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The vitamin B content of kale greens.

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THE VITAMIN B CONTENT OF KALE GREENS

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INTRODUCTORY

When listing greens for a special diet, the general custom is to name spinach. Experimenters have used spinach in determining the vitamin content of a green.

There is a green on the market known as kale, which is preferred by many because of its more palatable flavor. If kale were given as full recognition on a diet list as spinach, it would be consumed in greater quantities because of its more favorable flavor, thereby accomplishing the purpose of the special diet more effectively.

That kale is cons med in great quantities and must be in general favor with the public is evidenced by the report of the following wholesale produce dealers of Louisville, Kentucky:

The United Produce Company, Louisville, Kentucky, states "A fair estimate would be that there are about five hundred and fifty barrels per day, every day in the year received in Louisville".

Stinson & Martin, Louisville, Kentucky state, "The amount of kale greens sold in Louisville from January first to July first totals 1500 bushels daily."

W. G. Heimerdinger, Louisville, Kentucky reports, "The total for the year is about one hundred and ninety-three (193) carloads with an average of eight hundred (800) bushels per car or a total of one hundred and fifty-four thousand four hundred (154,400) bushels which are sold through this market and consumed in Louisville and nearby territory."

The mineral content of kale greens versus spinach is quoted below as given by Mary Swartz Rose:[22]

	Calcium Shares	Phosphorous Shares	Iron Shares
Spinach	12.22	6.48	30.12
Kale	40.08	5.93	26.09

	Vitamin A	Vitamin B	Vitamin C
Spinach	+++	+++	+
Kale	?	?	?

The calcium content of kale is seen to be over three times the amount present in spinach and the phosphorous and iron content fall only a few points below spinach. Thus far kale compares favorably with spinach.

The purpose of this experiment as presented in this thesis is to determine the vitamin B content of Kale as compared with the vitamin B content of spinach.

HISTORICAL

"It is now well established that what in the past has been called vitamin B is not an entity, but comprises more than one nutritionally essential substance."[1]

Various writers from time to time, and notably Mitchell (1919) in a critical discussion, called attention to the fact that not all of the published evidence was consistent with this simplifying assumption; but only slowly (slowly at least as things go in the rapidly moving history of the vitamins) did there accumulate such a preponderance of experimental evidence for the multiple nature of vitamin B as to command the universal acceptance of the students of this problem.

Emmett and Luros, in 1920[2], reported the results of a systematic investigation in which identical food materials were fed for the prevention of polyneuritis in pigeons and for the support of growth in rats. Furthermore, these comparative feeding experiments were repeated with portions of the same food materials which had been heated at different temperatures and under different conditions. They concluded that the anti-neuritic vitamin seemed to be less stable to heat and alkali than the rat-growth-promoting vitamin.

Fund and Dubin, in 1921[3], recorded very briefly some experiments upon fractional adsorption which led them to believe that autolyzed yeast contained two water soluble vitamins, of which the antineuritic was the more easily adsorbed by fuller's earth.

Levene and Muhfeld (1923)[4] found evidence of the multiple nature of vitamin B in the fact that their experiments upon the concentration of this factor resulted in altering the relative antineuritic and growth-promoting activities of their materials.

Kinnersley and Peters (1925), who also were working primarily from the point of view of isolation of the antineuritic vitamin, reported the seperation of a highly antineuritic substance which did not cause increase in the weight of pigeons after cure of the polyneuritic symptoms. This was interpreted as supporting the view of the multiple nature of vitamin B.

In the same year, this view was discussed tentatively by McCollum, Simmonds and Becker (1925)[5].

In 1926[6], Hauge and Carrick published further evidence of the fact that antineuritic and growth-promoting potencies do not always run parallel, and in the same year the subject was greatly clarified by three contributions from the United States Public Health Service and two from the laboratory of McCollum, all of which emphasize the important fact that the growth of rats (and there is no reason to doubt that this is true of mammals generally) requires <u>both</u> the antineuritic and another, more heat-stable substance which along with the antineuritic substance had hitherto been covered by the term vitamin B.

In the course of an investigation begun as a study of the influence of dietary deficiencies upon experimental tuberculosis in the albino rat, Smith and Hendrick (1926)[7] found that a diet containing 40 per cent of rolled oats, with adequate amounts of casein, salts, and vitamin A containing fats, was not adequate to support normal growth of young rats; but became so when supplemented by yeast which had autoclaved for six hours at fifteen pounds pressure, a procedure believed to destroy the antineuritic vitamin of the yeast and, furthermore, that neither autoclaved yeast alone nor Seidell vitamin B picrate alone would meet the growth-promoting vitamin B requirement of rats; but that they supplemented each other in such away that together they met this vitamin B requirement. This work of Smith and Hendrick was immediately confirmed by Seidell (1926)[8] who, as the result of experiments with both rats and pigeons, suggested that rats (and probably other mammals) need both the antineuritic substance and the other,more heat-stable factor of vitamin B, while pigeons need only the antineuritic vitamin; and for this reason he recommended that the rat be used for determinations of vitamin B and the pigeon, on account of its simpler needs, for the studies of the antineuritic vitamin.

Closely following the contribution of Smith and Hendrick, appeared the independent and practically simultaneous work of Goldberger, Wheeler, Lillie and Rogers (1926)[9] who found a similar supplementary relationship in meeting the growth requirements of young rats, between autoclaved yeast (as the source of the heat-stable factor) and as alcohol-extract of corn meal (as the source of the antineuritic substance). This work was discussed in its bearings upon other investigations of Goldberger and his associates upon human pellagra and the more or less analogous condition known as black-tongue in dogs. Goldberger and Tanner (1925)[10] had postulated the existence of a previously unrecognized dietary factor concerned in the prevention of pellagra (tentatively designated as the "P-P factor" or "pellagra-preventing vitamin"), and this was now held to be identical with the heat-stable factor of vitamin B, the rats whose rations lacked this factor being described by Goldberger, Wheeler, Lillie and Rogers[9] as developing a pellagra-like condition after about nine weeks of this dietary deprivation.

Meanwhile Laird (1926), in McCollum's laboratory, found in the testing of numerous extracts obtained from natural foods by means of different (usually acidulated) solvents, repeated instances in which antineuritic and growth-promoting potencies did not run parallel, and some cases in which growth was not promoted by either of the extracts but was induced by a mixture of them.

Salmon (1927)[11], working with soy beans and velvet beans and with the leaves of velvet beans and of rape, found that the seeds were relatively more potent for the prevention of polyneuritis and the leaves for the promotion of growth. He also described a partial separation upon fuller's earth. The alcohol-extract, in the form of a water solution, was treated for the removal of ether-soluble substances and of substances precipitable by lead acetate, before the treatment with fuller's earth. The leaves were extracted with acidulated water instead of alcohol. The continuation of this work has been described in a later paper by Salmon, Guerrant and Hays (1927), who report that fuller's earth adsorbs both factors; but, under certain conditions, the antineuritic more completely. They describe the preparation, from velvet bean leaves, of a fraction practically freed from the antineuritic but still rich in the other factor.

Meanwhile Chick and Roscoe (1927)[12] reported a series of experiments in which various extracts and preparations of yeast and of wheat embryo were fed singly and in combination with results which demonstrated still further the existence in the vitamin B complex of more than one factor essential to growth; and Hassan and

Drummond (1927)[13], in the course of study of the possible relation between vitamin B and protein metabolism, independently obtained evidence of the presence of two growth-promoting factors in what had been known as vitamin B.

