T H E S I S

on

A STUDY OF THE VITAMIN C CONTENT OF OREGON BOSC PEARS

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Introduction

Since the recognition of vitamin G (B_2) as an essential factor in human nutrition, comparatively few tests have been carried out on the presence of this vitamin in foods. Primarily because of this fact, the present piece of research was undertaken.

Historical

The multiple nature of vitamin B has been proved and amplified many times since Goldberger and Lillie (1), and Smith and Hendrick (2) gave satisfactory evidence that vitamin B involved two factors of importance for young rats. One of these, now known as vitamin G, Goldberger called the P-P vitamin, adducing much evidence that it was concerned in the prevention and cure of human pellagra.

During the past five years evidence has been presented indicating the further division of the vitamin B complex until, at the present time, four factors are not only recognized but have been more or less separated, and the probability of at least one more is apparent. Williams and Waterman (3) showed in their experimental work that pigeons required something not needed by young rats, since normal weight of pigeons could not

be maintained on a diet that supported normal growth in rats. These investigators found this unknown factor, referred to as B_3, to be present in whole wheat. Kennedy and Palmer (4), Hunt (5), and Roader (6) presented evidence for the division of the vitamin B complex into at least three components, all necessary for the nutrition of the rat. Later, Roader (7) gave more convincing evidence for the separate existence of three factors, when she discovered it possible to concentrate two of them, and found that the third must still be added to enable the animals to grow to maximum adult size. This third factor she first called vitamin B_3, but recently has renamed it B_4 (8), since the pigeon growth factor is now designated as B_3.

The most recent publications concerned with the multiplicity of the vitamin B complex indicate the probability of still another factor. Chick and Copping (8) have provisionally called this "Factor Y" and believe it is not identical with vitamins B_2, B_3 or B_4. This "Factor Y" appears to be the only one which will withstand both heat and alkali.

Vitamin G in Foods. While revaluation of foods in regard to their vitamin B content has been limited, some significant studies concerned with such a problem have been carried out. Hunt (5), after investigating the

vitamin B complex as contained in wheat and corn, found that all the rats receiving no other source of vitamin G developed symptoms indicative of pellagra, indicating that wheat and corn are both low in this factor. Akroyd and Roscoe (9) made an investigation of wheat and maize kernels for their vitamin G content. They concluded, first, that the vitamin G content of wheat and maize is poor, that of maize being on the whole lower, and second, that the germ and bran are better sources than the endosperm. Less extensive experiments carried on by these same workers indicated that dried peas have a low vitamin G content; that dried meat and egg yolk are fair sources; and that dried ox liver, yeast and whole milk are excellent sources of this factor. Work by Haugo and Beadle (10) indicated that white bread contains some vitamin B_1 but that it is deficient in G, and that whole wheat bread not only contains vitamin B_1 but has an ample supply of vitamin G for growth. Hagle (11), in testing honey for vitamin content, found it devoid of both vitamin B_1 and vitamin G. Hoagland and Snyder (12) carried on a group of experiments for the purpose of determining the relative amounts of vitamin G in commercial beef extract. Results showed that 20% of dried lean beef furnished sufficient vitamin G for fair growth and 25% for excellent growth. Artmayer (13) found vitamin G to be a limiting

factor in red kidney beans and lacking in polished rice. Almroyd (14) showed that "raw" and "parboiled" rice were both poor sources of vitamin C, and also that millet is low in this vitamin. Work of Munsell (15) indicated that watermelon contains a small quantity of vitamin C. Hoagland (16) carried on a series of experiments to determine the relative quantities of vitamin C in beef, pork, lamb, beef spleen, beef liver, pork liver, and beef kidney. From the results it appeared that beef, pork and lamb contain approximately the same quantities of vitamin C. Spleen, liver and kidney all furnished enough of this vitamin for excellent growth.

Purpose of This Study

The selection of the bosc pear for investigation concerning the vitamin C content was made primarily because it was desired to add to the data now accumulating in several departments at Oregon State College regarding this product. Extensive studies have been made by the Agricultural Experiment Station on production and marketing. The Chemistry Department has finished recently a complete chemical analysis of this variety. In addition, a Purnell research project dealing with methods of baking pears has been carried on this year in the department of Foods and Nutrition.

No work has been published on the vitamin G content of bosc pears; hence, this study hopes not only to supplement these other studies conducted on this campus but also to add to the accumulating knowledge of the distribution of this vitamin in foods.

Experimental

Animals

Albino and pied rats were used in the experiment. These animals were raised in our own stock colony or in the colony belonging to the Animal Nutrition Department of the Agricultural Experiment Station at Oregon State College. Our stock animals have, until this year, been fed for a number of generations on the Smith and Bing modification of Sherman's diet #13 (17) with the addition of 5 grams of lean meat three times a week, 15 grams of lettuce daily and 9 grams of yeast weekly, during pregnancy and lactation. This year some variations have been made in our stock diets and, as a result, some of the animals have been reared on the above diet, others on Sherman's unmodified diet (18), and still others on Steenbock's stock diet (19). All of these diets have been found adequate for satisfactory growth and reproduction.

The animals were weaned when twenty-one days old and at twenty-eight days of age were placed on

experimental diets. This is the procedure followed by Kennedy and Palmer (4), Williams, Waterman and Curin (20). Range of weights at the beginning of the experimental period was from 28 to 63 grams, the average weight being 39 grams.

The experimental period continued for ten to eleven weeks, from the beginning of the fifth week to the end of the fourteenth or fifteenth week of the rat's life, this being the period during which the rat grows most rapidly if given an adequate diet. Some experimental animals were also continued for a period of 50% longer time to ascertain whether any additional effect might be observed by such a procedure.

The rats were kept in individual metal cages with raised screen bottoms to prevent coprophagy. Daily care consisted in recovery of food, giving of fresh water, feeding of supplements, and making observations. Basal rations were given freely but careful food weights were made. The animals were weighed weekly, or oftener when necessary, and the amount of food consumed was determined and recorded for the same weekly intervals. Supplements were fed daily and immediate consumption was obtained by withholding basal diets.

The animals were distributed on various experimental diets with weight, sex and ancestry all taken into

consideration in order that any influence due to these factors might be equalized in each experimental series. At least six animals were fed each type of experimental ration, and in most cases more.

All rats were kept upon the basal diet alone until growth had ceased or a slight loss in weight had occurred. This period ranged from seven to sixteen days. Following this depletion period the animals received the basal diet with or without the addition of supplements, according to the experimental series in which they were placed.

Basal Diets

Two basal diets were used in the experiment.

Diet A, evolved by Munsell (15) of the U. S. Bureau of Home Economics, is as follows:

casein (B and G free)	18%
dextrinized starch	38
whole white corn (ground)	30
butterfat	8
cod liver oil	2
Osborne and Mendel salt mix.	4
	100%

Diet B, used in the Sherman laboratory as a diet free from both vitamins B_1 and G (37), was used in some of the series:

casein (B and G free)	18%
dextrinized starch	66
butterfat	8
cod liver oil	2
Osborne and Mendel salt mix.	4
agar	2
	100%

Diet A is not completely freed from vitamin G but has been shown to be sufficiently lacking in this vitamin to produce the symptoms, after a long period of time, usually attributed to G avitaminosis; and it is well adapted to experimental work in which the additive effect of a food material is being tested. Its selection in preference to other vitamin G-free diets was made because our laboratory does not have facilities for extensive purification, and up to the present time no one has found an entirely satisfactory method that does not involve great expense.

Preparation and Purification of Constituents of Basal Diets

Casein. Evans and Burr (21) and later Palmer and Kennedy (4) found better growth in a diet in which commercial casein was used than with the purified product, indicating that the former probably carried some of the growth promoting vitamin. There are several methods

recommended by various nutrition laboratories for the purification of casein. Osborne, Wakeman and Ferry (22), Sherman and Spohn (23) extract with alcohol. Palmer and Kennedy (4) also follow a method of alcohol extraction after having prepared their casein from raw milk by the grain curd method, HCl being used as the precipitating agent. Chick and Roscoe (24) purify caseinogen by washing in acetic acid and then extracting with alcohol.

The method adopted for our use was evolved by Steenbock, Sell and Nelson (25) and has been used in other laboratories. A preliminary test was made in our own laboratory to determine the efficiency of this method in obtaining a product comparable to that purified for vitamins B and G in the Harris laboratories. The results of this test showed our casein to be of like purity.

Dextrinized Starch. Carbohydrates may be added to the diet in the form of starch or dextrin. Laboratories vary in this procedure. Osborne, Wakeman and Ferry (22) use starch and have demonstrated by many experiments that it furnished carbohydrate in a form adequate for the normal nutrition of the rat. Sherman (23), Kennedy and Palmer (4), Williams and Waterman (3) and Steenbock and Gross (26) dextrinize their starch. Chick and Roscoe (27) use rice starch. The method used in our laboratory is that described by Palmer and Kennedy (4) in which the

dextrin is made from cornstarch by autoclaving the starch after moistening with a 1% citric acid solution.

Butterfat was prepared by melting fresh creamery butter, produced at Oregon State College dairy, at a temperature below 60° C., decanting and filtering through absorbent cotton in order that the salts and water present might be removed. Palmer and Kennedy (28) and Osborne, Wakeman and Ferry (22) used this method.

Salt Mixture. Osborne and Mendel Salt mixture (29) was used in the basal diets. This is one of the well recognized salt mixtures for meeting the mineral requirement of the rat.

Cod liver oil (Squibb's) was used throughout the experiment in the basal diets as a source of vitamins A and D. Laboratories differ as to their methods of adding this factor to the diet. Akroyd, Chick and Roscoe (9 and 27) give daily doses of 0.05 to 0.1 grams. Naryanan and Drummond (30) give 5 to 6 drops twice a week. Sherman and Sandels (32) included it as 3% of the basal diet. Our method of feeding, incorporating it as 2% of the basal diet is followed by Williams and Waterman (33), Artmayer (13), Munsell (15), Quinn and Brabeck (34) and Sherman and Spohn (23).

White Corn. The white corn (Champ White Pearl) used in the experiment was obtained from the Portland Seed

Company, Portland, Oregon, and was ground in our own laboratory. The corn was incorporated as a source of vitamin B_1; and while vitamin G is not entirely lacking, its content has been shown to be very low. Munsell (15) found it sufficiently low in this vitamin to produce recognized vitamin G deficiency symptoms.

Bosc Pears. The reasons for the selection of bosc pears as a possible source of vitamin G have previously been stated. Because of the other experimental work being done on pears in our institution, we were able to obtain a product harvested and ripened under careful supervision and standard conditions.

The bosc pears used in the experiment were harvested, ripened and stored under the direction of the Department of Horticulture, in accordance with the procedure now used for market practices. The pears were ordered through Mr. Henry Hartman and were harvested by the Pinnacle Packing Company of Medford, Oregon. All the pears had been harvested by September the thirteenth and placed in storage at the Southern Oregon Branch Experiment Station at a temperature of 32° F. The lot from which these experimental pears came was shipped by express, arriving in Corvallis on November the fourth.

