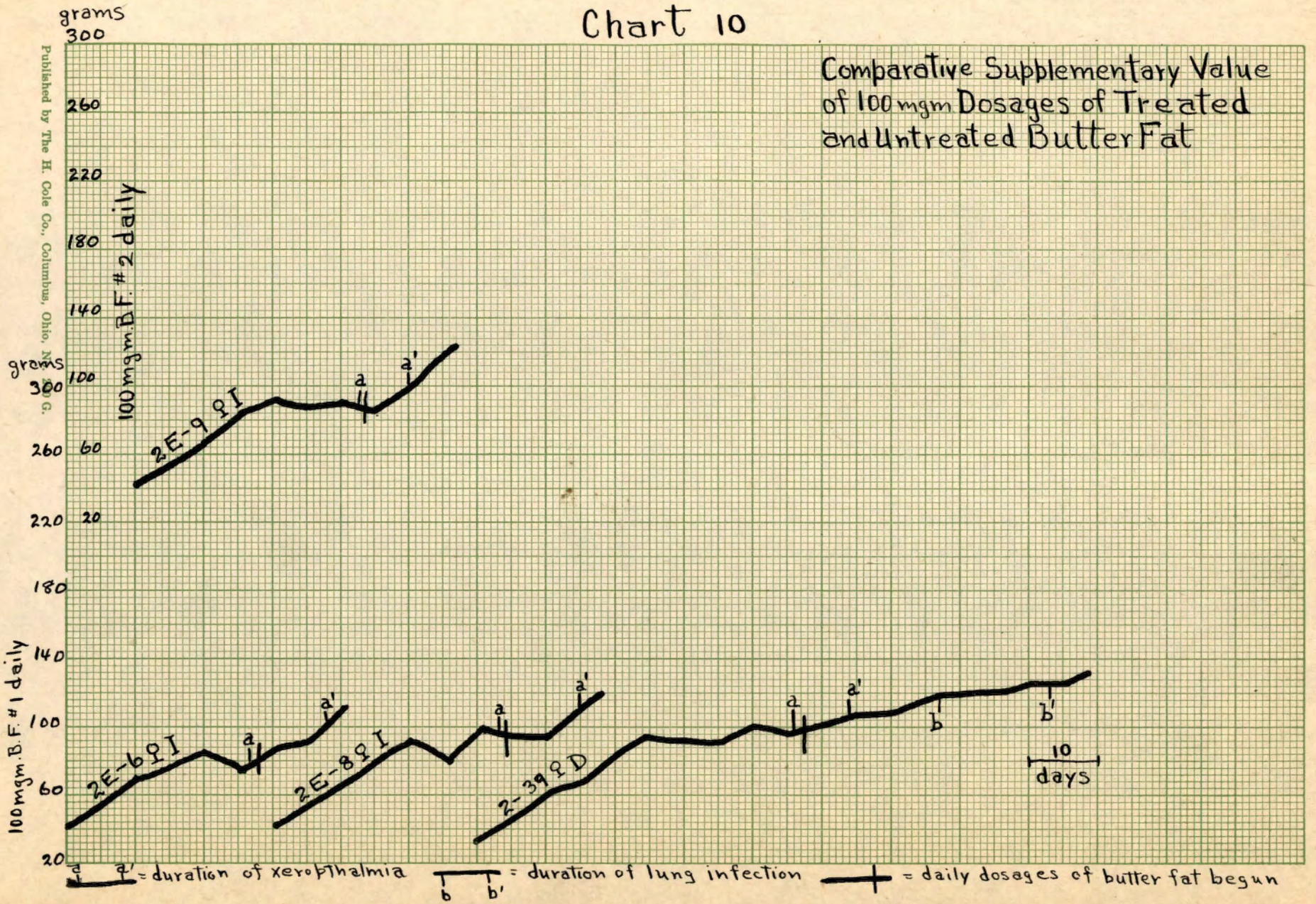


Chart 11

Comparative Supplementary Value of 100 mgm. Dosages of Treated and Untreated Butter Fat

