Total Amino Acids in Developing Worker Honey Bees *(Apis mellifera* L.)

The free amino acid content in the hemolymph or blood of insects is high in comparison with that of other animals, and the amino acid pool in insect cells is similar to that in the hemolymph[1]. Several investigators[2–8] have examined amino acids in honey bee (*Apis mellifera* L.) hemolymph. However, no complete quantitative data are available concerning these amino acids during development and aging of the bee. CHEN[9] has emphasized that valuable information concerning biochemical processes which accompany morphological changes could be gained from amino acid analysis at different embryonic stages. The purpose of this investigation was to examine the total (free and protein-bound) amino acid content in eggs, larval hemolymph, pupal fluid, and adult worker honey bee hemolymph to provide quantitative data on changes in individual amino acid levels as development proceeds.

In May 1967, a laying queen was confined by a queen excluder cage to an area of a brood comb in a colony. All samples were obtained from this colony so the age of the bees could be accurately determined. The first eggs were found after 24 h, and ages of all subsequent samples were determined by assigning these first eggs the age of 1 day. Thereafter, samples of worker brood were removed from the colony at 24-h intervals.

[1] M. FLORKIN and C. H. JEUNIAUX, in *The Physiology of Insecta* (Ed. M. ROCKSTEIN; Academic Press, New York 1964), vol. 3, p. 109.
[2] A. P. DE GROOT, Experientia *8*, 192 (1952).
[3] N. FUJII, Miyazaki Univ. Fac. Agric. Bull. *7*, 1 (1962).
[4] Z. GLINSKI and T. KOSTARZ, Med. Weterynar., Poland *24*, 183 (1968).
[5] P. F. LUE and S. E. DIXON, Can. J. Zool. *42*, 205 (1967).
[6] P. F. LUE and S. E. DIXON, Can. J. Zool *45*, 595 (1967).
[7] J. J. PRATT, Ann. ent. Soc. Am. *43*, 573 (1950).
[8] D. I. WANG and F. E. MOELLER, J. Invertebr. Path. *15*, 202 (1970).
[9] P. S. CHEN, in *Advances in Insect Physiology*, (Eds. J. W. L. BEAMENT, J. E. TREHERNE and V. B. WIGGLESWORTH; Academic Press, New York 1966), vol. 3, p. 56.

Total amino acids in developing worker honey bees (*Apis mellifera* L.)

Amino acid (μmole/ml)	Source of sample 1-Day eggs	2-Day eggs	3-Day eggs	4-Day eggs	5-Day larvae	6-Day larvae	7-Day larvae	8-Day larvae	9-Day larvae	10-Day larvae
Lysine	0.034	0.083	0.065	0.081	0.142	0.181	0.641	0.715	0.429	1.28
Histidine	0.008	0.018	0.018	0.022	0.043	0.062	0.253	0.281	0.161	0.476
Arginine	0.016	0.039	0.032	0.042	0.068	0.097	0.420	0.420	0.220	0.742
Aspartic acid	0.045	0.105	0.087	0.109	0.240	0.253	0.995	1.19	0.655	2.32
Threonine	0.021	0.052	0.041	0.055	0.078	0.099	0.404	0.495	0.270	0.918
Serine	0.025	0.068	0.057	0.069	0.113	0.140	0.504	0.565	0.275	1.16
Glutamic acid	0.062	0.173	0.136	0.156	0.189	0.300	1.32	1.35	0.808	3.38
Proline	0.101	0.150	0.122	0.099	0.125	0.312	1.16	1.50	0.868	1.56
Glycine	0.029	0.079	0.075	0.086	0.127	0.174	0.693	0.738	0.400	1.96
Alanine	0.031	0.077	0.057	0.068	0.104	0.123	0.500	0.590	0.295	1.41
Valine	0.022	0.059	0.048	0.049	0.120	0.183	0.600	0.813	0.396	1.36
Methionine	0.004	0.019	0.006	—	0.018	0.038	0.225	0.186	0.180	0.488
Isoleucine	0.019	0.048	0.093	0.046	0.072	0.082	0.347	0.429	0.241	1.14
Leucine	0.031	0.073	0.062	0.073	0.129	0.171	0.725	0.857	0.469	1.92
Tyrosine	0.011	0.024	0.022	0.028	0.054	0.098	0.437	0.538	0.293	0.932
Phenylalanine	0.015	0.033	0.027	0.032	0.057	0.077	0.324	0.407	0.232	0.718
Half cystine	—	—	—	—	—	—	—	—	—	0.070