The work of Palmer and Kennedy (1927-1928)[14], while chiefly presented from a somewhat different angle, seems also to furnish a large amount of evidence of the multiple nature of vitamin B. Evans and Burr (1928)[15] have recently furnished a clear-cut demonstration.

Williams and Waterman (1927-1928)[16] hold that vitamin B is of a tripartite nature, involving the antineuritic factor required by both rats and pigeons, a second factor required by rats.

Evidence of more quantitative nature was obtained by Sherman and Axtmayer (1927), who measured, by means of the averages of large numbers of quantitatively conducted feeding experiments, the relative richness, in the two factors required by rats, of whole wheat on the one hand and of milk or of autoclaved yeast on the other. That heating in the autoclave may destroy the antineuritic potency of yeast while leaving it still a good source of the other factor had been shown by previous workers... The present investigation established a marked supplementary relationship between wheat and milk, wheat being relatively richer in the antineuritic, and milk relatively richer in the more heat-stable factor. This work thus furnished a basis for the quantitative reinvestigation of the previously recorded "vitamin B values" of foods.

Hunt (1928)[17] found corn (maize) to be very similar to wheat in its content of the two factors required for growth of rats.

Eddy (1927, 1928)[18] found that the previously reported low vitamin B value of cooked or canned spinach is due to paucity of the antineuritic factor in a material relatively rich in the more heat-stable vitamin; and also that bananas are relatively rich in the more heat-stable vitamin than in the antineuritic factor.

Hogan and Hunter (1928)[19] have very recently reported that just as by heating the antineuritic factor can be more or less selectively destroyed, so the more heat-stable factor may be selectively destroyed by exposure to ultraviolet irradiation.

Moreover a food which is a good source of both may still be markedly richer in one than the other, as the investigations above cited have shown to be true in the case of milk, and of typical seeds and green leaves.

Milk and green leaves, good sources of both these factors, are relatively richer in the more heat-stable factor. Whole grain cereals and legumes, fair-to-good sources of both factors, are relatively richer in the antineuritic vitamin. Thus both milk and green leaves here show a valuable supplementary relationship to seeds with respect to vitamin B, as had previously been shown to be the case with respect to vitamin A.

The discussion of the evidence of the multiple nature of vitamin B has involved emphasis upon the fact that heating in the autoclave destroys the antineuritic vitamin but not the other factor, and consequently frequent references to the latter as the more heat-stable factor have been made. This fact might readily tend to give an exaggerated impression of the susceptibility of the antineuritic vitamin to destruction upon heating.

Something should therefore be said of the quantitative studies of the stability or lability of the antineuritic substance, because of the nutritional signigicance of this property in relation to vitamin values of foods which have been heated or stored under different conditions.

In dry state, at practically neutral reaction, the antineuritic vitamin is quite stable to heating at 100°, showing no measurable destruction or inactivation after 48 hours of such heating; whereas the same material heated in water for 6 hours at 100° showed a slight diminution of potency (Sherman and Spohn, 1923). Although this work was done before the differentiation of the vitamin B complex, it is certain that it was the antineuritic substance whose stability was measured, both because the so-called antipellagric vitamin is so much more stable and because the work of Sherman and Axtmayer has shown plainly that cow's milk is richer in this vitamin than in the antineuritic substance, so that the latter must have been the limiting factor in the rat growth experiments of Sherman and Spohn.

In other experiments (Sherman and Grose, 1923; Sherman and Burtis, 1926)[26] the rate of destruction of "vitamin B" was measured at different temperatures at a fixed temperature of heating of 100°. While it has not yet been shown whether the antineuritic substance is the limiting factor of the vitamin B complex of tomato juice, yet in view of the much greater stability of the other vitamin, it seems practically certain that the experiments here cited may be taken as affording at least approximate measures of the rate of destruction or inactivation of the antineuritic vitamin under the conditions described.

The development of methods for the separate quantitative measurement of values of foods as sources of the two separate factors is now well advanced.

Foods which, by means of rat feeding experiments, have been shown to be good sources of vitamin B as previously understood are, therefore, now seen to be good sources of both the antineuritic substance and the more heat-stable vitamin.

If, on the other hand, the food, when tested for undifferentiated vitamin B, has not given good results, it is not necessarily poor in both of these essentials, but may conceivably be a good source of one or the other of them, and should therefore, be tested for each separately.

"Thus the multiple nature of vitamin B is established by an abundance of evidence, and evidence of several different kinds.

"It no longer appears profitable to perform experiments or to publish papers in which vitamin B (in the sense in which the term has hitherto been used) is treated as an entity."[1]

EXPERIMENTAL

The experiments were conducted according to the methods of Sherman and MacArthur[20] as follows: Healthy, young rats of known nutritional history were placed at 24 to 30 days of age and weighing generally from 50 to 60 grams in wire cages proveded with screen bottoms and given a vitamin B free diet with water ad libitum.

The laboratory technique was that of Perry[23]. (1) The rats were weighed weekly and the weights and history of each animal were recorded in a loose leaf note-book. (2) The food was made into soft paste to prevent scattering and (3) the cages were sterilized weekly. Water was given by an inverted bottle with a glass tube which projected into the inside of the cage. The duties of this experiment were carried out on Sundays and holidays as regularly as on week days.

The kale was purchased fresh on the market daily and freshly cooked leaves were prepared daily by thoroughly washing and cooking in a glass beaker[25] with enough additional water to prevent sticking just prior to feeding. The time of cooking was regulated by the tenderness of the greens, usually about 15 minutes. The daily amount of fresh green kale fed each animal varied from 0.2 gram to 1.0 gram daily.

The object of using cooked kale instead of the raw leaves was to discover the vitamin B potency of the foodstuff in question as it is consumed by the individual.

Male and female rats were used interchangeably in this determination of vitamin B since the average difference in weights of the two is so small as to be negligible according to the statement of Sherman and Burtis [26].

DIETS The basal diet contained casein 19, starch 63, salt mixture 4[(a)], butter 10, agar agar 2 and cod liver oil 2 parts. The basal diet was adjusted to accommodate supplements for the fractional determination of vitamin B according to the diets of C. H. Hunt[17]. The supplements to the basal diet were: whole, coarsely ground wheat[(b)] as the source of vitamin F (Sherman's thermo-labile, antineuritic factor of B) and auto-claved yeast[(c)] as the source of vitamin G (Sherman's thermo-stable, antipellagric vitamin and Goldberger's P-P factor of vitamin B.) Sherman's nomenclature, vitamin F and vitamin G will be used to designate the fractions of vitamin B in this work.

Kale greens of unknown vitamin B content were combined with the basal diet in such a manner as to study it as a probable source of the vitamin F and G factors of vitamin B combined; as a source of vitamin F alone and as a source of vitamin G alone according to diets #1 and 4, 5, 6 respectively as shown in table 1. Two controls were conducted: first, canned spinach of known vitamin B content[21] was supplemented in the basal diet in the same manner as kale according to diets #2, 10 and 11 respectively as shown in table 1, and second; yeast foam tablets plus the basal diet according to diet #3 and whole wheat supplemented by auto-claved yeast as in diet #9 according to table 1.

a) Osborne and Mendel Salt Mixture:[24]

$CaCO_3$	134.8	grams
$MgCO_3$	24.2	"
Na_2CO_3	34.2	"
KI	0.020	"
K_2CO_3	141.3	"
H_3PO_4	103.2	"
HCl	53.4	"
$MnSO_4$	0.079	"
H_2SO_4	9.2	"
Citric Acid H_2O	111.1	"
Fe citrate $1\frac{1}{2}$ H_2O	6.34	"
NaF	0.248	"
$KAl(SO_4)_2$	0.245	"

Mixed carefully and dried at 100°C for three hours in an electric oven with intermittant stirring.

b) Whole wheat coarsley ground was obtained from a reliable whole-grain house, D. Lubber's, Broadway, Louisville, Kentucky.

c) The yeast foam tablets were obtained from Northwestern Yeast Company, 1750 N. Ashland Ave., Chicago, Illinois. To auto-clave, the yeast was placed in enamel pans to a depth of about $\frac{1}{2}$ inch and auto-claved for 4 hours at 15# steam pressure.

