EXPERIMENTS ON THE VITAMIN B (B_1) CONTENT OF WHEAT AND ITS MILLING PRODUCTS

by

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INTRODUCTION

Numerous studies on the occurrence of vitamins in foods have shown that wheat, one of our most important food cereals, is a good source of vitamin B (B_1). The modern diet appears to contain a decreasing amount of vitamin B (B_1) with a slight deficiency of such resulting in nutritional disorders. Certain differences of opinion are held as to the distribution of the vitamin B (B_1) in wheat and its milling products. The establishment of the separate identities of vitamin B (B_1) and vitamin G affords at least a partial solution to the question of the multiple nature of the old vitamin B. The following investigation has been undertaken for the purpose of making a quantitative study of the determination of vitamin B (B_1) in wheat and its milling products by means of growth experiments with young rats.

REVIEW OF LITERATURE

Cowgill (7) recently summarizing the trends in current researches on the vitamin B complex says, "The nomenclature of vitamins has undergone modifications since Funk introduced the term vitamine. McCollum and Kennedy proposed that the fat-soluble factor necessary for growth be called fat-soluble A, and the growth promoting water-

soluble substance, water-soluble B. Drummond suggested that the final "e" be dropped, the group term, therefore being spelled vitamin, a spelling which carries no implication as to the molecular structure of these dietary essentials. The work of Goldberger and associates and others has shown that what was formerly designated vitamin B consists of at least two physiologically active substances and these have been called by American workers, vitamins B and G, and by the English investigators, B_1 and B_2. More recently evidence has been brought forward pointing to the existence of still other B factors, which in accordance with the English practice, might be designated vitamins B_3, B_4 etc. The term vitamin B complex is a convenient one to use to designate the mixture of these B factors as occurring in natural foods."

In this study the thermolabile antineuritic factor will be termed vitamin B (B_1), the thermostable "pellagra preventing" factor vitamin G, and a mixture of vitamin B factors the vitamin B complex.

No attempt will be made here to review the extensive literature concerning the physiological role of the antineuritic vitamin. Various investigators (2, 4, 6, 7, 10, 13, 14, 15, 16, 21, 22, 23, 26) have found vitamin B (B_1) indispensable for normal appetite and growth, for metabolic functions, for reproduction and lactation, and in pre-

venting neuromuscular disorders. The physiological value of wheat germ in its effect on the appetite and growth of California school children demonstrated the necessity of using more whole grain cereals. Morgan and Barry (13) believe that the modern diet appears to contain a decreasing amount of vitamin B (B_1) as a result of the substitution of fruits, vegetables, and milk for whole cereals. Sebrell and Elvove (22) agree with Sandels (21) that an acute type of polyneuritis is associated with a shortage rather than a complete absence of the antineuritic vitamin. Sure, Kik and Smith (26) in a recent report of their work on the specific effect of vitamin B (B_1) on growth say, "The application of our work to the human can be anticipated in borderline diseases associated with malnutrition, i.e., people with a fair appetite may still be in need of vitamin B (B_1) for optimum well being, since the exact physiological role of vitamin B (B_1) in the organism is not yet clearly understood." Sandels (21) reports a definite gradation of symptoms correlated with the extent of the deficiency of the vitamin in the food supplied. A slight deficiency of such may be responsible for the widespread prevalence of digestive disorders, anorexia, nervousness and lassitude.

Previous studies on the occurrence of vitamins in various foods have shown that the cereals contain comparatively abundant supplies of vitamin B (B_1). Plimmer and his

co-workers (17, 18, 19), using maintenance tests with pigeons and adopting the same standard of comparison throughout, have compiled the vitamin B (B_1) value of pulses, nuts, cereal foods, fruits and vegetables. Numerous investigators confirmed Eijkman's early discovery that an antineuritic substance existing in rice polishings cured nutritional polyneuritis of fowls. Since rice polishings and wheat embryo have been found exceedingly rich in vitamin B (B_1) it has been generally assumed that vitamin B (B_1) resides chiefly in the embryo. Contrary to this opinion, Osborne and Mendel (14) showed that the vitamin B complex is not confined to any one part of the wheat kernel, but is distributed throughout the entire grain. Their observations were later confirmed by Bell and Mendel (1), who calculated the percentage distribution of the vitamin in different milling products on the basis of the fraction of the entire kernel represented by each of the milling products. Croll and Mendel (9) found that nearly all of the vitamin B (B_1) of maize or corn is located in the embryo. Croll (8) later found that whole unpolished "brown" rice and both the endosperm and embryo ends of the unpolished rice kernel contained vitamin B (B_1). Since rice and wheat carry vitamin B (B_1) distributed throughout the entire kernel and maize does not, a general statement regarding the uniformly similar distribution of vitamin B (B_1) in cereals would not be justified.

Goldberger and his associates (24) showed in a fairly conclusive manner that both vitamins B (B_1) and G are needed for growth. Salmon (20) verified this, since the two fractions separated from velvet bean leaves and seeds gave good growth to rats only when combined. Sherman and Axtmayer (23) were able to show a definite supplementation of autoclaved yeast by wheat, since a mixture of 0.4 gram autoclaved yeast and 0.4 gram wheat fed daily gave better growth than 0.8 gram of either fed separately.

Whole wheat has long been recognized as an excellent source of the antineuritic vitamin. Bourquin (3) using ground whole wheat as the source of vitamin B (B_1) concluded that a diet containing 20 per cent whole wheat showed the greatest possibility for a satisfactory basal diet when autoclaved yeast was fed as a source of vitamin G. The Illinois Agricultural Experiment Station (12) reported recently that the inclusion of 25 per cent whole wheat provided adequate vitamin B (B_1) for growth of youngsters if sufficient vitamin G was incorporated in the basal diet. Hunt (11) and Osborne and Mendel (14) both found that 15 per cent of whole wheat supplied sufficient vitamin B (B_1) for normal growth. Bell and Mendel (1) found 15 per cent of spring wheat or 40 per cent winter wheat to be necessary for normal growth. Croll and Mendel (9) showed that corn does not differ greatly from wheat in the vitamin B (B_1) content since for normal rat growth 20 - 30 per cent of corn

was the minimum requirement. Workers agree that for satisfactory reproduction and lactation more of the cereal product must be incorporated into the diet.