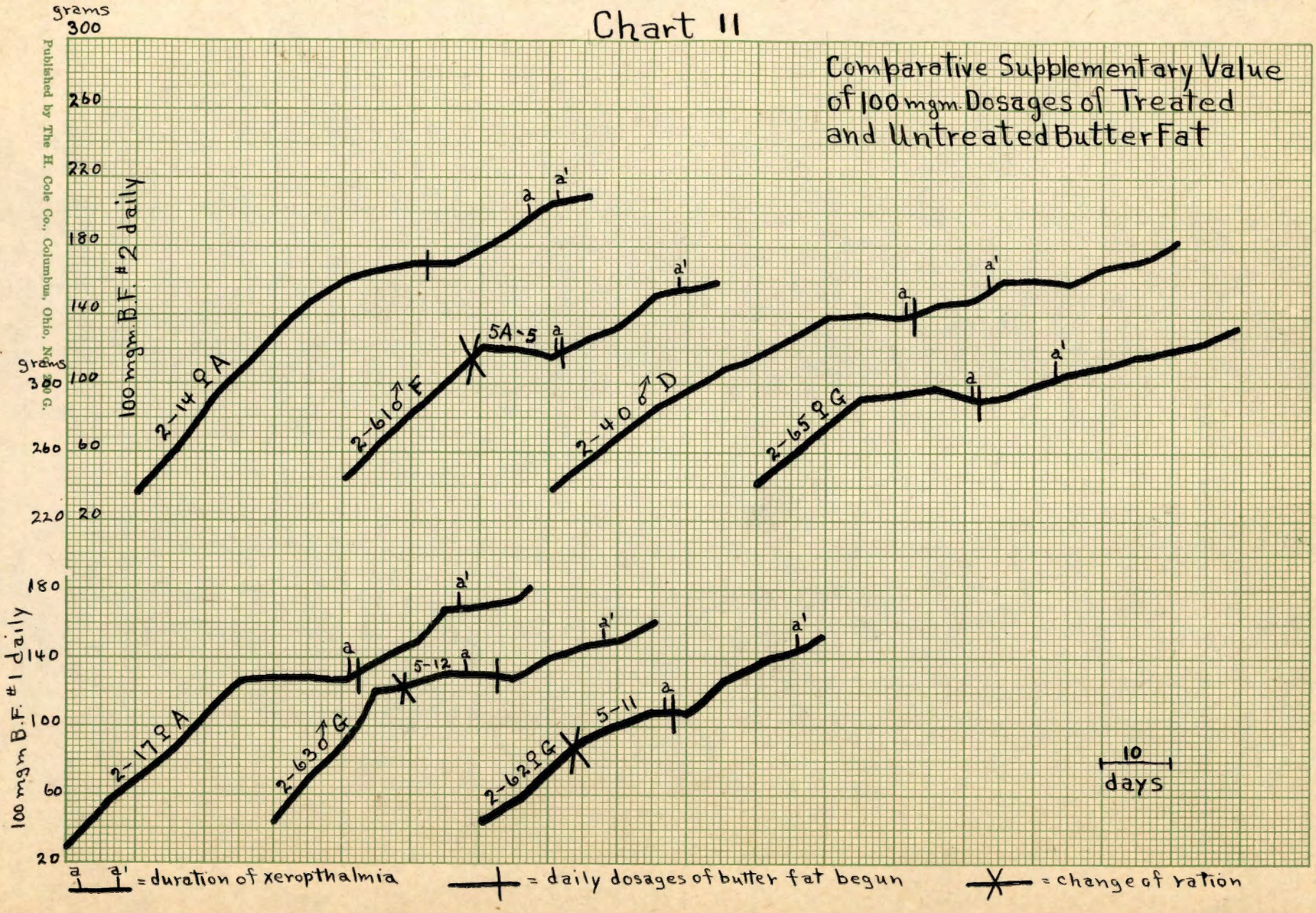


Chart 12

Comparative Supplementary Value of 120 mgm. Dosages of Treated and Untreated Butter Fat