DESCRIPTION OF DIETS

Table 1

Diet #1	Cooked kale greens plus basal diet. Experimental Studied as the probable source of both the F and G factors of vitamin B
Diet #2	Canned Spinach plus basal diet Control Foodstuff of known vitamin B content[21] ready cooked as source of both F and G factors of vitamin B
Diet #3	Yeast foam tablets plus basal diet Control a second foodstuff of known vitamin B content as source of both F and G factors of vitamin B
Diet #4	Cooked kale greens plus basal diet Experimental A second experimental kale diet run to observe the growth curve during summer months as compared with diet #1 run during winter months.
Diet #5	Kale greens plus cracked whole wheat plus basal diet Experimental Unknown vitamin content supplemented by F factor present in whole wheat. An attempt to discover the supplementary factor in kale.

DESCRIPTION OF DIETS

Table 1

(Continued)

Diet #6	Cooked kale greens plus auto-claved yeast plus basal diet	
	Experimental	
	Unknown vitamin content supplemented by the G factor of vitamin B	
Diet #7	Cracked whole wheat plus basal diet	
	Control	
	To observe growth curve when only F factor of vitamin B is present in the diet.	
Diet #8	Auto-claved yeast plus basal diet	
	Control	
	To observe growth curve when only G factor of vitamin B is present in the diet.	
Diet #9	Cracked whole wheat plus auto-claved yeast plus basal diet	
	Control	
	Supplemented ration.	
	To observe growth curve of normal vitamin B content.	
Diet #10	Cooked spinach plus auto-claved yeast plus basal diet	
	Control	
	Comparison of growth curves of kale with spinach when supplemented with G fraction of vitamin B	

DESCRIPTION OF DIETS

Table 1

(Continued)

Diet #11	Cooked spinach plus whole wheat plus basal diet
	Control
	Comparison of growth curves of kale and spinach when supplemented with F factor of vitamin B

Diet #1

Cooked Kale Greens and Basal Diet

Casein	1.0	grams
Butterfat	0.75	"
Starch	2.8	"
Salt mixture	0.2	"
Cooked kale	0.25	" (1 gm. 12/11/28)
Total	5.00	grams

Experimental Period

November 5, 1928 - January 5, 1929

Weights of Experimental Animals

Nov. 5, 1928	Rat ♀ #1048	Rat ♂ #1050	Rat ♀ #1065	Rat ♀ #1066	Rat ♀ #1062	Rat ♂ #1067
Nov. 5	25.5	34.3	49.2	33.0	21.5	44.4
" 10	35.5	42.4	62.25	40.75	41.5	53.4
" 17	43.25	43.8	54.4	42.5	45.0	57.4
" 24	43.0	49.4	62.5	45.5	47.4	54.1
Dec. 1	41.9	48.0	55.0	45.0	46.8	53.0
" 8	42.0	50.0	52.0	47.8	45.0	52.6
" 15	38.4	49.0	52.5	40.4	35.5	45.5
" 22	45.0	53.5	69.0	43.0	*12/16/28	47.8
" 29	49.0	55.5	66.0	43.0		45.5
Jan. 5	43.0	55.5	60.0	41.0		46.0

HISTORY OF RATS ON DIET #1

Cooked Kale Greens and Basal Diet

The rats on this diet became less active, fur ruffled, but otherwise normal after one week on the experimental diet. They had developed no snuffles nor lazy motions of any one member of the body. Second and third weeks rats appeared the same and normally active. Week of 11/24/28 -- 12/1/28 rats ate about one-half of their ration. From 12/1/28 -- 12/5/28 rats became fractious, snappy and fur stringy. All ratw on this diet began to show typical symptoms of polyneuritis[26] at this date. Rat #1062, 12/6/28 Head retracted. 12/13/28 Spastic gait, head retracted. 12/15/28 Cartwheel, drags hind leg and shows convulsive seizures when handled. 12/16/28 died. (Only animal lost in the group of six animals over an experimental period of 9 weeks.)

The remaining rats in the group suffered signs of polyneuritis, but never appeared in them as severely as in #1062. The consumption of food varied from 1/2 to 1/3 ration remaining with less and less food uneaten at close of experimental period.

1/5/29 Experimental period closed.

Fur smoother altho still yellow and stiff. Rats were nervous and undersized. In proportion to the length of the body, these animals had smaller chests and showed a tendency to have longer leg bones relativelyto their body length than did the animals on control diet #3 of the same age. However, these rats survived wide range of temperature[27] as shown in following temperature chart.

TEMPERATURE READINGS

Date		AM °C	PM °C
Nov.	23	10	19
	24	10	--
	25	18.5	15
	26	15	18.5
	27	15	17.5
	28	17	17
	29	16	--
	30	17	17
Dec.	1	18.5	17
	2	11	15.5
	3	18.5	22
	4	19	22
	5	17	21
	6	15	20
	7	15	20
	8	20	16
	9	--	15
	10	14.5	19
	11	14	20
	12	20	24
	13	20	21
	14	19	24
	15	20	18
	16	--	17
	17	21	21
	18	14	21.5
	19	18	20
	20	17.5	21

Diet #2

Canned Spinach and Basal Diet

Casein	1.0	grams
Butterfat	0.75	"
Starch	2.8	"
Salt mixture	0.2	"
Canned Spinach	0.25	" (1 gm. 12/11/28)
Total	5.00	grams

Experimental Period

November 5, 1928 - January 5, 1929

Weights of Experimental Animals

Date	Rat ♂ #1052	Rat ♀ #1053	Rat ♂ #1054	Rat ♂ #1060
Nov. 5'28	52.5	34.0	38.0	50.5
" 10	60.4	40.4	41.8	59.8
" 17	53.5	*11/16/28	34.4	59.0
" 24	61.1		38.7	55.8
Dec. 1	50.4		35.7	53.3
" 8	46.0*		33.0*	48.5*
" 15				
" 22				
" 29				
Jan. 5				

* = animal died

HISTORY OF RATS ON DIET #2

Canned Spinach plus Basal Diet

These rats became less active, fur ruffled, but otherwise normal after one week on experimental diet. Had developed no snuffles nor lazy motions of any one member of the body.

Typical polyneuritic conditions developed one week earlier (11/24/28) than they occurred in the rats on diet #1. 12/6/28 rat #1060 developed lazy movements and hunched back. 12/8/28 Rat #1060 nose bleed. 11/16/28 Rat #1053 died. (Autopsy showed gas in intestines). 12/9/28 -- 12/10/28 rats #1052, 1054 and 1060 died. Showed general loss of weight with mis-shapen bodies and convulsive seizures and cartwheel, spastic gait etc., as rat #1062 on diet #1.

1/5/29 Close of experimental period.

All ratw on this diet died of polyneuritis at close of 5th experimental week.