Immediately upon arrival, the pears were placed in a ripening chamber and ripened under the direction of Mr.

Oscar Dowd and Mr. G. L. Rygg of the Department of Horticulture. Temperature of the ripening chamber was held at approximately 65° F. The ripening process took six days.

At the end of the period the pears were delivered to Winkley's Creamery where they were held at approximately 32° F. for two days, and were then delivered preparatory to the drying process.

The drying of the pears was done under the direction of Mr. Thomas Onsdorff of the Horticultural Products Department. In preparation the pears were cut lengthwise into quarters. They were then laid on metal screens, with the skin side down so that the inside would not be in contact with the metal. Next, they were placed in an Oregon Tunnel Recirculation Prune Dryer, which had been preheated, and were dried at a temperature averaging 160° F. (never in excess of 165° F.). Dry air was kept moving by a circulating draft at about 600 feet per minute. The pears were allowed to dry for a period of 64 hours. After drying they were tightly sealed in number 2 1/2 cans and opened only for immediate use.

Chemical analysis was made on the pears by Mr. D. E. Bullis of the Chemistry Department of Oregon State College. Results of ash and moisture analysis are shown in the following:

Moisture on flesh	80.17%	Iron	.000139%
Ash	.330	Magnesium	.00799
Alkalinity of ash	4.53	Phosphorus	.0156
Insoluble and silica	.00030	Sulfur	.00367
Sodium	.00534	Chlorine	.00067
Potassium	.156	Crude fiber	1.43

The fruit was cored but not peeled. A composite sample of one quarter of each of 25 fruits was used for the above analyses, except for crude fiber when one quarter of each of 10 fruits was used. All percentages were calculated on the fresh fruit basis.

The moisture content of the base pear taken from samples dried by the above outlined process was found to be 9.67%.

Autoclaved yeast was used as a known source of vitamin G for comparison with pear in this factor. Goldberger and Lillie (1) observed that there was no destruction of the vitamin G content of yeast when heated in the autoclave at 15 pounds pressure for two and one-half hours while the B_1 factor was destroyed to a considerable extent. Smith and Hendrick (2) autoclaved dried brewers' yeast for six hours at fifteen pounds pressure to obtain a substance free from vitamin B_1 but containing vitamin G. Hunt (5) obtained desired results when autoclaving for four hours at 15 pounds pressure.

The most recent experimental work on autoclaved yeast as a source of vitamin G indicates that the hydrogen ion concentration has some influence upon the retention of the G factor. Williams and Waterman (20) carried on a series of experiments determining the effect of varying the pH of the yeast before autoclaving. After six hours autoclaving at 15 pounds pressure in a very weak acid (pH 4.5) considerable amounts of vitamin B_1 apparently remained. Alkaline autoclaving effectively destroyed the vitamin B_1 but serious losses of vitamin G were also found. Thus, while their work showed that hydrogen ion concentration was important, they established no better methods for treating yeast to destroy maximum amounts of B_1 and retain a maximum quantity of vitamin G.

Reader (6) heated yeast extract for one hour at 120° C. at pH 9.0 to retain vitamin G and showed, through rat-growth experiments, that this factor was still present in yeast. Successful destruction of the G factor was only accomplished by this investigator after a six hour extraction with ether.

Chick and Roscoe (36) found approximately 50% destruction of vitamin G when heating yeast for two hours at a temperature of 98-100° C. and at a pH 8.3. They further found that yeast at the normal hydrogen ion concentration (pH about 5.0) suffers no determinable loss in

vitamin G content after being steamed for two hours at 98-100° C.

From this evidence we have concluded that autoclaving dry yeast for five hours at 15 pounds pressure at the original hydrogen ion concentration (pH 6.0) is sufficient for destroying the vitamin B_1 factor and for retaining the G vitamin sufficient for normal growth when fed at a level of 0.5 grams daily.

That this method of autoclaving is effective was further determined in our own laboratory in a series of preliminary tests when symptoms of polyneuritis were readily developed on a purified diet (Diet B) supplemented by this autoclaved yeast. The fact that the addition of autoclaved yeast to the basal diet (Diet A) supplements it to the extent that normal growth is obtained further indicates that our method of autoclaving was satisfactory.

Dried Yeast. The dried yeast, used in the experiment, was obtained from the Fleischmann Yeast Company, New York City. The analysis of this yeast was as follows: moisture 7.44%, protein 48.5%, fat 1.59%, glycogen 28.0%, cellulose, gum, etc. 6.38%, ash 8.09%. The hydrogen ion concentration was found to be pH 6.0. This was changed to pH 5.0 after autoclaving for five hours at 15 pounds pressure. 0.5 grams of this original

yeast furnished growth when added to a basal diet otherwise complete.

Plan of Experimental Series

The animals were divided into ten groups with six to eleven rats in each series. While the small number of animals in each group prevents the drawing of definite conclusions, the results obtained at least allow certain predictions to be made.

Two groups of negative controls were established, not only because it was desired to secure symptoms of G avitaminosis but primarily to test the G deficiency of the basal diet A.

Series I. Diet A alone.

Series II. Diet B alone.

To prove that our basal diet A was adequate with the exception of a source of vitamin G, three positive control series were established.

Series III. Basal diet A plus 0.5 grams of autoclaved yeast fed as a daily supplement. This level has been found sufficient for supplying vitamin G by Akroyd and Roscoe (38), Axtmayer (13), and Chick and Roscoe (36).

Series IV. Basal diet A plus a daily supplement of 0.5 grams autoclaved yeast and 0.5 grams dried pear (equivalent to 4.15 grams

fresh pear). The pear was added, in this case, for the purpose of determining if the basal diet supplemented by autoclaved yeast was adequate in itself for supporting maximum growth.

Series V. Basal diet B plus 0.5 grams of dried yeast fed as a daily supplement.

The remainder of the experimental series was concerned with the feeding of various levels of dried pear as a source of vitamin G as daily supplements to the basal diet A. The pear was fed with the purpose of determining, if possible, the amount of this food needed to produce in a rat, on a diet otherwise devoid or nearly free from vitamin G, a three grams gain per week over a period of eight weeks. This is the measure for a unit of vitamin G that has been established in Sherman's laboratory at Columbia University (39). In the event that the basal diet proved not to be sufficiently devoid of the G vitamin to maintain a stationary weight throughout the experimental period, at least an additive effect might be observed which would indicate the relative richness of bosc pear as a source of vitamin G. Thus, the experimental series were arranged as follows:

Series VI. Diet A plus 0.5 grams dried bosc pear (equivalent to 4.15 grams fresh pear) fed daily.

Series VII. Diet A plus 0.75 grams dried pear (6.23 grams fresh) as a daily supplement.

Series VIII. Diet A plus 1 gram dried pear (8.29 grams fresh) fed daily.

Series IX. Diet A plus 1.5 grams dried pear (12.44 grams fresh pear) fed daily.

RESULTS

Discussion

Series I - Basal Diet A. (Table I.)

In observing Table I it will be noted that there is a wide variation in the gains made during the 8-week experimental period. It is shown that animal ♀ 1021 gained only 3 grams and that animal ♀ 1025 gained 26 grams during this period or that 1021 made only 11.54% of the gain made by animal 1025. Such a wide variation is difficult to explain. The animals were of the same sex; they were littor mates; they were subjected to exactly the same laboratory conditions; and their diet remained the same throughout the period. Furthermore, the difference cannot be accorded to the wide variation in food intake since the average food consumed by 1025 was 39.37 grams, while that consumed by 1021 averaged 37.50 grams or 90.2% of the food intake of 1025. The explanation

will apparently have to be sought by observing the trend of weights after the 8-week period. Below are given the weights of the two litter mates both placed on basal diet A and carried for 16 weeks, a period twice as long as that established as experimental.

No. sex	Depletion period		Weight at end of 8-week period	Weight at end of 12-week period	Weight at end of 16-week period
	Beginning weight	End weight			
♀ 1021	41	47	50	58	59
♀ 1025	36	45	71	78	79

Calculating the average gain made by the two animals, it is found that 1021 made 41.8% of the gain made by 1025 over the 16-week period. Comparing this figure with 11.5% for the original experimental period, it may be observed that apparently the weight of animal 1025 tends to be leveled off and more nearly approaches the low rate of gain characteristic of 1025. Figures show that practically the same gain was made by the animals during the 8- to 16-week period.

The fact that the average gain for this series is approximately 2 grams (1.92) indicates that: (1) the constituents of the diet are not entirely freed from vitamin G, (2) that some other growth promoting factor is present, or (3) that the experimental animal has bodily reserves of a growth promoting vitamin.

There are at least two possible sources for vitamin G in the basal diet, the casein and the white corn. Goldberger and his associates (40) showed that casein still may have pellagra-preventing value after being leached in acidulated water for a week with the water changed daily. Chick and Roscoe (24) believe that they can render their casein practically free from vitamin G only after prolonged extraction with 50 or 70% acidified alcohol, drying and roasting for 3 days at 120° C. It was shown in this laboratory that a purified diet, in which casein was the only possible source of the G vitamin, produced a loss in weight of 8.4 grams and death within a six week period, thus indicating that the diet was practically free from vitamin G. It has previously been mentioned that Munsell (15) found white corn to be not entirely lacking in vitamin G, but sufficiently devoid of it to produce G deficiency symptoms.

Even though the weight curves of animals on basal diet A show a little more growth than maintenance throughout the experimental period, observation of the other experimental series will show that growth was restricted to a point decidedly below normal, and that when supplemented with autoclaved yeast, a proved source of vitamin G, the rate of weight increase was 86.94% greater than on basal diet A unsupplemented.

Symptoms of vitamin G deficiency recorded by investigators have been somewhat varied. Goldberger and Lillie (1) published their observations in 1926 in which they record: arrest of growth; a tendency for the lids of the eyes to adhere together, and often an accumulation of dried secretion on the margins of the lids; a loss of fur which usually began in irregularly distributed patches, often first noticed on the side or over the top of the head, the sides or front of the neck or in the regions of the shoulders, in some cases resulting in almost complete denudation; the development of dermatitis with or without the loss of fur on various parts of the body; definite reddening and thickening of the ears with a yellowish incrustation of dried serum. In a few cases, it was observed that the animals presented a linear fissuring or ulceration at the angles of the mouth. In a somewhat larger number of cases there was observed a lesion at the tip of the tongue. Diarrhea was noted in two cases. The shortest period recorded by these investigators in which dermatitis appeared was approximately seven weeks.

Chick and Roscoe (27) described the condition of rats on a diet deficient in vitamin G in the following manner: "The body weight was usually maintained, but the animals were in poor condition; they were thin and

active, with rough coat and with urine slightly blood stained. Loss of hair took place and red inflamed patches of skin appeared on the nose and on the backs of the fore feet, which later became edematous. There was a marked conjunctivitis and in some cases the ears also became red and thickened. The inflamed areas spread and often involved the hind legs and whole areas around the nose and mouth; sometimes the chest became bare and inflamed. The animals survived 2 to 3 weeks or longer in this condition before death occurred. On post mortem examination there was no definite macroscopic signs except a very unhealthy condition of the whole alimentary tract, especially in the small intestines which showed signs of atrophy of the mucus membrane and often contained blood-stained mucous."

