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The nutritional requirements of the firebrat, Thermobia domestica (Packard).

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THE NUTRITIONAL REQUIREMENTS OF THE FIREBRAT, THERMOBIA DOMESTICA (PACKARD)

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INTRODUCTION

The firebrat, Thermobia domestica (Packard), is a small rapidly moving Thysanuran insect. The order Thysanura belongs to the Apterygota, a group of primitive insects having no winged ancestors. The adult insect is long, slender and wingless, attaining an average body length of 8-10 mm. and maximum length of approximately 12 mm. There are 3 long filiform appendages approximately 8-9 mm. in length at the caudal end of the body. A pair of styli is found on the ventral sternites of the 7th, 8th and 9th abdominal segments. The antennae are very long and many jointed, while the mouth parts are formed for chewing. The body is covered with scales which have dusky markings on the upper surface, giving the insect a brown-banded appearance.

In so far as is known, this species is confined entirely to heated habitations of man, particularly in warm moist areas around steampipes and boilers. When present in large numbers, these pests are of considerable economic importance, attacking starchy products and protein materials such as glue (Spencer 1930). Most damage is done by their attacks upon wall paper, card files, book bindings, starched clothing and stocks of paper on which glue or paste has been used as sizing.

This study is an attempt to determine some of the basic nutritional requirements of the firebrat, *Thermobia domestica* (Packard).

REVIEW OF LITERATURE

Since the nutritional work on insects to date has been done on a relatively few species, it is exceedingly difficult to arrange this information for analysis. Many of the workers experimenting in this field do not give the basic constituents of the diets used - which limits the value of the data obtained. This is particularly true in cases where the foods employed have overlapped in respect to the organic and inorganic elements. The possibility of microorganisms in the insect gut elaborating certain dietary constituents has been mentioned by many writers, but the fact remains however, that little is known about this subject, further complicating the available knowledge. Therefore, to facilitate the handling of the information, the writer has placed the insects into groups with similar feeding or social habits as follows:

A. Household Insects

B. Social Insects

C. Insects Affecting Man and Domestic Animals

D. Insects Attacking Grain and Grain Products

E. Insects Feeding on Fruits

F. Miscellaneous Insects

Household Insects

In early experiments by Wollman (1926) on the cockroach, Blatella germanica (L.), it was noted that nymphs fed on sterilized food, developed normally and produced a continuous series of generations for five years. This led the author to believe that the nymphs did not need vitamins. This opinion was reversed by Melampy (1937) who claimed that vitamin B-complex of dried yeast is required by B. germanica. He further stated that the essential part of this vitamin is heat stable. Among the foods used, ground whole wheat with meat products gave successful results. McCay (1938) agrees with Melampy in that certain essential factors found in yeast are necessary for normal development of the German cockroach. These factors are partially soluble in water and in lipid solvents. McCay also claimed that no demonstrable need could be found for the fat-soluble vitamins A and D. Bowers and McCay (1940) found no necessity for the vitamin A nor was any evidence found that it could be synthesized by Blatella. This work on vitamins was furthered by Gier (1947). He claimed that intestinal bacteria of Periplaneta americana L. may produce enough proteins or growth promoting vitamins from foods to keep the insects alive.

Crowell and McCay (1937) successfully reared the webbing clothes moth, Tineola bisselliella (Hummel) on a diet of casein, lactalbumin, Harris B concentrate and fish meal ash. The value of the latter was considered questionable. Purified casein alone was considered unsatisfactory for the larvae, but lactalbumin supplemented this satisfactorily. Vitamin B-complex was considered essential for development while the fat soluble vitamins were not. No definite need for fat could be found by these workers.

Slow growth was obtained by Moore (1943) with two species or varieties of the black carpet beetle, Attagenus, on a synthetic medium of vitamin free casein, starch, minerals, extracted fish meal, benzene extract of liver, glycerol, choline, nicotinic acid, thiamin, riboflavin, pyridoxin and pantothenic acid. If any of the latter five of the B-complex group was omitted, growth ceased and did not continue again until the missing vitamin was supplied.

Whittemore (1938) working with Thermobia domestica (Packard) claims that carbohydrate in some form is necessary for development. He considered that starch was not the form since amylolitic anzymes were apparently absent. Fats were considered necessary for development, proteins were essential and salts served as a supplement to and inadequate diet. Concerning the vitamins, he concluded that vitamin C is unnecessary, one or more fractions of B-complex are essential and A is doubtful. Remington (1948) successfully reared several generations of T. domestica on whole wheat flour, with no retardation of maturation and reproduction. Powdered yeast (vitamin B-complex) placed in the culture was not eaten by the insects. However, whole wheat flour does contain some elements of B-complex vitamins.

From this evidence it may be assumed that vitamin B-complex or certain of its components are necessary for the development of various species of cockroaches. No need for vitamins A or D is shown. However, the possibility of intestinal bacteria elaborating vitamins was not excluded. A necessity is also indicated for carbohydrates and proteins. The webbing clothes moth needs elements of proteins and vitamin B-complex but not the fat soluble vitamins. The black carpet beetle must have elements of fats, carbohydrates, proteins, minerals and especially vitamin B-complex to develop. *Thermobia domestica* must have carbohydrates, proteins and vitamin B-complex in some form. Vitamins C and A are probably not essential, while the need of salts and fats are doubtful.

In general it may be stated that for this group of insects, the evidence overwhelmingly favors the necessity of the B-complex group in addition to carbohydrate and proteins as essential for growth.

Social Insects

Phillips (1927) experimenting with simple and complex carbohydrates utilized by adult honeybees, concluded that the following could be used: glucose, levulose, sucrose, trehalose, maltose, and melezitose. Galactose, mannose, lactose, raffinose, dextrin, starch and inulin gave negative results. Meanwhile Bertholf (1927) found that the larvae of honeybees differ from the adults in their ability to use dextrin, galactose and lactose and they, like the adult, can metabolize two rare sugars, melezitose and trehalose. Searching for food to replace pollen, Haydak (1933) reared the larvae on a series of diets including dried yeast, fresh whole milk, skim milk powder, whole egg, egg yolk and egg white. The broods of these colonies however, were quite variable in number. The best results were obtained with dried yeast and the poorest with egg white. Haydak (1936) substituted meat scraps and commercial casein mixtures with good results. But, cottonseed meal, ground dried blood, whole oats and whole wheat flour gave poorer development. He was able to keep adults alive for a considerable period of time on carbohydrates alone, but for development, the elements found in pollen are essential.