Diet #3

Basal Diet and Yeast Foam Tablets

Casein	1.0 grams
Butterfat	0.75 "
Starch	2.8 "
Salt mixture	0.2 "
Yeast	0.25 " (1 gm. 12/11/28)
Total	5.00 grams

Weights of Experimental Diets

Date	Rat ♂ #1056	Rat ♂ #1057	Rat ♀ #1059	Rat ♀ #1061
Nov. 5'28	30.0	52.0	30.0	28.5
" 10	37.4	60.8	33.5	34.4
" 17	43.0	60.7	41.8	37.4
Dec. 1	63.2	83.8	57.4	53.7
" 8	77.0	97.0	47.0	63.0
" 15	92.0	105.3	12/9/28*	63.0
" 22	98.0	110.0		66.0
" 29	104.0	129.0		69.0
Jan. 5	113.0	141.0		81.0

HISTORY OF RATS ON DIET #3

Yeast plus Basal Diet

Snuffles and loss of weight (temperature).*

Control animals - normal albino rats in appearance and activity.

* weak - forepaws and nose bleeding preceding day.

Diet #4

Kale Greens and Basal Diet

Casein	1.8 grams
Starch	6.0 "
Salt Mixture	0.4 "
Butterfat	1.0 "
Agar Agar	0.1 "
Cod Liver Oil	0.2 "
Kale Greens	0.5 "
Total	10.0 grams

Experimental Period - 4/30/29 - 7/1/29

Weights of Experimental Animals

Rat #1223 ♂

May 18, '29	37.5 grams
" 20	45.5 "
" 25	67.0 "
June 1	72.0 "
" 7	75.0 "
" 17	69.0 "
" 23	72.0 "
July 1	81.0 "

HISTORY OF RATS ON DIET #4

Kale Greens plus Basal Diet

Very slight neuritic condition observed. Rat was less nervous, larger, sleeker, whiter fur with keen eyes.

Diet #5

Kale Greens and Whole Wheat and Basal Diet

Casein	1.8	gram
Starch	4.0	"
Salt mixture	0.5	"
Butter fat	1.0	"
Cod Liver Oil	0.2	"
Whole Wheat	2.5	"
Kale Greens	0.5	"
Total	10.0	grams

Experimental Period -- 4/29/29 - 6/8/29

Weights of Experimental Animals

Date	Rat ♂ #1206	Rat ♀ #1207	Rat ♂ #1208	Rat ♀ #1209	Rat ♂ #1210
4/29/29	74.5	76.5	48.0	44.0	51.5
5/4/29	97.5	95.0	75.5	72.5	78.5
5/11/29	106.0	108.7	87.0	84.3	92.2
5/18/29	119.0	118.0	102.5	95.5	106.5
5/25/29	131.0	118.0	117.0	107.5	119.0
6/1/29	133.5	120.0	123.0	114.0	125.0
6/8/29	162.0	142.0	147.0	133.0	154.0

HISTORY OF RATS ON DIET #5

Kale Greens plus Cracked Whole Wheat plus Basal Diet

Rats in this group ate 1/2 food fed first week. Second week - 5/16/29 little food remained. 5/18/29 on no food remained. These rats remained normal in appearance and activity.

1. Fur sleek.
2. Keen eyes.
3. Active.
4. Proper proportion.
5. No deaths.

Diet #6

Kale Greens and Auto-claved Yeast and Basal Diet

Casein	1.5	grams
Starch	6.0	"
Salt mixture	0.4	"
Butterfat	1.0	"
Agar Agar	0.2	"
Cod Liver Oil	0.2	"
Auto-claved Yeast	1.0	"
Kale greens	0.5	"
Total	10.8	grams

Experimental Period -- 4/29/29--6/8/29.

Weights of Experimental Animals

Date	Rat ♀ #1201	Rat ♀ #1202	Rat ♀ #1203	Rat ♀ #1204	Rat ♂ #1205
4/29/29	43.2	32.4	55.0	69.7	68.0
5/4/29	56.2	49.0	70.5	81.5	96.0
5/11/29	50.0	46.0	66.0	73.5	94.0
5/18/29	45.0	46.5	56.5	64.0	85.0
5/25/29	43.0	46.8	55.0	54.7	84.0
6/1/29	44.0	50.5	59.0	44.5*	86.0
6/8/29	45.0	54.5	64.5		83.0

* died 6/3/29

HISTORY OF RATS ON DIET #6

Kale Greens plus Auto-claved Yeast plus Basal Diet

One-half food eaten first week. By the third week no food remained. By fourth week (5/16/29) rats became slightly week, although, otherwise normal in appearance. 5/27/29 condition weaker, fur stringy and all food eaten. 6/2/29 Group much weaker with rats #1202, #1203 and #1204 dragging hind legs and convulsive seizures when handled. 6/3/29 #1204 died - previously nervous, snappy, stringy fur, weak, slow motion, legs longer in proportion to length of body.

Diet #7

Whole Wheat and Basal Diet

Casein	1.8	grams
Starch	4.0	"
Salt mixture	0.4	"
Butterfat	1.0	"
Agar Agar	0.2	"
Cod liver oil	0.2	"
Auto-claved yeast	1.0	"
Total	10.0	grams

Experimental Period -- 4/29/29-6/8/29

Date	Rat ♂ #1211	Rat ♂ #1212	Rat ♀ #1213	Rat ♂ #1214	Rat ♂ #1215
4/29/29	29.8	32.0	71.5	59.0	68.5
5/4/29	49.0	48.6	88.0	85.0	95.8
5/11/29	65.7	61.0	106.5	92.0	108.0
5/18/29	79.0	73.5	125.0	104.5	121.0
5/25/29	96.0	86.5	122.5	112.5	132.5
6/1/29	102.0	96.0	122.0	118.0	138.0
6/8/29	122.0	115.0	141.0	136.0	158.0

HISTORY OF RATS ON DIET #7

<u>Whole Wheat plus Basal Diet ------</u>

First two weeks 1/2 food eaten - by third week all eaten.

5/16/29 Rats all normal in appearance

5/27/29 " " " " "

6/2/29 Gain in weight, eyes not clear, not normally active.

Diet #8

Auto-Claved Yeast and Basal Diet

Casein	1.9	grams
Starch	6.3	"
Salt mixture	0.4	"
Butterfat	1.0	"
Agar Agar	0.2	"
Cod liver oil	0.2	"
Auto-claved yeast	1.0	"
Total	10.0	grams

Experimental Period -- 4/29/29-6/7/29

Weights of Experimental Animals

Date	Rat ♀ #1216	Rat ♂ #1217	Rat ♀ #1218	Rat ♀ #1219	Rat ♀ #1220
4/29/29	41.0	67.0	57.8	24.0	33.5
5/4/29	54.0	88.5	76.6	32.5	45.8
5/11/29	54.5	94.0	78.5	26.5	45.0
5/18/29	47.5	81.5	76.0	*	39.0
5/25/29	37.5	75.0	67.5		*
6/1/29	*	69.0	66.0		
6/7/29		58.0	62.5		

HISTORY OF RATS ON DIET #8

-- Auto-claved Yeast plus Basal Diet

All food eaten by second week (5/10/29)

Rat #1219 runt.

Rat #1219 died - sick weakling - runt.

5/16/29 little food remaining.

Rat #1216 Slightly weak, snuffles.

Rat #1217 Normal in appearance.

Rat #1220 Slightly weak.

5/22/29 #1216 Fur stringy-thinner-weak-emaciated.

