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Experimental Studies on the Developmental Mechanism of Bar Eye in *Drosophila melanogaster*

IV. Facet-increasing Effect of Amino Acids and Peptides

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Recently many organic substances have been found which have effect in increasing the facets of the Bar eye in *Drosophila melanogaster* (OGAKI, KAJI and TANAKA, 1953; KAJI, 1954, 1955a, b, 1956; OGAKI, 1956). As far as the experiments of the author (KAJI, 1955a, b, 1956) are concerned, it has been ascertained that the substances which proved to issue marked effect commonly possess the amide linkage in their chemical structures. From this fact, it can be assumed that the amide linkage may bear significance in increasing the facet number. In order to confirm this supposition were carried out the following experiments in which varied amino acids, their derivatives and peptides were tested as the facet-increasing agent.

In the experiments were used two strain of *Drosophila melanogaster*: inbred Bar (*B*) and *Oregon* wild type. Test of the substances was made in the same way as was done in our previous experiments (KAJI, 1954). Larvae of Bar stock in fifty hours age were raised on agar molasses medium containing the chemical substance to be tested. Throughout experiments the flies were kept at 25°C. Facet number of the flies were counted by means of the Sump method.

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Experiments

1. *Amino Acids and their Derivatives*

Thirty-one different amino acids and their derivatives were tested for their effect in increasing the facet number. They were glycine, glycinamide, DL-alanine, alanine amide, DL-serine, DL-threonine, DL-valine, L-leucine, L-isoleucine, sarcosine, L-aspartic acid, L-asparagine, L-glutamic acid, L-glutamine, L-arginine, L-ornithine, DL-citrulline, L-lysine hydrochloride, L-cystein, L-cystine, DL-methionine, L-phenilalanine, L-tyrosine, L-histidine, L-histamine hydrochloride, L-proline, L-hydroxyproline, DL-tryptophane, glycocyamine, creatine and creatinine. Some of these substances have been examined in our previous experiments (OGAKI, KAJI and

TANAKA, 1953; KAJI, 1954, 1955a), and the data of those experiments were again used in the following description. In order to facilitate the comparison of effectiveness of the respective substances, whole results of the experiments are shown in tabular form (Table 1). As is apparent in the table, most of the substances tested proved to be effective in increasing the facets of the Bar eye. But the increase of the facet number brought about by these substances was not necessarily uniform, but varied according to respective substances. Effect of such amino acids and their derivatives as L-arginine, DL-citrulline, L-lysine, L-histidine, L-hydroxyproline, glycocyamine, creatinine, alanine amide and glycinamide was marked as compared with that of other substances. Especially, L-hydroxyproline exerted the most marked effect, the increase of the facet number brought about with 2 per cent solution of this chemical being 182.8 on an average. In an extreme case facets of the male flies treated with this solution counted as many as 278. This average number is about 2.6 times that of the control Bar facets which are 70 in male flies. Effect of the other substances was always less than that of L-hydroxyproline. Among the substances tested, there were several substances the chemical structures of which were closely related. For instance, the molecular structures of L-aspartic acid, L-glutamic acid, DL-alanine and glycine correspond to those of L-asparagine, L-glutamine, alanine amide and glycinamide respectively.

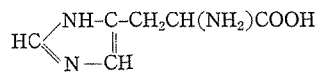
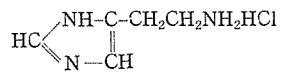
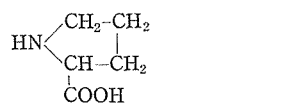
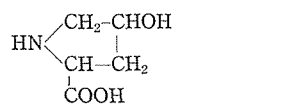
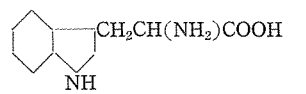
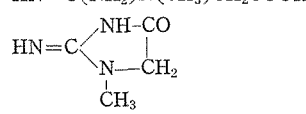
A common difference which can be distinguished between these two groups of substances is that the amide linkage is absent in the former group, while it is present in the latter one. Between the two groups of substances, there was a considerable difference in their effect in increasing the facet numbers. As apparent in the Table, effect of the former group was always far weaker than that of the latter group which possesses the amide linkage in their structure. Namely, the average increase produced by the substances in the former group was less than 115, whereas the increase brought about by the substances in the latter group was always more than 180. Therefore, significance of the amide linkage for the increase of the facet number is again demonstrated.