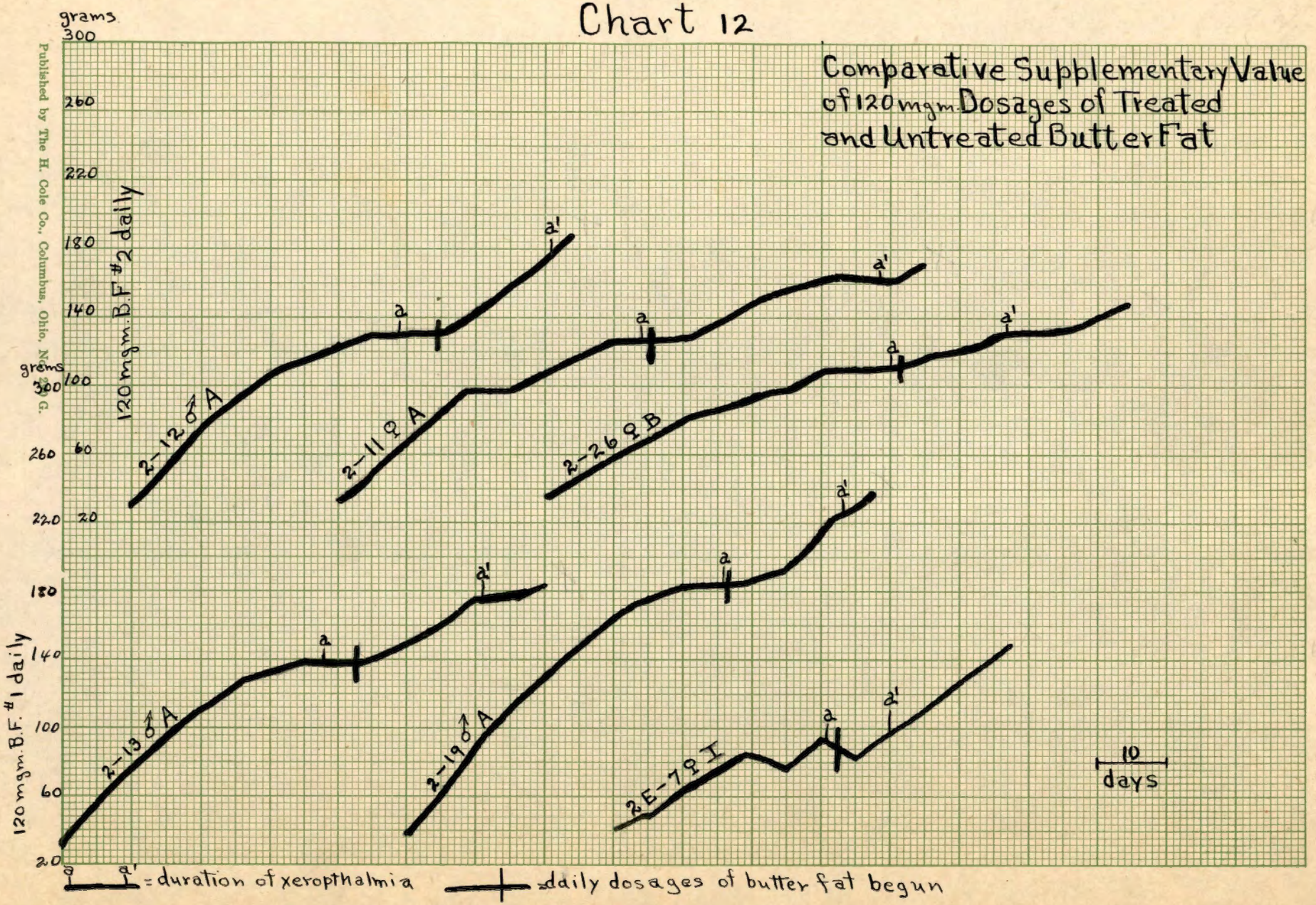


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Chart 13

Comparative Supplementary Value of 120 mgm. Dosages of Treated and Untreated Butter Fat