Chick and Roscoe (24) later reported that on a basal diet supplemented with Peter's antineuritic concentrate as a source of vitamin B_1 but free from vitamin G, weights of young rats were kept nearly constant for three months or more, with fair appetite and no outward symptoms with the exception of skin abnormalities similar to those previously described. Sherman and Sandels (32) reported that when vitamin G was restricted within certain limits, pellagra-like symptoms appeared similar to those described by Goldberger and Chick and Roscoe, but

they further reported that in cases of almost complete deprivation of this vitamin, death frequently occurred without the development of conspicuous skin symptoms.

Severe symptoms of G avitaminosis recorded by these investigators have not been observed in our laboratory but some of the characteristic symptoms have been noted. During the 8-week experimental period, the only discernible reactions to G deficiency have been the low rate of growth, sluggishness of movements and apparently a lowering of vitality. As the animals have been carried on over a period of several months, other characteristics have appeared. The skin becomes dry and the fur loses its luster and falls out easily. Sluggishness becomes even more apparent as the period lengthens. Ears, tails and feet have taken on rather a bluish cast. Autopsies performed at the end of the 8-week experimental period show no definite signs of deficiency except that all organs appear to be undersized, just as the animal is retarded in growth. The fact that we did not obtain symptoms found by previous investigators may be explained by a store of vitamin G in the animal body, by a low vitamin G content of the corn diet, or by the presence of some other factor associated with the G vitamin.

Series II. Basal Diet B (lacking vitamins B_1 and G). (Table II.)

This series was introduced for the purpose of testing the casein as a possible source of vitamin G. Results in Table II show that a rapid decline in weight (an average of 8.4 grams) resulted when animals were restricted to this diet, and that death occurred within a 6-week period. This would indicate that our method of casein purification was satisfactory and that the animals reserve store of vitamin G was soon exhausted if placed on a diet lacking this factor. From these results it would appear that corn is probably the only constituent of basal diet A which contains small amounts of vitamin G.

Series III. Basal Diet A plus 0.5 grams of autoclaved yeast. (Table III.)

Observations of the weight charts and tables for this series show that the addition of 0.5 grams of autoclaved yeast to basal diet A supplies a growth factor to such an extent that normal growth is obtained. Average gains for this series show a weight increase of 14.69 grams per week over the 8-week period, which is well within the standard established by many authorities for normal growth. Further observations of these figures will show that the average growth on this series (14.69 grams) was

seven times greater than that obtained from feeding basal diet A unsupplemented (1.92 grams), further indicating the efficiency of autoclaved yeast in providing vitamin G when fed at a level of 0.5 grams.

Series IV. Basal Diet A plus 0.5 grams of autoclaved yeast plus 0.5 grams pear. (Table IV.)

The inclusion of this series in the experimental plan was for the purpose of determining whether the addition of 0.5 grams of autoclaved yeast to the basal diet A produced maximum growth or whether further addition of vitamin G, as supplied by pear, would produce added increase in weight. At the present time indications are that the addition of 0.5 grams of dried pear have a supplementary effect, producing an added amount of growth. Average gain obtained from this series was 19.56 grams, while that obtained from basal diet A with 0.5 grams of autoclaved yeast as the only supplement was 14.69 grams. The growth on basal diet A plus autoclaved yeast was only 74.4% of that produced when 0.5 grams of pear were added. This fact indicates that the pear is supplying some limiting factor in the diet which is a growth factor. Such a conclusion as this can hardly be drawn due to the fact that we are comparing four animals in series IV with nine animals in series III. Six litter mates are still

being continued on this diet in order that the number of cases will be more comparable. Indications from this group, which has been on the diet for a period of 5 weeks, would seem to show that the addition of pear to this positive control group (basal diet A plus 0.5 grams autoclaved yeast) has no supplementary effect. The average gain per week from these two series at the end of the 5-week period was identical (21.40 grams). This would indicate that the maximum amount of vitamins B_1 and G was supplied in the positive control group, all essential growth factors being present. Drawing conclusions from a period of this length of time is the practice advocated by Akroyd and Roscoe (9) in which they disregard the first week's growth and use average weekly growth during the following 4 weeks as the standard for comparison. Sherman (40) also states that numerous unpublished data indicate that an experimental period terminating at the end of 4 or 5 weeks gives better results. This was found to be particularly advisable when fractions of food products rather than the natural materials were being tested for their vitamin G values.

Series V. Basal Diet B plus 0.5 grams of dried yeast. (Table V.)

Since this basal diet substituted with 0.5 grams

of dried yeast has been found satisfactory for normal growth. This series was introduced as a check against other positive control series.

Dried yeast as a source of vitamin B_1 has been recognized for some time, and its value as a source of vitamin G is now apparent. In 1929 it was reported by Akroyd and Roscoe (9) to contain 5 to 10 Akroyd-Roscoe vitamin G units per gram (the amount required daily to maintain a weekly gain of 11 to 14 grams). Quinn, Whalen and Hartley (41) found 10 to 30 Bourquin units per gram (2 to 5 grams gain weekly). The figure for average gain of animals in series V, 15.05 grams, indicates that this diet adequately supplied vitamin G.

Series VI. Basal Diet A plus 0.5 grams of dried pear. (Table VI.)

The pear was added daily to the basal diet as a source of vitamin G according to the methods outlined under experimental procedure. Observations of Table X (Summary Table) show that the negative control group on the unsupplemented diet A made an average gain per week of 1.92 grams. When 0.5 grams daily dosage of pear was fed in addition to this basal diet, the average gain was 4.70 grams per week or approximately a gain 2.5 times greater than the basal diet alone.

Series VII. Basal Diet A plus 0.75 grams dried pear. (Table VII.)

The growth figures of this group are of value for comparison with those of both the basal diet unsupplemented, and when supplemented by 0.5 grams of dried pear. It is shown that about four times as much gain was made by animals of this series as by those of series I (basal diet A). The average gain of series I (1.92 grams) is contrasted with 7.5 grams for this series. Observation of average weight for series VI (4.70 grams) and series VIII (7.5 grams) indicate that with the feeding of a fourth more pear 1.5 times the amount of gain is obtained.

Series VIII. Basal Diet A plus 1 gram dried pear. (Table VIII.)

In comparing the growth figures of this group with those of previous groups it may be seen that the rate of increase is not in the same proportion as that found between series VI and VII. The assumption would seem to be that at this level the point where vitamin C is adequately supplied is more nearly approached, and on a higher level the growth deficiencies exhibited by the lack of the vitamin would undoubtedly be less. This view may be strengthened by the fact that the animals in both series consumed approximately the same amount of food. Varia-

tions in results in series VII and VIII may have been influenced by the wide variation in weights at weaning and at the 28-day period when animals were placed on a depletion diet. Differences in the growth rate might also have been due to the differences in the diet of the mother, as variation in stock diets did occur which have previously been discussed. These are factors which could not very well be controlled in our laboratory due to the fact that the number of available animals was not large enough to discriminate carefully as to ancestry and weight. It is felt, however, that more animals need to be included in this experimental series before a final understanding of the results can be obtained.

Series IX. Basal Diet A plus 1.5 grams of dried pear. (Table IX.)

This experimental series serves to strengthen many facts which have previously been observed. It is found by a study of figures concerned with weight gains that the increase is about five times that made on basal diet A (9 grams in contrast to 1.92 grams). It is also seen that it is about twice that made by animals when 0.5 grams of dried pear supplemented the basal diet (9 grams in contrast to 4.7 grams). Further, it is observed that animals in this series made a gain about

two-thirds as great as that made by the positive control series III (9 grams and 14.69 grams).

Summary of Results. (Table X and Chart X.)

Table X and Chart X illustrate the comparative results obtained upon each of the diets used in this investigation. It will be noted that increasing the amount of pear evidently supplied increasing amounts of vitamin G and more nearly approached the vitamin G content of autoclaved yeast. Since weight increase is in proportion to the level of pear fed, it is possible to approximate a unit of vitamin G contained in bosc pear. The average gain for the four levels of feeding is found to be 4.96 grams above the gain made on the basal diet A alone. The 1.0 gram level of feeding is the middle level of the series established. Therefore, 1.0 gram of pear supplementing basal diet A will produce approximately 4.96 grams in weight. Translating this into Bourquin units (3 grams gain per week), it is found that 0.59 grams of dried pear (4.64 grams of fresh pear) contain a unit of vitamin G.

A second evaluation may be made between bosc pear and autoclaved yeast in providing vitamin G. Normal growth equal to that obtained when 0.5 grams of autoclaved yeast supplemented basal diet A (14.69 grams) might have been attained if approximately 3 grams of dried pear

(2.97) or 24.87 grams of fresh pear were fed as a daily supplement to basal diet A.

Conclusions

1. It has been demonstrated that corn contains vitamin B_1 since no polyneuritis developed when fed as 30% of the diet.

2. The white corn used in the experiment was not entirely free from vitamin G. A gain in weight approximating 1.9 grams per week was obtained over the 8-week experimental period.

3. Autoclaved yeast, obtained by autoclaving dry yeast for 5 hours at 15 pounds pressure at the original hydrogen ion concentration (pH 6.0), when fed at 0.5 gram level supplements white corn in vitamin G and normal growth can be obtained.

4. When vitamin B_1 is adequately supplied by white corn, vitamin G is furnished by bosc pear in the same ration as the quantity supplied in the diet. The greater the amount of pear the greater the gain.

5. Under the conditions of this investigation it appears that 0.59 gram of dried pear (4.64 grams of fresh pear) contains a Bourquin unit of vitamin G.

6. It is believed that normal growth might have been obtained had the pear been fed at a higher level. Approximately 3 grams of dried pear (2.97 grams) or

24.87 grams of fresh pear would be necessary to produce growth equal to that obtained from the corn basal diet supplemented by 0.5 grams of autoclaved yeast (14.69 grams).

SUMMARY

1. The supplementary effect of bosc pear in providing vitamin G to a basal diet in which vitamin B_1 was supplied by corn has been studied.

2. Severe symptoms of G avitaminosis recorded by investigators were not obtained during the 8-week experimental period, but increase in growth was low (1.92 grams per week).

3. Increased amounts of pear as a supplement to the basal diet in which vitamin B_1 was supplied resulted in increases in growth rate.

4. A tentative figure is suggested as a unit of vitamin G in bosc pear.

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Table I.

The Effect of a Diet Low in Vitamin G.