Cook and Scott (1933) were able to rear termites, *Termopsis angusticollis* Hagen on a diet consisting of sugar, proteins, salts, vitamins A, B, D, and G. All these substances were believed necessary for normal development. The termites were able to exist for considerable periods on pure cellulose, but for normal growth, salts, sulfur, phosphorous and organic nitrogen must be available.

Smith (1945) working with ants of the genus Camponotus, showed that imagines of colonies fed on vitamin free food were smaller than those supplied with vitamins. His conclusions were that the bacterial flora found in the gut of nurse ants and their larvae elaborate enough vitamins to permit somewhat abnormal larval development to proceed.

From this limited evidence, it may be concluded that honeybees are able to utilize a wide variety of simple and complex sugars. The adults and larvae differ only slightly in this respect. The pollen substitutes giving the best results were casein and yeast and these or pollen are necessary for development.

Termites and ants are somewhat similar in respect to food requirements, requiring certain vitamins for development. There is the possibility that bacterial flora in the gut are the source of some or all of the required vitamins.

Insects Affecting Man and Domestic Animals.

Mickelbacker, Hoskins, and Herms (1932) showed that larvae of Lucilia sericata Meig. grow well on a diet of technical casein, in agar solution to prevent drying. Highly purified casein was deficient, but additions of yeast, salts and butter or cod-liver oil gave good results except for pupal irregularities. Additions of small amounts of cystine to the above mixture resulted in perfect pupae, rapid growth and high pupation.

Hobson (1935) experimented with this same species and found it necessary to add vitamin B (B-complex) to blood cultures to obtain development with aseptic larvae. His conclusions were that when larvae grow in cultures where bacteria are present, the bacteria are able to synthesize needed vitamins. He further stated that purified cholesterol supplied some essential factor required by the larvae.

Other work on L. sericata has shown that carbohydrates are essential for continued life of the adult fly, and protein for development of the ovaries (Dorman, Hale and Hoskin, 1938). Hill, Bell and Chadwick (1947) reared the black blowfly, Phormia regina Meig. on a medium of powdered casein, brewer's yeast and powdered agar in proportions of 30:3:1. To this mixture, lanolin and a modified Belar solution containing phosphate were added.

The test conducted by Glaser (1923) on Musca domestica L., Stomoxys calcitrans L. and Lyperosia irritans L. indicate that the intestinal bacteria of adults flies may be a possible source of vitamins. This possibility of bacterial influence on nutrition of flies is again submitted by Simmons (1939). Working with the larvae of Hypoderma lineatum (DeVilliers), he was able to isolate the enzymes glycogenase, lipase, trypsin and erepsin as being secreted by the larvae. Lactase, maltase, invertase and rennin were found also, but were believed to be produced by bacteria.

Subbarrow and Trager (1940) rearing larvae of Aedes aegypti (L.) were able to grow them normally under sterile conditions in a medium of killed yeast, riboflavin and two fractions of liver extract, designated as barium filtrate and barium precipitate. The larvae also grew at a normal rate in a medium of killed yeast, flavin-purine complex, vitamin B_2, pantothenic acid and glutathione. Replacement of the flavine-purine complex by pure riboflavin resulted in slower growth, but nevertheless some larvae reached the adult stage. Using this same species, Buddington (1941) developed larvae of Aedes to maturity on alcohol sterilized yeast under sterile conditions. He was unable to get growth beyond the 5th instar on autoclaved yeast. Nicotinic acid and ascorbic acid did not replace the heat stable factor in yeast. His conclusion was that "larvae need for development to maturity under aseptic conditions, (1) thiamin hydrochloride (B_1),

(2) riboflavin (B_2) and (3) a heat stable factor in yeast and liver extract."

Bates (1947) found that brewer's yeast added to tap water was a stimulus for egg hatching in Haemagogus mosquitoes and was very consistent for good larval development.

Most of the work done on the flies shows a need for casein, a probable need for carbohydrates, brewer's yeast and fat. Again the bacterial flora in the gut must be considered as a possible source of vitamin elaboration. The mosquitoes must have some of the elements of vitamin B-complex when reared under aseptic conditions.

Insects Attacking Grain and Grain Products.

This large group of insects has been the source of many interesting experiments. One of the early workers (Richardson, 1926) tested several kinds of milled flours on the Mediterranean flour moth, Ephestia kuhniella Zeller. Using milled flour alone, and supplemented with purified casein or salt mixture, he found that development proceeded equally well. His conclusions were: that vitamin B (B-complex) is largely but not entirely contained in the wheat kernel, that highly milled flour produces growth deficiency because of low concentrations of vitamin B (B-complex) and that vitamin C is not essential for the normal growth and development of insects.

Sweetman and Palmer (1928) experimenting with Tribolium confusum Duval, concluded that vitamin B (B-complex) is essential and no demonstrable need could be found for vitamin A. This evidence was supported by Bushnell (1938) using a diet of yellow corn meal. He concluded that yellow corn meal with additions of yeast enhanced production of eggs markedly. In the larval stages, growth occurred more rapidly on the yeast corn medium and a greater percentage of individuals survived the metamorphic period. Chiu and McCay (1939) were able to successfully rear the larvae of T. confusum on a diet largely made up of sucrose, but vitamin B (B-complex) was necessary for normal development. Casein added to the diet, 20-25%, caused an increase in beetle population. Tests conducted with vitamins A and D indicated that neither are necessary for growth of the larvae. Adding yeast to the patent flour diet of Tribolium adults, Lund and Bushnell (1939) obtained a large increase in egg production. The total egg production of yeast fed beetles totalled 742.1 ± 47.4. (S.E.) eggs per female; those not fed on yeast totalled 490.0 ± 37.17 (S.E.) eggs per female.

Gay (1938) claimed that Dermestes vulpinus Fab. required one or more components of yeast, while Fraenkel, Reid, and Blewett (1941) showed that some sterol must be present in the food for normal growth. However, the latter writers admit the presence of symbiotic organisms in the larvae and it was believed that these organisms may be involved in the elaboration of some essential food components.