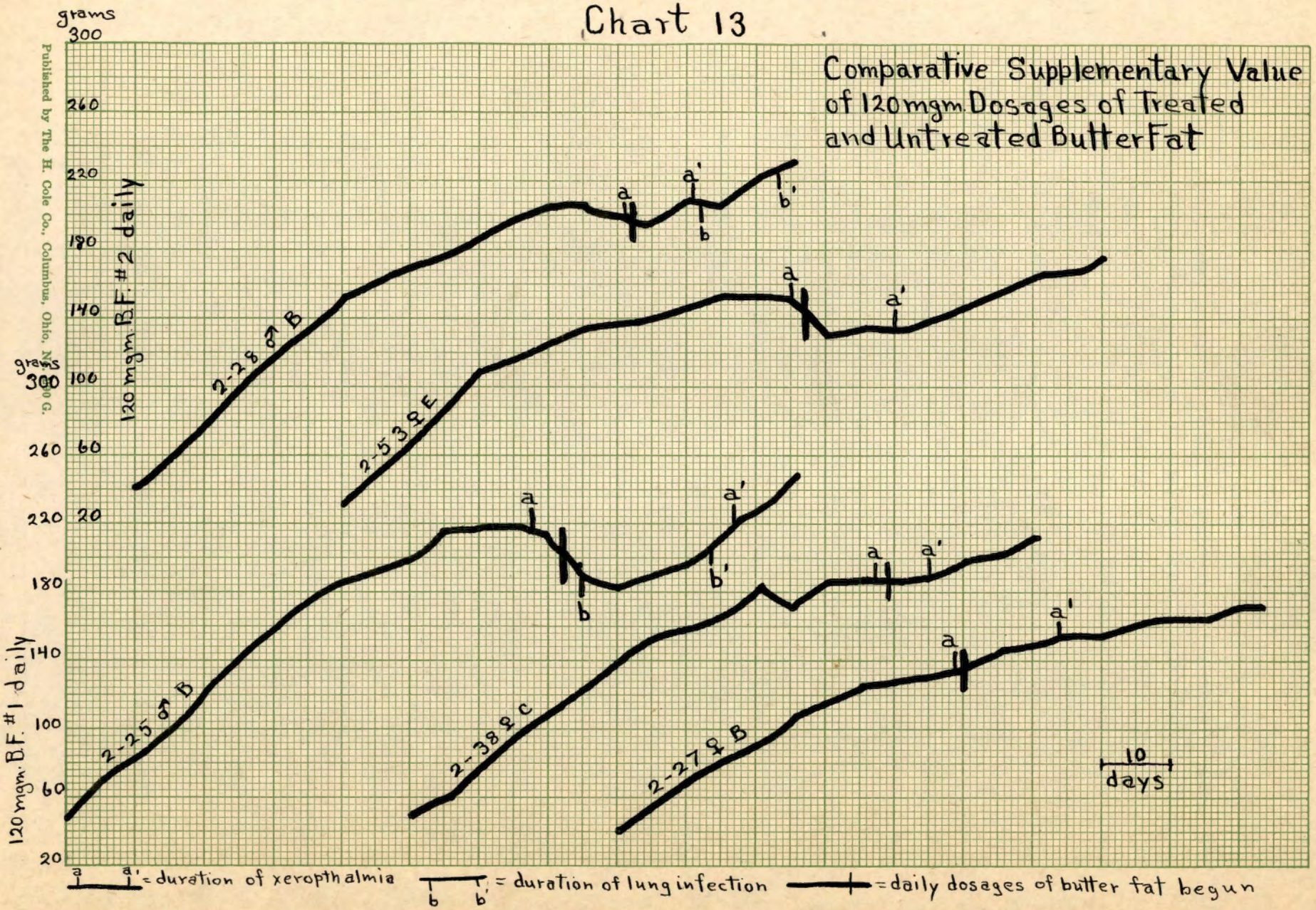

Chart 14

Comparative Supplementary Value of 140 mgm. Dosages of Treated and Untreated Butter Fat