The Effect of Basal Diet A -- Vitamin B_1 Supplied in White Corn

Sex and number	Depletion period			Eight week period					
		Body weight (grams)		Body weight (grams)			Food intake		Av. gain
	Length (days)	Beginning	End	Final	Gain	Av. gain per wk.	Food consumed	Av. food per wk.	per gram food per week
♀ 1021	14	41	47	50	3	0.37	300	37.50	.009
♂ 1023	14	38	48	67	9	1.12	292	36.50	.030
♀ 1025	14	36	45	71	26	3.25	315	39.37	.088
♀ 1068	10	36	48	74	26	3.25	301	37.50	.086
♂ 1074	8	35	36	52	16	2.00	246	30.75	.065
♂ 1084	13	48	55	63	8	1.00	249	31.12	.032
♂ 1092	13	54	67	87	20	2.50	240	30.00	.083
Average	12.2	41	49.4	66	15.4	1.92	277	34.53	.056

Chart 1

Basal Diet A

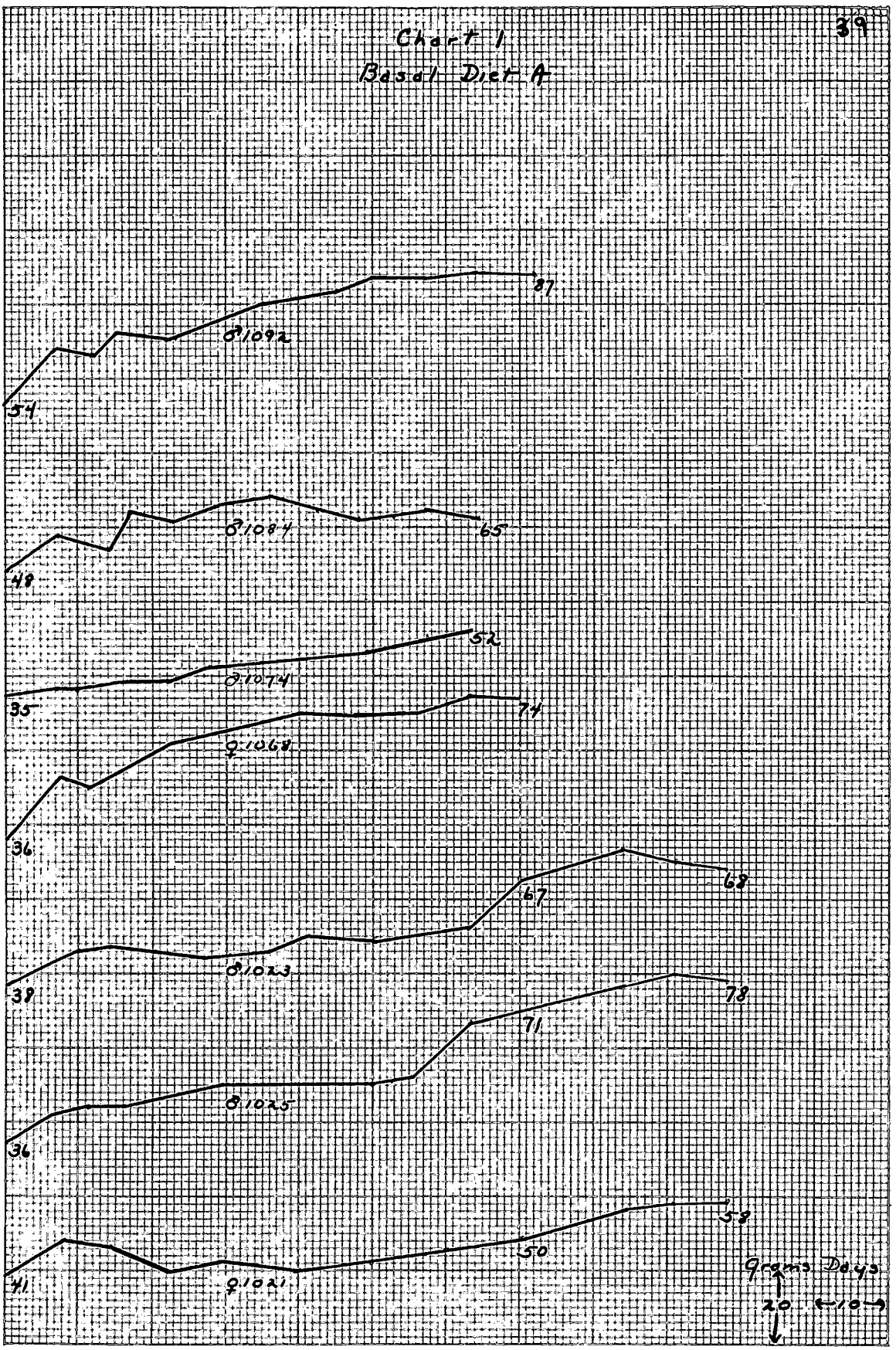


Table II.

The Effect of a Diet Devoid of Vitamin B Complex.

The Effect of Basal Diet B -- Purified Diet Alone.

Sex and number	Body weight (grams) Initial	End	Gain or loss	Av. gain or loss per wk.	Length of period	Food intake (grams) Food consumed	Av. food per wk.	Av. gain or loss per gram food per wk.
♀ 1017	55	46	-9	-1.80	5 wks.	172	34.40	-.059
♀ 1018	54	38	-16	-4.00	4 "	122	30.50	-.131
♀ 1058	39	36	-3	-0.75	4 wks. 4 days	90	21.75	-.034
♂ 1024	47	38	-9	-1.50	6 wks.	123	20.50	-.073
♀ 2007	40	35	-5	-0.83	6 "	146	24.33	-.034
Average	47	38	-8.4	-1.77	5.1	130.6	26.29	-.066

Chart 2
Basal Diet B

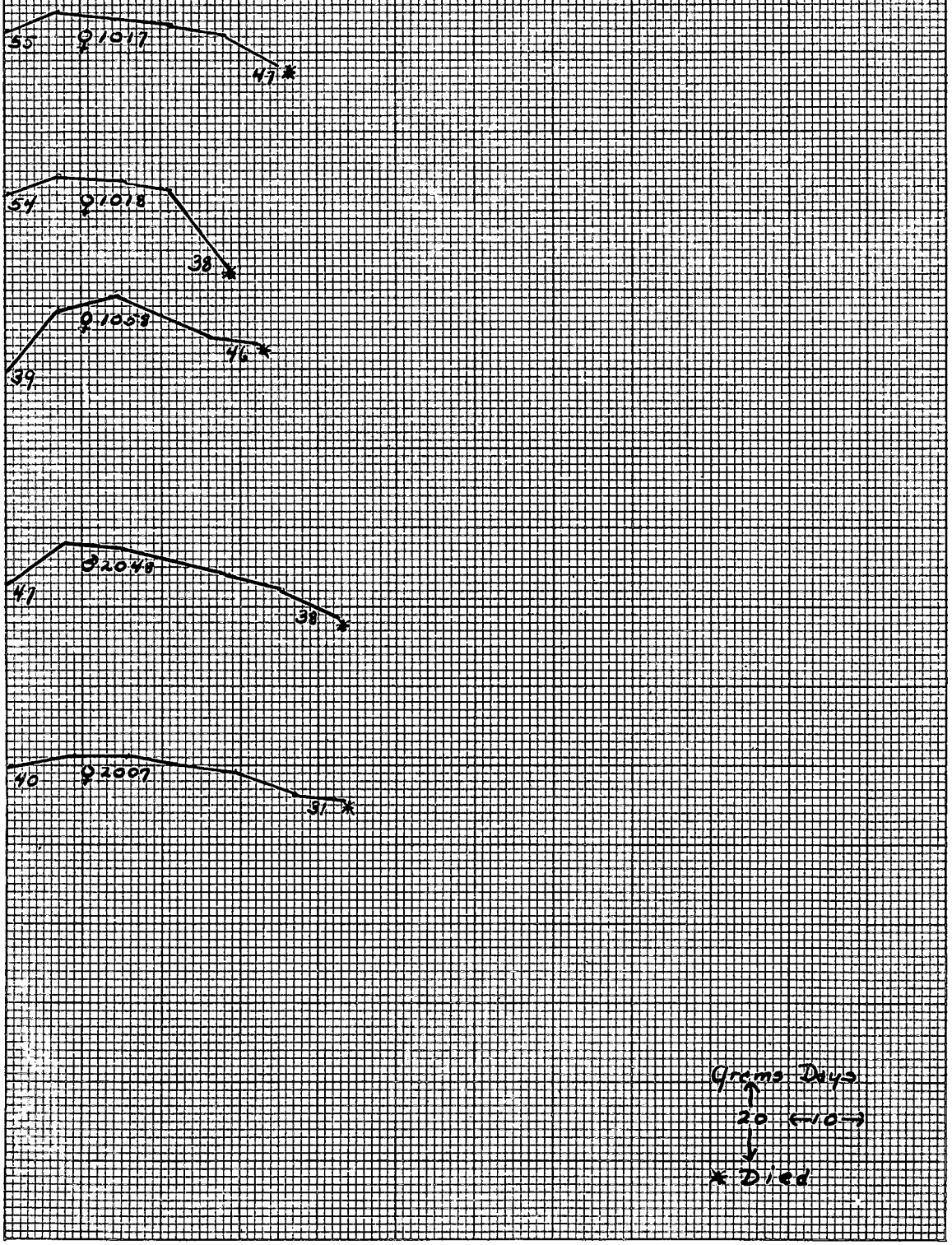


Table III.

The Effect of Basal Diet A Supplemented by 0.5 Grams of Autoclaved Yeast as a Source of Vitamin G.

Sex and number	Depletion period			Eight week period					
		Body weight (grams)		Body weight (grams)			Food intake		Av. gain
	Length (days)	Beginning	End	Final	Gain	Av. gain per wk.	Food consumed	Av. food per wk.	per gram food per week
♂ 1022	--	40		111	71	9.00	560	70.00	.129
♀ 1024	--	38		178	130	16.00	595	74.35	.215
♀ 1026	--	38		158	120	15.00	592	74.00	.202
♀ 1048	7	37	44	185	141	17.62	493	61.62	.283
♀ 1069	10	34	45	164	119	14.87	535	66.87	.222
♀ 1075	8	33	38	130	92	10.25	398	49.75	.206
♂ 1080	11	47	51	207	156	19.50	596	74.50	.261
♀ 1086	14	43	48	148	100	12.50	358	44.75	.278
♀ 1091	13	60	68	194	126	15.75	529	66.01	.238
♂ 2033	16	39	54	191	137	17.12	576	72.00	.237
♂ 2038	16	34	44	194	150	18.75	590	75.00	.250
Average	12	42	49	169	122	16.03	561.11	70.55	.237

Chart 3a.