5/24/29 #1216 Decidely hunch back - ribbed tail, emaciated.

5/26/29 #1216 died - neuritic.

5/27/29 #1220 died - same history #1216

Three losses.

Diet #9

Cracked Whole Wheat and Auto-claved Yeast and Basal Diet

Casein	1.8	grams
Starch	4.0	"
Salt mixture	0.5	"
Butterfat	1.0	"
Agar Agar	0.2	"
Whole Wheat	2.5	"
Auto-claved yeast	0.4	"
Total	10.4	grams

Experimental period: 5/25/29 -- 7/8/29

Weights of Experimental Rats

Date	Rat ♂ #1221	Rat ♀ #1222
5/25/29	45.5	54.0
6/1/29	61.0	54.0
6/7/29	73.5	80.0
6/17/29	106.0	93.0
6/23/29	114.5	97.0
7/1/29	125.5	102.0
7/8/29	130.0	107.0

HISTORY OF RATS ON DIET# 9

Cracked Whole Wheat plus Auto-claved Yeast plus Basal Diet

Rats normal in appearance; eyes keen, fur sleek, very active and normal in size.

Diet #10

Spinach and Auto-claved Yeast and Basal Diet

Casein	1.5	grams
Starch	6.0	"
Salt mixture	0.4	"
Butterfat	1.0	"
Cod liver oil	0.2	"
Auto-claved yeast	1.0	"
Spinach	0.5	"(later 1.0 gm.)
Total	10.6	grams

Experimental Period -- 6/17/29 - 8/5/29.

Weights of Experimental Animals

Date	Rat ♀ #1224	Rat ♀ #1225	Rat ♂ #1226	Rat ♂ #1227
6/17/29	32.0	18.0	33.0	31.0
6/24/29	45.0	*6/20/29	44.0	45.0
7/1/29	49.0		46.0	49.0
7/8/29	45.5		45.5	43.0
7/15/29	51.5		46.0	42.0
7/22/29	48.5		48.0	44.0
7/29/29	46.0		47.0	45.0
8/5/29	44.0		48.0	45.5

HISTORY OF RATS ON DIET #10

Spinach plus Auto-claved Yeast plus Basal Diet

1st week 1/2 food eaten

2nd week 3/4 food eaten

Remainder of experimental period all of food was eaten.

3rd week fur became stringy and yellow. Slight symptoms of polyneuritis, otherwise rats remained practically normal until conclusion of experimental period.

Diet #11

Spinach, Whole Wheat and Basal Diet

Casein	1.8	grams
Starch	4.0	"
Salt mixture	0.5	"
Butterfat	1.0	"
Cod liver oil	0.2	"
Whole Wheat	2.5	"
Spinach	0.5	"
Total	10.5	grams

Experimental Period: 6/17/29 -- 8/5/29.

Weights of Experimental Animals

Date	Rat ♂ #1228	Rat ♂ #1229	Rat ♀ #1230	Rat ♀ #1231	Rat ♂ #1232
6/17/29	32.0	29.0	31.0	33.0	34.0
6/24/29	44.0	46.0	47.0	39.0	42.0
7/1/29	52.0	50.0	57.0	42.5	60.0
7/8/29	65.0	66.0	68.0	47.0	70.0
7/15/29	72.5	78.0	79.0	60.0	85.0
7/22/29	84.0	86.0	87.0	78.5	95.0
7/29/29	92.0	95.0	96.0	87.7	107.0
8/5/29	101.0	105.0	105.0	97.0	119.0

HISTORY OF RATS ON DIET #11

Spinach plus Whole Wheat plus Basal Diet

1st week 1/2 food eaten.

2nd week all of food eaten.

Rats were normal in appearance and activity.

DISCUSSION

In the experimental work of the winter of '28 (Nov. 5, 1928 to Dec. 29, 1928) it was noted that, even tho there was a sudden and wide range of temperature,[27] the experimental animals on the cooked kale greens diet plus the basal diet survived the test period of nine weeks with one death occurring in the seventh week.

One of the control diets, canned spinach plus the basal diet, had one death to occur at the close of the fifth week with no survivals at the end of the test period of nine weeks. Polyneuritis appeared in both groups although less severely in the cooked kale group than the canned spinach group.

The growth curve of the rats on the diet containing cooked kale greens more nearly approaches the normal growth curve of the control animals than does the growth curve of the rats on the canned spinach. (Chart I).

It was concluded that vitamin B was present to some extent in cooked kale greens. Possibly this food was richer in one of the vitamin B "complexes" or that cooked kale lost some of the potency of the vitamin F fraction if it were present in the green kale.

In order to ascertain which fraction was more abundent, if any, in the cooked kale as consumed by the human race, a more elaborate experimental program was outlined and the work carried on from April 29 to July 1, 1929.

According to Chart II, kale greens show better growth curve than spinach, but evidently the B fraction present in kale greens has not been supplemented by the addition of the vitamin G fraction in the form of auto-claved yeast as has the control of whole wheat plus auto-claved yeast and, therefore, from comparison of the data on diets #6, #9 and #10 in Chart II, the rats on diet #6 if reduced to simple terms, means G(kale) plus G (auto-claved yeast) without the resultant normal growth curve. However, it may also be observed from this chart that vitamin F is not lacking entirely.

Chart III shows kale greens and basal diet with a better growth curve resulting. Vitamin B is not as yet supplemented by its complementary fraction to yield a normal growth curve as in the control diet #7. The spinach diet #8 is still taking heavy toll. No deaths occurred in the diet #4 with kale greens.

The improvement of the growth curves in Chart IV are probably due to the addition of whole wheat to all three diets. Granting that the whole wheat may have present some vitamin G which would supplement the curve representing the growth of the animals on kale greens, diet #5 consisting of kale greens plus whole wheat plus the basal diet, shows greater growth than the control, diet #9 consisting of whole wheat plus auto-claved yeast plus basal diet, which clearly indicates that kale greens is well supplemented by the whole wheat and is itself potent in the opposite, thermo-stable factor, vitamin G since the growth curve of the control diet #9 is much less advanced.

Another contrast may be observed in the study of Chart #6.

	Average gain for experimental period.
Diet #10--Spinach, ACY*, B. D.-	13.8
Diet # 9--Whole wheat, A C Y, B D	79.0
Diet # 6--Kale, A. C. Y., B. D.	4.6
Diet # 7--Whole wheat, B. D.	82.2
Diet # 8--A. C. Y., B. D.	0.4 loss
Diet # 4--Kale and B. D.	33.5
Diet #11--Spinach, Whole Wheat	74.0
Diet # 9--Whole Wheat, B. D., A. C. Y	79.0
Diet # 5--Kale, Whole wheat, B. D.	88.7

* = auto-claved yeast

- = basal diet

In all the diets listed containing kale greens, there is a gain in weight. Kale greens supplemented by auto-claved yeast or vitamin G shows an average gain of 4.6 grams for the group. Spinach supplemented by auto-claved yeast shows an average gain of 13.8 grams for the group.

Kale greens as the source of vitamins F and G show an average gain of 33.5 grams for the group. The control, whole wheat plus autoclaved yeast as the source of vitamins F and G respectively shows an average gain of 79.0 grams for the group while kale greens and whole wheat, the apparent source of vitamins G and F respectively, show an average gain of 88.7 grams for the group, a difference of 9.7 grams between kale supplemented by whole wheat and the control diet, whole wheat supplemented by auto-claved yeast, the known source of the vitamins F and G respectively [17].