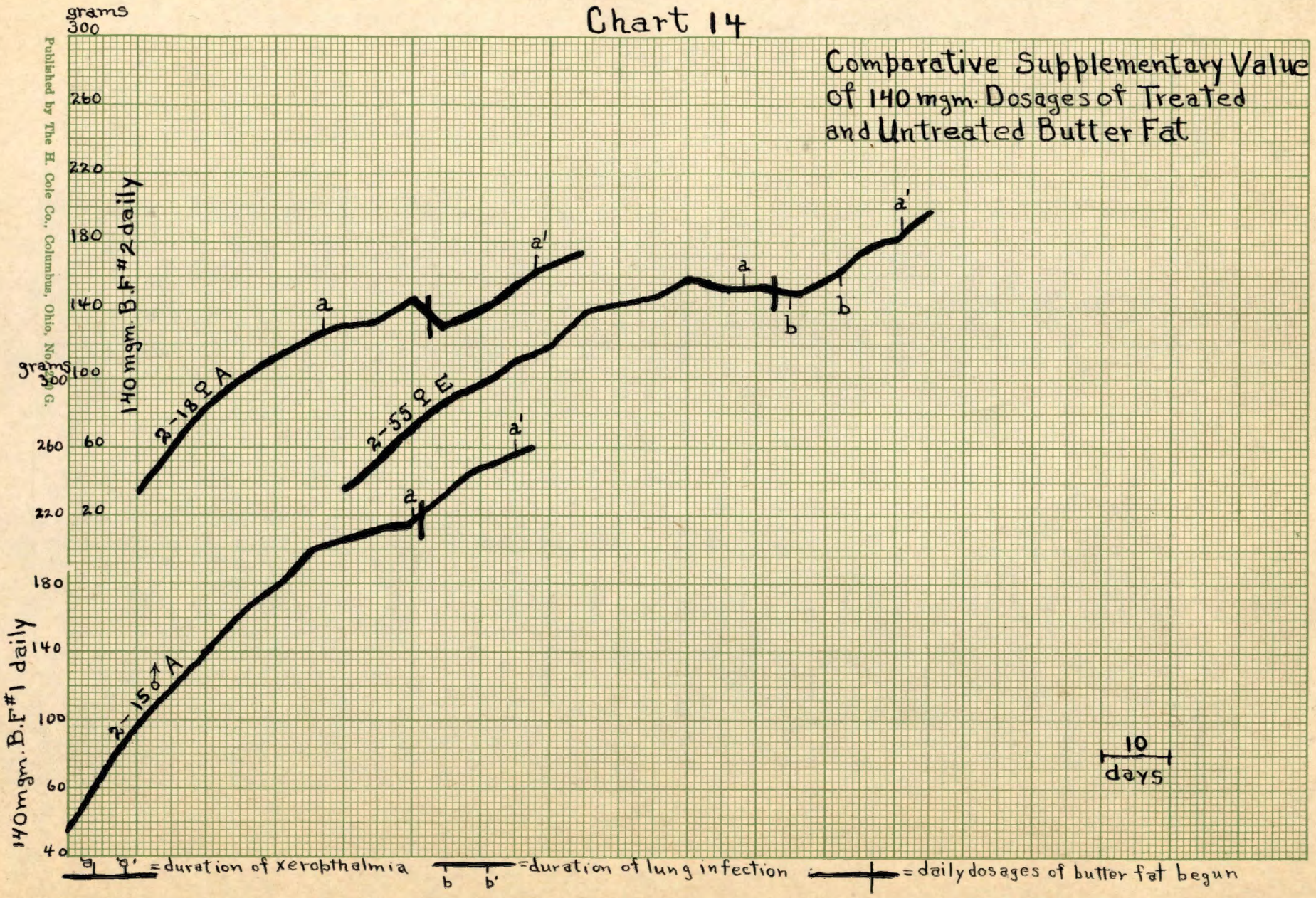

Fig. 1.

Fig. 2.

Fig. 1. is a photograph of 2-43 ♀ D, a negative control.

Fig. 2. is a photograph of 2-39 ♀ D, 33 days after being given 100 mgm. butter fat daily. These animals are litter mates. At the time the pictures were made their respective weights were 95 g. and 147 g. and they were 110 days old. The animals were the same distance from the camera so their relative size is as it appears in the pictures.

Table II

Autopsy Findings

Rat Number	Abcessed tongue	Abcessed ear and sinuses	Lung infection	Kidney infection	Body fat	Supplement
2-16 ♀ A	+ +	+	+ +	+	- -	Negative Controls
2-22 ♀ B	- -	-	-	-	-	
2-37 ♀ C	+	+	-	-	- -	
2-43 ♀ D	+	+	+	+	- -	
2-57 ♀ F	+ +	+	+	+	- -	
2-64 ♂ G	+	-	-	-	- -	
2A-6 ♂ H	+	+	+	+	- -	
2-59 ♂ F	-	-	-	-	+	40 mgm. # 1
2E-3 ♂ J	-	-	-	-	-	
2-31 ♂ C	-	-	-	-	+ +	40 mgm. # 2
2-60 ♀ F	-	-	-	-	+	
2E-1 ♂ J	-	-	-	-	-	
2-20 ♂ B	-	-	+	-	+ +	60 mgm. # 1
2-29 ♂ B	-	-	-	-	+	
2-34 ♀ C	+	-	-	-	+ +	
2-45 ♀ D	-	-*	-	-	+	
2-52 ♂ E	-	-	-	-	+ +	
2A-3 ♂ H	+	-	-	-	+	
2A-4 ♂ H	+	-	-	-	+	
2E-2 ♂ J	-	-	-	-	+	
2-21 ♂ B	-	-	-	-	+	60 mgm. # 2
2-24 ♂ B	-	-	-	-	+	
2-36 ♂ C	-	-	-	-	+	
2-44 ♀ D	+	-	+	-	+	
2-48 ♂ E	+	-	-	-	+	
2-49 ♀ E	-	-*	-	-	+	
2A-5 ♀ H	+	-	-	-	+ +	
2A-9 ♂ H	+	-	-	-	+	

* Bloody nose, mucous in upper respiratory tract.

(Table II continued.)

Rat Number	Abcessed tongue	Abcessed ear and sinuses	Lung infection	Kidney infection	Body fat	Supplement
2-30 ♂ C	-	- *	-	-	+ +	80 mgm.
2-33 ♂ C	-	-	-	-	+ +	# 1
2-42 ♀ D	-	-	-	-	+ +	
2-46 ♂ E	-	-	-	-	+	
2-54 ♂ E	-	-	+	-	+	
2-58 ♂ F	-	-	-	-	+	
2A-7 ♂ H	+	+ *	+	-	+	
2E-5 ♂ J	-	-	-	-	+	
2-23 ♀ B	-	-	-	-	+	80 mgm.
2-32 ♂ C	-	-	+	-	+++	# 2
2-35 ♂ C	-	-	-	-	+	
2-41 ♀ D	-	-	-	-	+	
2A-8 ♀ H	-	-	-	-	+	
2E-4 ♂ J	-	-	-	-	+	
2-17 ♀ A	-	-	-	-	+	100 mgm.
2-39 ♀ D	-	-	-	-	+	# 1
2-62 ♀ G	-	-	-	-	+	
2-63 ♂ G	-	-	-	-	+++	
2E-6 ♀ I	-	-	-	-	+	
2E-8 ♀ I	-	-	-	-	+	
2-14 ♀ A	-	-	-	-	+	100 mgm.
2-40 ♂ D	-	-	-	-	-	# 2
2-61 ♂ F	-	-	+	-	+	
2-65 ♀ G	-	-	-	-	+	
2E-9 ♀ I	-	-	-	-	+	
2-13 ♂ A	-	-	-	-	+	120 mgm.
2-19 ♂ A	-	-	-	-	+	# 1
2-25 ♂ B	-	- *	+	-	+++	
2-27 ♀ B	-	- *	+	-	+	
2-38 ♀ C	-	-	-	-	+	
2E-7 ♀ I	-	-	-	-	+	
2-11 ♀ A	-	-	-	-	+	120 mgm.
2-12 ♂ A	-	-	-	-	+	# 2
2-26 ♀ B	-	-	-	-	+	
2-28 ♂ B	-	-	-	-	+	
2-53 ♀ E	-	-	-	-	+	
2-15 ♂ A	-	-	-	-	+	140 mgm.
						# 1
2-18 ♀ A	-	-	-	-	+	140 mgm.
2-55 ♀ E	-	-	-	-	+	# 2