Basal Diet A + 0.6 grams Autoclaved Yeast

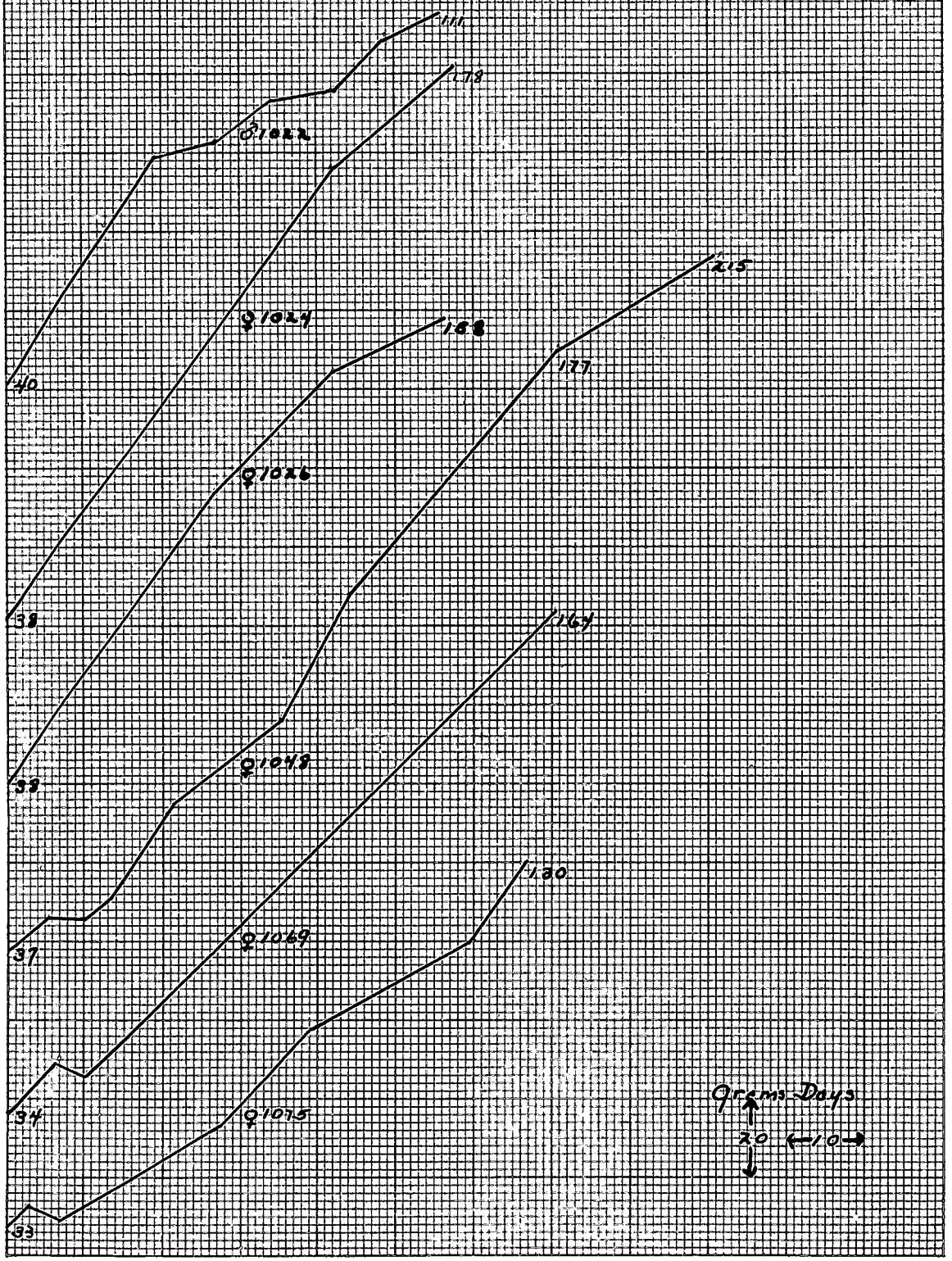


Chart 34

Basal Diet A + 0.5 Grams Autoclaved Yeast

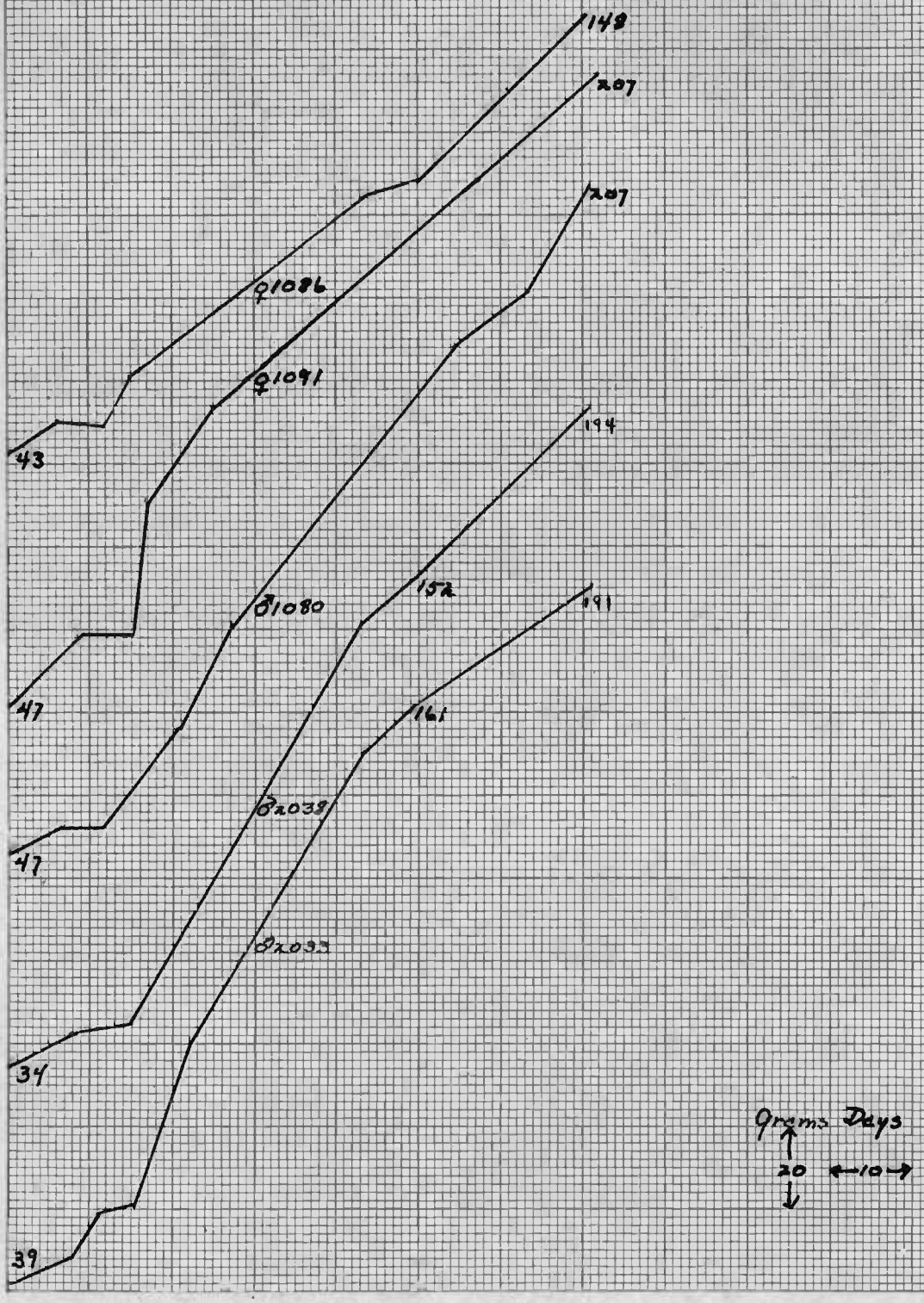


Table IV.

The Effect of Basal Diet A Supplemented by 0.5 Grams of Autoclaved Yeast and 0.5 Gram Dried Pork (1.15 Grams Fresh) as a Source of Vitamin G.

Sex and number	Forcible period: Body weight (Gms): Beginning	Forcible period: Body weight (Gms): End	Forcible period: Gain	Free feed period: Body weight (Gms): Final	Free feed period: Gain	Free feed period: Gm. gain per day	Free feed period: Feed consumed	Free feed period: Gm. feed per day	Gm. gain per gm. feed
♂ 1088	6	43	60	221	102	30.25	303	70.37	.207
♂ 1091	14	61	40	217	110	21.00	333	35.37	.334
♂ 1097	14	62	30	214	102	20.50	450	62.25	.330
♀ 1099	12	63	74	206	132	20.00	651	80.37	.232
♂ 2034	16	33	53	202	149	18.62	459	57.37	.325
♀ 2035	15	67	51	196	145	18.12	512	64.00	.283
♀ 2037	20	33	49	190	141	17.63	588	73.50	.240
♀ 2039	16	55	49	204	155	19.37	503	63.00	.307
Average	13.7	41.6	53.37	206.25	152	18.99	511	66.57	.299