Amino acid (μmole/ml)	Source of sample 11-Day pre-pupae	12-Day pupae	13-Day pupae	14-Day pupae	15-Day pupae	16-Day pupae	17-Day pupae	18-Day pupae	19-Day pupae	20-Day emerging bees
Lysine	1.05	0.980	0.922	0.855	0.884	1.35	0.578	0.104	0.140	0.292
Histidine	0.391	0.344	0.354	0.333	0.303	0.491	0.212	0.016	0.027	0.043
Arginine	0.563	0.560	0.546	0.550	0.522	0.793	0.344	0.046	0.068	0.126
Aspartic acid	1.77	1.74	1.72	1.62	1.65	2.40	1.02	0.102	0.140	0.205
Threonine	0.652	0.676	0.659	0.634	0.669	0.882	0.388	0.052	0.064	0.098
Serine	0.818	0.904	0.862	0.852	0.864	1.13	0.539	0.061	0.079	0.110
Glutamic acid	2.66	2.64	2.44	2.32	2.24	3.40	1.44	0.133	0.199	0.314
Proline	1.16	1.09	1.08	1.08	1.09	1.55	0.686	0.138	0.017	0.393
Glycine	1.48	1.41	1.39	1.35	0.981	1.95	0.860	0.080	0.107	0.191
Alanine	1.13	1.07	1.04	1.00	1.02	1.52	0.615	0.071	0.087	0.116
Valine	1.09	1.04	0.938	0.944	0.990	1.32	0.582	0.062	0.072	0.131
Methionine	0.262	0.346	0.344	0.322	0.279	0.496	0.194	0.015	0.033	0.058
Isoleucine	0.852	0.774	0.766	0.740	0.842	1.03	0.472	0.058	0.070	0.102
Leucine	1.48	1.45	1.37	1.36	1.35	1.93	0.888	0.092	0.115	0.162
Tyrosine	0.680	0.714	0.620	0.700	0.705	0.972	0.455	0.059	0.081	0.061
Phenylalanine	0.540	0.536	0.534	0.522	0.562	0.726	0.346	0.049	0.057	0.091
Half cystine	—	—	—	—	—	0.082	0.066	—	—	0.025

Approximately 1 ml of fluid or hemolymph from each age group of honey bees was collected in a sterile glass test tube and frozen prior to analysis. Eggs were placed in a tube and macerated. Hemolymph from larvae was obtained by gently puncturing the insects with a sterile hypodermic needle. The fluid which exuded from the wound was drawn into a capillary tube and then expelled into a test tube. Fluid from pupae and hemolymph from adults were best obtained by severing the head with a scalpel and drawing the liquid which exuded from the thorax into a capillary tube.

Each sample was lyophilized, flushed twice with nitrogen, and hydrolyzed with 6 *N* HCl in sealed tubes. After the tubes were heated for 24 h at 108 °C, the hydrolysate was removed, filtered, and dried. Distilled water was added twice during the evaporation procedure to remove excess HCl. After the final evaporation, the volume of the residue was adjusted to 25 ml with citrate buffer, ph 2.2. The sample was then examined with a Beckman Model 121 amino acid analyzer[10].

The total amino acid levels were lowest in eggs of all ages, in hemolymph from 5- and 6-day-old larvae, in fluid from 18- and 19-day-old pupae, and in the hemolymph of emerging adult bees (Figure). Hemolymph from 10-day-old larvae and fluid from 16-day-old pupae contained the greatest amounts of amino acids. Amino acid levels from day 10 larvae through day 16 pupae were consistently high. Obviously, these compounds were involved in protein synthesis during pupation which occurred at day 11 and in preparation for emergence of the adult at day 20. The sharp decreases in amino acids that occurred at days 9, 11, 17, and 18 reflected the morphological events of pupation and emergence which were occurring.