Fraenkel and Blewett (1943a) have done the greatest amount of nutrition work on insects attacking grain products. The larvae of Tribolium confusum Duv., Silvanus surinamensis L., Stegobium paniceum L., Lasioderma serricorne Fab., Ptinus tectus Boeild. and Dermestes vulpinus Fab. were successfully reared by them on a synthetic medium, consisting of casein 50, glucose or starch 50, yeast 5, cholesterol 1, McCollums salt mixture 1, water 15 parts and yeast. When the yeast was omitted, the larvae entirely failed to grow. Again (1943b, 1943f), using the same insects plus Ephestia kuhneilla Zell. and Ephestia elutella Hb., they concluded that, "four main factors are of importance in the nutrition of insects: (1) requirements of carbohydrates, (2) quantitative and qualitative requirements of sterols, (3) moisture content of food and (4) qualitative and quantitative requirements of the vitamins of the B group."

The findings of Martin and Hare (1942) differ slightly from those listed above. Larvae of Tenebrio molitor L. reared on a diet of not less than 50% carbohydrate and not less than 15% nor more than 25% protein resulted in good development. Fats, were not required and in excess of 3%, inhibited growth. The diet used consisted of casein, fat, carbohydrate, salt mixture, cholesterol and yeast. If the yeast was removed, normal larval growth failed. If thiamine, riboflavin, pyridoxine, nicotinic acid and pantothenic acid were all present, good growth resulted. If any were omitted, results were negative. Vitamins A, D, C, E, K and choline gave negative results.

The nutritional requirements of insects feeding on stored grain and their products are very similar. B-complex appears to contain essential vitamins. Carbohydrates and proteins are necessary and possibly fat, depending on the species. The possibility that minerals are needed must not be overlooked, but the evidence concerning these is somewhat vague.

Insects Feeding on Fruits

Loeb and Northrop (1916) were unable to develop *Drosophila* to the pupal stage on a diet of casein, edestin, egg albumin, milk or certain amino acids. They were however, able to raise 12 successive generations under aseptic conditions upon a medium of sterile yeast, citric acid and water. Yeast was found to be an essential element for the existence of *Drosophila*. The number of flies developing on yeast may be increased by the addition of banana, casein or sugar

(Northrop 1917). He concluded that yeast is a necessary element and that yeast contains twice the weight of banana in necessary substances available as food. Guyenot (1917) shows definitely that no artificial food is complete unless the substances contained in the filtered autolysate of yeast are added to it. While Baumberger (1919) working with this same genus, decided that the nucleoprotein of yeast must be present and the complex nitrogenous material, lecithin was unnecessary for normal development. Bacot and Harden (1922) using newly hatched larvae of Drosophila in a sterile medium concluded that vitamin C was not essential for growth. In their work, butter fat (vit. A) and yeast (vit. B-complex) added to the diet, gave normal growth irrespective of the presence of lemon juice (vit. C). But, with respect to butter fat and yeast, yeast was essential while butter fat was not, thus indicating that while vitamin B-complex is necessary, vitamin A is not.

Dean (1938) rearing apple maggots found that of the carbohydrates, only lactose and starch failed to support life. Sucrose and honey were distinctly better. The mediums used were two parts of protein to one part of sugar. On protein tests, only casein proved of no value, while proteoses-peptones and yeast were outstandingly effective. The addition of salts did not effect oviposition markedly. Hall

(1938) found a diet of yeast, honey and raisins fed dry, with water supplied separately, was entirely satisfactory for the adults of the apple maggot.

Of the insects feeding on fruits, all appear to require elements of the B-complex group, but not the other vitamins under aseptic conditions. The value of other foods is questionable for Drosophila, while the apple maggot needs carbohydrates and proteins of some kind.

Miscellaneous Insects.

Chiu and McCay (1939) experimenting with the bean weevil, Acanthoscelides obtectus (Say) concluded that vitamin A and D, or D in a fat free diet, permitted larval growth, but not full development. The presence of vitamin B-complex was required.

Bottger (1940) found no starch hydrolyzing enzymes in the European corn borer, Pyrausta nubilalis Hub., but sucrose and protein splitting enzymes were isolated.

Summary

Carbohydrates in some form are essential for the growth of the majority of the insects considered, with the possible exception of mosquitoes.

Fats appear to be necessary in the metabolism of certain insects and not in others. However, little work has been done on this material and enough evidence is not available to draw any definite conclusion.

Proteins either in simple or complex form are considered an essential dietary element by most writers; the possible exception being the immature mosquitoes.

Salts may or may not be needed. The work done with these elements is very limited.

Vitamin B-complex components have been found to be the most essential of the vitamins tested. The evidence concerning this material is very conclusive as to its necessity for growth, development and longevity. Vitamin A does not appear to be essential in the insect world with the possible exception of ants and termites.

Microorganisms in many insects must be considered as a possible source of food and vitamin elaboration. A great deal of work has yet to be done in this field and in the total field of insect nutrition before an adequate fund of knowledge is available.

PROCEDURE

A stock culture of insects was obtained from Charles A. Remington at Harvard University, after losing the original culture obtained at the University of Massachusetts. The original culture was lost because of inadequate moisture conditions. The stock culture was maintained in a controlled temperature cabinet of 37° C. (Sweetman, 1934; Adams, 1933) in an open flat bottomed porcelain pan. Evaporating pans with water placed in the cabinet served to provide the necessary humidity of 70-85% (Sweetman, 1934, 1938). The relative humidity was measured with a wet and dry hygrometer and was checked from time to time by a commercial hair hygrometer.

Small bits of cotton were placed in the stock culture pan for oviposition of eggs. This greatly facilitated the handling of the eggs, since the females will oviposit all eggs in the cotton and not under the paper towel tray or in food. The insects being negatively phototropic, no lights were used in the cabinet.

