THE EFFECTS OF VITAMINS B AND G UPON THE MEALWORM

TENEBRIO MOLITOR L.

by

AGNES JEANNE LYON

B. S., Kansas State Agricultural College, 1929

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

KANSAS STATE AGRICULTURAL COLLEGE

1930

TABLE OF CONTENTS

	Page
INTRODUCTION	2
REVIEW OF LITERATURE	5
History of Vitamin B	5
Nutritional Requirements of Insects	8
METHODS	13
DATA	14
Experiments to Control Diet	14
Data on the Nutritional Requirements of Insects	15
DISCUSSION	32
SUMMARY	36
ACKNOWLEDGMENTS	37
LITERATURE CITED	38

INTRODUCTION

Every year the feeding habits of insect pests cause enormous losses to cultivated plants, domestic animals and human life. It would seem, therefore, that the problem of the nutrition and the metabolism of insects should be regarded as a key both to the successful control of injurious insects and to the progress of the industries dependent on the products of useful insects. The greater part of the work done so far on the feeding habits or the digestion on insects is purely descriptive, anatomical or histological. Very little work has been done on insect nutrition from the standpoint of chemistry and physiology. This paper has for its purpose the presentation of the influence of a diet or diets upon the growth of the larval stage of an insect.

With an increasing understanding of the complexity of food constituents and the nutritional requirements of higher animals the science might be accelerated if it had a more complete evolutionary foundation. Bayliss holds that increasing complexity of organisms in the course of evolution is associated with lessened ability to synthesize compounds of which they consist, lower animals should be expected to have the simpler and more easily understood food needs. So far, actual experiments do not permit expansion of this point of view, but the work of Wulzen on the nutrition of planarian worms may be a forerunner of future ventures into this field.

The rather contradictory results so far obtained in regard to the vitamin requirements of insects may be due to the use of the same principles and methods of study on insects as are used in the case of higher animals. It has been assumed that the vitamins required by higher animals and by insects must be the same, and only vitamins from the sources usual in higher animals have been tested. The differences in physiology between insects and higher animals are so great that it is permissible to suggest that the vitamins required by insects may be essentially different from those necessary for higher animals, and that their sources may be also very different. It would be of interest to try to separate vitamins specific to insects, if they exist. They may be identical with those already known and in the case of insects feeding on flour they apparently are the same. The diets of insects differ so much that their vitamin requirements may differ widely.

The object of this work is to present the results from a study of the nutritional requirements of the common mealworm _Tenebrio molitor_ Linnaeus. The general plan of the experiments has been a study of the effects on growth of modification of food materials obtained by extraction and of rations consisting of purified food stuffs. Especial attention has been given to the effects of vitamins B and G.

Tenebrio molitor is a nearly cosmopolitan member of the family Tenebrionidae belonging to the order Coleoptera. The eggs are deposited singly or in bunches in the meal or cereal. The eggs hatch in from ten to fourteen days depending on the temperature. The tiny white mealworms begin to feed at once and soon turn a glossy waxy yellow characteristic of the larval stage. The larval stage may last three months or more and the worms are twenty-five millimeters or more in length when full grown. The pupal stage lasts from one to two weeks depending on the temperature. The adult is a shining black beetle and about fifteen millimeters in length. The larval stage is a period of growth and for this reason the growth of the larva upon different foods may be taken as an index of the adequacy of the foods for the insect.

REVIEW OF LITERATURE

History of Vitamin B

Takaki Kanehiro, Director General of the Japanese Navy, in 1887 recognized beri-beri as a dietary disease and changed the food for Japanese sailors to eliminate beri-beri.

Eijkman (1889), Director of the Hygienic Laboratory, Dutch East Indies, observed a disease in fowls which he considered analogous to beri-beri. Upon investigating into its ethiology, he found that it could be produced experimentally in fowls on a diet of polished rice. He called the disease polyneuritis gallinarum. The theory he advanced was that the starch in the cereal grain gave rise to toxins which exerted a deleterious effect upon the nervous system.

Grijns (1898) rejected the amylum-toxin theory and attributed the disease simply due to a dietary deficiency. Grijns also showed that a curative agent could be extracted from rice polishings, and that other natural foods contained this substance.

Funk (1911) separated out the active agent from rice bran by extraction with alcohol containing hydrochlorid acid. This cured polyneuritis induced in birds fed on a diet of polished rice. After confirming his observations during the next year he named the substance vitamin.

Emmett and Luros (1920) 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 antineuritic vitamin seemed to be less stable to heat and alkali than the rat growth-promoting vitamin.

Funk and Dubin (1921) briefly recorded some experiments upon fractional absorption which led them to believe that autolyzed yeast contained two water-soluble vitamins, of which the antineuritic was the more readily absorbed by fuller's earth.

Kinnersley and Peters (1925) isolated an 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.

When Goldberger, Wheeler, Lillie, and Rogers (1925) first postulated the existence of a substance P-P, pellagra preventive, it seemed to have slight connection with vitamin B. The next year they advanced the theory that water soluble B, that is the growth promoting substance, consisted of two factors; the one an antineuritic principle for which they retained the term vitamin B, and the other a pellagra preventive which they had already named P-P. The best chemical distinction made between the two was their differential behavior to heat; the vitamin B being thermolabile, the factor P-P being thermostable.

In 1926, Hauge and Carrick published further evidence of the fact that antineuritic and growth-promoting do not always run parallel. In the same year the subject was greatly clarified by three contributions from the laboratory of McCollum, all of which emphasized the important fact that the growth of rats requires both the antineuritic vitamin and another more heat-stable substance which belongs with the antineuritic substance hitherto covered by the name of vitamin B.

Chick and Roscoe (1927) 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. Hassen and Drummond (1927) in the course of a study of the possible relation between vitamin B and protein metabolism, independently obtained evidence of the multiple nature of vitamin B.

The existence of two factors was recognized by Sherman and Axtmayer (1927) who treated them as two primary vitamins rather than as subordinate parts of a vitamin complex. They suggested the names vitamin F for the antineuritic thermolabile factor and vitamin G for the thermostabile factor.

The term vitamin B now seemed to be the group name for vitamins F and G so controversy arose as to the standard nomenclature of these factors. The committee on vitamin B terminology of the American Association of Biological Chemists (1929) voted to recommend that (1) the term B be restricted to designate the more heat-labile antineuritic factor, (2) that the term G be used to denote the more heat-stable, water soluble, dietary factor called P-P, pellagra preventive, factor by Goldberger and associates, and which has to do with maintenance and growth. This is the nomenclature that has been followed in this work.