Chase and Sherman (5) fed graded allowances of ground whole wheat as a source of vitamin B (B_1) in proportion from .1 - 1 gram daily for an 8 week experimental period. Since increasing the allowance of vitamin B (B_1) did not increase the rate of gain in exact arithmetrical proportion quantitative comparison was made easier by adopting a unit of vitamin B (B_1) for a basis. A unit of vitamin B (B_1) is that amount which when fed daily will induce in the experimental animals an average weekly gain of 3 grams during an 8 week experimental period.

Chick and Roscoe (6) believe that if a unit of vitamin B (B_1) be defined as the dose required to restore normal (11 to 14 grams weekly) growth to a young rat, of which the growth has failed on a basal diet deficient only in vitamin B (B_1) and containing excess of vitamin B_2, the assay of a foodstuff consists in determining the minimal dose necessary for this degree of restoration.

PROCEDURE

This investigation was planned to quantitatively determine the antineuritic vitamin B (B_1) of wheat and its milling products according to the newer method as developed

by Chase (4) and Chase and Sherman (5). This method is essentially as follows:

Normal albino rats reared by mothers fed on the Sherman adequate stock diet of ground whole wheat, dried whole milk powder and sodium chloride, were separated at four weeks of age from their mothers. Since from preliminary experiments it has been shown that rats store vitamin B (B_1) these animals were fed only the basal vitamin B (B_1) free diet for a depletion period of about two weeks, or until they reached constant weight, as determined by frequent weighings. At the beginning of the experimental period the rats were placed in individual round cages with a raised wire screen bottom to prevent access to excreta. A representative number of males and females of approximately the same average weights were then continued on the basal vitamin B (B_1) free diet to serve as negative controls, some were given an adequate diet to serve as positive controls, while others were fed in addition to the basal diet different per cents of the milling product to be tested. Fresh distilled water was always available. The cages were cleaned frequently and spilled food recovered. The rats and food were weighed once a week with records kept as to average weekly weight, weekly gain, food consumption and notes on the condition of the animal. Weight curves for averages of the data obtained quantitatively expressed the relative levels of vitamin

B (B_1) feeding and the resulting weights of test animals under systematically controlled conditions.

In this particular study young rats of Wistar stock, after depletion, were placed on the various diets. Fresh distilled water and a weighed amount of diet were given ad libitum. The cages were cleaned every other day, and were sterilized once a week together with food and water cups. The milling products to be tested were incorporated in varying amounts to the basal diet lacking vitamin B (B_1) but adequate and optimal in all other respects. The gains and lengths of survivals made by animals receiving various per cents of different milling products were compared directly with those made by negative controls receiving only the basal vitamin B (B_1) free diet, and also with the positive controls receiving adequate diets in which the yeast was not autoclaved to destroy the vitamin B (B_1). Chase and Sherman (5) in order to facilitate quantitative comparisons, adopted as a "unit" of vitamin B (B_1) that amount which when fed as a daily allowance to a standard test animal sufficed to support 3 grams per week of gain with no apparent symptoms of polyneuritis during an 8 week experimental period. Daily doses of milling product cannot be conveniently used here, so the milling product due to its bulk must be incorporated directly into the basal vitamin B (B_1) free diet after the manner of Bell and Mendel (1).

The gain of 3 grams per week is a favorable guide since it ensures fairly healthy test animals--yet so far below normal growth that there is ample room for response to an increased intake of the vitamin. Chick and Roscoe (6) adopted a unit of vitamin B (B_1) which will restore growth to young rats depleted of their vitamin B (B_1) store and induce a normal weekly increase in weight of about 11 - 14 grams.

The basal vitamin B (B_1) free diet as developed by Sherman and Spohn (25) and modified by Chase (5) consists of:

Casein (freed of vitamin B (B_1) and G)	18%
Autoclaved yeast	15%
Osborne and Mendel salts	4%
Cod liver oil	2%
Butterfat	8%
Cornstarch	53%

The casein was freed from vitamin B (B_1) by cold extraction with 60 per cent (by weight) alcohol. Four hundred grams of casein were treated with two liters of 60 per cent alcohol, stirred for one-half hour, allowed to stand 5 1/2 hours, filtered with suction and thoroughly washed with one liter of 60 per cent alcohol. It was again treated with two liters of 60 per cent alcohol, and stirred for another half-hour. After standing 18 hours it was filtered again, washed with one liter of 60 per cent alcohol and air dried.

The dried bakers' yeast was spread in a uniform layer in open petri dishes, placed in tightly covered pans, and autoclaved at 15 pounds pressure for at least three hours to destroy the vitamin B (B_1). The autoclaved product was cooled in the autoclave, crumbled while still warm and powdered. The salt mixture was prepared according to the method of Osborne and Mendel (14). The same brand of cod liver oil of a good grade was used throughout the experiment. The butterfat was prepared by melting butter at a temperature below 50° C. and separating the fat from the curd, salt and water. The cornstarch was a commercial grade of good quality.

The supplements chosen to provide the vitamin B (B_1) were wheat and its milling products. A country run of hard winter wheat containing 11.5 per cent protein as obtained from Salina, Kansas, was milled by the Department of Milling Industry at this college. The fractions of wheat and the streams comprising each are as follows:

(1) Patent flour ------- sizings flour; 1st, 2nd, 3rd and 4th middlings flours. "Middlings flours" implies the streams derived mostly from the endosperm.

(2) First clear -------- 5th middlings flour; 1st, 2nd and 3rd break flours; 1st and 2nd tailing's flours; reel flour.

(3) Low grade ---------- 4th break flour and low grade stream. This grade may be used for graham cracker making or for baking when color is of no consequence.
or
Second clear

(4) Standard middlings - very fine bran, some endosperm and a little of the germ. A farm animal feed.
or
Shorts

(5) Bran ---------- mostly the outer covering of the wheat.

(6) Germstock ---------- similar to shorts but containing practically all of the germ.

(7) Whole wheat -------- the entire cleaned kernel ground fine.
or
Graham

The approximate percentage of the milling product to incorporate into the basal vitamin B (B_1) free diet was estimated from Bell and Mendel's (1) findings of the adequate minimum of a given milling product to furnish sufficient of the vitamin B complex for normal growth. In this study the different per cents of each milling product were tried with the object of determining the minimum quantity necessary to supply sufficient vitamin B (B_1) for a level of gain of 3 grams per week with no apparent symptoms of polyneuritis during an 8 week experimental period. The quantity of

protein in the particular per cent of wheat product being used was calculated, using whole numbers largely, and the casein correspondingly diminished. The remainder of the wheat was allowed to supplant an equivalent amount of starch. The formulae for the series of diets used are given in Table I.