* Bloody nose, mucous in upper respiratory tract.

General Condition

The duration of xeropthalmia and lung infection is indicated on the individual growth curves for the rats. See Charts 1 - 14.

The autopsy findings showed marked uniformity especially on rats receiving the higher dosages of butter fat. More variation was displayed between rats at different levels of butter fat intake than between rats on different types (#1 and #2) of butter fat fed at the same level.

Two of the negative controls did not show the characteristic lung and kidney infection displayed by the others. These animals died of diarrhea accompanied by a hemorrhagic condition of the intestines and bladder, one also had a complication of abcesses on the neck. All other controls showed pus sacs on the base of the tongue, and in the inner ear and sinuses, in addition to lung and kidney infection and extreme emaciation. The amount of pus found and the extent of lung infection, while varying among the controls, were much greater than any found in the rats receiving butter fat supplements to the diet.

The smaller dosages of butter fat (40, 60, and 80 mgm.) were not as effective supplements as were the higher dosages. While the relative amounts of body fat compared favorably with the amounts present in animals

receiving higher dosages, and while there was no diseased condition of the kidney, there was more evidence of infection. About 20% of these rats showed abcesses at the base of the tongue, although in only one case was there found any pus in the ears and sinuses. Only 5 out of the 35 rats showed any evidence of lung infection.

Among the rats receiving the higher dosages of butter fat (100, 120, and 140 mgm.) the response was more uniform. None of these animals showed pus in the ears and sinuses or at the base of the tongue. The kidney condition was normal in all cases and only 3 out of the 23 rats showed any evidence of lung infection. The amount of body fat present was average or above.

During the period when most of the rats were in a depleted condition a deterring factor was introduced in the form of a respiratory infection. Coughing and sneezing was contagious and in spite of such precautions as isolation and the sterilization of cages and food and water containers, it spread throughout the entire colony including the stock animals. The growth curves of many of the animals show considerable variation in the time required to respond with the expected gains in weight, after the treatment with butter fat was begun. On referring to Charts 1 - 14 it will be seen that in most instances periods of failure to gain were identical with the duration of this current infection. This condition made it difficult

to judge accurately the effectiveness of the dosages of butter fat given and may account for the few cases in which, on autopsy, the upper respiratory tract was filled with mucous and the nose was bloody.

Possibilities of Vitamin A Storage in Animals Studied.

It is of interest to note the different possibilities for vitamin A storage exhibited by the rats studied. None of the animals used in the experiment had received cod liver oil directly, their only source having been that obtained indirectly through the mother in case she had received cod liver oil during pregnancy or lactation.

Until Sept. 18, 1929 our policy had been to give to breeding stock the following diet, indicated in Table III as Stock Diet A. (Whole wheat - 58, skim dried milk - 20, dried alfalfa - 5, Crisco - 5, NaCl - 1, and $CaCO_3$ - 1.) During periods of pregnancy and lactation female rats were given daily supplements of: 3-4 drops of cod liver oil, one small lettuce leaf, 500 mgm. of yeast and a small portion of lean meat. The liver and alfalfa of this stock ration and the cod liver oil and lettuce given as supplements are all dependable sources of vitamin A.

Subsequent to this date we have used as a stock ration a commercial preparation of calf meal supplied by the G.P.L. Cooperative Exchange, Buffalo, N.Y., having the following composition according to the manufacturer's statement. (Linseed oil meal - 15, ground malted barley - 10, wheat flour - 22, oat flour - 15, dried skim milk - 15, yellow corn meal - 20, steam bone meal - 1, ground limestone - 1, and salt - 1.) In Table III this diet is referred to as Stock Diet B.

The same supplements were given during periods of pregnancy and lactation as described above, with the exception that cod liver oil was omitted. The chief source of vitamin A in this ration has been the yellow corn meal of the Stock Diet and the lettuce given as a supplement.

All rats used in these experiments were born between Dec. 5, 1929 and Jan. 26, 1930.

Table III indicates the possibilities for storage of vitamin A as related to the stock diet used and the daily supplements given during the previous pregnancy and lactation periods of the rats that produced the litters used in these experiments. This table also gives data on the average duration of the fore-period and the average gain in weight during this time, for each litter used.