The food given to the culture consisted of a mixture employed by Haydak (1938) and used successfully by Sweetman of the University of Massachusetts. This mixture consists of:

Yellow Corn Meal . . . .4 parts
Whole Wheat Flour. . . .2 parts
Skim Milk Powder . . . .2 parts
Bran . . . . .2 parts
Dried Powdered Yeast . .1 part

Haydak's mixture was considered as containing the basic constituents necessary for growth and development of _T. domestica_. All foods were thoroughly ground with a mortar and pestle, carefully mixed and reground so that the young nymphs could not pick individual particles. The crystalline vitamin A, when added to the food, was first dissolved in ether, poured onto the food and the mixture desiccated and ground.

The cotton in the pan was inspected daily for eggs which were transferred, in the cotton, to a small beaker to await eclosion. The number of eggs laid daily varied, but usually thirty or more were present. On hatching, 30 nymphs were transferred to a petri dish and experimental foods added. The food being placed in a small piece of paper towel, folded to form a tray, prevented scattering of the particles. All food employed was in dry form and checked daily to see that no molds had formed. None were observed during the experimental period.

The food constituents of Haydak's mixture should be approximately as follows: carbohydrates, 74 percent; fats, 4 percent; proteins, 16 percent; vitamin B-complex, vitamin A and mineral salts. (Anon., 1943, Sherman, 1946). The latter three were in amounts of less than 3 percent. Using this information as a basis of nutritional requirements purified foods containing single foods and combinations used in the experimental diets, were purchased from the Nutritional Biochemical Corporation of Cleveland, Ohio.

PRELIMINARY EXPERIMENTS

Humidity

Initial experiments were conducted to determine the average humidity ranges for successful egg development and nymphal growth.

Using a humidity range of approximately 40-60 percent, the developmental time of 15 lots of eggs averaged 14 days, approximately 70-75 percent humidity required 11.5-12 days and 75-85 percent averaged 11.5-12 days. The latter two compared favorably with Sweetman (1934, 1938). Higher humidities caused condensation on the metal parts, thermometers and the glass inner door of the cabinet. This moisture dripping into the pans and glassware containing the insects, created an unworkable situation.

The humidity range of approximately 75-85 percent and temperature of 37° C. was satisfactory for growth and sexual maturity of *Thermobia*. Using Haydak's mixture as food, the average time from eclosion to egg laying was 53 days. This compares very favorably with results obtained by Sweetman (1938) and Adams (1933).

Food.

The first series of dietary test were conducted to determine the maximum growth attained, the maximum period of survival and the length of time for reproduction. Since maturation of the insects was desired, an arbitrary test period of 105 days was selected as ample. The foods used in the preliminary experiments are listed below.

Powdered Purified Starch

Dry Vitamin Test Casein with the following analysis -

	Percent
Protein	92.7
Ash	3.5
Fat	0.2
Moisture	3.6

Powdered Brewer's Yeast U.S.P. with the following vitamin content in micrograms per gram -

Thiamine	150
Riboflavin	65
Niacin	475
Pantothenic acid	125
Pyridoxine	30
Folic acid	22
Biotin	2.2
Choline	3600
Inositol	4500

Crystalline Vitamin A

The percentages of these substances used in the various diets are shown in Table I.

Table I.

The percentage composition of the purified diets in the preliminary test with *Thermobia domestica*.

Approximate Percentage of Food Elements.

Diets.

Foods	II	III	IV	V	VI	VII	VIII	IX	X	XI
Casein	0	100	0	0	18	50	0	15	0	15
Starch	0	0	0	100	82	0	82	70	82	70
Yeast	0	0	100	0	0	50	18	15	18	15
Vit. A	0	0	0	0	0	0	0	0	.04	.04

Discussion of Experiments.

Cannibalism among developing and adult *Thermobia* is of common occurrence (Sweetman, 1934). They will not only eat the cast skins and dead bodies of their own kind, but will attack the weak and dying.

A control experiment with no food (Diet II) was set up to determine the maximum time that unfed nymphs could survive by cannibalism alone. The results (Table II) indicate that the bodies of newly hatched nymphs do not contain sufficient food elements to stimulate appreciable growth, and length of survival was 14 days.

The control composed of Haydak's mixture (Diet I) was adequate for growth, survival and reproduction (Table II, Fig. I). Maturation was considered to have been attained when the ovipostor became visible from a dorsal view (Sweetman, 1938). This occurred when the females were abuut 7^+ mm. in length. Reproduction frequently occurred shortly thereafter.

Casein (Diet III) appeared very unsatisfactory for survival, although the life span of a few nymphs was extended considerably, (Table II, Fig. I). The length of the nymphs was only slightly increased over those offered no food (Diet II), indicating that casein was inadequate for growth.

Yeast (Diet IV) was inadequate for growth, giving similar results to Diet II. The survival compared closely with

Table II.

The survival and growth of *Thermobia domestica* nymphs on Diets I to V.

	Number surviving Days										Final size - (length) mm.	Greatest length of survival Days
Diets	5	10	15	20	30	45	60	75	90	105		
I	28	28	28	28	28	28	24*	24	22	22	8-9	105+**
II	28	8	0								3	14
III	28	13	6	2	2	2	1	1	0		3^{+}-4	89
IV	29	21	19	15	7	3	3	1	0		3	85
V	26	26	24	20	12	6	2	1	1	1	4	105+