TABLE I

SERIES OF DIETS AS TESTED

	PERCENTAGES						
No. Diet	Milling Product	Casein	Starch	Yeast*	O.& M. salts	Cod liver oil	Butter fat
1. Positive		18	53	15	4	2	8
2. Negative		18	53	15	4	2	8
3. Patent flour	60	11		15	4	2	8
4. " "	30	15	26	15	4	2	8
5. " "	10	17	44	15	4	2	8
6. First clear	20	15	36	15	4	2	8
7. " "	10	17	44	15	4	2	8
8. Low grade	5	17	49	15	4	2	8
9. " "	2	18	51	15	4	2	8
10. Middlings	3	18	50	15	4	2	8
11. "	2	18	51	15	4	2	8
12. "	1	18	52	15	4	2	8
13. Bran	4	17	50	15	4	2	8
14. "	3	18	50	15	4	2	8
15. "	2	18	51	15	4	2	8
16. Germstock	3	18	50	15	4	2	8
17. "	2	18	51	15	4	2	8
18. "	1	18	52	15	4	2	8
19. Whole wheat	10	17	44	15	4	2	8
20. " "	8	17	46	15	4	2	8
21. " "	5	17.5	48.5	15	4	2	8
22. Purified wheat germ	1	18	52	15	4	2	8

* Yeast - unautoclaved for the positive diet, No. 1., and autoclaved for all other diets.

TABLE II

RATS ON POSITIVE DIET (DIET 1)

Rat No.	Wt. at 4 weeks grams	Depletion days	Weight at end of depletion grams	Weekly gains - grams 1	2	3	4	5	6	7	8	Net gains grams	Weight at end of survival grams	Survival days	Total food intake grams	*Remarks
5311M	36	13	54	24	31	34	26	20	14	22	4	175	229	56K	791	-
5423	49	12	73	36	29	35	31	26	21	25	12	215	288	56K	1037	-
5422	53	12	71	38	28	24	41	26	16	18	10	201	272	56K	914	-
5477	43	15	45	21	33	26	26	22	16	16	17	177	222	56K	837	-
5488	41	13	68	35	25	16	26	25	14	20	21	182	250	56K	795	-
5562	39	12	57	14	44	28	22	22	31	21	12	194	251	56K	921	-
5330F	45	16	73	32	9	30	18	8	6	8	-4	107	180	56K	1191	-
5345	41	12	56	31	23	23	17	9	18	4	3	128	184	56K	928	-
5346	38	12	53	30	24	19	23	7	12	10	4	129	182	56K	825	-
5362	40	14	46	27	37	7	39	24	26	22	14	196	242	56K	907	-
5418	50	12	59	37	16	12	24	8	11	6	9	115	174	56K	649	-
5447	40	11	55	29	26	20	15	12	9	10	10	131	186	56K	946	-
5587	36	11	50	24	19	23	18	15	8	6	4	117	167	56K	787	-
Av.	42.4	12.7	58.5	29.1	26.5	22.9	25.1	17.2	15.2	14.5	8.9	159	217.5	56K	887	-

* - = Non-polyneuritic
K = Killed after 56 days on adequate diet.

TABLE III

RATS ON VITAMIN B (B_1) FREE DIET (DIET 2)

Rat No.	Wt. at 4 weeks grams	Depletion days	Weight at end of depletion grams	Weekly gains - grams 1	2	3	4	5	6	7	8	Net gains grams	Weight at end of survival grams	Survival days	Total food intake grams	*Remarks
5307M	52	23	87	-4	-5	-9						-18	69	17	62	-
5313	36	13	61	-7	-2	-13	6					-16	45	22	153	P
5337	50	16	75	-3	-7	-7	-8					-25	50	24	199	-
5356	53	15	66	-4	-12	-1						-17	49	17	85	-
5417	51	13	70	4	6	-1	-15	-15				-21	49	33	232	-
5473	40	15	58	-6	-6	-10						-22	36	15	89	-
5329	50	14	75	-5	1	-3	-11	4				-14	61	35	225	-
5397	45	12	64	-6	-4	2	-16					-24	40	28	190	-
5426	46	13	68	-4	-1	-11	-10					-26	42	26	186	-
5479	43	15	57	-12	-2	+2						-12	45	20	79	-
5517	43	15	76	-2	-5	-2	-19					-28	48	27	229	-
Av.	46.3	14.9	68.8	-4.5	-3.4	-4.8	-10.4					-20.3	48.5	24	157	-

* P = Polyneuritic
  - = Non-polyneuritic

TABLE IV

RATS ON VITAMIN B (B_1) FREE DIET PLUS 60 PER CENT PATENT FLOUR (DIET 3)

Rat No.	Wt. at 4 weeks grams	Depletion days	Weight at end of depletion grams	Weekly gains - grams 1	2	3	4	5	6	7	8	Net gains grams	Weight at end of survival	Survival days	Total food intake grams	*Remarks
5310M	39	13	63	17	25	25	12	1	-16	-10	-14	40	103	56K	725	Sl.P.
5312	36	13	52	16	13	15	4	-9	-19			20	72	41	649	Sl.P.
5341	42	12	58	17	17	18	19	-4	2	4	-7	66	124	56K	711	Sl.P.
5490	48	14	83	14	-3	0	5	-3	5	2	-2	18	101	56K	281	Sl.P.
5491	46	14	64	12	6	1	3	0	-5	2	6	25	89	56K	267	Sl.P.
5316F	36	13	54	20	23	14	5	-10	-3	-10	-18	21	75	56K	543	P.
5323	49	18	69	1	3	4	15	0	1	-7	-7	10	79	56K	330	Sl.P.
5492	44	14	75	10	6	3	-3	-11	-4	0	2	3	78	56K	266	P.
5493	38	14	63	7	5	4	7	-6	-12	-9		-4	59	44	197	Sl.P.
5494	49	13	64	17	-3	17	3	2	-9	-6	-3	18	82	56K	274	Sl.P.
5544	47	11	73	3	10	4	14	3	4	2	1	41	114	56K	350	-
Av.	43.1	13.5	65.3	12.2	9.3	9.5	7.6	-3.4	-5.1	-3.2	-4.7	23.4	88.7	56K	418	Sl.P.