Nutritional Requirements of Insects

The nutritional requirements of insects have been studied by investigators in order to better understand their environments and to determine their usefulness as experimental animals in other types of study such as genetics and economic control. Attractive characteristics are the small amount of food required, short life cycles, and simple laboratory requirements for rearing.

Most of the work done on this problem refers to the fly Drosophila, which feeds on yeast developing in fermenting fruit and similar substances. Delcourt and Guyenot (1911) reported a method of rearing aseptic cultures of flies upon potato and dead brewers' yeast. Potato alone produced a few, stunted, and sterile adults. Guyenot (1913 and 1917) showed as the result of some extensive and carefully arranged experiments with Drosophila ampelophila on various synthetic media that no artificial food is complete unless the substances contained in filtered autolysate of yeast are added. A series of experiments to isolate the active elements of the autolysate was without success. It was concluded that it was a vitamin of yeast the absence of which retards the development of larvae and causes their death. He suggested the similarity of the essential fraction of yeast to Funk's vitamin. Loeb and Northrop (1916) verified these findings regarding the value of yeast and its extracts in the diets of this insect. Baumberger (1919) reported that the essential protion of yeast was its nucleo-protein, but the growth-promoting factor may have been present in his preparation. Bacot and Harden (1922) used a purified basal diet of caseinogen, starch, salts, and cane sugar, all extracted with alcohol, and reared aseptic cultures of flies when yeast or its alcoholic extract together with butter fat were added. The results showed that the insect requires for its complete development the presence of vitamin B (yeast), but not that of the vitamin C (lemon juice). Preliminary experiments with vitamin A did not show definitely whether the vitamin was needed or not, but it seemed as though it might not be needed.

The nutritional requirements of blow flies, _Calliphora vomitoria_ have been investigated by Bogdanow (1908) and Wollman (1919). The latter bred the larvae on brains sterilized at 130° C. for 45 minutes. Contrary to expectations the larvae developed much better than if fed on meat sterilized at 115° C. In a control experiment to determine whether the sterilization was sufficient to destroy all of the vitamins in the brains rats were used. The results showed that not all of the vitamins were destroyed and Wollman concluded that the larvae of the flesh-fly are able to concentrate vitamins while feeding on a diet deficient in them.

Insects infesting cereals and their products offer great possibilities for experimental use because of the nature of their food habits. Portier (1919) was able to rear Tenebrio molitor larvae as rapidly on food sterilized at 130° C. as on food that was not sterilized. His theory was that the larvae of this insect have in the epithelial tissue of the digestive tract symbiotic microörganisms. Passerini (1925) found that the larvae of Tenebrio molitor can not develop on wheat flour made of grain deprived of husks, but do develop rapidly if even small amounts of husks or other vitamin containing foodstuffs are added. Chapman (1924) has conducted extensive research with the confused flour beetle, Tribolium confusum Duval. He used purified diets consisting of corn starch, various proteins, salts, and wheat germ or vitamin B containing factor. A diet containing starch, wheat gluten, wheat germ, vitamin B in dextrin, and salts gave almost the same rate of development as the normal food. A diet of the same kind, but without the wheat germ gave almost normal development up to the time of pupation, but then the larvae all died. The vitamin extracted from the wheat germ did not give the results that the wheat germ gives, so that the latter must be of importance also as a source of protein. None of the basal rations of Chapman's work contained fat. Sweetman and Palmer (1928) found that the addition of fat considerably accelerates growth. Vitamin A did not seem to be essential as crisco was as efficient as butter fat. In Chapman's work the single purified proteins were probably incomplete and the wheat germ covered an amino acid deficiency. This meant that vitamin B was not demonstrated to be unessential and further evidence to this effect was afforded by the fact that only rarely did an adult emerge on a purified ration lacking wheat germ or its extract. Sweetman and Palmer using yeast and alcoholic extracts of wheat germ demonstrated that the confused flour beetle is very sensitive to even as small an amount as 0.5 per cent of a source of the yeast vitamins (B and G). He found that these vitamins are essential to normal growth and reproduction.

Richardson (1926) working with the Mediterranean flour moth, Ephestia kuehniella, reports that whole wheat flour extracted with chloroform produced only a few larvae which developed into moths. More careful extraction removed all of the growth-promoting substances so that all of the larvae died at an early age. If the extract was returned the larvae grew normally. When whole wheat was extracted with chloroform or anhydrous ether and supplemented with an ether extract of egg-yolk the larvae grew normally. The ether soluble part of butter did not permit normal growth. Butter oil permitted growth at a somewhat reduced rate. Highly milled patent wheat flour retarded the development, but an addition of an alcoholic extract of yeast caused normal growth and weight. These experiments seem to indicate that ether and chloroform both remove from whole wheat the substance or substances similar or identical to vitamin A. The same vitamin A contained in butter and egg-yolk promotes growth. Steenbock and Coward (1927) have pointed out that wheat may contain a small amount of this vitamin. Richardson's experiments may not have been as carefully controlled as they might have been.

So far, it seems that vitamins C and D are not essential in insect nutrition. The work of Guyenot, Northrop, and Bacot and Harden shows that Drosophila requires vitamin B. The investigations of Chapman, Richardson, Sweetman and Palmer shows that vitamin B is necessary to two cereal infesting insects. It has not been conclusively proved whether vitamin A is essential or not. Sweetman and Palmer report that it is not necessary in the case of the confused flour beetle, <u>Tribolium confusum.</u>

METHODS

Larvae approximately 14 to 17 millimeters in length of the species <u>Tenebrio molitor</u> Linnaeus were used in this experiment. The larvae were obtained from the General Biological Supply House at Chicago.

The experiments were kept in the basement and at a minimum temperature of between 70-80° F. Each mealworm was placed in a one-ounce tin salve box together with about two grams of food. Moisture was supplied every other day by placing a film of distilled water in the lid of each box.

At the beginning of the experiment each larva was measured by means of a scale tracing wheel used on drawings made six times natural size using a micro-photographic apparatus. Thereafter measurements were made every ten or fourteen days. The larvae were examined daily for moults.