Chart 4a

Basal Diet A + 0.5 grams Autoclaved Yeast + 0.5 grams Dried Beer

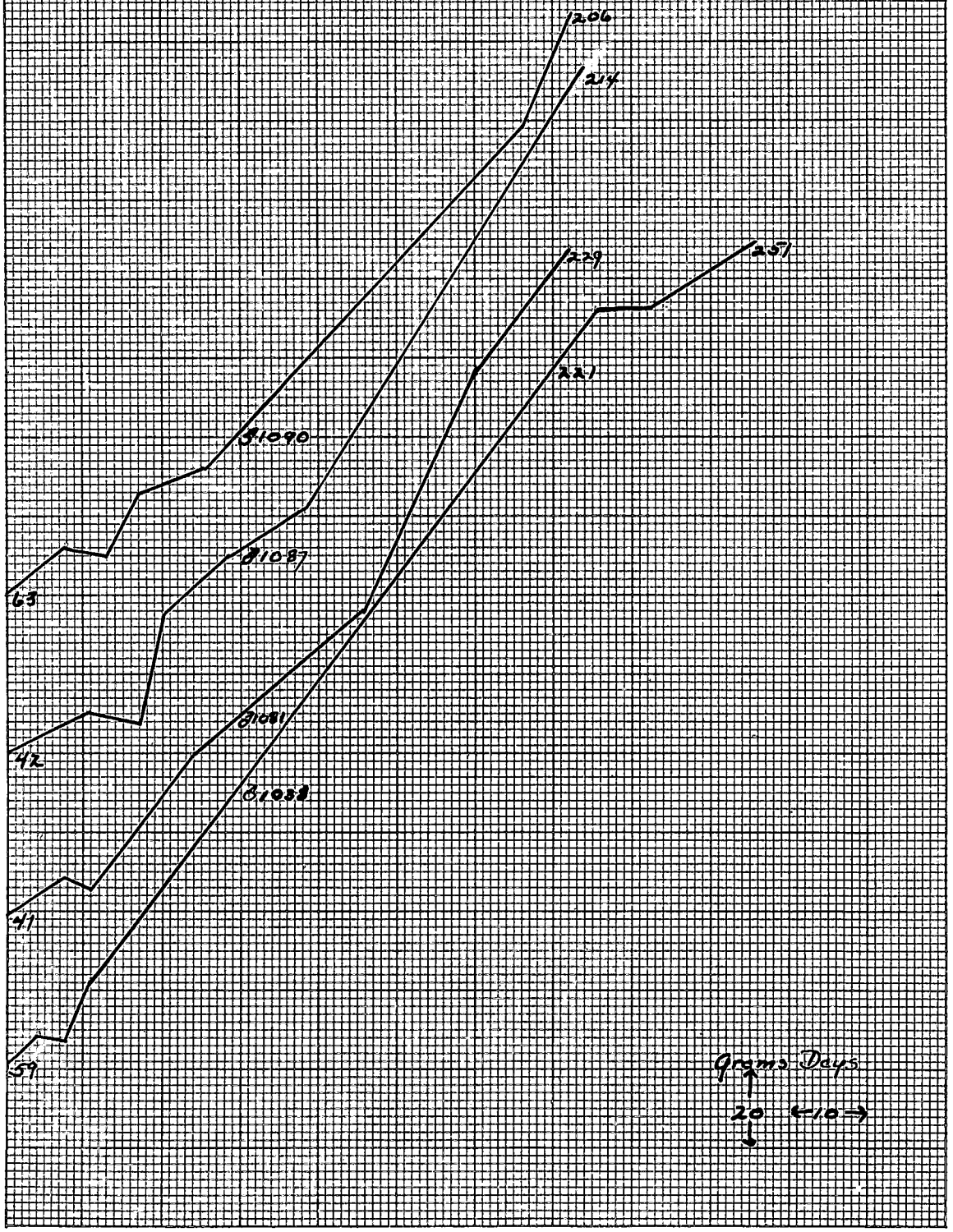


Chart 46

Basal Diet A + 0.5 Grams Autoclaved Yeast + 0.5 Grams Dried Pork

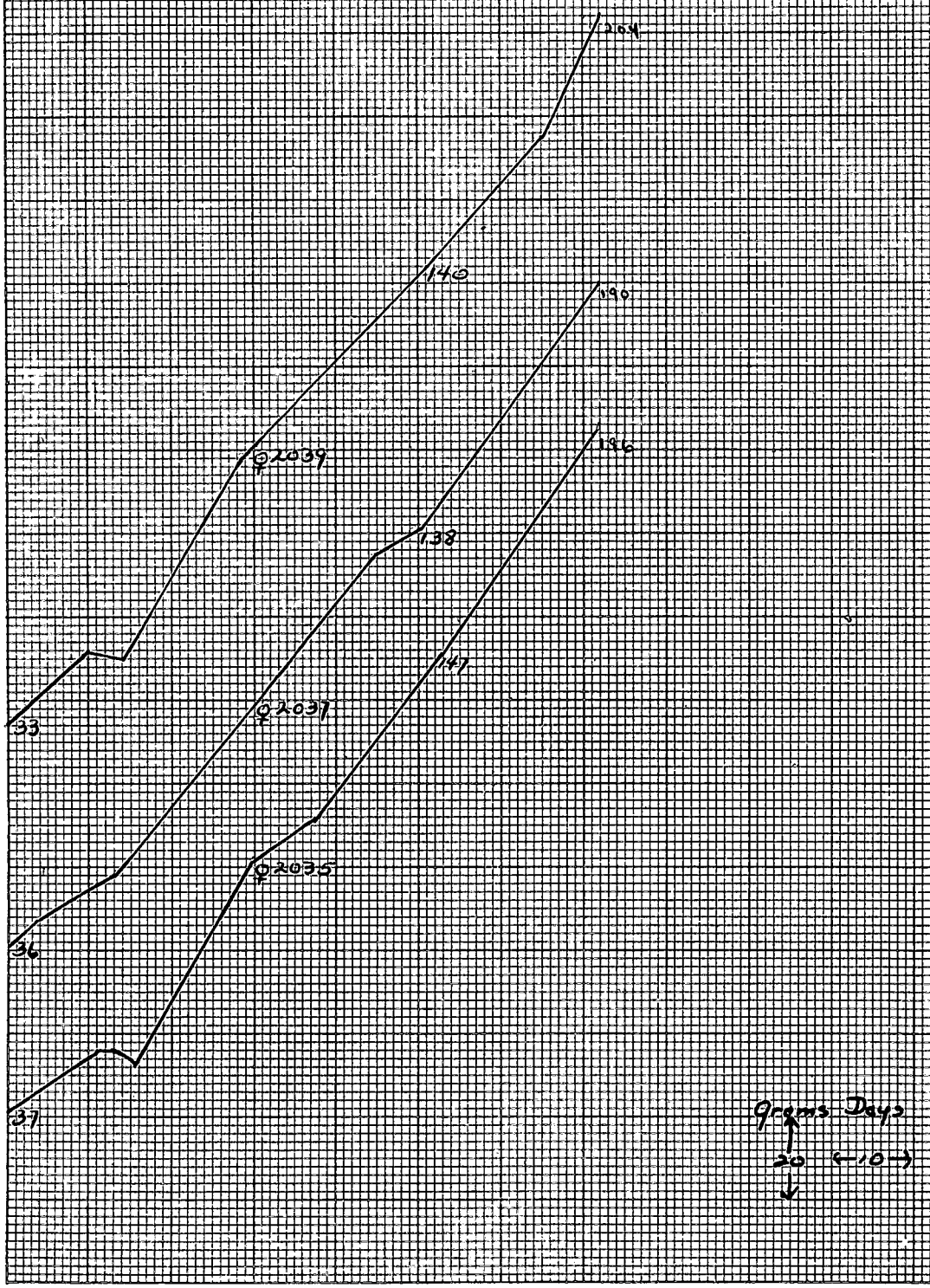


Table V.

The Effect of Basal Diet B Supplemented by 0.5 Grams of Dried Yeast as a Source of Vitamin B Complex.

Sex and number	Depletion period: Length (days)	Depletion period: Body weight (grams): Beginning	Depletion period: Body weight (grams): End	Eight week period: Body weight (grams): Final	Eight week period: Body weight (grams): Gain	Eight week period: Body weight (grams): Av. gain per wk.	Eight week period: Food intake: Food consumed	Eight week period: Food intake: Av. food per wk.	Eight week period: Av. gain per gram food per week
♂ 1028	--	42		152 (7 wks.)	110	17.14	492	70.00	.244
♀ 1012	--	36		114	78	9.75	558	69.75	.140
♀ 1009	--	38		160	122	15.25	510	63.75	.240
♀ 1004	--	36		157	121	15.12	502	65.25	.231
♀ 1006	--	34		153	119	14.87	472	59.00	.252
♀ 1055	15	45	49	152	93	11.62	410	51.25	.226
♀ 2009	15	40	48	129 (7 wks.)	81	11.57	416	59.42	.201
Average		36	48.5	148	106	15.05	480	62.65	.219

Chart 5a

Basal Diet B + 0.5 grams Dried Yeast

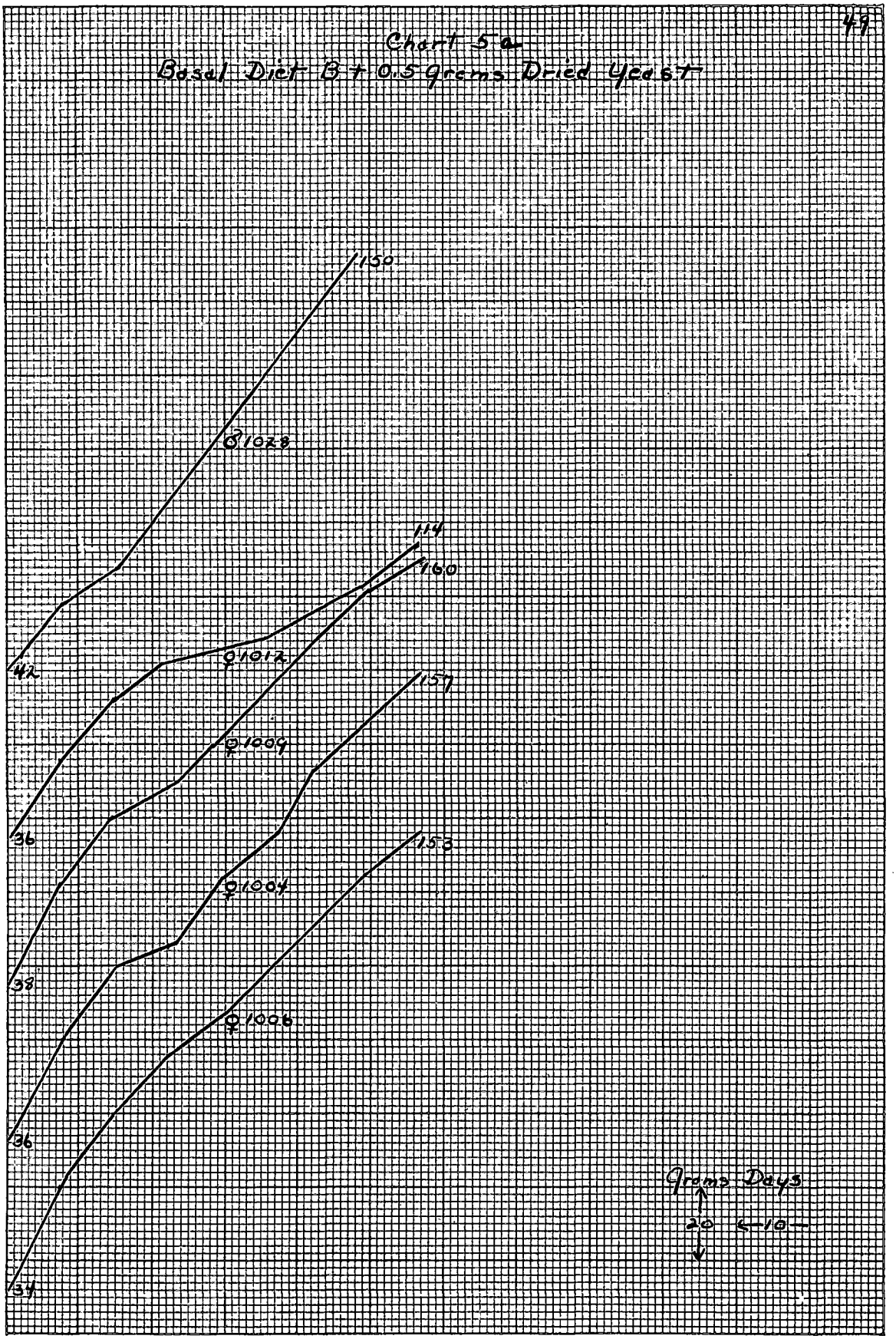
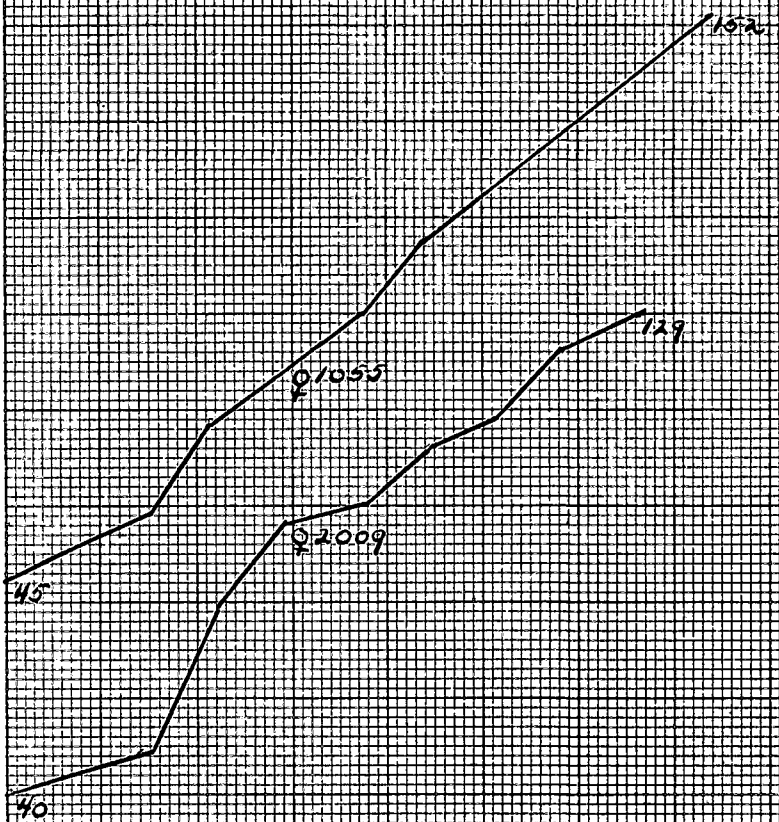


Chart 5b

Basal Diet B + 0.5 Grams Dried Yeast



Grams Days

20 ←10→

Table VI.