Table III

Nutritional History of Animals Used in relation to Length of Fore-period and Gains in Weight during this Period.

Stock Diet A	Stock Diet B	Litter used	Litter mate designation	Duration of Fore-period (days)		Average gains in Fore-period (grams)	
				♂	♀	♂	♀
Until the 14th day of lactation for 1st litter	Beginning on 15th day of lactation for 1st litter.	2nd	A	45	40	132	109
Until 12th day of lactation for 2nd litter.	Beginning on 13th day of lactation for 2nd litter.	3rd	B	59	56	163	91
Until 7th day of pregnancy for 1st litter.	Beginning on 8th day of pregnancy for 1st litter.	2nd	C	57	67	205	124
Until a few days before the 1st pregnancy. (No supplements had been given.)	Beginning a few days before the 1st pregnancy.	2nd	D	48	48	96	85
Until 9th day of pregnancy for 2nd litter.	Beginning on 10th day of pregnancy for 2nd litter.	3rd	E	55	56	165	131
Until 11th day of pregnancy for 2nd litter.	Beginning on 12th day of pregnancy for 2nd litter.	3rd	F	34	37	93	67
Until a few days before the 1st pregnancy. (No supplements had been given.)	Beginning a few days before 1st pregnancy.	1st	G	25	28	72	57
Until 7th day of pregnancy for 2nd litter.	Beginning on 8th day of pregnancy for 2nd litter.	3rd	H	39	39	105	93
Until a few days before first pregnancy.	Beginning a few days before 1st pregnancy.	1st	I		32		50
This group of rats was obtained from the U.S. Bureau of Home Economics and previous to this experiment were fed Sherman's Stock Ration.			J	29		28	

Table IV

Average Duration of Fore-period and Gains in Weight On Vitamin A-free Rations as Secured by Other Investigators.

Investigators	Stock Diet Used	Duration of Fore-period	Gains during Fore-period ♀	♂
Steenbock and Coward (1927)	C	4-5 weeks	47	54
Macy, et al. (1927)	D	34 3 days	59	68
Steenbock, Nelson and Black	C	4-6 weeks	55	80
Dutcher, Honeywell and Dahle	E	35-40 days	Average for ♀ and ♂ 22	
Sherman and Storms (1925)	D	40 days	38 for ♂	

Stock Diet C.

Steenbock Nelson and Black (1924). Yellow corn - 76, linseed oil meal - 16, crude casein - 5, ground alfalfa - 2, NaCl - 0.5, and $CaCO_3$ - 0.5. Fresh whole milk ad libitum.

Stock Diet D.

Sherman and Munsell (1925). Dried whole milk - 1/3, ground whole wheat - 2/3, and NaCl - 2% weight of wheat.

Stock Diet E.

Dutcher, et al. (1927a). Wheat - 45, corn - 17, rolled oats - 4.9, linseed meal - 8, bran - 2.5, dried meat scraps - 5, powdered skim milk - 10, NaCl - 1, $CaCO_3$ - 1.5, ferric citrate - 0.1, and alfalfa meal - 5.

Steenbock's stock ration (C) has the following sources of vitamin A: yellow corn, crude casein and the fresh whole milk. Sherman's stock ration (D) has as its only source of this vitamin the dried whole milk. Dutcher, et al, include in their stock ration (E) alfalfa meal and corn. Presuming this to be yellow corn, these are the only possible sources of vitamin A. The probable explanation of the longer duration of the fore-period and the larger gains in weight of our test animals is the more abundant sources of vitamin A in our Stock Diet A. Our Stock Diet B corresponds more closely to the breeding rations used by these other investigators.