* Sl.P. = Slightly polyneuritic
P. = Polyneuritic
\- = Non-polyneuritic
K = Killed after 56 days on diet

TABLE V

RATS ON VITAMIN B (B_1) FREE DIET PLUS 20 PER CENT FIRST CLEAR (DIET 6)

Rat No.	Wt. at 4 weeks grams	Depletion days	Weight at end of depletion grams	Weekly gains - grams 1	2	3	4	5	6	7	8	Net gains grams	Weight at end of survival grams	Survival days	Total food intake grams	Remarks*
5443M	44	18	71	21	12	3	-3	1	-5	-10	6	25	96	56K	317	Sl.P.
5454	55	16	86	18	25	12	6	3	-7	4	4	65	151	56K	417	-
5445F	43	17	63	11	11	19	13	16	-14	-10	-3	43	106	56K	377	-
5446	40	17	57	10	8	6	14	9	-14	-7	7	33	90	56K	322	Sl.P.
Av.	45.5	17	69.3	15.3	14	10	8.3	7.3	-10	-5.8	4.3	41.5	110.8	56K	358	-, Sl.P.

Sl.P. = Slightly polyneuritic
- = Non-polyneuritic
K = Killed after 56 days on diet

TABLE VI

RATS ON VITAMIN B (B_1) FREE DIET PLUS 5 PER CENT LOW GRADE (DIET 8)

Rat No.	Wt. at 4 weeks grams	Depletion days	Weight at end of depletion grams	Weekly gains - grams 1	2	3	4	5	6	7	8	Net gains grams	Weight at end of survival grams	Survival days	Total food intake grams	Remarks*
5442M	46	15	64	2	3	4	14	15	-4	-6	3	31	95	56K	305	-
5452	47	15	66	14	16	-3	-4	9	-8	-4	1	21	87	56K	314	-
5437	45	20	89	11	20	10	0	4	-1	-7	23	60	149	56K	399	-
5441F	40	11	67	11	10	13	3	0	6	7	-1	49	116	56K	362	-
5439	42	13	67	0	1	-1	3	8	9	5	-2	23	90	56K	282	-
Av.	44	14.8	70.6	7.6	10	4.6	4	7.2	0.4	-1.0	4.8	36.8	107.4	56K	332	-

* - = Non-polyneuritic
K = Killed after 56 days on diet.

TABLE VII

RATS ON VITAMIN B (B_1) FREE DIET PLUS 2 PER CENT MIDDLINGS (DIET 11)

Rat No.	Wt. at 4 weeks grams	Depletion days	Weight at end of depletion grams	Weekly gains - grams 1	2	3	4	5	6	7	8	Net gains grams	Weight at end of survival grams	Survival days	Total food intake grams	Remarks*
5436M	48	21	90	19	16	10	8	3	8	7	9	80	170	56K	454	-
5455	53	17	78	23	15	11	15	5	14	7	31	121	199	56K	510	-
5468	46	20	70	17	5	6	11	-5	1	-8	8	35	105	56K	335	Sl.P.
5470	42	15	54	5	10	3	7	1	-6	-9	21	32	86	56K	308	Sl.P.
5474	37	15	51	2	0	6	18	15	26	20	11	98	149	56K	401	-
5583F	45	12	65	13	6	1	-1	4	20	17	19	79	144	56K	401	-
5582	47	12	67	9	10	2	0	4	10	8	19	62	129	56K	408	-
5592	46	10	56	10	9	13	2	14	18	15	5	86	142	56K	464	-
5601	50	16	75	2	-3	21	11	11	-3	15	22	76	151	56K	475	-
5606	45	15	64	8	3	-1	9	20	19	8	12	78	142	56K	390	-
Av.	45.9	15.3	67	10.8	7.1	7.2	8	7.2	10.7	8	15.7	74.7	141.7	56K	415	-

* Sl.P. = Slightly polyneuritic
\- = Non-polyneuritic
K = Killed after 56 days on diet.

TABLE VII

RATS ON VITAMIN B (B_1) FREE DIET PLUS 1 PER CENT MIDDLINGS (DIET 12)

Rat No.	Wt. at 4 weeks grams	Depletion days	Weight at end of depletion grams	Weekly gains - grams 1	2	3	4	5	6	7	8	Net gains grams	Weight at end of survival grams	Survival days	Total food intake grams	Remarks *
5321M	42	16	76	4	4	3	-14	1	4	-1	-11	-9	67	56K	300	P+
5322	37	16	65	-4	-13	4						-13	52	21	75	Sl.P.
5334	54	13	76	-6	-11	-11						-28	48	24	206	P+
5342	40	12	59	14	7	10	12	10	6	-7	-14	38	97	56K	446	Sl.P.
5549	35	12	55	13	14	12	1	-6	-2	-11	-18	3	58	55	284	P+
5340F	45	13	72	0	1	-5	-10	4	14	9	13	26	98	56K	385	Sl.P.
5499	37	13	56	4	5	-5	-2	-14				-12	44	32	118	P+
5495	48	15	72	-2	-4	-4	-3	-4	-12			-29	43	42	158	P+
5539	40	13	62	2	2	12	1	1	-2	0	2	18	80	56K	259	Sl.P.
5547	41	12	57	2	-6	-5	-6					-15	42	23	111	P+
Av.	41.9	13.5	65	2.7	-0.1	1.2	-2.6	-1.1	1.3	-2.0	-7.4	-21	62.9	42.1	234	Sl.P., P+

* Sl.P. = Slightly polyneuritic
P+ = Severely polyneuritic
K = Killed after 56 days on diet.