2. *Peptides*

Tweleve kinds of di- and tri-peptides such as glycil-glycine, glycil-L-leucine, glycil-L-aspartic acid, glycil-L-alanine, L-leucil-L-alanine, DL-leucil-glycine, L-alanil-glycine, L-alanil-L-alanine, DL-leucil-glycil-glycine, glycil-glycil-glycine, DL-alanil-glycil-glycine and L-alanil-L-alanil-L-alanine were examined. As is enumerated in Table 2, all the substances examined were shown to be effective in increasing the Bar facets, though degrees of the increase varied with respective substances. It will be said that these peptides exserted, as a rule, stronger effect than the amino acids and their derivatives used in the above mentioned experiments.

Out of the di-peptides tested, L-alanil-L-alanine used at a concentration of 3 per cent produced the highest effect, and the facets increased to 147.2 on an average. But effect of tri-peptides of L-alanine was by far stronger, i.e., L-alanil-L-alanil-L-alanine at a concentration of 3 per cent was found to increase the facets

Table 1. Effect of amino acids and their derivatives on the increase of facet number.

Substances	Formula	Conc. (%)	Facet number* (average in males)
Glycine	$CH_2(NH_2)COOH$	3.0	103.3
Glycinamide	$H_2NCH_2CONH_2$	0.5	180.9
DL-Alanine	$CH_3CH(NH_2)COOH$	2.0	102.0
Alanine amide	$CH_3CH(NH_2)CONH_2$	1.0	173.4
DL-Serine	$CH_2OHCH(NH_2)COOH$	2.0	117.1
DL-Threonine	$CH_3CH(OH)CH(NH_2)COOH$	2.0	72.0
DL-Valine	$(CH_3)_2CHCH(NH_2)COOH$	2.0	91.0
L-Leucine	$(CH_3)CHCH_2CH(NH_2)COOH$	2.0	86.7
L-Isoleucine	$CH_3CH_2CH(CH_3)CH(NH_2)COOH$	2.0	90.9
Sarcosine	CH_3NHCH_2COOH	1.0	117.0
L-Aspartic acid	$HOOCCH_2CH(NH_2)COOH$	2.0	114.9
L-Asparagine	$H_2NCOCH_2CH(NH_2)COOH$	3.0	130.4
L-Glutamic acid	$HOOC(CH_2)_2CH(NH_2)COOH$	3.0	74.7
L-Glutamine	$H_2NCO(CH_2)_2CH(NH_2)COOH$	3.0	128.3
L-Arginine	$H_2NC(=NH)NH(CH_2)_3CH(NH_2)COOH$	2.0	148.2
L-Ornithine	$H_2N(CH_2)_3CH(NH_2)COOH$	2.0	125.4
DL-Citrulline	$H_2NCONH(CH_2)_3CH(NH_2)COOH$	2.0	177.3
L-Lysine hydrochloride	$H_2N(CH_2)_4CH(NH_2)COOH\cdot HCl$	2.0	143.0
L-Cysteine	$HSCH_2CH(NH_2)COOH$	3.0	82.6
L-Cystine	$[-SCH_2CH(NH_2)COOH]_2$	2.0	81.0
DL-Methionine	$CH_3S(CH_2)_2CH(NH_2)COOH$	1.0	101.7
L-Phenilalanine	$\bigcirc CH_2CH(NH_2)COOH$	2.0	89.0
L-Tyrosine	$HO\bigcirc CH_2CH(NH_2)COOH$	2.0	89.8
L-Histidine		4.0	179.5
L-Histamine hydrochloride		3.0	113.2
L-Proline		1.5	111.3
L-Hydroxyproline		2.0	182.8
DL-Tryptophane		2.0	84.6
Glycocyamine	$H_2NC(=NH)NHCH_2COOH$	1.0	158.9
Creatine	$HN=C(NH_2)N(CH_3)CH_2COOH$	1.5	100.3
Creatinine		2.0	148.1

* The mean facet number of the untreated Bar (*B*) eye is 70 in male, and that of the wild type (*Oregon*) 680.

Table 2. Effect of various peptides on the increase of facet number.