Series A included 273 larvae started on June 1, 1930, and June 23, 1930. Series B included 150 larvae started on June 30, 1930.

A basal ration devised by Sweetman and Palmer for use with <u>Tribolium confusum</u> was used supplemented with alcoholic and aqueous extracts of whole wheat flour together with yeast, also yeast, and autoclaved yeast. The yeast was autoclaved at 15 pounds pressure for three hours. When yeast is mentioned reference is to the pure dried product of the Northwestern Yeast Company, Chicago. The basal ration is as follows:

B-free casein	28 per cent
Osborne Mendel Salts	4 per cent
Dextrin	65 per cent
Yeast	3 per cent
Crisco	10 per cent

Young white rats were used to check the potency of the yeast. Two rats were placed on the following diet:

B-free casein	18 grams
Polished rice flour	68 grams
Crisco	6 grams
Cod liver oil	4 grams
Hogan's salt mixture	4 grams
Water	150 grams
Yeast (In control)	6 grams

The yeast was fed to the control rat during the entire experiment, but it was not fed to the first rat until it was definitely losing weight. The rats were weighed every other day.

DATA

Experiments to Control Diet

In order to be assured that the yeast used in these experiments contained the vitamins under investigation in sufficient quantities, a control experiment was run using rats, since the effects of vitamins B and G on rats are already well known. The rats used were males of the same litter.

Rat Number 1 was fed on a vitamin B and G free diet consisting of polished rice flour, vitamin free casein, Hogan's salt mixture, Crisco, and cod liver oil; Rat Number 2 received in addition 6 grams of yeast. Within 20 days Rat Number 1 was definitely losing in weight and so his diet was changed to include yeast. Table I and the graph (Fig.1) will show the slow gain in weight of the first rat, the losing of weight, and the gain when given yeast. The same table and graph shows the steady increase in weight of the second rat until by the twentieth day the second rat was almost twice as heavy as the first rat. Figure 2 shows something of the relative sizes of the two rats on the twentieth day. This experiment shows that the yeast was active for vitamins B and G.

Data on the Nutritional Requirements of Insects

In all, twelve experiments were run. Experiments II, III, and IV consisted of 50 larvae on each of the three diets, basal ration plus whole yeast, basal ration plus autoclaved yeast, and basal ration without yeast. The basal ration used in any of the synthetic diets was made up of vitamin free casein, Osborne Mendel salts, fat and dextrin. Controls (Experiments V and VI) were respectively fed upon a natural food, whole wheat flour, and kept without food.

Table I. Weights of Rats to Check Diet

Date	Number 1 B and G lacking	Number 2 B and G present
June 28	31	30.5
June 30	38.3	38.3
July 2	45	46
July 4	49	57
July 7	52	71
July 9	52	75
July 12	52	83
July 14	51	83
July 16	53	90
July 18	49	93
July 21	57	98

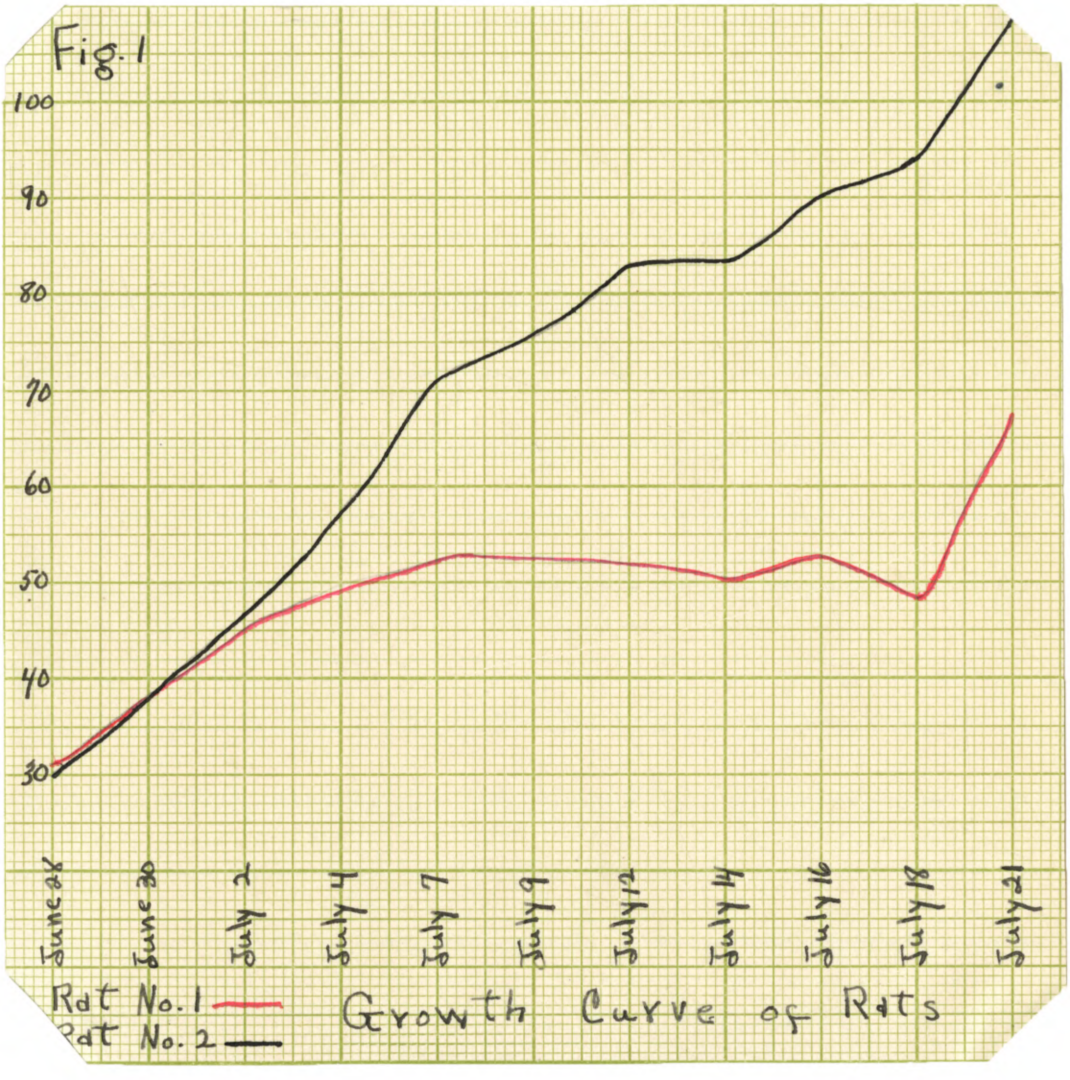
Fig. 1
100
90
80
70
60
50
40
30
June 28
June 30
July 2
July 4
July 7
July 9
July 12
July 14
July 16
July 18
July 21
Rat No. 1
Rat No. 2
Growth Curve of Rats

Figure 2.

