COMPARISON OF SOME AMINO ACID MIXTURES AND PROTEINS FOR THE DIET OF DROSOPHILA MELANOGASTER

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ABSTRACT.—The nutritive values of several proteins for the development of *Drosophila melanogaster* as measured by viability, adult size, and developmental time exceeded that of amino acid mixtures. The descending order of effectiveness of the test proteins was soy, lactalbumin, casein, supplemented gluten and peanut meal. One of the test amino acid mixtures supported development better than any which has been employed by others in previous studies. Of the nitrogen sources tested, the best development was observed when a mixture of acid hydrolyzed and intact casein was fed.

Drosophila melanogaster is among the insect species which have been cultured on diets containing amino acids as the sole source of nitrogen (Schultz, St. Lawrence and Newmeyer, 1946; Hinton, Noyes and Ellis, 1951). However, *D. melanogaster* larvae do not develop as well on amino acid diets as they do on intact protein diets (Sang, 1956, 1959, 1962; Geer, 1963). These data coupled with the results of experimentation on other insect species (Vanderzant, 1958; Cheldelin and Newburgh, 1959; Leclercq and Lopez-Francos, 1964; Naylor, 1964) indicate that nonhydrolyzed protein has a nutritive value for insect development not found in its constituent amino acids. An exception to this observation, which may possibly be extended to insects in general, is the onion maggot which develops more rapidly on an amino acid diet (Friend, Salkeld and Stevenson, 1959).

Investigators studying the growth-stimulating value of amino acids in rat diets have made conflicting observations. Sauberlich (1961) presented data which indicate that amino acid diets can equal diets of intact protein in nutritional value. However, reports by Breuer et al. (1963) and Salmon (1964) do not agree, but instead indicate that intact protein is superior for growth to amino acid mixtures in the rat diet. Thus, the majority of the studies of insect nutrition and rat nutrition agree on the superior dietary qualities of intact protein.

The present investigation compares the nutritive values of several intact protein and amino acid diets for *D. melanogaster* development. It also examines the relationship of the degree to which dietary protein has been hydrolyzed to its adequacy for development. The term development as used here includes not only the period of rapid larval growth but also the ability of the larva to metamorphose.

Methods and Materials

To avoid strain-specific nutritional differences, the larvae tested in this study were obtained by a three-way cross of the Canton-S, Riverside and Oregon-R strains. These strains had been made homozygous by a marker-inversion mating scheme (Geer, 1964). Canton-S females were crossed to Riverside males and the heterozygous female offspring were mated to Oregon-R males. Using the techniques described by Geer (1963) and Geer and Vovis (1965), eggs were collected from the females of the latter cross, sterilized and inoculated by aseptic methods onto the test media. Cultures were kept at 23.8± 1.1 °C. and 50% relative humidity with a 10 hour light to 14 hour dark day. Results of the experiments were assessed by comparing the larval developmental times, defined as days from egg to pupation; the larval viabilities, determined by the percentage of larvae to pupate and to reach adulthood; and the adult sizes, measured by female dry weight. These variables are dependent upon the larva's ability to utilize its diet to develop from a newly hatched larva to the adult or imago stage.

The amino acid test mixtures are given in Table 1, and the other nitrogen test sources are indicated with the ap-

Table 1.—Amino acid test mixtures. Individual amino acids are given in mg of amino acid per 100 ml of medium.