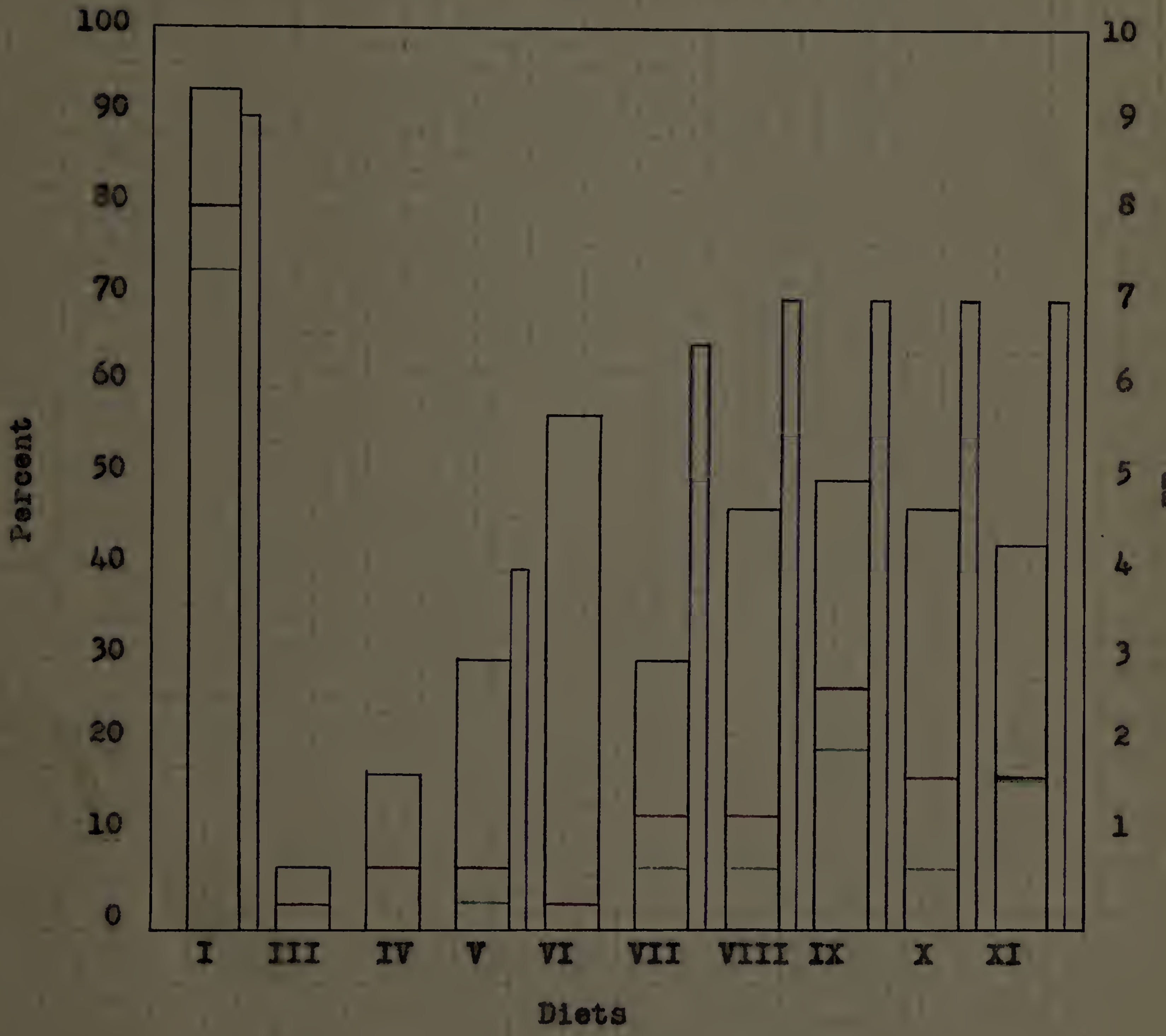
*Two accidently killed.

**First eggs laid in 53 days.

Figure I.

Percentage of nymphs alive at 35,70 and 105 days and size of nymphs at 105 days.

Percentage alive at 35 days - black
Percentage alive at 70 days - red
Percentage alive at 105 days- green
Size of nymphs in mm.--------- purple



the casein fed nymphs, but a much greater average length of survival was obtained with yeast (Table II, Fig. I).

Purified starch (Diet V) did not contain sufficient dietary elements to permit maturation, although length of survival and growth were increased. Starch was superior to casein or yeast but obviously lacking in nutritional elements (Table II, Fig. I).

The results obtained when casein, starch and yeast were used in combination, is given in Table III and Fig. I. The composition of these diets is given in Table I. Casein and starch (Diet VI) appeared favorable during the first three weeks but the number of nymphs surviving decreased rapidly thereafter (Table III, Fig. I). A slight increase in growth over Diets III, IV and V is evident, indicating that each of these materials contains essential growth elements. Yeast added to casein (Diet VII) further increased the size of the nymphs and the length of survival with 7 percent surviving the test period (Table III, Fig. I). Yeast added to starch (Diet VIII) gave an increase in size over that of Diets VI and VII, but did not increase survival (Table III, Fig. I). It is evident from these data that vitamin B-complex contains important growth factors for _T. domestica_. It must also be noted that the protein contained in the yeast, would have some effect on the results obtained.

Table III.

The survival and growth of *Thermobia domestica* nymphs on Diets VI to XI.

No reproduction occurred with any of these diets.

	Number surviving Days										Final size length mm.	Greatest length of survival Days
Diets	5	10	15	20	30	45	60	75	90	105		
VI	26	26	24	22	17	5	1	1	1	0	5	93
VII	27	26	23	15	11	8	5	2	2	2	6+	105+
VIII	30	28	28	27	16	10	5	4	2	2	7	105+
IX	28	24	24	23	18	13	9	7	7	6	7	105+
X	29	23	18	18	13	6	5	2	2	2	7	105+
XI	29	27	23	20	15	8	5	5	5	5	7	105+

When the three components, casein, yeast and starch are contained in the food (Diet IX), growth was similar to Diet VIII, but 20 percent of the nymphs survived the test period (Table III, Fig. I). Since none of the previous diets contained the combination of fats, mineral as ash, carbohydrates, proteins and vitamin B-complex a necessity for most of these constituents in the food of the developing nymph is suggested.

To determine if vitamin A was a necessary nutrient in the food of _T_. _domestica_, it was added to Diets VIII and IX, which had shown the greatest stimulus for growth (Diets X, XI). No appreciable improvement in development occurred and the percentage of survival was similar (Table III, Fig. I).

Summary of Preliminary Experiments.

1. Nymphs without food survived for 14 days with little increase in length.
2. Vitamin test casein, brewer's yeast and purified starch, when fed separately, were very inadequate for growth and survival of young firebrat nymphs.
3. Casein and starch indicated somewhat better growth, casein and yeast slightly more growth and survival, and starch and yeast still better growth.
4. Casein, starch and yeast in the mixture produced as

good or better growth and greater survival than any two of these foods in combinations. Neither growth nor survival equaled that produced by Haydak's mixture.