In chart #1, note the following cases of deaths reduced when kale is present: Diet #6 the number of rats at the beginning of the experimental period are 5, the number surviving this period, 4. Diet #8 same without kale, number of rats at the beginning of the experimental period are 5, number surviving this period, 2. The period of the latter is longer, but the deaths occur early in the period. Diet #2, number of rats at the beginning of the period 4, number surviving the period 0,

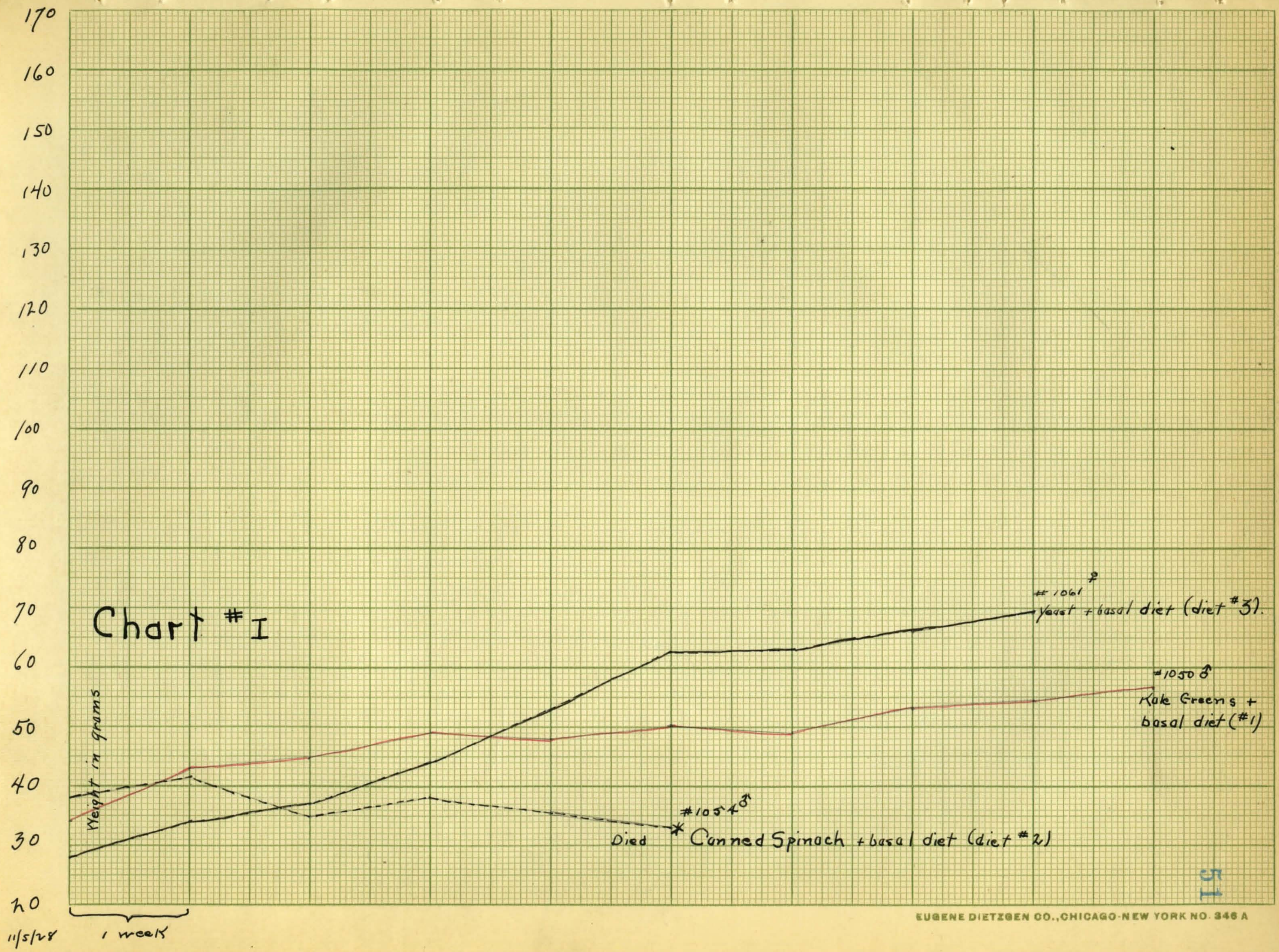

Chart #I
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
11/5/28
1 week
Weight in grams
#1061 ♀
Yeast + basal diet (diet #3).
#1050 ♂
Kale Greens + basal diet (#1)
#1054 ♂
Died
Canned Spinach + basal diet (diet #2)