Since a large number of the larvae in these experiments died, due to the development of mould in their cultures, another series of experiments (IX-XII) was begun using the same diets except that 5 per cent instead of 3 per cent yeast was used. Also there were 25 instead of 50 larvae on each diet. The larvae kept without food, Experiment VI, were regarded as a control to both series of experiments.

Since in the series of Experiments II-VI it was obvious that the larvae on whole wheat flour made better gains than any of those on synthetic diets two more experiments were begun to determine whether some factor in whole wheat flour, other than vitamins B and G supplied by yeast, but necessary for the optimum growth of the larvae could be extracted and added to the synthetic ration. In Experiment VII the basal ration was supplemented with 3 per cent yeast and 2 per cent dried aqueous extract of whole wheat flour. In Experiment VIII the basal ration was supplemented with 3 per cent yeast and 2 per cent dried alcoholic extract of whole wheat flour. These experiments were controlled by Experiment IX in which 5 per cent yeast was added to the basal ration so that the amount of yeast added to the control would equal the amount of extract added to the experiment thus eliminating the possibility of any effects of the extract being due to a quantitative increase in vitamins B and G.

Experiment XIII was undertaken in order to determine whether the presence of other insects in the culture would affect the rate of growth of the Tenebrio larvae. Tenebrio larvae are said to be in part predatory. According to Sweetman and Palmer the synthetic diet plus yeast, as used in these experiments, produces growth of Tribolium confusum equal to their growth on natural food, therefore, when it became apparent that the growth of Tenebrio larvae on this same food did not compare with their growth on natural food the question naturally arose as to whether the Tenebrio larvae might not thrive better in the presence of Tribolium, the Tribolium utilizing the synthetic diet and, in turn, furnishing food for the Tenebrio. In this experiment 20 Tenebrio larvae were placed in boxes of synthetic diet to each of which had been added 10 adult and 10 larval Tribolium.

The following tables show the length of each larva at the times of measuring, the gain or loss in millimeters during the intervals, the total gain during the experiment, the average gain and the per cent of individuals gaining.

Table II. Basal Ration Plus Yeast

Number	June 1 Length	June 14 Length	Gain or Loss	June 28 Length	Gain or Loss	July 7 Length	Gain or Loss	July 19 Length	Gain or Loss
2	18	20	2	23	5	p			
4	20	20.5	0.5	d					
5	24	28.5	4.5	d					
6	20.5	19	-1.5	d					
8	22.5	23.5	1	d					
9	20	25.5	5.5	25	5	p			
12	18	23	5	21	3	d			
13	16	20	4	24.5	8.5	26.5	10.5	p	
14	16.5	16.5	0	d					
22	13	12	-1	d					
24	19	20	1	23	4	23	4	p	
27	20.5	24.5	4.5	26	6	p			
30	22	22	0	d					
31	26	24	-2	23	-3	25	-1	24.5	-1.5
33	20.5	22	1.5	19	-1.5	d			
34	23	22	-1	d					
36	23	26	3	d					
39	19.5	23	3.5	p					
40	17	19	2	d					
41	13.5	15	1.5	13	-0.5	13	-0.5	10.5	-3
42	19	21	2	d					
44	24	23	-1	d					
46	16	18	2	20.5	4.5	22.5	6.5	p	
48	16	0	d						
49	20	23.5	3.5	24	4	p			
50	19.5	19	-0.5	d					
Total Gain			40.0		35.0		19.5		-4.5
Average Gain			1.54		3.2		3.9		-2.25
Per Cent Gaining			65.0		72.7		60.0		

During first two weeks 23 died and one pupated.

Table III. Basal Ration Plus Autoclaved Yeast

Number	June 1 Length	June 14 Length	Gain or Loss	June 28 Length	Gain or Loss	July 7 Length	Gain or Loss	July 19 Length	Gain or Loss
54	21	23	2	22	1	p			
55	23	23	0	d					
57	16.5	21	4.5	23	6.5	24	7.5	p	
62	22	21	-1	d					
64	19	21	2	23	4	24.5	5.5	p	
65	17	21.5	4.5						
66	22.5	24	1.5	26.5	4	27	4.5	30.5	8
67	26	27	1	p					
69	22.5	25	2.5	d					
70	21	20.5	-0.5	d					
73	24	28	4	d					
74	19	21.5	2.5	21	.2	22	3	24	5
75	25	23	-2	d					
76	23	23	0	d					
77	22	20	-2	d					
82	22	22	0	d					
83	24	25	-1	d					
84	19.5	17.5	-2	d					
86	24.5	24.5	0	d					
87	18	19	-1	d					
88	25	25	0	24	-1	23	-2	d	
89	18.5	21	2.5	22.5	4	24.5	6	p	
90	19	23	4	d					
91	25.5	26.5	1	d					
92	19.5	26	6.5	p					
94	21.5	21.5	0	d					
95	22	25	3	p					
96	16	18.5	2.5	d					
99	22	25	3	d					
100	22	22.5	0.5	d					
Total Gain			38		20.5		24.5		13
Average Gain			1.27		3		4.1		6.5
Per Cent Gaining			57		85		83.3		

During first two weeks 20 died.

Table IV. Basal Ration (without yeast)

Number	June 6 Length	June 22 Length	Gain or Loss	July 7 Length	Gain or Loss	July 19 Length	Gain or Loss
101	21.5	21	-0.5	20	1.5	p	
103	20	20.5	0.5	d			
105	18	23	5	27	9	p	
106	13	10.5	2.5	d			
108	19	16.5	2.5	d			
110	16	19	3	d			
113	18	19	1	14.5	3.5	d	
118	21	25	4	d			
120	23.5	24	0.5	d			
122	22	23.5	1.5	d			
129	20.5	17.5	3	d			
141	21.5	19	2.5	d			
145	21.5	21.5	0	d			
149	21.5	23.5	2	25	3.5	24.5	3
Total Gain			7		8		3
Average Gain			0.5		1.6		3
Per Cent Gaining			57		60		

During first two weeks 29 died and 7 pupated.