5. Vitamin A added to diets of starch and yeast, and casein; and starch and yeast appeared to be of no value.

EXPERIMENTS

In the experiments, the vegetable proteins zein and gluten were substituted for the casein to determine the value of plant versus animal protein. All the foods listed below with the exceptions of skim milk powder and whole wheat flour, were in purifide form and obtained from the Nutritional Biochemical Company.

Whole wheat flour - composition according to Sherman (1946) -

	Percent
Protein	7
Fat	2
Carbohydrate	72
Salts	present
Vit. B-complex	present

Skim milk powder - composition according to Sherman (1946) -

Protein	36
Fat	1
Carbohydrate	52
Salts	present
Vit. A.	present

Dry gluten - with the following analysis -

	Percent
Protein----------	82.5
Ash--------------	1.5
Fat--------------	10
Moisture---------	6

Dry Zein - with the following analysis -

Protein----------	91.5
Ash--------------	0.4
Fat--------------	.1
Moisture---------	8

Dry vit. B-complex Test Diet - with the following analysis -

Vit. Test Casein-	18
Sucrose----------	68
Vegetable oil----	10
U.S.P. Salt mixture------------	2-4

The percentages of the purified substances used in various diets are shown in Table IV.

Discussion of Experiments.

The nutritional adequacy of Haydak's mixture wad demonstrated in the preliminary tests (Diet I, Table II, Fig. I)

Table IV.

The percentage composition of purified diets in the advanced tests with *Thermobia domestica*.

Percentage of Food Elements.

Foods	Diets									
	XVI	XVII	XVIII	IX	XX	XXI	XXII	XXIII	XXIV	XXV
Gluten	100	0	0	82	0	50	0	15	0	0
Zein	0	100	18	0	50	0	15	0	0	0
Starch	0	0	82	18	0	0	70	70	0	0
Yeast	0	0	0	0	50	50	15	15	0	50
Vit. B-complex test diet	0	0	0	0	0	0	0	0	100	50

and advanced tests (Diet XII, Table V, Fig. II). Growth, survival and age at reproduction were closely paralleled in both experiments.

Since the food trays were made from pieces of paper towel, this paper (Diet XV) was tested for acceptancy and food value. The results (Table V) compared closely with the group given no food (Diet II, Table II). None of the paper was eaten by the nymphs, although adult Thermobia were observed to feed on it under similar circumstances. It is thought that the nymphs had not developed sufficiently to feed on this paper.

Whole wheat flour (Diet XIII) did not contain the proper dietary requirements to permit maximum growth and only 13 percent survived the test period. (Table V, Fig. II). No reproduction occurred during the test. This data is in contrast to the conclusions given by Remington (1948). He obtained good growth and reproduction with whole wheat flour in his stock culture of T. domestica. This contradictory information may be somewhat explained by the findings of Sweetman (1948). He concluded that certain lots of whole wheat flour are nutritionally adequate for the nymphs while others are not.

Skim milk powder (Diet XIV) contained sufficient nutritive elements to permit 23 percent survival for the test period, but was obviously lacking in some essential factors for growth (Table V, Fig. II).

Table V.

The survival and growth of *Thermobia domestica* nymphs on Diets XII to XVII.

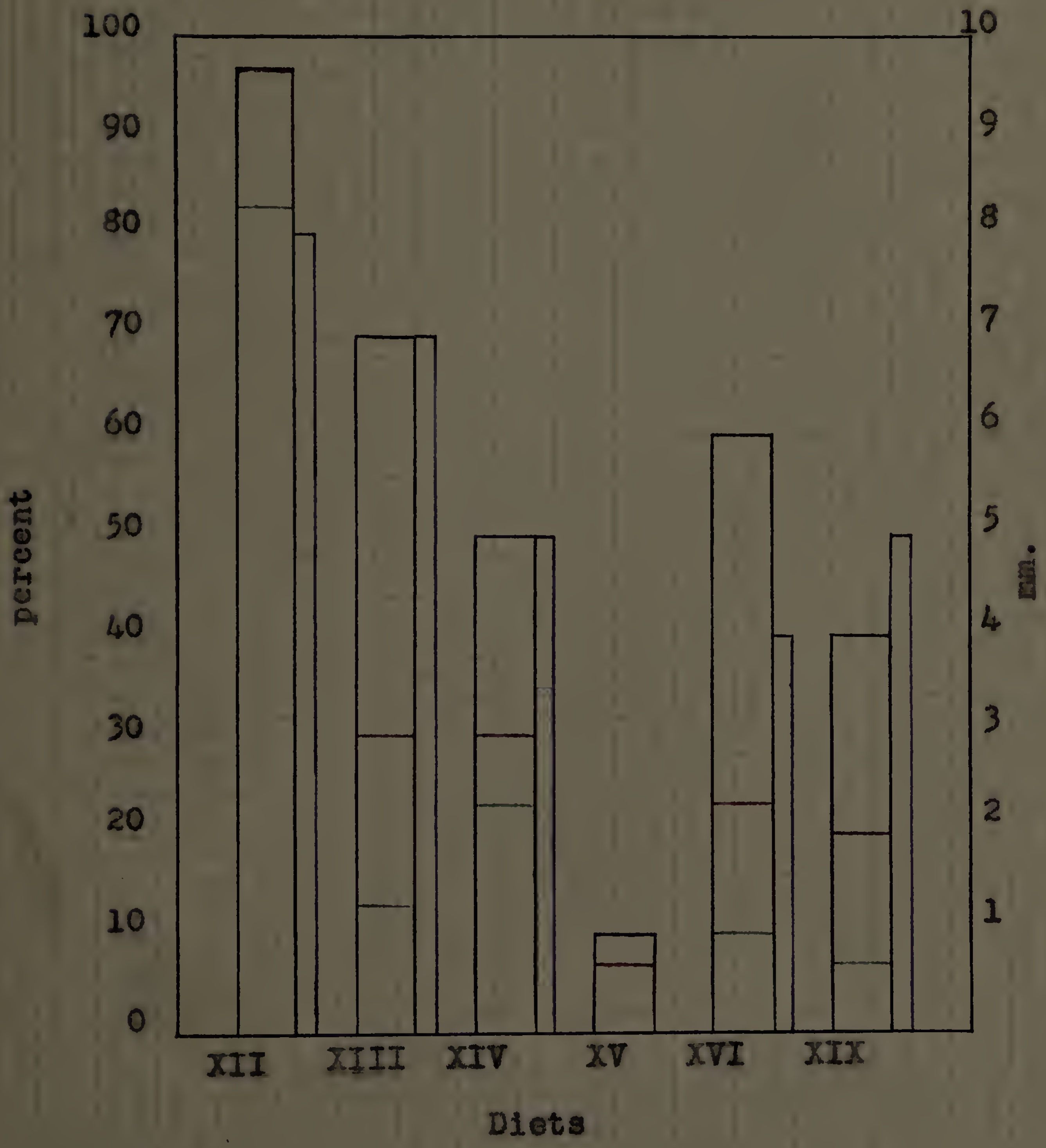
	Number surviving Days										Final size of length mm.	Greatest length of survival*
Diets	5	10	15	20	30	45	60	75	90	105		Days
XII	30	29	29	29	29	29	29	29	28	25	8	105+
XIII	27	27	26	25	21	15	12	7	5	4	7	105+
XIV	30	28	23	18	15	12	10	9	7	7	5	105+
XV	28	8	1	0							3	16
XVI	30	30	24	11	3	3	2	2	0		3+	87
XVII	30	27	17	7	1	0					2+	31