Mixture	A	B	C	D	E	F	G
L-Alanine	108.5	152.0	60.0	....	....	....	....
L-Arginine·HCl	55.9	179.0	80.0	80.0	80.0	80.0	80.0
L-Aspartic acid	122.1	198.0	60.0	....	....	....	....
L-Cystine	48.0	31.0	30.0	30.0	30.0	30.0	30.0
L-Glutamic acid	441.8	300.0	400.0	840.0	1,000.0	1,200.0	1,200.0
Glycine	174.5	110.0	40.0	40.0	40.0	40.0	40.0
L-Histidine·HCl	48.4	93.5	100.0	100.0	100.0	100.0	100.0
L-Hydroxyproline	38.8	....	....	....	....	....	....
DL-Isoleucine	126.0	119.0	300.0	300.0	250.0	200.0	300.0
L-Leucine	234.5	166.0	200.0	200.0	175.0	150.0	200.0
L-Lysine·HCl	133.7	227.0	190.0	190.0	170.0	150.0	190.0
DL-Methionine	33.9	36.0	80.0	80.0	80.0	80.0	80.0
DL-Phenylalanine	100.8	81.0	130.0	130.0	130.0	130.0	130.0
L-Proline	168.6	110.0	50.0	....	....	....	....
DL-Serine	126.0	116.0	50.0	....	....	....	....
DL-Threonine	75.6	127.0	200.0	200.0	200.0	150.0	200.0
L-Tryptophan	174.5	30.0	50.0	50.0	50.0	50.0	50.0
L-Tyrosine	124.0	91.0	80.0	80.0	80.0	80.0	80.0
DL-Valine	135.5	134.0	280.0	280.0	220.0	200.0	280.0

propriate data. Unless otherwise noted, the total nitrogen supplied by protein or amino acids in all test diets was 0.30 g per 100 ml of medium. The remainder of the diet consisted of yeast ribonucleic acid, sucrose, cholesterol, choline, B- vitamins and salts in the concentrations given by Geer and Vovis (1965). Amino acid test mixture A is essentially the same as those employed by Schultz et al. (1946) and Hinton et al. (1951); B resembles the amino acid composition of yeast protein; and C and D contain amino acids in the same proportions as found in amino acid mixtures used in rat diets by Salmon (1964).

Vitamin-free casein was obtained from the California Corporation for Biochemical Research, Assay Protein C-1 (soy) from Skidmore Enterprises and extracted peanut meal from the General Biochemical Corporation. Inorganic constituents were purchased from the Fisher Scientific Corporation. All other nutrients and vitamins were obtained from the Nutritional Biochemicals Corporation.

Results

The nutritive values of amino acid mixtures A-D are indicated in Table 2. D was a superior and C an inferior nitrogen source. Larvae cultured on D had a significantly shorter developmental period and exhibited higher rates of pupation and emergence than larvae cultured on A or B. There was no size difference between adults grown on A and D, but adults grown on B were much reduced in size.

The concentration of L-glutamic acid was much greater in D than in A, B or C. Mixtures E, F and G were formulated to test the effects of increasing the concentration of L-glutamic acid above the level in D. In E and F the total nitrogen content was maintained at 0.30 g per 100 ml of medium by reducing the concentrations of DL-isoleucine. L-leucine, L-lysine and DL-threonine as the concentration of L-glutamic acid was increased. In G the concentrations of all amino acids were maintained at the same level as in D while the concentration of L-glutamic acid was increased; consequently, the nitrogen content of G was 0.33 g per 100 ml of medium. Increasing the concentration of L-glutamic acid at the expense of the essential amino acids had little effect on viability but it lengthened the developmental time (Table 3). Adult size was also decreased. Increasing the L-glutamic acid concentration, but not at the expense of other amino acids, decreased adult size slightly and lengthened the developmental time by 0.6 day.

TABLE 2.—Development of larvae on amino acid diets.

Test mixture	Original no. of larvae	% Larvae to pupate	% Larvae to become adults	Developmental time ±S.D. (days)	Female dry weight (mg X 100)
A	1872	72.0	60.1	15.0 ±2.1	23.1
B	1640	48.7	34.3	18.5 ±3.6	14.5
C	1228	7.3	2.5	23.5 ±2.4	....
D	1965	92.1	86.9	12.3 ±1.4	23.1

The influence of the degree of hydrolysis of the protein on development was studied by feeding larvae either intact casein, enzyme digested casein, acid hydrolyzed casein or a mixture of acid hydrolyzed and intact casein (Table 4). Larvae cultured on intact casein, enzyme digested casein or the acid hydrolyzed-intact casein mixture were equally viable and were more viable than larvae cultured on supplemented acid hydrolyzed casein. Those larvae which were grown on the acid hydro-

TABLE 3.—Development of larvae on amino acid diets with different concentrations of L-glutamic acid.