Substances	Formula	Conc. (%)	Facet number (average in males)
Glycil-Glycine	$H_2NCH_2CONHCH_2COOH$	3.0	132.5
Glycil-L-Leucine	$H_2NCH_2CONHCH(COOH)CH_2CH(CH_3)_2$	2.0	105.9
Glycil-L-Aspartic acid	$H_2NCH_2CONHCH(CH_2COOH)COOH$	1.0	102.7
Glycil-L-Alanine	$H_2NCH_2CONHCH(CH_3)COOH$	1.0	123.7
L-Leucil-L-Alanine	$(CH_3)_2CHCH_2CH(NH_2)CONHCH(CH_3)-COOH$	1.0	133.7
DL-Leucil-Glycine	$(CH_3)_2CHCH_2CH(NH_2)CONHCH_2COOH$	1.0	110.2
L-Alanil-Glycine	$CH_3CH(NH_2)CONHCH_2COOH$	3.0	134.8
L-Alanil-L-Alanine	$CH_3CH(NH_2)CONHCH(CH_3)COOH$	3.0	147.2
DL-Leucil-Glycil-Glycine	$(CH_3)_2CHCH_2CH(NH_2)CONHCH_2CONH-CH_2COOH$	1.0	137.7
Glycil-Glycil-Glycine	$H_2NCH_2CONHCH_2CONHCH_2COOH$	3.0	145.3
DL-Alanil-Glycil-Glycine	$CH_3CH(NH_2)CONHCH_2CONHCH_2COOH$	3.0	218.5
L-Alanil-L-Alanil-L-Alanine	$CH_3CH(NH_2)CONHCH(CH_3)CONHCH-(CH_3)COOH$	3.0	239.0

as many as 239.0. In order to make more accurate comparison of the effects, these polypeptides were tested at the same molar concentration. That is, DL-alanine, L-alanil-L-alanine and L-alanil-L-alanil-L-alanine were tested at 0.14 molar solution. At this concentration all the chemicals were shown to have effect to increase the facets of the Bar fly, but the degrees of increase were not the same in respective substances. In the case of DL-alanine the average number of the facets of the treated fly was 95.2, in the case of L-alanil-L-alanine it was 132.7 and in the case of L-alanil-L-alanil-L-alanine it was counted as many as 239.0. From this result, it may be inferred that the facet-increasing influence of those substances which possess the amide linkage in their chemical structure becomes stronger in accordance with the increase of the peptide bonds. Since, however, no polypeptides possessing more than 3 peptide bonds were tested on account of the difficulty to gain them, it seems too hasty to regard this as a general tendency. From this result alone, however, it becomes evident that the substances which have the peptide linkage in their chemical structure issue always stronger influence in increasing the facet number of the Bar eye than those which have no peptide linkage such as amino acids and their derivatives.

Discussion

As is above described, all the amino acids and their derivatives tested in the present experiment proved effective in increasing the facet number of the Bar eye. But the number of increase actually brought about by these substances was not so

great. The maximum number of the facets of treated flies counted 278, and this was found in the treatment of L-hydroxyproline. On an average, the facet increase produced by most of these substances was always less than 150. Therefore, the facet-increasing effect of these amino acids is apparent but not so conspicuous as that of ureids and acid amides that has been proved by the previous experiments of the author (KAJI, 1954, 1955a, b, 1956). The flies treated with some of acid amides were possessed of the eye as large as the wild type.

On the other hand, degrees of the effect brought about by the amino acids and their derivatives tested in the present experiment were comparable to those that have been produced with other agents than chemical substances, such as low temperature (SEYSTER, 1919; KRAFKA, 1920; HERSH, 1930, etc.), increased supply of oxygen (MARGOLIS, 1939), semi-starvation of larvae (BODENSTEIN, 1939, 1941) and breeding in the alkaline medium (unpublished data of the author). With these agents the maximum number of facets of the Bar flies increases to some 200, the number being equal to that we have found in the present experiment with amino acids.

It should be mentioned that among the amino acids tested two different groups are distinguished in respect to their facet-increasing effect. In one group of amino acids which possess the amide linkage in their molecular structure, the effect was always greater than that of the other group which lacks the amide linkage. This result seems to indicate that the presence of amide linkage in the molecular structure bears significance for the increase of facets. Similarly in the case of peptides, significance of the amide linkage was shown by the following facts: 1) Facet-increase was always greater in the treatment of peptides than in that of amino acids and 2) tri-peptides always brought about greater increase than di-peptides. Yet, it is a question still to be examined whether the effect polypeptides is augmented in proportion to the increase of the number of peptide linkages in their molecule. At present, however, it will be safe to consider that the molecular structure which possesses the amide linkage may bear special significance for the increase of the facet number of the Bar fly.

Summary

Facet-increasing effect of a large number of amino acids, di-peptides and tri-peptides was tested. The result indicates that 1) although these substances commonly exert an influence in increasing the facets of the Bar eye, degree of the effect varied with respective substances, 2) the substances which possess the amide linkage in their molecular configuration issue greater effect than those which lack the amide linkage and 3) tri-peptides are more effective than di-peptides.

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