TABLE VIII

RATS ON VITAMIN B (B_1) FREE DIET PLUS 3 PER CENT BRAN (DIET 14)

Rat No.	Wt. at 4 weeks grams	Depletion days	Weight at end of depletion grams	Weekly gains - grams 1	2	3	4	5	6	7	8	Net gains grams	Weight at end of survival grams	Survival days	Total food intake grams	Remarks*
5509M	50	18	88	22	19	4	-7	-10	-1	-4	-8	15	103	56K	384	Sl.P.
5508	51	18	88	23	23	6	-15	-19	-23	-1	-6	-12	76	56K	403	P+
5520	49	15	82	23	20	7	-6	-6	19	-1	2	58	140	56K	412	-
5585	38	11	53	19	10	10	15	22	18	9	4	107	160	56K	491	-
5564F	38	10	62	18	15	11	1	-4	4	1	3	49	111	56K	353	-
5565	36	10	56	9	8	25	5	-1	-2	-5	7	46	102	56K	328	-
5563	39	10	58	12	17	6	2	0	6	1	-2	42	100	56K	335	-
5595	45	10	58	17	-2	-1	2	13	13	13	5	70	128	56K	414	-
5593	45	10	58	14	10	2	-5	7	13	9	6	56	114	56K	370	-
Av.	43.4	12.4	67	17.4	13.3	7.7	-0.9	0.2	5.2	2.4	1.2	47.9	114.9	56K	388	-

* Sl.P. = Slightly polyneuritic
P+ = Severely polyneuritic
\- = Non-polyneuritic
K = Killed after 56 days on diet

TABLE VIII

RATS ON VITAMIN B (B_1) FREE DIET PLUS 2 PER CENT BRAN (DIET 15)

Rat No.	Wt. at 4 weeks grams	Depletion days	Weight at end of depletion grams	Weekly gains - grams 1	2	3	4	5	6	7	8	Net gains grams	Weight at end of survival grams	Survival days	Total food intake grams	Remarks*
5335M	54	19	78	-1	9	-9	3	4	-7	-9	11	1	79	56K	410	P.
5347	49	16	73	-6	8	-8	6	10	0	8	9	27	100	56K	580	-
5352	42	13	58	6	-3	-2	1	4	7	3	-2	14	72	56K	375	Sl.P.
5349	47	13	69	-2	0	2	-4	-2	3	5	-4	-2	67	56K	400	P.
5567	44	12	70	5	4	2	-1	-2	1	9	12	30	100	56K	255	Sl.P.
5502F	43	14	71	8	2	4	-7	-8	8	17	12	36	107	56K	298	-
5526	41	11	61	16	3	-5	-17	-8				-11	50	31	161	P.
5528	46	10	61	-1	-1	0	5	1	6	9	3	22	83	56K	245	-
5530	45	12	62	-5	-1	-8	3					-11	51	22	99	P+
5531	45	12	68	-4	4	5	4	-3	9	13	16	44	112	56K	340	-
Av.	45.6	13.2	67.1	1.6	2.5	-1.9	-0.7	-0.4	3.4	6.9	7.9	15	82.1	50.1	316	- to P+

* Sl.P. = Slightly polyneuritic
P. = Polyneuritic
P+ = Severely polyneuritic
- = Non-polyneuritic
K = Killed after 56 days on diet

TABLE IX

RATS ON VITAMIN B (B_1) FREE DIET PLUS 2 PER CENT GERMSTOCK (DIET 17)

Rat No.	Wt. at 4 weeks grams	Depletion days	Weight at end of depletion grams	Weekly gains - grams 1	2	3	4	5	6	7	8	Net gains grams	Weight at end of survival grams	Survival days	Total food intake grams	Remarks*
5471M	40	16	65	8	7	2	-8	-14	9	17	6	27	92	56K	344	Sl.P.
5481	39	14	76	28	23	5	3	1	-2	3	10	71	147	56K	471	-
5514	44	18	76	13	13	8	-3	1	-4	-1	-10	17	93	56K	341	Sl.P.
5521	48	14	80	18	3	15	5	-2	5	-8	-6	30	110	56K	347	Sl.P.
5485F	38	14	65	19	13	7	2	-3	-6	3	2	37	102	56K	341	-
5484	42	14	72	17	15	7	5	7	4	15	4	74	146	56K	484	-
5524	46	14	76	16	1	8	-12	-12	-11	-9		-21	55	49	250	P+
5538	44	13	68	26	14	19	9	10	6	0	-2	82	150	56K	521	-
5525	45	14	76	8	-1	5	4	22	-1	3	-2	38	114	56K	322	-
Av.	42.9	14.6	72.7	17	9.8	8.4	0.6	1.1	0	2.6	0.3	39.4	112.1	56K	380	-, Sl.P.

* Sl.P. = Slightly polyneuritic
P+ = Severely polyneuritic
\- = Non-polyneuritic
K = Killed after 56 days on diet.

TABLE IX

RATS ON VITAMIN B (B_1) FREE DIET PLUS 1 PER CENT GERMSTOCK (DIET 18)

Rat No.	Wt. at 4 weeks grams	Depletion days	Weight at end of depletion grams	Weekly gains - grams 1	2	3	4	5	6	7	8	Net gains grams	Weight at end of survival grams	Survival days	Total food intake grams	Remarks*
5338M	58	23	84	-4	2	-2	18	22	34	-4	-12	54	138	56K	490	-
5348	47	14	70	-1	3	-4	-11					-13	57	24	287	Sl.P.
5351	44	14	58	-3	-5	-1	1					-8	50	24	107	Sl.P.
5501	40	14	64	5	3	2	5	4	14	10	16	59	123	56K	321	-
5512	47	17	82	-3	3	3	0	-12	1	4	11	7	89	56K	497	Sl.P.
5605	46	15	79	-4	-7	-6	-16	-1				-34	45	29	158	P+
5339F	51	18	81	2	-5	-7	15	7	1	4	2	19	100	56K	433	-
5357	50	14	68	0	-12	0						-12	56	15	102	Sl.P.
5498	37	15	61	2	1	2	-5	-13	22			9	70	37	145	Sl.P.
5529	46	10	61	3	1	0	11	2	16	4	6	43	104	56K	283	-
5532	44	12	62	3	-8	2						-3	59	21	212	Sl.P.
Av.	46.4	15.1	70	0	-2.2	-1	1.8	1.3	14.7	3.6	4.6	11	81	39.1	276	-, Sl.P.