Table V. Diet of Whole Wheat Flour

Number	June 9 Length	June 28 Length	July 7 Length	Gain or Loss	July 19 Length	Gain or Loss
168	20	20	p			
169	19.5	22.5	24.5	5	d	
174	18	23.5	21	3	p	
175	20	26	25	5	24	4
177	21	25	p			
182	18	16.5	18.5	0.5	21	3
184	14	14	d			
192	18	23.5	26.5	8.5	23.5	5.5
193	20	23	p			
195	14.5	21	22	7.5	p	
196	17.5	24.5	p			
Total Gain				29.5		12.5
Average Gain				4.9		4.16
Per Cent Gaining				100		(100)

During first two weeks 23 died and 2 pupated.

Table VI. Starvation

Number	June 9 Length	June 28 Length	July 7 Length	Gain or Loss	July 19 Length	Gain or Loss
200	17.5	17				
201	19	22	20.5	1.5	21.5	2.5
202	17	19.5	19	2	19	2
203	17	20	19.5	2.5	18.5	1.5
205	19.5	23	22.5	3	21.5	2
207	18	17	d			
211	18	21	21	3	19	1
213	16.5	18	15.5	1	17.5	1
214	15.5	17	15	0.5	12	3.5
215	12	13	12	0	d	
216	16.5	16	18	1.5	16	0.5
217	20	21	23	3	21	1
218	17.5	21	21	3.5	18	0.5
219	15.5	16	14.5	1	d	
220	18.5	20	20.5	2	21	2.5
221	19.5	22	21.5	2	20.5	1
222	18	20	20.5	2.5	20	2
223	20	18.5	p			
225	17	19	19.5	2.5	18.5	1.5
226	20	22	21	1	22	2
227	17.5	20.5	20	2.5	20	2.5
228	16	18	17	1	16.5	0.5
230	18	20	19.5	1.5	18	0
231	17	20.5	p			
232	19	19	20	1	18	1
Total gain				33.5		18.5
Average Gain				1.6		.97
Per Cent Gaining				81		79-

During first two weeks 8 died and 2 pupated.

Table VII. Basal Ration Plus Yeast Plus Aqueous Extract of Whole Wheat

Number	June 30 Length	July 12 Length	Gain or Loss	July 19 Length	Gain or Loss
250	18.5	18	-0.5	20	1.5
251	17	18	1	19.5	2.5
252	18	16.5	1.5	18.5	0.5
253	14.5	15.5	1	14.5	0
254	14	17	3	17	3
255	18	19.5	1.5	22	4
256	17	20.5	3.5	22	5
257	18.5	20	1.5	21	2.5
258	14	15.5	1.5	15.5	1.5
259	14.5	16	1.5	16.5	1.5
260	15	16.5	1.5	18	3
261	16.5	19.5	3	20	3.5
262	14	14.5	0.5	16	2
263	19	18	-1	20	1
264	18	18	0	16.5	-1.5
265	19.5	19.5	0	19	-0.5
266	17	20	3	19	2
267	15.5	20	4.5	20	4.5
268	14.5	15.5	1	15	0.5
269	17.5	18.0	0.5	19.5	2
270	16	18	2	19	3
271	18	21	3	20	2
272	18.5	22	3.5	21	2.5
273	17.5	19	1.5	19	1.5
274	14.5	14	-0.5	15.5	1
Total Gain			35		49
Average Gain			1.4		1.96
Per Cent Gaining			76		88

Table VIII. Basal Ration Plus Yeast Plus Alcoholic Extract of Whole Wheat

Number	June 30 Length	July 12 Length	Gain or Loss	July 19 Length	Gain or Loss
275	15	16.5	1.5	16	1
276	17	16	1	14.5	2.5
277	15	19.5	4.5	21	6
278	14	19	4	19	4
279	14.5	14	-0.5	14	-0.5
280	17	19	2	21	4
281	16	15	-1	16.5	0.5
282	17	18	1	17.5	0.5
283	17	19	2	19	2
284	12.5	13.5	1	13	0.5
285	15.5	18	2.5	19.5	4
286	15	15.5	0.5	15	0
287	18.5	16	2.5	20	1.5
288	14	14.5	0.5	13	-1
289	14.5	15	0.5	15	0.5
290	18	21	3	20	2
291	16	19	3	21.5	5.5
292	19	20.5	1.5	22.5	3.5
293	14.5	14	-0.5	15	0.5
294	13.5	16	2.5	17	3.5
295	14	13.5	-0.5	13	-1
296	14.5	17	2.5	20	5.5
297	18	20.5	2.5	21	3
298	16	18	2	19	3
299	16	16.5	0.5	19.5	3.5
Total Gain			32		50.5
Average Gain			1.28		2.02
Per Cent Gaining			76		80

Table IX. Basal Ration Plus Yeast.

Number	June 30 Length	July 12 Length	Gain or Loss	July 19 Length	Gain or Loss
300	17	18	1	19	2
301	16.5	17	0.5	17	0.5
302	19	18	-1	21	2
303	16	15	-1	17	1
304	15	16	1	15	0
305	16	17	1	15.5	-0.5
306	12	16	4	17	5
307	13.5	16	2.5	16.5	3
308	16	20	4	21	5
309	14.5	17	2.5	18	3.5
310	15	17	2	16	1
311	13	17	4	17.5	4.5
312	15	17	2	16.5	1.5
313	14.5	15.5	1	15.5	1
314	16.5	19.5	3	21	4.5
315	12	14	2	14	2
316	19	19	0	21.5	2.5
317	18	21	3	20.5	2.5
318	19.5	20.5	1	19.5	0
319	13	11	-2	9	-4
320	13	15.5	2.5	16	3
321	13	15.5	2.5	17	4
322	14	16	2	17	3
323	19	19	0	19	0
324	17	17.5	0.5	17.5	0.5
Total Gain			38.0		47.5
Average Gain			1.52		1.9
Per Cent Gaining			80		80