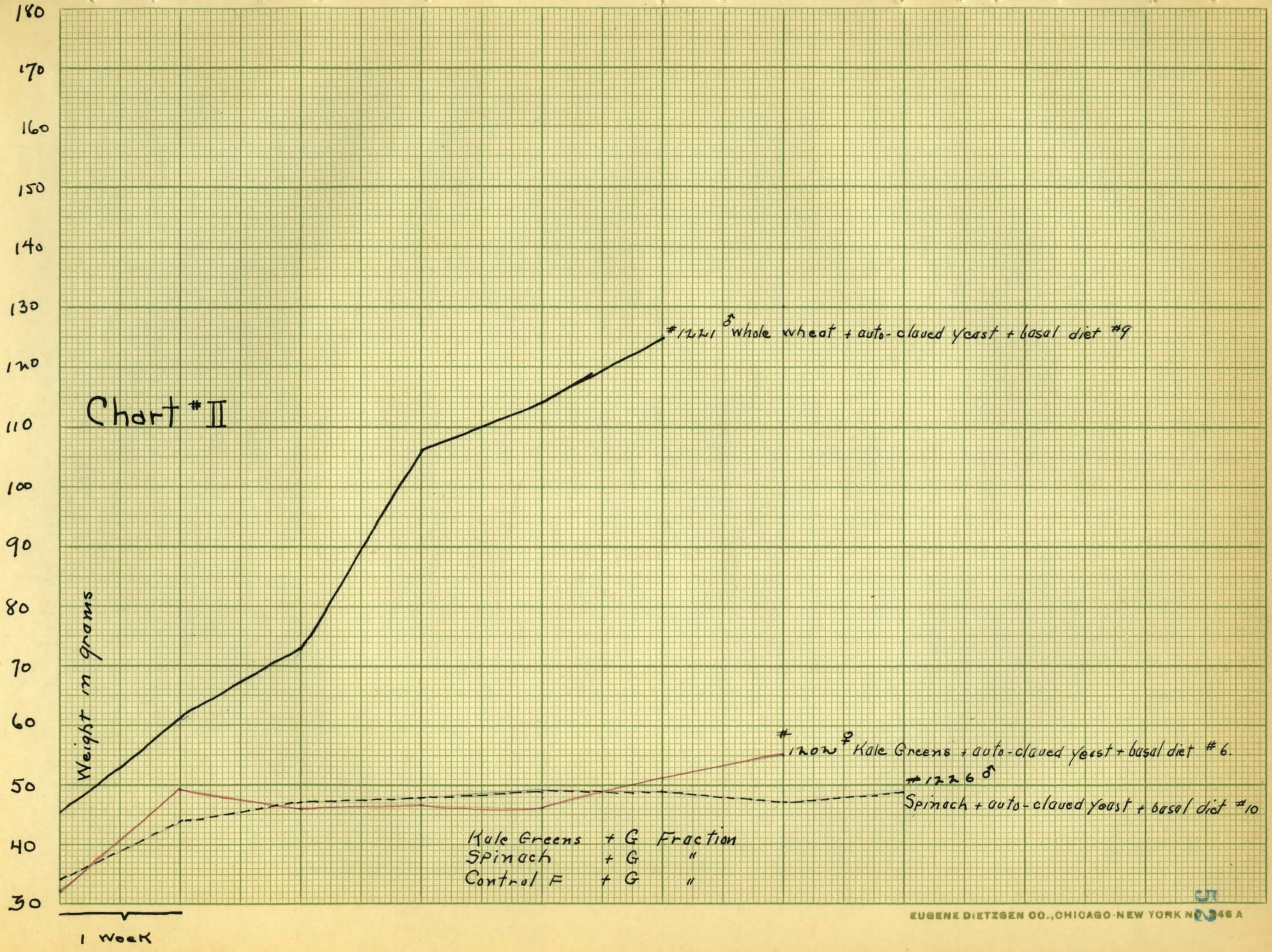

Chart # II
Weight in grams
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
1 Week
#1221 ♂ whole wheat + auto-claved yeast + basal diet #9
#1202 ♀ Kale Greens + auto-claved yeast + basal diet #6.
#1226 ♂
Spinach + auto-claved yeast + basal diet #10
Kale Greens + G Fraction
Spinach + G "
Control F + G "
EUGENE DIETZGEN CO., CHICAGO-NEW YORK NO. 346 A

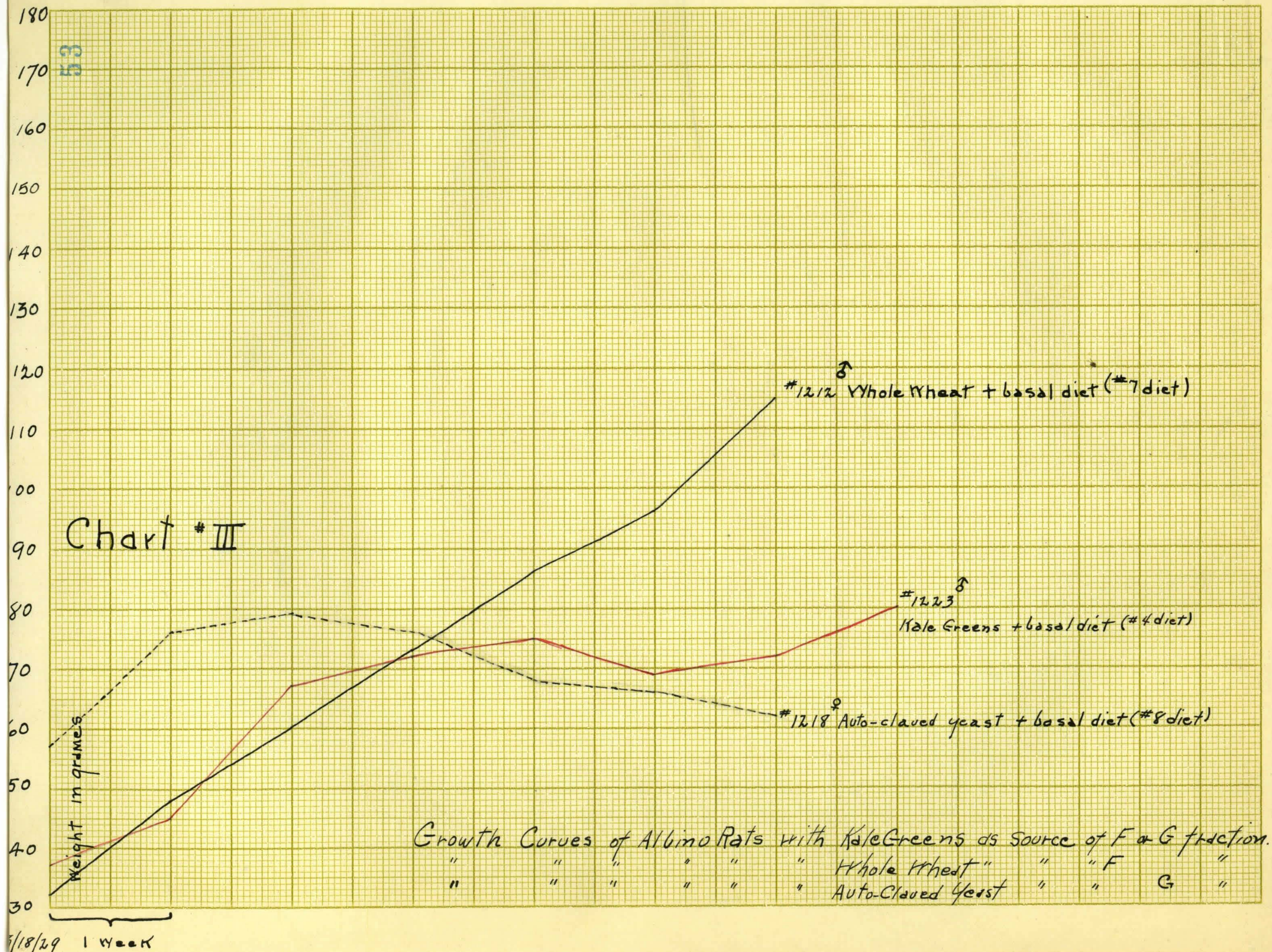
Chart # III
Growth Curves of Albino Rats with Kale Greens as Source of F & G fraction.
" " " " " " Whole Wheat " " "F "
" " " " " " Auto-Claved Yeast " " G "
#1212 ♂ Whole Wheat + basal diet (#7 diet)
#1223 ♂ Kale Greens + basal diet (#4 diet)
#1218 ♀ Auto-claved yeast + basal diet (#8 diet)
Weight in grames
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
5/18/29
1 week

