addition of reduced glutathione does not modify the shape of the curve in deuteroHb.

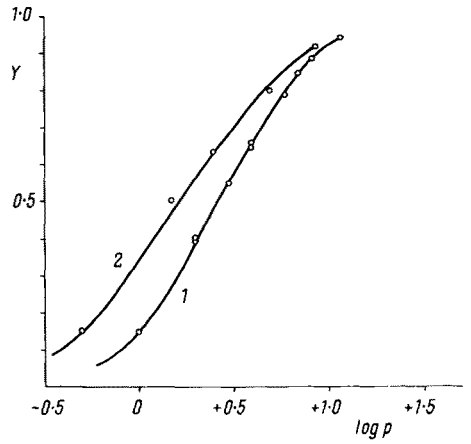

Fig. 2.–Oxygen equilibrium of reconstituted deutero Hb (2×10^{-4} *M* as Fe) curve 1 and meso Hb (1×10^{-4} *M* as Fe curve 2). 30°C; Tris buffer 0·1 *M*, pH 7·7.

Reconstituted mesoHb. The results obtained with meso-Hb (Fig. 2) are similar to those obtained with deuteroHb. For the curve reported in the Figure, the value of n in the Hill equation is 1·5.

The results reported above demonstrate that reconstituted protoHb shows the same heme-heme interaction as the native pigment. The behaviour of deutero- and meso-Hb points out the role of the vinyl groups of the porphyrin in heme-heme interaction.

The oxygen equilibrium of the reconstituted Hbs in different conditions of temperature, pH, etc. and the discussion of these results will be reported *in extenso* in another publication.

A. Rossi-Fanelli and E. Antonini

Institute of Biological Chemistry, University of Rome (Italy), January 21, 1958.

Riassunto

È stato dimostrato che nella protoemoglobina ricostituita l'interazione fra gli emi è identica a quella della emoglobina nativa mentre è fortemente diminuita nella deutero e mesoemoglobina.

Electrophoresis of the Venom and Haemolymph of the Scorpion *Buthus Judaicus* E. S.

During work on the toxicity of the black scorpion which occurs commonly in the Mediterranean regions of Israel, a study of the protein components of its venom was undertaken. Specimens of the venom were obtained by electric stimulation of laboratory-kept scorpions. The first clear drop was collected apart from the remainder of the secreted venom. The venom parts, denoted *A* and *B* respectively, were delivered into small quantities of distilled water and stored at 4°C until freeze drying. The total protein of the fresh venom as estimated by biuret reaction was 8·7 g/100 ml and amounted to about 50% of the dried venom. No marked difference in the protein content was observed between parts *A* and *B* of the venom. Electrophoretic separation at pH 8·6 revealed that the venom is composed of at least 6 protein fractions (Fig. 1 and 2), three of them of cathodic mobility. The relative concentrations of the protein fractions varied somewhat with different venom pools, the anodic fractions A_1 and A_2, as well as the cathodic fraction K_1, being affected in particular. These variations seemed to be caused by differences in composition of parts *A* and *B* of the venom. On the other hand, the protein pattern obtained after direct application of the venom from scorpion's telson to the filter paper was essentially the same as with freeze dried venom. No lipoproteins were detected on venom electropherograms stained with Oil Red 0, while a feeble spot reflecting protein-bound carbohydrate appeared at the site of the K_2 fraction after periodic acid-Schiff staining[1].

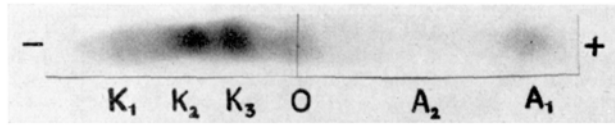

Fig. 1.–Paper electrophoretic separation of pooled *Buthus judaicus* venom. The venom was separated on Whatman 3 MM filter paper in the apparatus of Köiw, Wallenius, and Grönwall[2], in barbital buffer pH 8·6 and ionic strength 0·05. The strips were stained with Amido Black and scanned in an automatic photoelectric recorder (Spinco Analytrol). The relative concentrations of the fractions were: A_1: 11·0%, A_2: 9·9%, *O*: 21·1%, K_3: 19·8%, K_2: 23·1%, and K_1: 15·1%.