*First reproduction occurred at 60 days.

Figure II.

Percentage of nymphs alive at 35,70 and 105 days and size of nymphs at 105 days.

Percentage alive at 35 days - black
Percentage alive at 70 days - red
Percentage alive at 105 days- green
Size of nymphs in m.m.------- purple



Gluten (Diet XVI, Table V, Fig. II) gave poor survival and growth comparing closely with casein (Diet III). Neither gluten nor casein were sufficiently adequate nutritionally to permit growth beyond the early instars. Zein (Diet XVII) is more deficient for longevity and growth than either casein or gluten (Table V). An analysis of zein for amino acids has proved that this protein is deficient for animal survival (Sherman, 1946). The experimental results then, would indicate a probable necessity for certain essential amino acids in the diet of T. domestica.

Zein and starch (Diet XVIII, Table VI, Fig. II) permitted 10 percent survival but limited growth, while growth on gluten and starch (Diet XIX) compared closely with that of animal protein (Diet VI), although survival was greater. Diets XVIII and XIX are sufficient to permit limited growth of a few insects, but not maturation, during an average life cycle (Table VI, Fig. II).

Fair survival and limited growth of the nymphs resulted employing zein and yeast (Diet XX). Gluten and yeast (Diet XXI) was similar to zein and yeast for growth, but the survival was not as great (Table VI, Fig. III). These two diets, compared with casein and yeast (Diet VII, Table III), showed that the zein-yeast combination was more favorable for survival and the casein-yeast more favorable for growth although

Table VI.

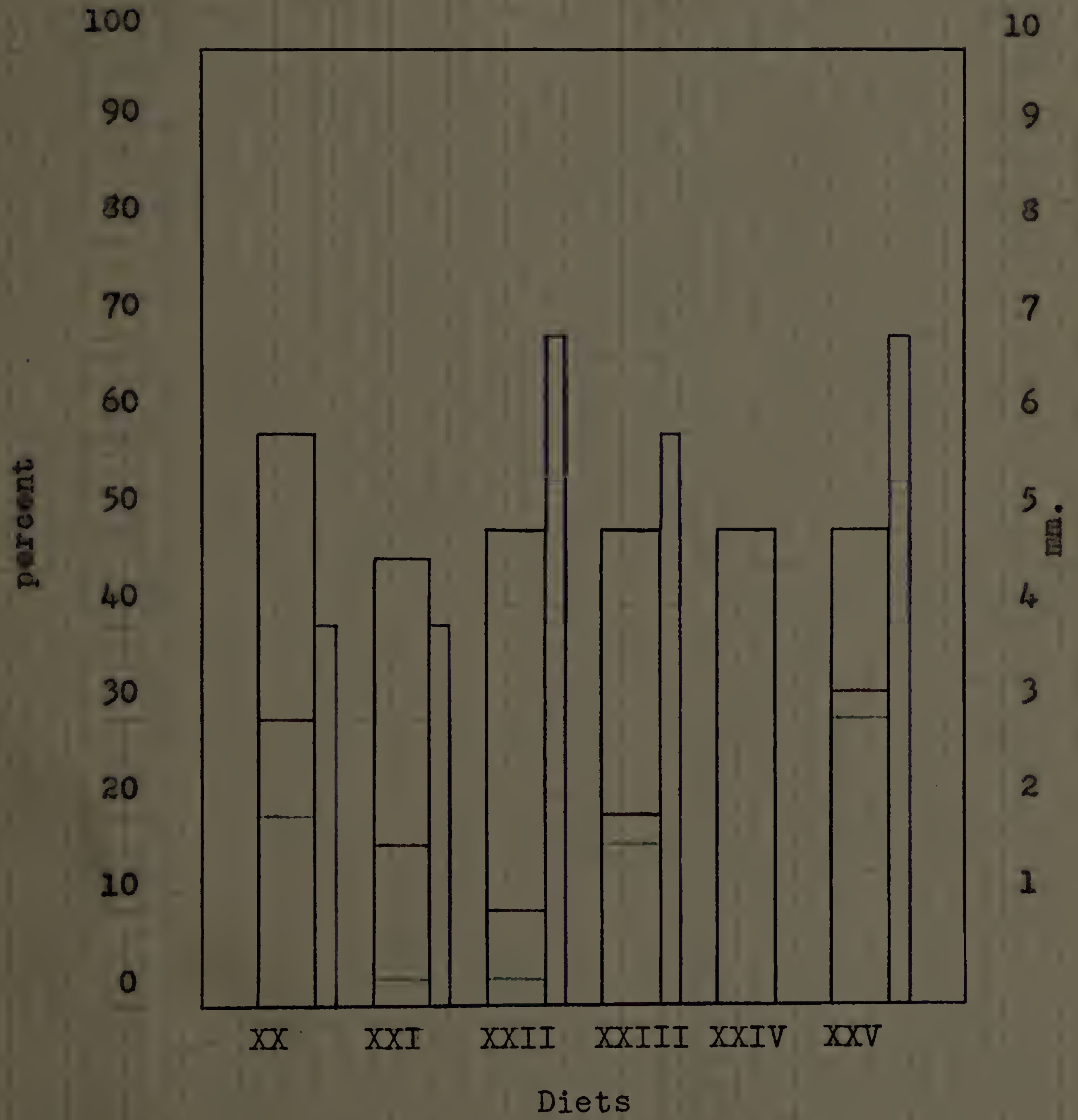
The survival and growth of <u>Thermobia domestica</u> on Diets XVIII to XXIII.