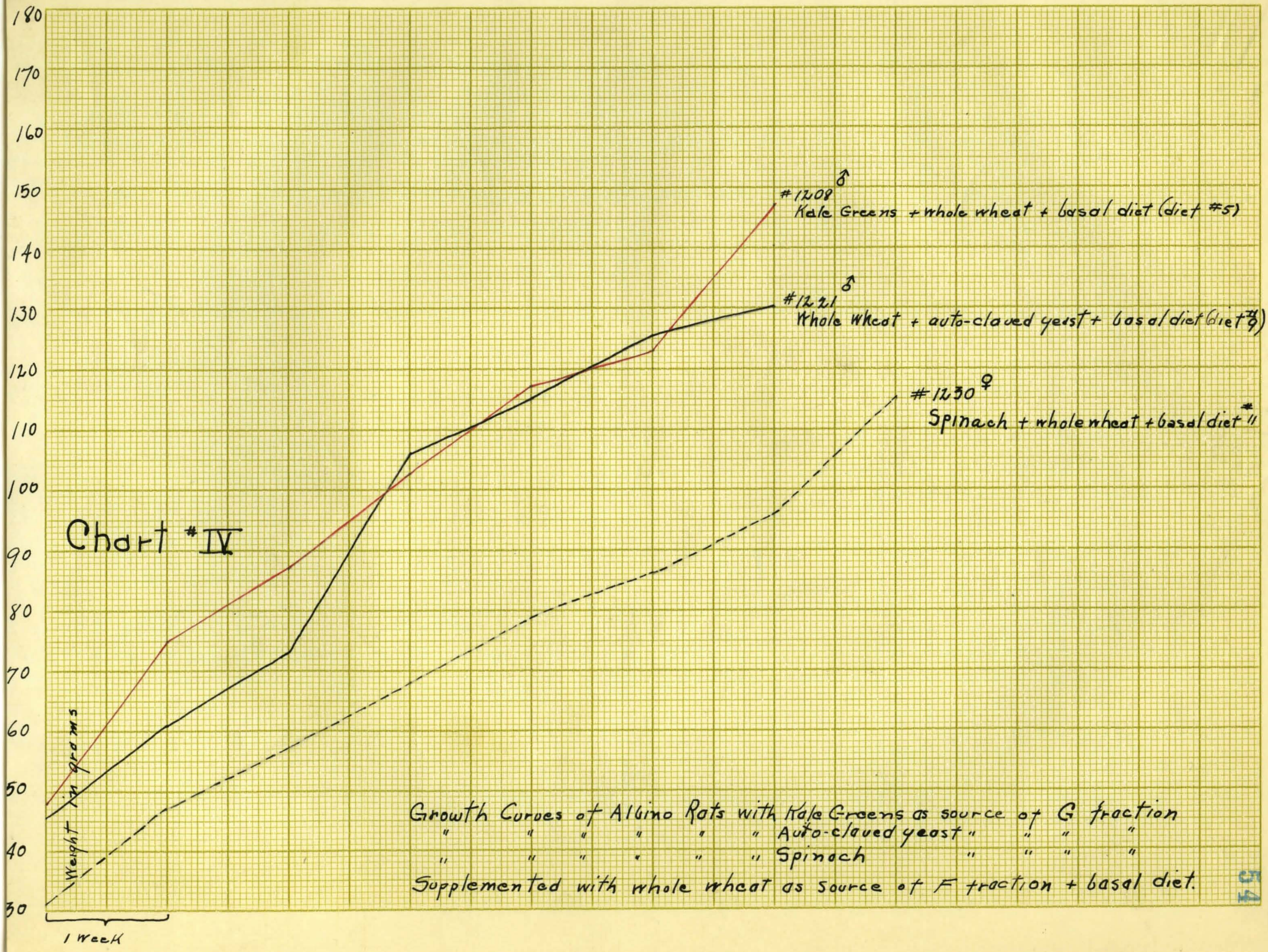
Chart #IV
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
Weight in grams
1 Week
#1208 ♂
Kale Greens + whole wheat + basal diet (diet #5)
#1221 ♂
Whole wheat + auto-claved yeast + basal diet (diet #7)
#1230 ♀
Spinach + whole wheat + basal diet # "
Growth Curves of Albino Rats with Kale Greens as source of G fraction
" " " " " " Auto-claved yeast " " "
" " " " " " Spinach " " " "
Supplemented with whole wheat as source of F fraction + basal diet.

Weights

Chart #6

Diet #1 Rat Nos.	Initial Wt. in grams	Final Wt. in grams	Gain gms	Average gain of the group gms
Kale,b.d				
1048	25.5	43.0	17.5	
1050	34.3	55.5	21.2	
1065	49.2	60.0	10.8	
1066	33.0	41.0	8.0	
1062	21.5	35.5 *6 wk	14.0	
1067	44.4	46.0	1.6	12.5
#2 Canned spinach b.d.				
1052	52.5	46.0 *5 WK	6.5 loss	
1053	34.0	40.4 *2 "	6.4	
1054	38.0	33.0 *5 "	5.0 loss	
1060	50.5	48.5 *5 "	2.0 loss	7.1 loss
#3 yeast, bd.				
1056	30.0	113.0	83.0	
1057	52.0	141.0	89.0	
1059	30.0	47.0 *5wk	17.0	
1061	28.5	81.0	52.5	60.4
#4 Kale, b.d.				
1223	37.5	71.0	33.5	33.5
#5 Kale Wh wheat,b.d.				
1206	74.5	162.0	87.5	
1207	76.5	142.0	65.5	
1208	48.0	147.0	99.0	
1209	44.0	133.0	89.0	
1210	51.5	154.0	102.5	88.7
#6 Kale auto-cl yeast, b.d.				
1201	43.2	45.0	1.8	
1202	32.5	54.5	22.0	
1203	55.0	64.5	9.5	
1204	69.7	44.5	25.3 loss	
1205	68.0	83.0	15.0	4.6

* Died

Weights

Chart #6 (continued)

Diets & Rat Nos.	Initial Wt. in grams	Final Wt. in grams	Gain in gms.	Average gain of group (gms)
#7				
Wh wheat, b.d.				
1211	29.8	122.0	92.2	
1212	32.0	115.0	83.0	
1213	71.5	141.0	69.5	
1214	59.0	136.0	77.0	
1215	68.5	158.0	89.5	82.2
#8				
Auto-claved yeast, b.d.				
1216	41.0	37.5 *5wk	4.5 loss	
1217	67.0	58.0	9.0 "	
1218	57.8	62.5	4.7	
1219	24.0	26.5 *3wk	2.5	
1220	33.5	39.6 *4wk	6.1	0.44 loss
#9				
Wh wheat, auto-cl yeast, b.d.				
1221	34.0	130.0	96	
1222	45.0	107.0	62	79.0
#10				
Spinach, auto-cl yeast, b.d.				
1224	32.0	44.0	12.0	
1225	18.0	weakling died		
1226	33.0	48.0	15.0	
1227	31.0	45.5	14.5	13.8
#11				
Spinach, wh wheat, b.d.				
1228	32.0	101.0	69.0	
1229	29.0	105.0	76.0	
1230	31.0	105.0	74.0	
1231	33.0	97.0	64.0	
1232	34.0	119.0	85.0	74.0

Vitamin B Content of Kale Greens

Chart #1

11/5/-12/29/28 Diet No.	Amt. per day	No. of rats	Average initial wt.	Wkly average chg in wt.	Time of survival in dyas	No of rats surviving per.	Averg. gain in wt. of survivals
#1 Kale b.d.*	1.0	6	35	1.9	63	5	12.5
#2 canned spinach b.d.	1.0	4	43.6	-1.2	35	0	7.1 loss
#3 yeast b.d.	1.0	4	35.0	5.8	63	3	60.4
#4 Kale b.d.	1.0	1	37.5	3.3	49	1	33.5
#5 Kale wh wheat b.d.	1.0	5	58.8	16.2	42	5	88.7
#6 Kale auto-cl yeast b.d.	1.0	5	53.6	3.7	42	4	4.6
#7 wh wheat b.d.	1.0	5	52.1	15.5	42	5	82.2
#8 Auto-cl yeast b.d.	1.0	5	44.6	1.9	49	2	0.44 loss
#9 Wh wheat auto-cl yeast, b.d	1.0	2	49.0	14.0	42	2	79
#10 Spinach auto-cl yeast,b.d.	1.0	4	28.5	2	49	3	13.8
#11 Spinach wh wheat b.d.	1.0	5	32.0	13.0	49	5	74.0

b.d.= basal diet.

SUMMARY

1. Death does not occur in the albino rat when the sole source of vitamin B is cooked kale greens.

2. When cooked kale greens are supplemented by whole wheat to supply the vitamin F fraction, a normal growth curve results with an average gain of 9.7 grams above the normal control and 14.7 grams above the spinach control.

3. Cooked kale greens are potent in the thermostable factor of vitamin B, vitamin G and a fair source of the thermo-labile factor of vitamin B, vitamin F.

4. Cooked kale greens are a better source of vitamin F and an exceedingly better source of the vitamin G fraction of vitamin B than cooked spinach.

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