The venom was also separated preparatively by electrophoresis on filter paper. Preliminary assays of the eluted fractions on mice indicated that most of the toxicity resided in the cathodic fractions of the venom.

Fig. 2.–Descending boundary pattern of pooled *Buthus judaicus* venom analysed in Perkin-Elmer electrophoresis apparatus at total protein concentration of about 1%, barbital buffer pH 8·6, ionic strength 0·1 and 1°C. The venom was dialyzed for 24 h prior to analysis. The mobilities of the five fractions from left to right, are in $cm^2 \cdot V^{-1}s^{-1} \times 10^{-5}$: A_1: –3·36, A_2: –1·57, K_3: +0·58, K_2: +1·9 and K_1: +2·2. The arrow indicates the position of the boundary at start. The non-moving 0 fraction encountered in paper electropherogram was not discernible in the moving boundary pattern. The distended peaks of the cathodic fractions indicate their heterogeneity.

The protein pattern of *Buthus judaicus* haemolymph as shown in Figure 3 does not reveal any similarity with that of the corresponding venom. It is interesting that the albumin-like main protein fraction of the haemolymph comprised at least 3 components. On paper electropherogram, this fraction contained also 46% of haemolymph glycoprotein (total concentration 170 mg/100 ml[3]). Only traces of lipoprotein staining material were detected at the origin of haemolymph electropherogram.

[1] E. Köiw, G. Wallenius, and A. Grönwall, Scand. J. clin. Lab. Invest. *4*, 47 (1952).

[2] E. Köiw and A. Grönwall, Scand. J. clin. Lab. Invest. *4*, 244 (1952).

[3] M. R. Shetlar, J. V. Foster and M. R. Everett, Proc. Soc. exp. Biol. Med. N. Y. *67*, 125 (1948).

It is believed that the observed abundance of toxic cationic proteins in *Buthus judaicus* venom, along with other studies[4] on electrophoretic separation of scorpion venoms, provides a valuable hint towards elucidation of its toxic properties.

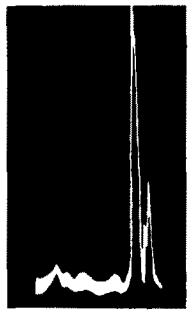

Fig. 3. – Ascending boundary pattern of *Buthus judaicus* haemolymph. Experimental conditions as with venom. The mobilities of the 6 fractions resolved are in cm^2. $V^{-1}s^{-1} \times 10^{-5}$; the relative concentrations in % of total haemolymph protein (9·2 g/100 ml) are given in parentheses. From right to left: A_1: −7·04 (14·9), A_3: −6·23 (48·9), α_1: −5·28 (7·1), α_2: −4·13 (9·6), β: −2·36 (7·9), and γ: −1·83 (11·6). The mobility and the relative concentration of the A_2 component could not be measured due to incomplete separation from A_1 peak.

ADINA WEISSMANN, A. SHULOV, and E. SHAFRIR

Department of Zoology and Department of Biochemistry, Hebrew University-Hadassah Medical School, Jerusalem (Israel), December 9, 1957.

Zusammenfassung

Die elektrophoretische Analyse des Skorpiongiftes von *Buthus judaicus* E.S. ergab den Gehalt von mindestens 6 Proteinfraktionen. Drei dieser Fraktionen waren bei pH 8,6 von kathodischer Beweglichkeit und von offenbar höchster Toxizität. Davon völlig verschieden war das elektrophoretische Bild der Eiweisse in der Skorpionhämolymphe.

[4] F. G. FISCHER and H. BOHN, Hoppe-Seylers Z. *306*, 269 (1957).

On the Significance of Amino Acids in the Larval Development of Khapra-Beetle, *Trogoderma granarium* Everts. (Coleoptera: Dermestidae)

The amino acid requirement of insects still remains a comparatively less explored field of study in insect physiology in spite of the increasingly accumulating information on various aspects of nutrition of insects. In recent literature however some experimental data are available on the effect of amino acids on development and metamorphosis of a few insect species. The present state of our knowledge has been reviewed by TRAGER[1], and LIPKE and FRAENKEL[2].