* Sl.P. = Slightly polyneuritic
P+ = Severely polyneuritic
- = Non-polyneuritic
K = Killed after 56 days on diet

TABLE X

RATS ON VITAMIN B (B_1) FREE DIET PLUS 8 PER CENT WHOLE WHEAT (DIET 20)

Rat No.	Wt. at 4 weeks grams	Depletion days	Weight at end of depletion grams	Weekly gains - grams 1	2	3	4	5	6	7	8	Net gains grams	Weight at end of survival grams	Survival days	Total food intake grams	Remarks*
5459M	48	11	71	14	22	1	9	2	7	39	15	109	180	56K	478	-
5465	50	14	62	8	-2	4	18	12	7	3	11	71	133	56K	339	-
5456F	55	16	83	10	10	4	-5	1	3	14	1	38	121	56K	330	-
5469	54	14	77	10	16	17	17	11	19	-13	7	103	180	56K	502	-
5516	47	18	80	11	9	13	2	9	-6	-1	3	40	120	56K	335	-
5515	47	18	82	10	2	17	18	5	15	-4	1	64	146	56K	424	-
5603	47	16	63	4	6	5	1	9	7	13	18	63	126	56K	388	-
5599	50	16	75	7	10	5	2	-2	1	-2	9	30	105	56K	358	-
5602	49	16	75	-4	3	-1	-3	5	4	11	30	45	120	56K	321	-
5600	50	16	82	4	5	8	-4	-1	20	16	15	63	145	56K	420	-
Av.	49.7	15.5	75	7.4	8.1	7.3	5.5	5.1	7.7	7.6	11	62.6	137.6	56K	390	-

* - = Non-polyneuritic
K = Killed after 56 days on diet

TABLE X

RATS ON VITAMIN B (B_1) FREE DIET PLUS 5 PER CENT WHOLE WHEAT (DIET 21)

Rat No.	Wt. at 4 weeks grams	Depletion days	Weight at end of depletion grams	Weekly gains - grams 1	2	3	4	5	6	7	8	Net gains grams	Weight at end of survival grams	Survival days	Total food intake grams	Remarks*
5320M	49	16	77	0	-6	-11						-17	60	21	150	-
5343	38	12	52	4	23	16	-11	12	18	-10	-29	23	75	56K	416	P+
5344	38	12	57	-2	8	9	-2					13	70	26	253	P+
5568	44	10	67	10	5	-4	-18	-6				-13	54	29	133	P+
5576	39	10	60	1	6	3	-1	-4	-5	-12		-12	48	48	221	P+
5575	45	10	63	6	4	-1	-21	-2				-14	49	29	125	P+
5324F	45	16	71	4	-3	-4	-11	5	9	0	3	3	74	56K	522	Sl.P.
5325	45	16	75	-1	3	3	-2	1	6	-15	-10	-15	60	56K	415	P+
5566	36	10	53	-1	5	-1	-7	-11				-15	38	32	111	P+
Av.	42.1	12.4	63.9	2.3	5	1.1	-9.1	-7	7	-9.3	-12	-5.2	58.7	39.2	261	P+

* Sl.P. = Slightly polyneuritic
P+ = Severely polyneuritic
\- = Non-polyneuritic
K = Killed after 56 days on diet

TABLE XI

SUMMARY OF DATA COLLECTED FOR ANIMALS ON THE VARIOUS DIETS (FIGURE 3)

No.	Diet		Wt. at 4 weeks grams	Depletion days	Weight at end of depletion grams	Weekly gains - grams 1	2	3	4	5	6	7	8	Net gains grams	Weight at end of survival grams	Survival days	Total food intake grams	Remarks *
1.	Positive		42.4	12.7	58.5	29.1	26.5	22.9	25.1	17.2	15.2	14.5	8.9	159	217.5	56K	887	-
2.	Negative		46.3	14.9	68.8	-4.5	-3.4	-4.8	-10.4					-20.3	48.5	24	157	-
3.	Patent flour	60%	43.1	13.5	65.3	12.2	9.3	9.5	7.6	-3.4	-5.1	-3.2	-4.7	23.4	88.7	56K	418	Sl.P.
4.	" "	30	45.6	13.6	70.4	3.6	-0.6	-7.2	-12.4	-4				-22.2	48.2	32.2	138	P†
5.	" "	10	44.9	14.6	68.2	-2.6	-6.8	-7.8	-3.2					-17.2	51	19.8	129	Sl.P. to P†
6.	First clear	20	45.5	17	69.3	15.3	14	10	8.3	7.3	-10	-5.8	4.3	41.5	110.8	56K	358	-, Sl.P.
7.	" "	10	44.3	12.2	65	2.2	0	-3.8						-11.3	53.7	24.7	191	P
8.	Low grade	5	44	14.8	70.6	7.6	10	4.6	4	7.2	0.4	-1.0	4.8	36.8	107.4	56K	332	-
9.	" "	2	47.2	17	71.2	-4.6	-9.8	-1.5						-19.4	51.8	21.6	157	P†
10.	Middlings	3	46.1	11.5	68.8	15.8	15.4	18	11.4	19.3	5.3	2.5	5.9	83.6	152.4	56K	456	-
11.	"	2	45.9	15.3	67	10.8	7.1	7.2	8	7.2	10.7	8	15.7	74.7	141.7	56K	415	-
12.	"	1	41.9	13.5	65	2.7	-0.1	1.2	-2.6	-1.1	1.3	-2.0	-7.4	-2.1	62.9	42.1	234	Sl.P., to P†
13.	Bran	4	50.3	14.3	76	14.3	12.8	11.8	15.3	8.6	8	7	3	78.8	154.8	56K	517	-
14.	"	3	43.4	12.4	67	17.4	13.3	7.7	-0.9	0.2	5.2	2.4	1.2	47.9	114.9	56K	388	-
15.	"	2	45.6	13.2	67.1	1.6	2.5	-1.9	-0.7	-0.4	3.4	6.9	7.1	15	82.1	50.1	316	- to P†
16.	Germstock	3	44	11.3	63.8	23	18.1	21.5	14.5	12.3	14.3	13.5	10.8	128.1	191.9	56K	587	-
17.	"	2	42.9	14.6	72.7	17	9.8	8.4	0.6	1.1	0	2.6	0.3	39.4	112.1	56K	380	-, Sl.P.
18.	"	1	46.4	15.1	70	0	-2.2	-1	1.8	1.3	14.7	3.6	4.6	11	81	39.1	276	-, Sl.P.
19.	Whole wheat	10	45.4	14.6	74.8	12.6	11.4	10.8	5	-2.2	4.6	10.2	12.2	64.6	139.4	56K	401	-
20.	" "	8	49.7	15.5	75	7.4	8.1	7.3	5.5	5.1	7.7	7.6	11	62.6	137.6	56K	390	-
21.	" "	5	42.1	12.4	63.9	2.3	5	1.1	-9.1	-7	7	-9.3	-12	-5.2	58.7	39.2	261	P†
22.	Purified wheat germ	1	43	15.2	70.4	10.8	1.8	2.6	0.8	-8	-15	-6		-8	62.4	47.6	265	P†

* Sl.P. = Slightly polyneuritic
P = Polyneuritic
P† = Severely polyneuritic
- = Non-polyneuritic
K = Killed after 56 days on diet.