The Effect of Basal Diet A Supplemented by 0.5 Grams Dried Pear (4.15 Grams Fresh Pear) as a Source of Vitamin C.

Sex and number	Depletion period: Length (days)	Depletion period: Body weight (grams): Beginning	Depletion period: Body weight (grams): End	First week period: Body weight (grams): Final	First week period: Body weight (grams): Gain	First week period: Av. gain per wk.	First week period: Food intake: Food consumed	First week period: Av. food per wk.	First week period: Av. gain per gram food per week
♂ 1040	9	43	59	75	16	8.00	300	37.50	.055
♂ 1047	9	40	48	97	49	6.12	299	37.37	.169
♀ 1066	10	41	54	93	39	4.87	356	44.50	.109
♀ 1076	8	32	34	75	41	5.12	294	33.50	.141
♂ 1083	11	40	49	106	57	7.12	328	41.00	.173
♂ 1086	12	47	49	73	24	3.00	265	33.12	.083
♀ 2032	10	40	53	107	54	8.75	280	35.00	.250
♀ 2039	16	36	45	83	38	4.75	279	34.87	.136
Average	11.25	39.9	48.94	88.62	39.75	5.22	288.11	36.01	.169

Chart 6

Basal Diet At .05 grams Per

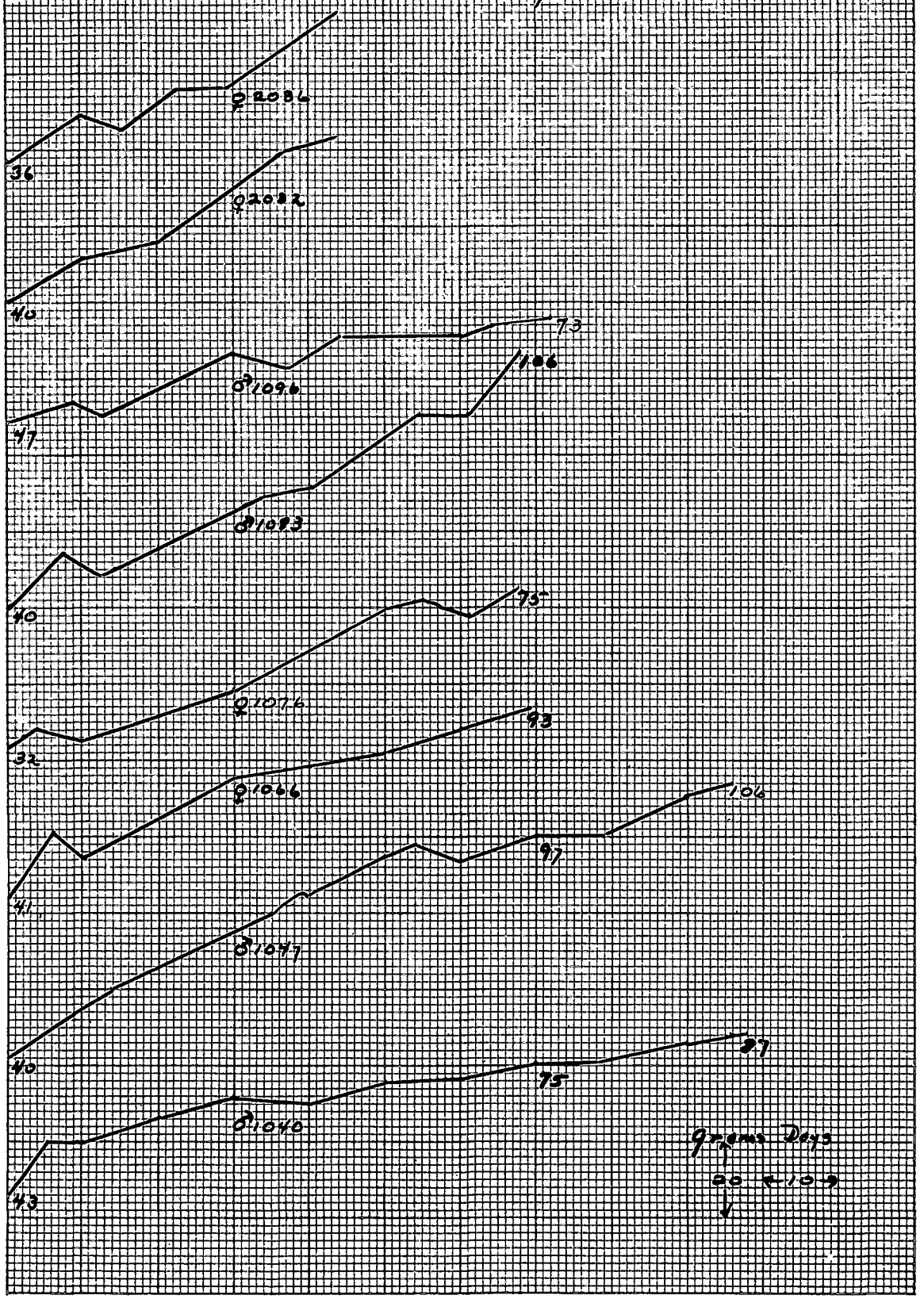


Table VII.

The Effect of Basal Diet A Supplemented by 0.75 Grams Dried Pear (6.23 Grams Fresh Pear) as a Source of Vitamin G.

Sex and number	Depletion period			Eight week period					
		Body weight (grams)		Body weight (grams)			Food intake		Av. gain
	Length (days)	Beginning	End	Final	Gain	Av. gain per wk.	Food consumed	Av. food per wk.	per gram food per week
♂ 1070	10	33	45	95	50	6.25	332	41.50	.128
♂ 1072	10	24	34	100	66	8.25	336	45.75	.180
♀ 1077	8	32	34	103	69	8.62	309	38.62	.223
♀ 1079	8	28	35	90	55	6.87	315	39.37	.174
♀ 1082	15	41	51	124	73	9.12	395	49.72	.183
♂ 1088	14	40	60	118	58	7.25	356	44.50	.163
♀ 1093	13	50	58	101	43	5.37	268	33.50	.160
Average	11	35.4	45.3	104	59	7.51	334.42	41.80	.173

Chart 7.

Basal Diet A + 75 grams Raw

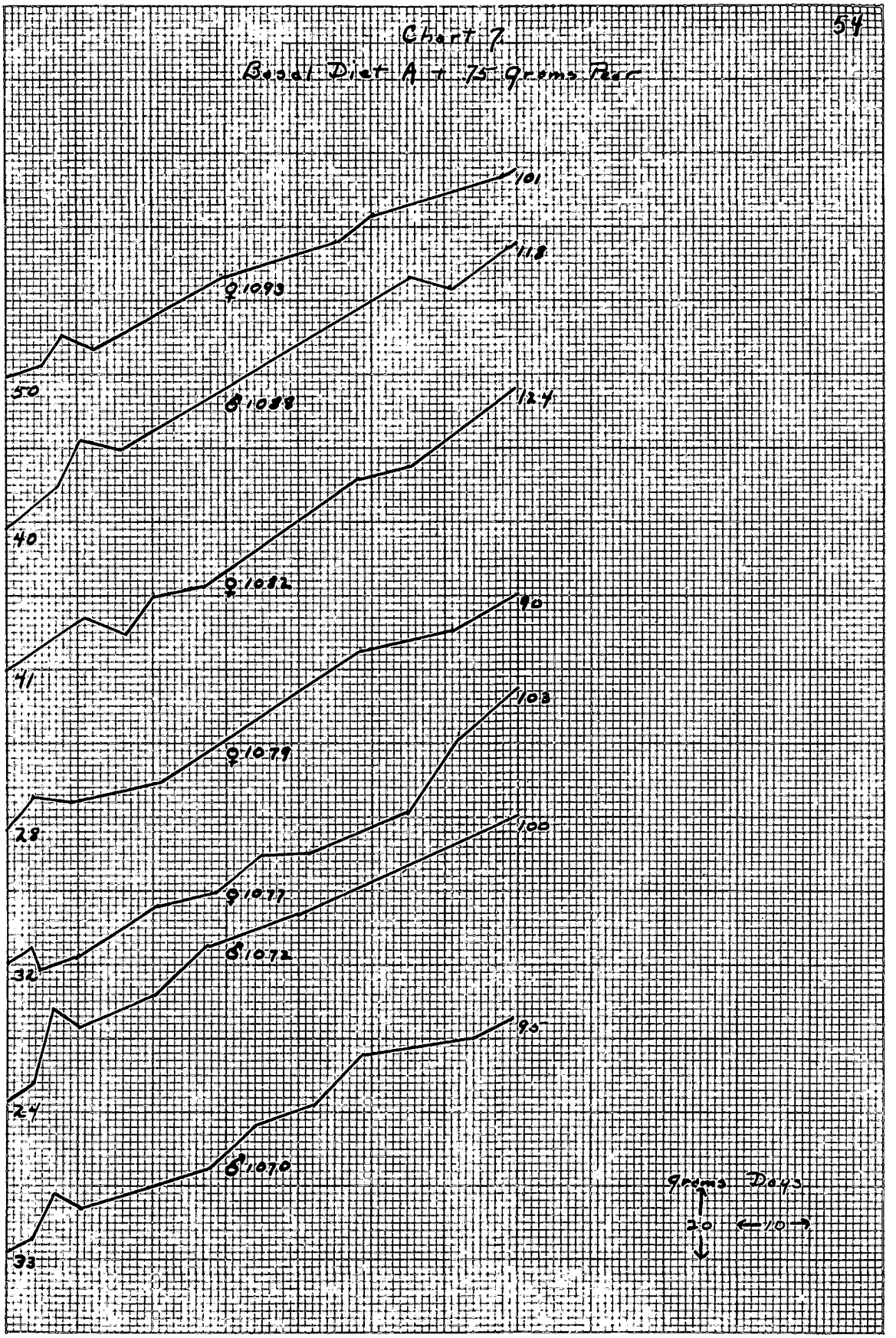


Table VIII.