In the present work which forms part of the research schedule on the nutritional studies on *Trogoderma granarium*, an attempt has been made to investigate the role of amino acids in the larval development of this beetle. The results obtained are briefly reported here while a detailed account will be published elsewhere. *Trogoderma* has earlier[3] been shown to be capable of developing well on a chemically defined medium containing casein as a source of food protein. The casein has now been replaced by a mixture of 19 amino acids in the proportion first suggested for rats by ROSE *et al.*[4] and later also used for *Tribolium confusum*[5]. The all amino acids-diet for *Trogoderma* has been further modified to incorporate nucleic acid, maize starch (in place of glucose) and lard. The diets lacking lard or nucleic acid were nutritionally very poor. A necessity for these two ingredients was not observed earlier[3] in a casein diet.

Briefly the composition of the basic all-amino acids-diet was as follows:

Amino acid mixture*	1 part
Maize starch	4 parts
Cholesterol	0·021 parts
Lard	0·025 parts
Nucleic acid	0·025 parts
Osborn-Mandel salt mixt.	0·084 parts
Vitamins of B group	25 µg/g of diet except choline chloride and biotin which were used at the rate of 500 µg and 0·01 µg/g of diet respectively.

* The 19 amino acids used were: L-Arginine, L-Histidine HCl, DL-Isoleucine, L-Leucine, L-Lysine, DL-Methionine, DL-Phenylalanine, DL-Threonine, L-Tryptophane, DL-Valine, DL-Alanine, DL-Aspartic acid, L-Cystine, L-Glutamic acid, Glycine, L-Hydroxyproline, L-Proline, DL-Serine and L-Tyrosine.

The tests were performed in small shell vials containing 2 g of diet and 30 newly hatched larvae at a constant temperature of 36°C and about 50% relative humidity.

In the absence of any one of the first 10 amino acids listed above, the larvae failed to grow or pupate. They were however able to survive without gaining weight for a long period of 22 days when the average weight per larva was from 0·3 to 0·4 mg in all diets lacking in one of the 10 essential amino acids. The corresponding weight in the control was 1·92 mg. The larvae were allowed to remain in the diets and examined after 40 days when 33% larvae were alive without arginine or valine, 50% without isoleucine or leucine, 63% without lysine or phenylalanine, 83% without histidine, methionine or threonine and 100% without tryptophane. The average weight was more or less the same as recorded after 22 days and no pupae were formed except in control (10 amino acid diet). In the absence of any one or all the remaining 9 amino acids larvae developed normally although a 19-amino acids-diet was somewhat superior to a mixture of 10 amino acids. It is therefore apparent that for *Trogoderma* the first 10 amino acids are essential while others are not so vitally important.

The amino acid requirement of *Trogoderma* closely resemble those of other insects like *Tribolium*[5], *Aedes*[6], *Attagenus*[7] or *Drosophila*[8] and a vertebrate(rat) but differs

[1] W. TRAGER, *Nutrition in Insect Physiology* (Ed. by K. D. ROEDER; John Wiley and Sons, Inc. 1953, p. 350).

[2] H. LIPKE and G. FRAENKEL, Ann. Rev. Ent. *1*, 17 (1956).

[3] N. C. PANT, Ind. J. Ent. *18*, 259 (1956).

[4] W. C. ROSE, M. J. OSTERLING, and M. WOMACK, J. biol. Chem. *176*, 753 (1948).

[5] A. LEMONDE and R. BERNARD, Canad. J. Zool. *29*, 80 (1951). – G. FRAENKEL and G. E. PRINTY, Biol. Bull. *106*, 149 (1954).

[6] D. GOLBERG and B. DEMELLION, Biochem. J. *43*, 379 (1948).

[7] W. MOORE, Ann. ent. Soc. Amer. *39*, 513 (1946).

[8] T. HINTON, D. T. NOYES, and J. ELLIS, Physiol. Zool. *24*, 379 (1951).