Chart 15

Effect of Vitamin A Deficiency on the Oestrus Cycle

													B.F. Dosage									
2-34	♀C	● Δ ● ●	Δ ● Δ Δ	Δ ● Δ Δ	● ●	3 days	● + ● ●	● ●	● ● ● ●	● Δ ● ●	3 days	● ● ● ●	60 mgm #1	● ● ● ●	+ + Δ + +	● + ●	3 days	+ Δ + +	+ Δ Δ Δ	Δ Δ Δ Δ		
2-38	♀C			Δ ● Δ Δ	● + ● ●	● + ● ●	● ● ● ●	3 days	● ●	● ● +	●	2 days	120 mgm #1	17 days	+ Δ + +	+ Δ + Δ	+ Δ Δ Δ					
2-39	♀D		+ + +	● ● ●	● ●	● ● ●	3 days	● ● ●	● ●	● ●	● ●	● ● ●	100 mgm #1	4 days	● ● ●	● ●	+ ● + +	3 days	+ Δ + +	+ Δ + Δ	Δ + Δ Δ	
2-56	♀E			● ●	● ●	● ●	● ●	3 days	● ● ● ●	● ● ● ●	● ●	● ● ● ●	80 mgm #2	11 days	● ● ● ●	● ● ● ●	● ● ●	20 days	Δ Δ Δ Δ	Δ ● Δ Δ	● Δ ● ●	
2-60	♀F	+ + + +	+ + + +	+ + + +	+ + + +	3 days	+ + + +	+ + + +	+ + + Δ +	● + ● +	4 days	● ● ● ●	40 mgm #2	7 days	● + ● +	● + ● +	● ● ● ●	20 days	▲ ● ▲	● Δ ● ●	● + ● ●	
2-55	♀E			● ● ● ●	● ● ● ●	● ● ●	● ● ● ●	3 days	● Δ ● ●	● ● ● ●	● ● ● ●	2 days	120 mgm #2	18 days	+ + +	+ Δ + +	+ Δ + Δ					
	Negative Controls																					
2-16	♀A		● + ● ●	● ● ● ●	● ● ● ●	● ● ● ●	3 days	+ ● + ●	● ● ●	● ● ● ●	● ● ● ●	3 days	● ● ● ●	● ● ● ●	● ● ●	● ● ●						
2-37	♀C		+ + +	+ + + +	+ + + +	+ Δ + +	3 days	+ ● + +	+ ● + +	● ● ●	● ● ● ●	3 days	● ● ● ●	● ● ●	● ● ● ●							

+ = leucocytes
Δ = epithelial cells
● = cornified cells

Effect on Oestrus Cycle.

Results confirmatory to those of Evans (1928) were obtained. The smears gradually resumed their normal appearance as supplementary feeding was continued. No relation was found between the amount of butter fat given and the time required for the cycle to return to normal.

Table IV

Effect of Vitamin A Deficiency on the Hemoglobin Content of the Blood

Rat Number	Grams Hemoglobin per 100 cc.													
							Supplementary Feeding							
2-11 ♀ A							120 mgm. # 2	15.08	12 days	14.90	13 days	16.23	27 days	14.73
2-28 ♂ B		17.14	14 days	16.91	14 days	16.91	120 mgm. # 2	21 days	13.61	3 days	14.03			
2-36 ♀ C	16.52	20 days	16.91	6 days	16.46	1 day	120 mgm. # 1	22 days	13.05					
2-39 ♀ D			19.21	12 days	18.52	6 days	120 mgm. # 1	5 days	19.39	6 days	17.59	24 days	15.25	5 days	13.19
2-46 ♂ E			20.04	11 days	15.60	4 days	80 mgm. # 1	18.52	6 days	17.81	24 days	16.91	5 days	14.20	
2-65 ♀ G					19.68	5 days	100 mgm. # 2	6 days	16.23	6 days	17.59	6 days	16.01	28 days	15.45
Negative Controls														
2-16 ♀ A	20.32	13 days	22.90	5 days	18.04	6 days	21.35							
2-37 ♀ C	20.01	9 days	17.36	5 days	16.91	6 days	17.81							
2-57 ♀ F	17.59	13 days	18.26	5 days	17.14	6 days	18.80							
2-64 ♂ G	19.38	13 days	19.68	5 days	18.26	6 days	17.14							

Effect on Hemoglobin Content of Blood.

In most cases an increase was noted in the percentage of hemoglobin. Rats that had exhibited an increased hemoglobin content of the blood in the fore-period, and whose diets were then supplemented with higher dosages of butter fat responded with a return to normal hemoglobin values. Thickening of the blood in control animals, together with its darkened color and the increased rate of coagulation, indicate that the high hemoglobin values obtained on progressive deprivation of vitamin A can be explained on the basis of dehydration.

SUMMARY AND CONCLUSION.

Sixty-seven rats were placed on vitamin A-free diets and observed over different periods of time varying from 50 to 90 days.

As their reserve of vitamin A was depleted some were given daily, varying amounts of butter fat from milk which had been treated with mineral oil to remove the onion flavor and odor, others were given equal amounts daily of butter fat from milk which had received no treatment with mineral oil.

Observations were made on the effect of these butter fat supplements on the general condition of the animals, on the oestrus cycle, and on the hemoglobin content of the blood. At the termination of the experiment all of the animals were autopsied.

The effects of vitamin A deficiency on the oestrus cycle confirmed the findings of Evans. On supplementing the basal diet with either form of butter fat, the normal cycle gradually was resumed.

Hemoglobin determinations showed a constantly high percentage in the case of continued deprivation of vitamin A. When the animals were supplied with either form of butter fat as a source of vitamin A the hemoglobin returned to its normal level.

Judging from these results as well as from improvements in the general condition of the animals, and from the autopsy findings, it was concluded that the mineral oil treatment of milk for the removal of the onion flavor and odor, had no effect on the vitamin A content of the butter fat.

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