Test mixture	Original no. of larvae	% Larvae to pupate	% Larvae to become adults	Developmental time ±S.D. (days)	Female dry weight (mg X 100)
D	1008	96.0	89.3	13.1 ±1.4	21.8
E	1312	93.7	89.7	13.2 ±1.6	18.6
F	1107	90.3	83.7	14.4 ±1.2	18.4
G	1207	91.3	87.9	13.7 ±1.5	18.6

TABLE 4.—Development of larvae on intact, enzyme digested, and acid hydrolyzed casein.

Nitrogen source	Original no. of larvae	% Larvae to pupate	% Larvae to become adults	Developmental time ±S.D. (days)	Female dry weight (mg X 100)
Intact	686	93.7	85.6	8.8 ±1.0	39.9
Enzyme digest	704	93.0	86.6	9.3 ±0.6	28.7
Acid hydrolysate[1]	733	75.3	72.0	15.0 ±1.5	25.4
Acid hydrolysate + intact[2]	729	96.3	84.4	7.4 ±0.6	37.3

[1] Supplemented with 50 mg of L-tryptophan and 30 mg of L-cystine per 100 ml of medium.
[2] Supplemented with 25 mg of L-tryptophan per 100 ml of medium.

lyzed-intact casein mixture developed more rapidly than larvae fed the other test diets. The second most rapid larval development was on intact casein. The rate of development was slower with enzyme digested casein and slowest with supplemented acid hydrolyzed casein. Adult size was correlated with the length of the larval growth period. The acid hydrolyzed-intact casein mixture and the intact casein diets yielded the largest adults and the enzyme digested casein and acid hydrolyzed casein diets were less effective in this respect.

The optimal ratio of intact casein to acid hydrolyzed casein in the larval diet was determined by feeding ratios of 1 to 9, 3 to 7, 1 to 1, 7 to 3, and 9 parts of dietary nitrogen in the form of intact casein to 1 part of dietary nitrogen supplied as acid hydrolyzed casein (Table 5). Using developmental time and larval viability as the criteria, 7 parts of intact casein to 3 parts of acid hydrolyzed casein was the optimal ratio. In terms of adult size, intact casein was superior to the acid hydrolyzed-intact casein mixtures.

The nutritional qualities of proteins other than casein were studied (Table 6). The larvae grew much more rapidly and the adults were larger when fed soy

TABLE 5.—Development of larvae on mixtures of acid hydrolyzed and intact casein.

% Nitrogen supplied as acid hydrolyszed casein[1]	Original no. of larvae	% Larvae to pupate	% Larvae to become adults	Developmental time ±S.D. (days)	Female dry weight (mg X 100)
0	550	93.8	69.5	8.3 ±1.0	41.4
10	633	91.4	79.2	8.0 ±0.9	35.3
30	674	96.6	86.0	7.3 ±0.7	33.7
50	606	97.1	82.3	7.7 ±0.7	36.3
70	551	95.6	84.0	8.6 ±0.8	32.7
90	604	94.8	81.2	9.1 ±0.6	25.2
100	725	92.0	77.9	9.3 ±1.0	22.8

[1] The diets were supplemented with L-tryptophan in amounts proportional to the amount of acid hydrolyzed casein contained in each diet. In addition, the diet containing only acid hydroloyzed casein was supplemented with 30 mg of L-cystine, 20 mg of DL-phenylalanine, and 20 mg of L-histidine • HCl per 100 ml of medium.

TABLE 6.—Development of larvae on several protein diets.