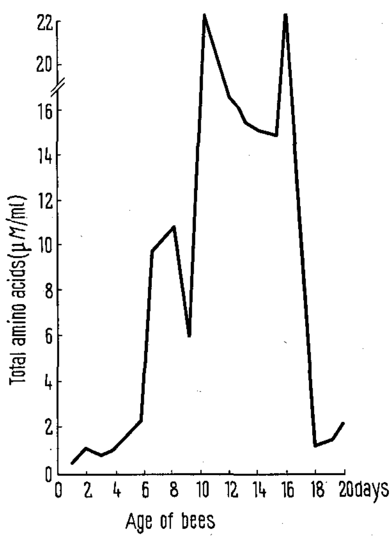
The Table shows total amino acids present in developing worker honey bees. LUE and DIXON[6] found proline, glutamic acid, serine, glycine, and alanine nonessential to bees. The highest values we found were those for glutamic acid, aspartic acid, and proline. CHEN and HADORN[11] reported a high concentration of proline in the blood of *Ephestia* spp. but could offer no explanation for this. Proline, aspartic acid, and glutamic acid were interrelated in the silkworm[12], and this appeared to be true for the honey bee also. Those amino acids present in the lowest concentrations were 1/2 cystine, histidine, methionine, and phenylalanine. MILES[13] found low concentrations of free cysteine-cystine in the hemolymph of *Eumecopus punctiventris* Stål. All amino acid levels increased from day 18 to day 20 (emerging bees).

It has rarely been possible to associate changes in individual amino acids with specific developmental processes[14] and no such correlation could be made in this study. However, we anticipate that correlations will be made in the future with improved methodology and better understanding of the developmental processes of insects[15].

Zusammenfassung. Glutaminsäure, Aspartinsäure und Prolin wurden in Eihomogenaten und Haemolymphe der Honigbiene (*Apis mellifera* L.) gefunden.

MARTHA GILLIAM and
W.F. MCCAUGHEY

Entomology Research Division,
Agricultural Research Service,
US Department of Agriculture, 2000 E. Allen Road,
Tucson (Arizona 85719, USA),
and
Department of Agricultural Biochemistry,
University of Arizona,
Tucson (Arizona, USA), 13 July 1971.



Total amino acids in developing worker honey bees.

[10] Mention of a proprietary product in this paper does not constitute an endorsement of this product by the USDA.
[11] P. S. CHEN and E. HADORN, Rev. Suisse Zool. *61*, 437 (1954).
[12] T. ITO and N. ARAI, J. Insect Physiol. *12*, 861 (1966).
[13] P. W. MILES, Austral. J. biol. Sci. *22*, 1271 (1969).
[14] R. L. CHAPUT and J. N. LILES, Ann. ent. Soc. Am. *62*, 742 (1969).
[15] Acknowledgments. This investigation was supported by Cooperative Agreement Grant No. 12-14-100-9062(33) from the Entomology Research Division of the USDA and was done in cooperation with the University of Arizona Agricultural Experiment Station, Tucson. We thank Mrs. KAREN JACKSON, Mrs. GAIL SPRATT and Mr. STEPHEN TABER III for technical assistance.

Redox Enzyme Models from Polymerized Aminoacyladenylates

The difficulties of ascertaining enzyme reaction mechanisms by direct means of investigation has often led to model system studies[1,2]. Our studies of mechanism in the flavin mono-nucleotide (FMN) reduced nicotinamide ademine dinucleotide (NADH) model system[3,4] for reduced pyridine dinucleotide flavoenzyme dehydrogenases produced an initial reaction rate which was 10^{-6} the rate of the oxidation of NADH by the NADH dehydrogenase[5] from bovine heart mitochondria. WANG[6] had proposed that the enhancement of reaction rate for heme-oxygen interaction in hemoglobin was the result of the prosthetic group residing in a protein region of low dielectric strength. Studies on heme-catalyzed oxidations following the incorporation of heme into