The Effect of Basal Diet A Supplemented by 1 Gram Dried Pear (8.29 Grams Fresh Pear) as a Source of Vitamin G.

Sex and number	Depletion period			Eight week period					
		Body weight (grams)		Body weight (grams)			Food intake		Av. gain
	Length (days)	Beginning	End	Final	Gain	Av. gain per wk.	Food consumed	Av. food per wk.	per gram food per week
♀ 1044	9	40	49	89	40	5.00	281	35.12	.142
♀ 1049	7	33	35	115	80	10.00	336	42.00	.238
♂ 1067	10	40	52	110	58	7.25	326	40.75	.128
♀ 1085	13	45	55	115	60	7.50	292	36.50	.206
♂ 1089	13	40	48	88	40	5.00	216	27.00	.190
♀ 1095	12	48	60	85	25	3.12	261	32.62	.095
Average	10.6	41	49.6	100	53.7	6.31	285.3	35.66	.171

Chart 8

Basal Diet A + 1 gram Peas

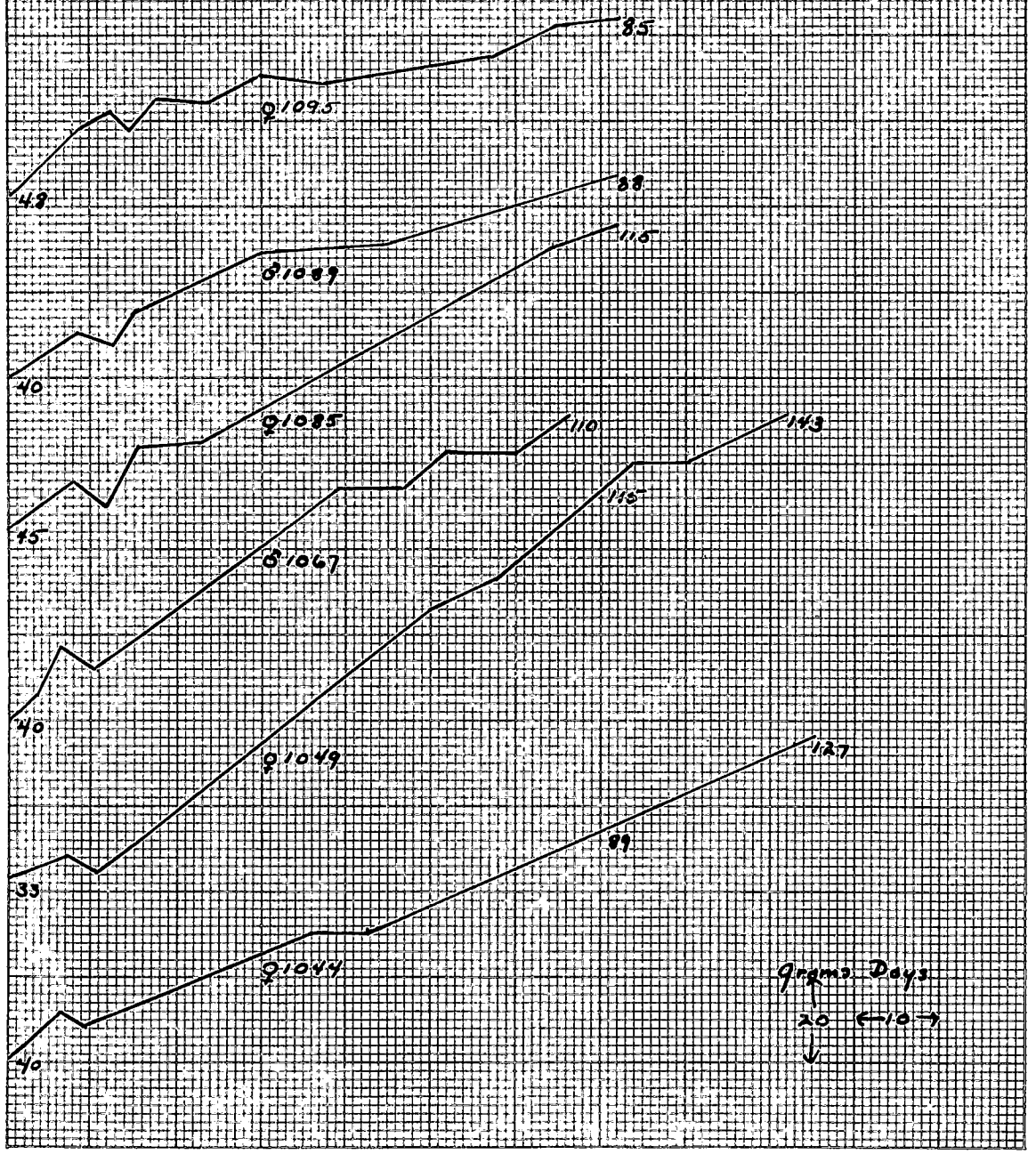


Table IX.

The Effect of Basal Diet A Supplemented by 1.5 Grams Dried Pear (12.49 Grams Fresh Pear) as a Source of Vitamin G.

Sex and number	Depletion period			Eight week period					
		Body weight (grams)		Body weight (grams)			Food intake		Av. gain
	Length (days)	Beginning	End	Final	Gain	Av. gain per wk.	Food consumed	Av. food per wk.	per gram food per week
♀ 1045	9	39	50	92	42	5.25	263	32.87	.159
♂ 1046	7	41	42	136	94	11.75	367	45.87	.256
♀ 1071	10	30	44	117	73	9.12	425	53.12	.171
♂ 1073	8	36	40	110	70	8.75	420	52.50	.166
♀ 1078	8	29	34	118	84	10.50	355	44.37	.236
♀ 1094	13	54	54	123	69	8.62	346	43.25	.200
Average	9.2	38	44	116	72	9.00	362.7	45.35	.198

Chart 9

Basal Diet A + 15 grams Peas

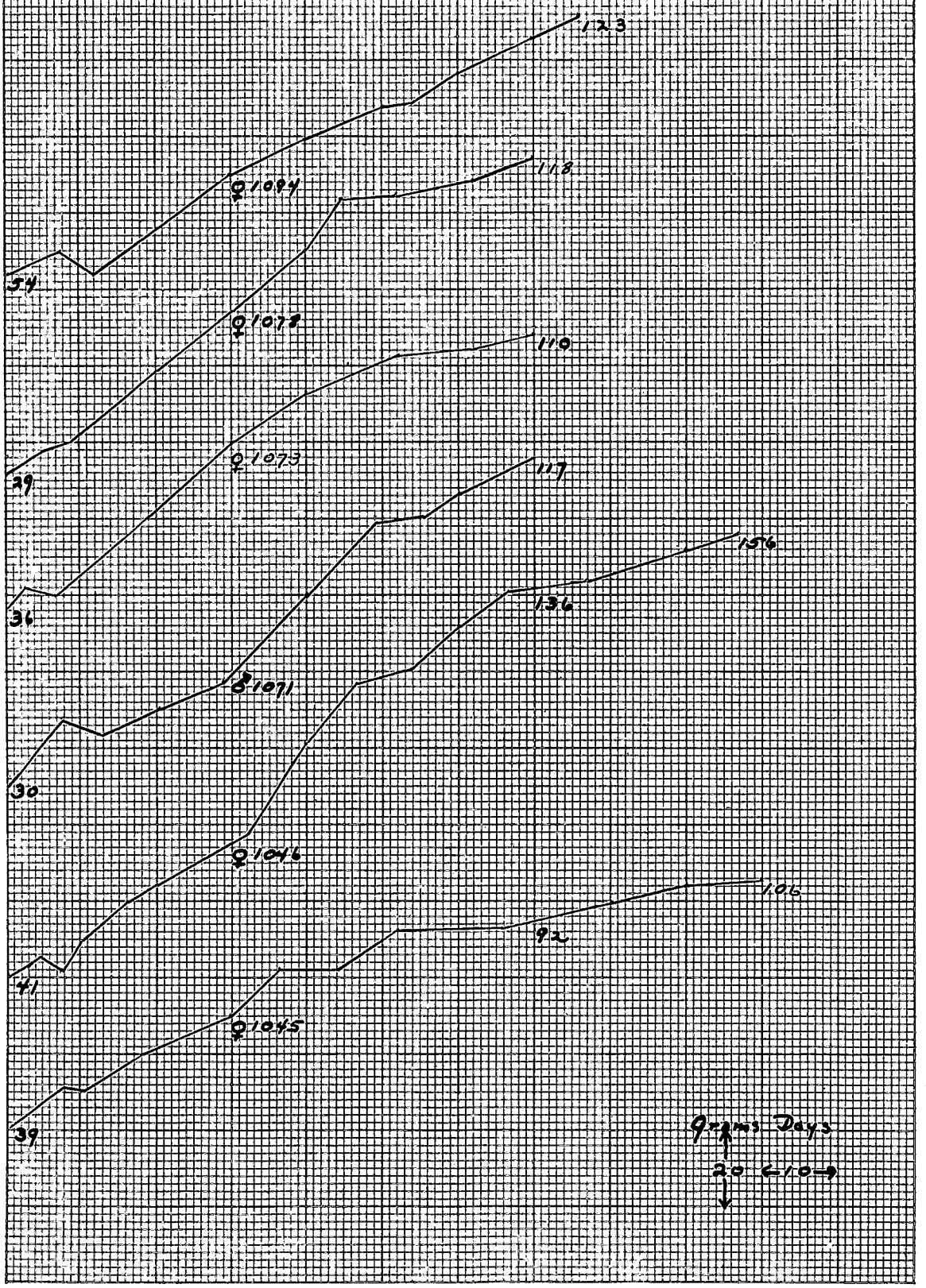


TABLE X.

Summary of Results of Feeding Experiments Showing

Effect of Bosc Pear as a Source of Vitamin C.

Series no.	No. of rats	Average length of depletion period (days)	Average body weight (grams) Initial	End of depletion period	Av. net gain or loss	Av. gain or loss per week	Food intake (grams) Av. for period	Av. per week	Av. net gain or loss per gm. food per wk.
I	7	12.29	41	49.42	15.42	1.92	277.57	34.53	.056
II	5	---	47	---	-8.40	-1.77	130.60	26.29	-.066
III	11	12.00	42	49.00	122.00	16.03	561.11	70.55	.237
IV	8	13.7	41.6	53.37	152.00	18.99	511.00	66.57	.299
V	7	15.00	36.0	48.50	106.00	15.05	480.00	62.65	.219
VI	8	11.25	39.9	48.94	39.75	5.22	288.11	36.01	.250
VII	7	11.0	35.4	45.26	59.14	7.51	334.42	41.80	.173
VIII	6	10.6	41.0	49.66	53.70	6.31	285.33	35.66	.171
IX	6	9.2	38.0	44.00	72.00	9.00	362.70	45.33	.198

Chart 10

Summary