TABLE XII

AN ATTEMPTED ESTIMATE OF THE DISTRIBUTION OF VITAMIN B (B_1) IN MILLING PRODUCTS OF KANSAS WINTER WHEAT

Product	Min. % adequate in diet Sherman standard	Concentration of vitamin as referred to whole wheat: B (B_1) this study Sherman standard	B (Complex) Bell and Mendel normal growth	B (B_1) Plimmer maintenance pigeons (b)
Whole wheat	7 to 8	1	1	1
Patent flour	60	0.12 to 0.14	0.0 to 0.10 (?)	
First clear	20 or less	0.35 to 0.40	1 to 0.67 (?)	
Low grade	5 or less	1.5	(a) 1 (Second clear) 2 (Low grade)	
Middlings	1.5 to 2	4	4	(a) 1.3
Bran	2.5 to 3	2.5	2	(a) 1.3
Germstock	1.5 to 2	4	(a)	(a) 7

(a) The wheat and the milling fractions were not identical.

(b) A basal diet was used, composed of fish meal, white flour and white rice.

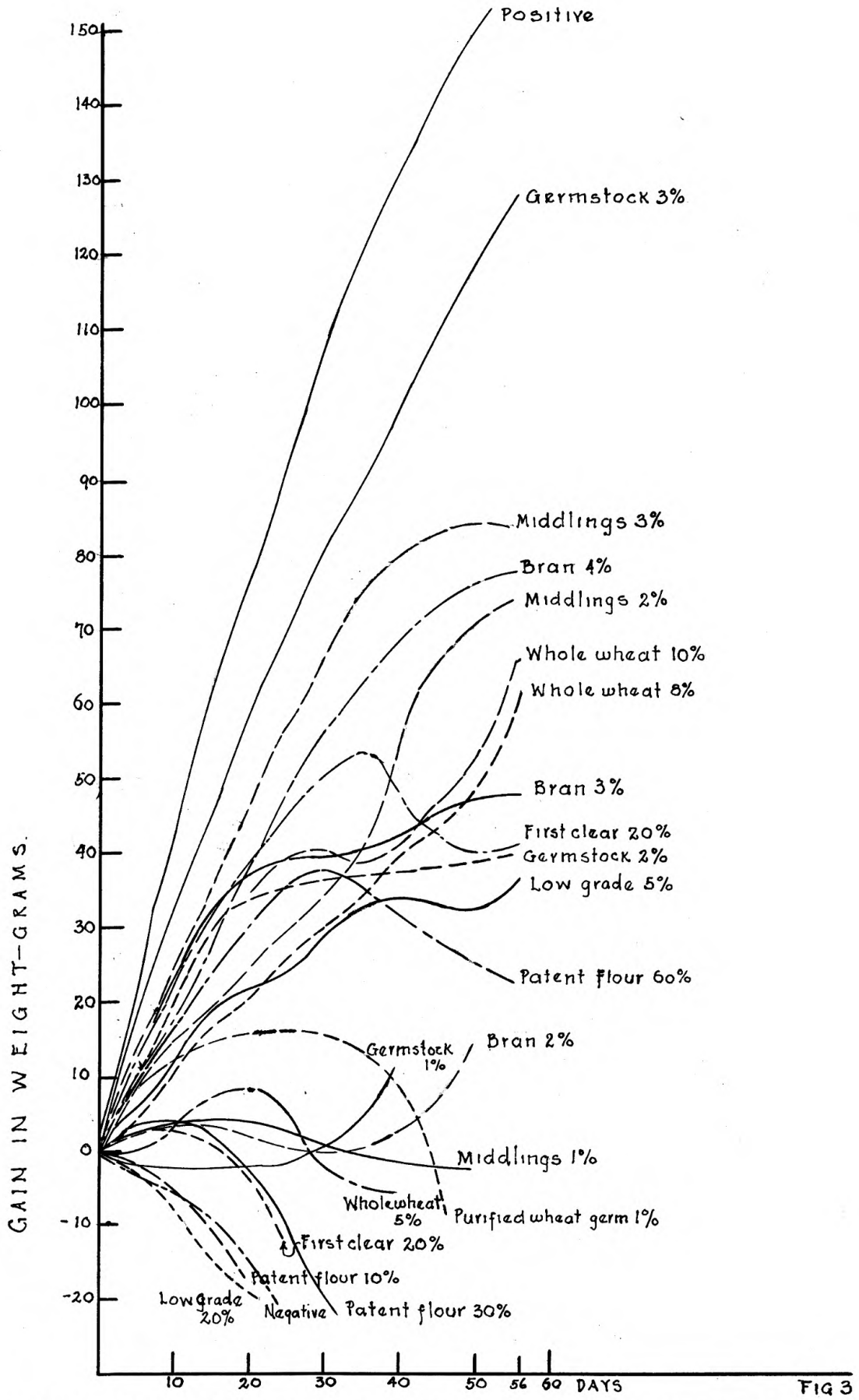

FIG 3

AVERAGE GAIN CURVES OF ANIMALS FED ON THE VARIOUS DIETS

Fig. 1. Typical polyneuritic animal on diet containing 1 per cent middlings.

Fig. 2. Typical healthy animal on positive diet.

DISCUSSION

The summaries in Tables XI and XII and Figure 3 show the results obtained. The individual gains for the most significant groups of animals are included in Tables II to X.

Rats, four weeks of age and weighing 35 to 55 grams, after a depletion period usually of 12 to 17 days, weighed on the average 69 grams at the beginning of the experimental period. Increases in gains in weight summarized in Table XI show corresponding increases in total food consumption.

Polyneuritis occurred quite regularly among the animals deficient in vitamin B (B_1), with a definite gradation of symptoms correlated with the extent of the vitamin deficiency in the food supplied. Polyneuritic symptoms varied in severity from the curved spine, spastic gait and head retractions to a complete loss of coordination followed by convulsions. An autopsy usually revealed crowded chest organs, an unhealthy condition of the alimentary canal, and intestinal hemorrhage. Animals on a diet deficient in vitamin B (B_1) did not survive the 8 week experimental period. The amount of vitamin which allows minimal growth according to the Sherman standard, was frequently found insufficient for protection from the polyneuritic symptoms. Figure 1 shows a typical polyneuritic animal after 7 weeks

on a diet containing 1 per cent middlings and deficient in vitamin B (B_1). Figure 2 shows a typical healthy animal of the same age on a positive diet, adequate and planned to be optimal in all respects.