Protein	Original no. of larvae	% Larvae to pupate	% Larvae to become adults	Developmental time ±S.D. (days)	Female dry weight (mg X 100)
Lactalbumin	1016	97.2	93.0	9.2 ±0.7	35.2
Peanut meal	972	85.7	83.0	12.3 ±1.3	36.0
Soy	1025	87.2	84.7	7.3 ±0.7	48.2
Gluten[1]	1110	87.2	84.9	8.6 ±0.8	34.7

[1] Supplemented with 50 mg L-glutamic acid, 30 mg of L-histidine • HCl, 100 mg of L-lysine • HCl, 30 mg of DL-threonine, and 20 mg of DL-valine per 100 ml of medium.

protein. However, viability of larvae fed lactalbumin was greater than that of the larvae fed the other proteins. The viabilities of larvae fed peanut meal and supplemented gluten were equal to the viability of soy-fed larvae, but less than lactalbumin-fed larvae.

Discussion

Glutamic acid appears to be of primary importance in the diet of *D. melanogaster* larvae. This is evidenced by the inferior nutritive value for development of amino acid test mixture C. Except for a lower glutamic acid concentration, C includes a greater number of nonessential amino acids than D. Apparently, glutamic acid does more than replace nonessential amino acids in diet D. Also, reductions of the concentrations of four essential amino acids in E, F and G had little effect on development when glutamic acid was increased. These observations on the development of larvae fed amino acid mixtures suggest that glutamic acid, in addition to its role in the synthesis of nonessential amino acids, has a sparing effect on the nutritional requirements for the essential amino acids. A glutamic acid sparing effect has been observed, in fact, in the nutrition of several insects. Naylor (1963) found that glutamic acid can partially replace essential amino acids in the diet of *Tribolium confusum*. Hodgson, Cheldelin and Newburgh (1956) showed that either aspartic acid or glutamic acid is required in the diet of *Phormia regina*. The suggestion of Hinton (1959) that glutamic acid can substitute for glycine in the diet of *D. melanogaster* agrees with the present observations.

Judgment of the adequacy of a protein for development depends upon the criteria which are used. For example, when larval developmental time is used as the criterion, soy, casein, supplemented gluten, lactalbumin and peanut meal are effective as dietary nitrogen sources in that descending order. Lactalbumin is the superior protein if larval viability is the criterion. If the protein diets are judged by their effect upon adult size, the descending order of dietary effectiveness is soy, lactalbumin, supplemented gluten, casein and peanut meal. The use of a number of criteria, which is perhaps the best approach, indicated that the sequence of effectiveness is soy, lactalbumin, casein, supplemented gluten and peanut meal in that order. That soy protein outranks lactalbumin and casein for the development of Drosophila agrees with the results of previous work with protein hydrolysates (Geer, 1963) on *D. melanogaster*.

Experimental data are consistent with the concept that amino acids do not support insect development as well as intact proteins do. This is true for a variety of insects. Naylor (1964) found that four different proteins had a development-accelerating effect for *T. confusum*. Vanderzant (1958) showed that acid hydrolyzed casein was inferior to intact casein in the diet of *Pectinophora gossypiella*. Similar observations have been made for *P. regina* (Cheldelin and Newburgh, 1959) and *Tenebrio molitor* (Leclercq and Lopez-Francos, 1964). To possibly explain the intact protein effect, Naylor (1964) has suggested that there may be some dietary value in peptide linkages or that there may be special peptides in certain proteins which stimulate insect development. There is no conclusive evidence for either of these hypotheses.

The current experimentation indicates that a source of free amino acids may contribute to the nutritive value of a diet containing an intact protein. The evidence is supplied by the observation that development is more rapid and survival greater on a diet containing a mixture of acid hydrolyzed and intact casein than on one containing intact casein alone. Before any generalizations can be made, however, the work needs to be repeated with different proteins and also with other organisms.

Much effort has been directed toward the replacement of intact protein with amino acid mixtures in the diets of many organisms. The pertinent literature has been reviewed by Almquist (1951) and Greenstein and Winitz (1961). *D. melanogaster* belongs to the growing list of organisms for which intact dietary protein has been found to be superior to amino acid mixtures.

The fact that the development of *D. melanogaster* fed amino acid mixture D surpassed that of larvae fed mixture A is of some importance. Since A and variations thereof have been used when amino acid mixtures have been employed in previous studies of Drosophila nutrition, the superiority of D provides Drosophila research workers with a better amino acid diet for nutritional and biochemical investigations.

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