Table X. Basal Ration Plus Autoclaved Yeast

Number	June 30 Length	July 12 Length	Gain or Loss	July 19 Length	Gain or Loss
325	17	17	0	17	0
326	19	23	4	24	5
327	19.5	21	1.5	20	0.5
328	16.5	18	1.5	17	0.5
329	17	18	1	17	0
330	18	20	2	18	0
331	17	21	4	23	6
332	16	19	3	22	6
333	17	18	1	20	3
334	10.5	12	1.5	13.5	3
335	13	13.5	0.5	14	1
336	12.5	15.5	3	15	2.5
337	17	18	1	19.5	2.5
338	14	16.5	2.5	17	3
339	16.5	21	4.5	22.5	6
340	19.5	21	1.5	23	3.5
341	17	18	1	19	2
342	18	17	-1	16	-2
343	18	18.5	0.5	21	3
344	13	13	0	10	-3
345	14.5	13.5	-1	14.5	0
346	19	19	0	21	2
347	18	19.5	1.5	18	0
348	17.5	18	0.5	19	1.5
349	19	16.5	-2.5	18	-1
Total Gain			31.5		45
Average Gain			1.26		1.8
Per Cent Gaining			76		64

Table XI. Basal Ration

Number	June 30 Length	July 12 Length	Gain or Loss	July 19 Length	Gain or Loss
350	15	18	3	18.5	3.5
351	16	dead			
352	12.5	14.5	2	15	2.5
353	14.5	15	0.5	16	1.5
354	20	19	-1	d	
355	18	18	0	18	0
356	18	17	-1	18.5	0.5
357	15	17	2	17	2
358	17.5	18	0.5	19	1.5
359	20.5	21	0.5	22	1.5
360	16	15	-1	d	
361	14	14.5	0.5	15.5	1.5
362	17	19.5	2.5	19	2
363	15.5	17	1.5	18	2.5
364	15	16	1	18.5	3.5
365	19	20	1	22	3
366	19	22.5	3.5	21.5	2.5
367	14	15.5	2	15	1
368	13.5	15.5	2	17	3.5
369	13.5	13.5	0	15	1.5
370	14.5	15	0.5	15	0.5
371	17.5	19	1.5	21	3.5
372	17	20	3	20	3
373	15.5	16.5	1	19	3.5
374	19.5	22.5	3	23.5	4
Total Gain			28		48.5
Average Gain			1.17		2.2
Per Cent Gaining			79		99

Table XII. Ration of Whole Wheat Flour

Number	June 30 Length	July 12 Length	Gain or Loss	July 19 Length	Gain or Loss
375	15.5	21.5	6	23	7.5
376	12	16.5	4.5	18	6
377	19	21.5	2.5	24	5
378	18	20	2	21	3
379	13	16	3	18	5
380	16.5	20	3.5	21.5	5
381	13	16	3	17	4
382	18	21	3	22	4
383	17.5	20.5	3	22	4.5
384	12.5	13	0.5	16.5	4
385	18.5	22	3.5	25.5	7
386	20	22	2	24.5	4.5
387	15.5	17	1.5	18	2.5
388	16.5	21.5	5	23	6.5
389	14	17	3	18	4
390	13	18	5	19	6
391	19	23	4	22.5	3.5
392	13	15.5	2.5	17	4
393	13.5	16.5	3	18	4.5
394	15.5	18	2.5	20.5	5
395	15	18.5	3.5	20	5
396	20	24.5	4.5	25	5
397	14	17	3	20	6
398	17.5	22	4.5	21	3.5
399	13	17	4	19	6
Total Gain			82.5		121
Average Gain			3.3		4.84
Per Cent Gaining			100		100

Table XIII. Basal Ration Plus Yeast

Number	June 23 Length	July 7 Length	Gain or Loss	July 19 Length	Gain or Loss
		With *Tribolium confusum*			
1	20	24	4	24	4
3	18	19.5	1.5	21.5	3.5
4	18	20	2	21	3
5	17	20	3	20.5	3.5
6	20	21	1	21	1
7	19	20.5	1.5	20.5	1.5
8	18	21	3	24.5	6.5
10	18	25	7	25.5	7.5
11	19	19	0	21	2
14	21	24	3	26	5
15	21	22.5	1.5	p	
16	14	17.5	3.5	23.5	9.5
17	15	16	1	16.5	1.5
18	17	21	4	26	9
19	20	24	4	25.5	5.5
20	16	18.5	2.5	25.5	9.5
21	19	21	2	24.5	5.5
22	20	25	5	p	
23	21	21	0	24	3
25	19	26.5	7.5	22	3
Total Gain			57.0		84.0
Average Gain			2.85		4.66
Per Cent Gaining			90		100
		Without *Tribolium confusum*			
26	18	20	2	23	5
28	17	19	2	23	6
29	20	23	3	26.5	6.5
30	16	19.5	3.5	23	7
32	20	22	2	p	
34	20	24.5	4.5	p	
35	20	22	2	25.5	5.5
36	21	23	2	21.5	0.5
37	19	22	3	22	3
38	18	21.5	3.5	26.5	8.5
39	23	p			
40	19	23	4	p	
42	21	p			
43	23	26	3	30	7
44	29	28	-1	p	
45	25	25	0	p	
47	27	p			
48	22	p			
50	26	28	2	p	
Total Gain			35.5		48
Average Gain			2.49		5.3
Per Cent Gaining			86.6		100

DISCUSSION

During the first two weeks the indications for the three first experiments (II, III, and IV) were that the larvae having whole yeast in their diet were able to get along better than those not having this substance in their diet. This time, two weeks, was, however, insufficient for conclusions and before another measurement could be taken most of the larvae had died. The death of the larvae was probably due to first too little humidity then to too much, which caused the development of mould. The fact, however, that some larvae in each group lived to pupate indicates that it is possible for larvae about 16 millimeters or longer to grow and pupate on any of the diets.

The mixed culture experiment so far has shown nothing conclusive. The larvae have grown well having made an average gain of 4.66 millimeters during a period of almost a month. The controls made a gain just a little better, 5.3 millimeters so the indications are that the presence of *Tribolium confusum* in the culture has neither aided nor harmed the Tenebrio larvae.

The larvae without food have made an average gain of .97 millimeters during a period of a month and a half, but since length was the only measurement taken it would be hard to say just what caused the gain. It may have been that the larvae were ready to shed their skins and thus the tissue was already laid down ready for elongation, or there may have been considerable food in their alimentary tracts at the beginning of the experiment, or again the larvae may have grown longer by a decrease in diameter. A statistical treatment of this group compared with the group on the basal ration showed that the difference between the gains made by the two groups of larvae was significant and that the larvae do better on even a synthetic diet without known growth promoting factors than without food, a condition not found to be the case in mammals.