	Number surviving Days										Final size - length mm.	Greatest length of survival
Diets	5	10	15	20	30	45	60	75	90	105		Days
XVIII	30	29	28	23	19	12	9	6	4	3	4	105+
XIX	29	29	29	23	12	10	8	5	3	2	5	105+
XX	30	29	29	28	22	12	10	9	8	6	4	105+
XXI	30	28	28	25	16	9	6	4	4	1	4	105+
XXII	30	30	30	30	16	13	9	1	1	1	7	105+
XXIII	30	29	27	20	17	11	8	6	6	5	6	105+

Figure III.

Percentage of nymphs alive at 35,70 and 105 days and size of nymphs at 105 days.

Percentage alive at 35 days - black
Percentage alive at 70 days - red
Percentage alive at 105 days- green
Size of nymphs in m.m.------- purple



both were definitely inadequate.

Starch, yeast and zein (Diet XXII) was adequate for only one individual to mature (Table VI, Fig. III). The evidence indicated that zein was inferior in nutritional elements to casein (Diet IX, Table III). Gluten, starch and yeast (Diet XXIII) produced 17 percent survival (Table VI, Fig. III), but growth was retarded.

A comparison of the relative nutritive value of the three proteins indicates that casein is the better of the three in respect to growth, survival and maturation. The results suggest that a high fat content is of little value in the diet of _T. domestica_ since gluten, with a fat content of 10 percent, was less adequate than casein with a fat content of 0.2 percent.

Vitamin B-complex test diet (Diet XXIV) was very deficient for growth, survival and maturity, but when supplemented with yeast (Diet XXV), was much improved (Table VII, Fig. III). One third of the test animals matured and some reproduction occurred on the 105th day. This suggests that the vitamin B-complex in the diet was essential for reproduction. This was the only one of the purified diets on which reproduction occurred. Comparing Diet XXV with that of casein, yeast and starch (Diet IX), as to component parts, the former has the larger amount of fat and also salts. Furthermore, DietXXV contains glucose, while Diet IX has starch, a complex carbohydrate. The fat did not

Table VII.

The survival and growth of *Thermobia domestica* on Diets XXIV and XXV.

Diets	Number surviving (Days) 5	10	15	20	30	45	60	75	90	105	Final size length mm.	Greatest length of survival Days
XXIV	30	30	29	28	21	6	2	0			3+	65
XXV	30	28	21	19	16	13	11	9	9	9	7+	105*+

*First reproduction occurred at 105 days.

appreciably effect the growth of the nymphs, since gluten with its higher fat content gave poorer development than casein. The apparent difference then would rest with the salts and simple carbohydrates versus complex carbohydrates. The possibility of the young firebrat being unable to digest and assimilate carbohydrates in complex form must be considered, as must the necessity for salts in normal development. More experimentation with regard to these two factors is needed, before a definite conclusion can be reached.

CONCLUSIONS

A. Carbohydrates.

The growth rate of T. domestica on diets containing starch was not as great as on a diet containing glucose, indicating that complex carbohydrates may not be assimilated by the young nymphs. The apparent absence of amylolitic enzymes appears to be in contrast to the feeding habits of this insects i.e., on starchy products. More work is needed before the presence or absence of the enzymes can be established.

B. Proteins.

Protein in some form was an essential component of a complete diet. Proteins alone were very deficient for growth and survival, but when supplemented with other substances such as sucrose, minerals and vitamin B-complex, were adequate for growth and reproduction. The variation in the results obtained with the proteins gives some indication that certain of the amino acids may be required for development of the nymphs.

C. Fats.

Fat in excess of one percent was considered unessential in the diet of T. domestica. Experiments with protein materials containing 10 percent fat content gave no better results than those containing .2 percent. However, since the fat was not added separately to the diets, the quantitative and qualitative requirements of this substance are still doubtful.

D. Mineral Elements.

Diets containing minerals in the form of ash (of unknown composition) gave poorer results than those with U.S.P. salt mixture no. 2, indicating a possible necessity for minerals as a supplement in the diet.

E. Vitamins.

One or more of the B-complex fractions appeared to be necessary since no reproduction occurred in its absence.

Vitamin A was considered unessential, since the purified diet that gave reproduction did not contain this vitamin. Furthermore, when added to other diets, this vitamin gave no noticeable improvement in growth or survival.

Vitamins C, D, E and K were considered unnecessary since they were not present in the purified diet most adequate for survival, growth and reproduction.

The conclusions concerning the vitamins are believed to be well established and in keeping with the results obtained by other workers in the field of insect nutrition.

SUMMARY OF EXPERIMENTS

1. The nymphs were unable to utilize paper towel as a source of cellulose.
2. Neither whole wheat flour nor skim milk powder was an adequate food for normal survival and development of *T. domestica* under laboratory conditions. Whole wheat flour permitted maturation of a few insects, but not reproduction.
3. Gluten and zein were inadequate for growth and development with the former more sufficient than the latter.
4. Both gluten and starch, and zein and starch were deficient diets, permitting limited growth and survival. The former diet was somewhat superior for growth over the latter, whereas survival on these two diets was similar.
5. Zein + yeast and gluten + yeast permitted limited growth with poor survival. The zein + yeast mixture was better for survival than gluten + yeast. The nutritive value of these foods for growth was equal.
6. Starch + yeast + zein and starch + yeast + gluten produced better growth than any of the purified diets above. The latter diet was the better for survival and the former for growth.

7. The vitamin B-complex test diet was a deficient diet for growth and survival.

8. Vitamin B-complex test diet supplemented with yeast is a satisfactory diet for growth and reproduction. However, survival was less with this diet than with Haydak's mixture.

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