When non-autoclaved bakers' yeast was used in the positive diet as a source of vitamin B (B_1) no polyneuritis occurred. All of the animals, as shown in Table II and Figure 3 made high gains with a correspondingly large intake of food for the 8 week survival period.

On the basal vitamin B (B_1) free diet (Table III) the negative controls declined rapidly and died within an average of 24 days. The two surviving beyond 30 days might have been practicing coprophagy. The majority died, evidently of starvation accompanied by intestinal hemorrhage, before the characteristic symptoms of polyneuritis occurred. These observations are in agreement with Sandels (21) and Chase (4) who found that when vitamin B (B_1) is absent or very low in the diet, the animals die of inanition before polyneuritis occurs.

Patent flour, as shown in Tables IV and XI, contains definite but small measurable amounts of vitamin B (B_1). The animals receiving 60 per cent of patent flour made the minimal gain of 3 grams per week, but were not protected from the typical symptoms of polyneuritis. On diets containing 30 and 10 per cent patent flour respectively

(Table XI) the average survival periods of 20 to 32 days were accompanied by average net gains of -17 to -22 grams and severe polyneuritis.

On 20 per cent first clear (Tables V and XI) the animals made good gains the first half of the survival period with a decline in weight the last half. Here again the amount of vitamin which allowed more than approximate minimal growth proved insufficient for protection from slight polyneuritis. With 10 per cent first clear the animals survived an average of only 24 days and were polyneuritic.

The animals on 5 per cent low grade, as noted in Tables VI and XI, made slightly more than minimal growth with no apparent polyneuritic symptoms. With 2 per cent low grade the rats survived an average of 22 days with a net gain of -19 grams.

An allowance of 2 per cent middlings furnished more than enough vitamin B (B_1) for minimal growth, with just enough in most cases to protect from polyneuritis. Tables VII and XI compare the results of an adequate diet containing 2 per cent middlings with those of an inadequate diet of 1 per cent middlings.

The average of the animals on 3 per cent bran (Tables VIII and XI) were above the Sherman standard, with nearly all of those on 2 per cent bran surviving the full period with an average net gain of 15 grams, but showing poly-

neuritic symptoms. A diet containing 4 per cent bran resulted in too high gains to be of value in this study.

As seen in Tables IX and XI, the animals receiving 2 per cent germstock were slightly above the Sherman standard for growth, with those on 1 per cent slightly below, and both groups showing polyneuritic symptoms. Three per cent germstock proved definitely too much.

All animals (Tables X and XI) receiving 8 per cent whole wheat made more than minimal growth with no signs of polyneuritis. The 10 per cent allowance of whole wheat did not result in a corresponding increase in net gains. Rats on 5 per cent whole wheat appeared severely polyneuritic after their average survival period of 39 days. From this data an estimate of 6 to 7 per cent whole wheat should prove sufficient for minimal gain and protection of rats over an 8 week survival period.

One per cent of purified wheat germ, a commercial product, allowed a slightly longer survival period but less net gain and more severe polyneuritis than 1 per cent germstock (Table XI).

Composite growth curves as shown in Figure 3, using the average weekly and net gains in grams and the average survival period in days, were prepared for comparisons of all the diets used. From this data, estimates of the minimum percentages of the milling products of wheat adequate in

the diet, using the Sherman standard, were listed in tabular form in Table XII. With 7 to 8 per cent of whole wheat taken as the minimum requirement for growth and used as the standard for comparison, the concentration of the vitamin B (B_1) was estimated for the products used and compared in Table XII to other data on the vitamin content of wheat and its milling products. Middlings, bran and low grade were richer than the whole wheat in decreasing order as named. Patent flour and first clear were less rich than the whole wheat. These findings are not widely divergent from those of Bell and Mendel on the vitamin B complex. The findings of Plimmer et al cite a slightly lower figure for bran and middlings and a somewhat higher figure for wheat germ. Germstock, similar to the middlings fraction but containing practically all of the germ, was about four times as rich as whole wheat.

The vitamin B (B_1) content of patent flour is important on account of the wide use of this highly milled product. Bell and Mendel (1) considered the vitamin B content of patent flour to be very low, questioning its presence in a measurable amount. Plimmer and his co-workers (17) considered patent flour to be almost without vitamin B (B_1). For their 26 week maintenance experiments with pigeons, in addition to the food measured for vitamin B (B_1) content, they say, "The remainder of the diet can be white

rice, or white flour, or fishmeal or any foodstuff without vitamin B." From this study, patent flour has been shown to contain definite, but small measurable amounts of vitamin B (B_1). This is physiologically significant in view of the widespread use of highly milled patent flours in the American diet.

SUMMARY AND CONCLUSIONS

A study was made to quantitatively determine the anti-neuritic vitamin B (B_1) of Kansas winter wheat and its milling products by means of growth experiments with young rats, according to newer methods.

Various amounts of the milling products were incorporated to a basal vitamin B (B_1) free diet including vitamin G, to determine at what level minimal growth was obtained. A gain of at least 24 grams with no apparent symptoms of polyneuritis during an 8 week experimental period, was adopted as a favorable guide as recommended by Sherman and co-workers. This suggestion was not entirely confirmed in this study in that this gain usually did not protect the animal from polyneuritis. On diets deficient in vitamin B (B_1) a definite gradation of symptoms was correlated with the extent of the vitamin deficiency in the food supplied.

Composite growth curves were prepared for comparisons of all the diet used. From this data it was estimated that

minimal growth resulted when 7 to 8 per cent of whole wheat, 60 per cent of patent flour, 20 per cent or less of first clear, 5 per cent or less of low grade, 1.5 to 2 per cent of middlings and also of germstock, and 2.5 to 3 per cent of bran were used as the sole source of vitamin B (B_1) in the diet.

The approximate concentration of vitamin B (B_1) in each product was calculated on the basis of the minimal amount of whole wheat adequate in the diet. Patent flour contained small measurable amounts of the vitamin; first clear was about 0.4 as rich; low grade was 1.5 as rich; middlings and germstock were 4 times as rich and bran 2.5 as rich as the whole wheat.

The results of this study are applicable to humans in that the modern diet tends to be deficient in vitamin B (B_1) due to the use of less cereal grains and those the more highly milled products. The presence of small but measurable amounts of vitamin B (B_1) in patent flour is therefore physiologically significant.

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