A comparison of the larvae on a ration of whole wheat flour with those upon the basal ration showed the former made by far the better gains. When the data for Experiments XI and XII were treated statistically the difference divided by the error of difference gave 10 which is very significant.

Statistical treatment of data was made according to the following formulas:

$$\sigma = \sqrt{\frac{\Sigma x^2 - \Sigma x(Mx)}{n}}$$

$$Em = \pm \frac{.6745 \ \sigma}{\sqrt{n}}$$

$$E \text{ diff} = \sqrt{(Em_1)^2 - (Em_2)^2}$$

$$\frac{\text{Difference}}{\text{Error of Diff}} = \frac{Mx_1 - Mx_2}{\text{Error of difference}}$$

In which

σ = Standard deviation

Σ = Summation

M = Mean

Em = Error of mean

E diff = Error of difference

n = Number of individuals

x_1 = Gain in length of larvae on starvation whole wheat flour

x_2 = Gain in length of larvae on basal ration

In the series of experiments (VII-XI) the growth of all of the larvae on synthetic diets was practically the same. This indicates that vitamins B and G are not essential for growth and development of Tenebrio molitor.

The factor or factors that make for accelerated growth of larvae on whole wheat flour either were not extracted with alcohol or water or were not extracted in sufficient amounts to be effective as the larvae with and without these extracts (Tables VII, VIII, and IX) made practically the same gains.

It would seem that *Tenebrio molitor* can not be used as a test animal for vitamin research, at least not for standardizing vitamins for mammalian diets. It may be that this larva does not need vitamins at all, or that it has symbiotic microorganisms, or is able of itself to synthesize vitamins. No studies have been undertaken to determine which is the case.

Factors not controlled or only partially controlled were temperature and humidity. The experiments were kept in the basement which had a mean temperature of between 70-80° F. The humidity was regulated to a certain extent. The experiments were subject to variations in both temperature and humidity and these factors would make a difference in the growth of the larvae.

In three preliminary experiments each involving 10 larvae on diets of whole wheat flour, gluten flour with yeast, and gluten flour without yeast the growth of all the larvae was practically uniform. As the gluten flour is finely milled this would indicate that the size of the particles of food is probably not the controlling factor in the greater growth of the Tenebrio larvae on the natural diets.

These experiments were performed on larvae 14 millimeters or longer and it would be interesting to try using smaller larvae preferably just hatched and to carry them through to pupation. It would, also, be of interest to try more extractions of whole wheat flour and varying amounts in the food to further determine the factors making for accelerated growth of larvae on this food. Experiments VII, VIII, IX, X, XI, XII, and XIII are being continued.

SUMMARY

1. Experiments were conducted on 450 Tenebrio molitor larvae in order to determine whether vitamins B and G are necessary for growth and development of this larvae.

2. The rate of growth of larvae on synthetic diets used in the experiments was checked by controls upon natural diet and under conditions of starvation.

3. An experiment was conducted on 20 Tenebrio larvae to determine the effects of a mixed culture, Tenebrio and Tribolium, on the larvae.

4. All of the experiments are still in progress.

5. The best growth was made on a diet of whole wheat flour. The factors in whole wheat flour producing this superior growth have not been determined.

6. The data indicate that vitamins B and G are not necessary to the growth and development of this larvae.

7. The data on the mixed culture group shows no appreciable difference in growth indicating that although

the larvae are predatory the presence of another insect does not alter their food to such an extent as to cause a difference in growth.

ACKNOWLEDGMENTS

This problem was undertaken in collaboration with Dr. Minna E. Jewell of the Zoology Department of the Kansas State Agricultural College. The author is also indebted to Dr. E. J. Wimmer for assistance during a part of the experiment; and to Mr. George B. Wagner of the United States Department of Agriculture, Manhattan, Kansas, for valuable aid in securing food and for suggestions during the experiment.

LITERATURE CITED

Back, E. A., and Cotton, R. T.
1926. Biology of the saw-toothed grain beetle Oryzaephilus surinamensis L.
Jour. Agr. Research, 33:435-452.

Bacot, A. W., and Harden, A.
1922. Vitamin requirements of Drosophila. I. Vitamins B and C.
Biochem. Jour., 16:148-152.

Bailey, C. H.
1925. Chemistry of wheat flour.
Amer. Chem. Soc. Monograph Ser., 0:324 p., il.

Baumberger, J. P.
1917. The food of Drosophila melanogaster Meigen.
Nat. Acad. Sci. Proc., 3:122 p.

1919. A nutritional study of insects with special reference to microorganisms and their substrata.
Jour. Expt. Zool., 28:1-25.

Bayliss, W. M.
1920. Principles of general physiology.
3rd Edition.
London and New York.

Bogdanow, E. A.
1906. Ueber das Züchten der Larven der gewöhnlichen. Fleishfliege, (Calliphora vomitoria) in sterilisierten Nährmitteln.
Arch. ges. Physiol., 113:97-105.

1908. Ueber das Abhängigkeit des Wachstums der Fliegenlarven von Bakterien und Fermenten und über Variabilitat und Verebung bei den Fliegenlarven.
Arch. Anat. Physiol., 1908, Suppl., 173-200.

Chapman, R. N.
1918. The confused flour beetle Tribolium confusum Duval.
Minn. State Entomologist Rept., 17:73-94.

Chapman, R. N.
1919. Insects in relation to wheat flour and wheat flour substitutes.
Jour. Econ. Ent., 12:66-70.

1924. Nutritional studies on the confused flour beetle Tribolium confusum Duval.
Jour. Gen. Physiol., 6:565-585.

Chick, H., and Roscoe, M. H.
1927. On the composite nature of the water-soluble B vitamin.
Biochem. Jour., 21:698.

Committee, Amer. Soc. Biol. Chem.
1929. Vitamin B terminology.
Science, 69:1784, 276.

Delcourt, A., and Guyénot, E.
1911. De la possibilité d'étudier certains Diptéres en milieu défini.
Compt. rend. Acad., cli, 255; Bull. Sc. France et Belg., 1911, xlv, 249.

Eijkman, C.
1897. Ein Versuch zur Bekämpfung Beri-beri.
Virchow's Archiv., 141:197.

Emmett, A. D., and Luros, G. O.
1920. Water soluble vitamins.
Jour. Biol. Chem., 43:265-286.

Funk, C.
1911-1912. On the chemical nature of the substance which cures polyneuritis in birds induced by a diet of polished rice.
Jour. Physiol., 43:395.

1912-1913. Further studies on experimental beri-beri; the action of certain purines and prymidine derivatives.
Jour. Physiol., 45:489.

Funk, C., and Dubin, H. E.
1921. The vitamins of yeast and their role in animal nutrition.
Proc. Soc. Exper. Biol. Med., 19:15.

Glaser, R. W.
1923. The relation of microorganisms to the development and longevity of flies.
Amer. Jour. Trop. Med., 4:85-109.

Goldberger, J., and Tanner, W. F.
1925. A study of the pellagra preventive action of dried beans, casein, dried milk and brewer's yeast with consideration of the essential preventive factors involved.
U. S. Public Health Report, 40:54.

Goldberger, J., Wheeler, G. A., Lillie, R. D., and Rogers, L. M.
1926. A further study of butter, fresh beef, and yeast as pellagra preventives, with consideration of the relation of factor P-P of pellagra of vitamin B.
U. S. Public Health Report, 41:297.

Grijns, G.
1901. Over polyneuritic gallinarum.
Geneesk. Tijdschr. V. Nederland-Ind., 41:1; vid. Arch. f. Schiffs-u-. Trop.- Hyg., V, 3:302.

Guyènot, E.
1913. Etudes biologiques sur une mouche, Drosophila ampelophila Loew.
C. R. Soc. Biol., 74:97, 178, 223, 270, 332, 389, 443.

1917. Recherches expèrimentales sur la vie aseptique et le dèveloppement d'un organisme (Drosophila ampelophila) en fonction du milieu.
Bull. Biol. Fr. Belg., 51:1-330.

Hassan, A., and Drummond, J. C.
1927. The relation of certain dietary factors in yeast to growth of rats on diets rich in proteins.
Biochem. Jour., xxi:653-661.

Hauge, S. M., and Carrick, C. W.
1926. A differentiation between the water soluble growth-promoting and antineuritic substances.
Jour. Biol. Chem., 69:403.

Herrick, Glenn W.
1921. Insects injurious to the household. (The Rural Science Series) The Macmillan Company, New York, 470 p.

Johansson, B.
1920. Der Gaswechsel bei Tenebrio molitor in seiner Abhangigkeit von der Nahrung. Acta Univ. Lundensis, n. ser., 16:5, 1-36.

Kanehiro, Takaki
1887. Health of the Japanese Navy. Lancet, 86.

Kinnersley, H. W., and Peters, R. A.
1925. Antineuritic yeast concentrates. Biochem. Jour., 19:820.

Kruse, H. D., and McCollum, E. V.
1929. Biochemical investigations of vitamin B. Physiol. Rev., 9:1, 126-239.

Laird, C. N.
1926. A comparison of the pigeon and the rat as test subjects for vitamin B. Am. Jour. Hyg., 6:201.

Levene, P. A., and Van der Hoeven, B.J.C.
1926. The concentration of vitamin B. III. Jour. Pharmacol. and Exp. Therap., 29:227-233.

Loeb, J., and Northrup, J. H.
1916. Nutrition and evolution. Second note. Jour. Biol. Chem., 27:309-312.

McCollum, E. V.
1923. The newer knowledge of nutrition. The Macmillan Company, New York. 2nd Ed., 449 p.

McCollum, E. V., and Kruse, H. D.
1926. Some observations on the extraction of the vitamin B from wheat germ. Amer. Jour. Hyg., 6:2, 197-200.

Northrop, J. H.
1917. The role of yeast in the nutrition of an insect (Drosophila). Jour. Biol. Chem., 30:181-187.

Osborne, T. B., and Mendel, L. B.
1919. The nutritive value of the wheat kernel and its milling product.
Jour. Biol. Chem., 37:557-601.

Passerini, N.
1925. Influenza della qualita delli agrumi sull' accressimento delle larve e sul metabolismo del Tenebrio molitor L.
Atti R. Acad. Lincei, Rendic., 6:1, 58-59.

Portier, P.
1919. Developpement complet des larves de Tenebrio molitor, obtenu au moyen d'une nourriture stérilisée a haute temperature (130° C.).
C. R. Soc. Biol., 82:59-61.

Richardson, Ch. H.
1926. A physiological study of the growth of the Mediterranean flour moth (Ephestia kuehniella, Zell.) in wheat flour.
Jour. Agric. Res., 32:895-929.

Schwardt, H. H.
1927. The effects of different foods on the larval periods of three grain insects.
Master's Theses, K.S.A.C., 18:43 p.

Sherman, H. C., and Axtmayer, J. H.
1927. A quantitative study of the multiple nature of vitamin B.
Jour. Biol. Chem., 75:207.

Steenbock, H., and Coward, K.H.J.
1927. Fat-soluble vitamins. XXVII. The quantitative determination of vitamin A.
Jour. Biol. Chem., 72:765-781.

Sweetman, M. D., and Palmer, L. S.
1928. Insects as test animals in vitamin research.-I, Vitamin requirements of the flour beetle, Tribolium confusum Duval.
Jour. Biol. Chem., 77:1, 33-52.

Swingle, H. S.
1925. Digestive enzymes of an insect.
The Ohio Jour. Sci., 25:5, 209-218.

Cotton, R. T., and St. George, R. A.
1929. The meal worms.
Technical Bulletin No. 95, U.S.D.A., 37 p.

Uvarov, B. P.
1928. Insect nutrition and metabolism. A summary of the literature.
Trans. of the Ent. Society of London, 76:255-343.

Weiss, H. B.
1925. Notes on the ratios of insect food habits.
Proc. Biol. Soc. Washington, 38:1-4.

Wollman, E.
1919a. Larves de mouches (Calliphora vomitoria) et vitamins.
C. R. Soc. Biol., 82:1208-1210.

Woodsedalek, J. E.
1917. Five years of starvation of larvae.
Science, 46:366.

Wulzen, Rosalind
1923. A study in the nutrition of an invertebrate, Planaria maculata.
In Univ. of Cal. Pub. in Physiol., 5:175-187.