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.

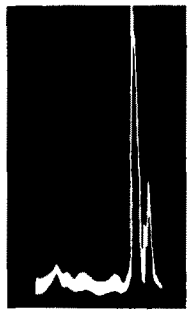

Fig. 3. – Ascending boundary pattern of *Buthus judaicus* haemolymph. Experimental conditions as with venom. The mobilities of the 6 fractions resolved are in cm^2. $V^{-1}s^{-1} \times 10^{-5}$; the relative concentrations in % of total haemolymph protein (9·2 g/100 ml) are given in parentheses. From 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.

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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.

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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

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from that of *Oryzaephilus*[9] which can do well without leucine, lysine, threonine or phenylalanine but requires cystine and glycine.

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Résumé

On décrit un milieu de composition chimique définie, se prêtant à l'analyse qualitative des acides aminés nécessaires au développement des larves de *Trogoderma*. A cet effet, dix acides aminés sont indispensables: l'arginine, l'histidine, l'isoleucine, la leucine, la lysine, la méthionine, la phénylalanine, la thréonine, la tryptophane et la valine. Le lard et l'acide nucléique sont également nécessaires à l'alimentation de ces larves.

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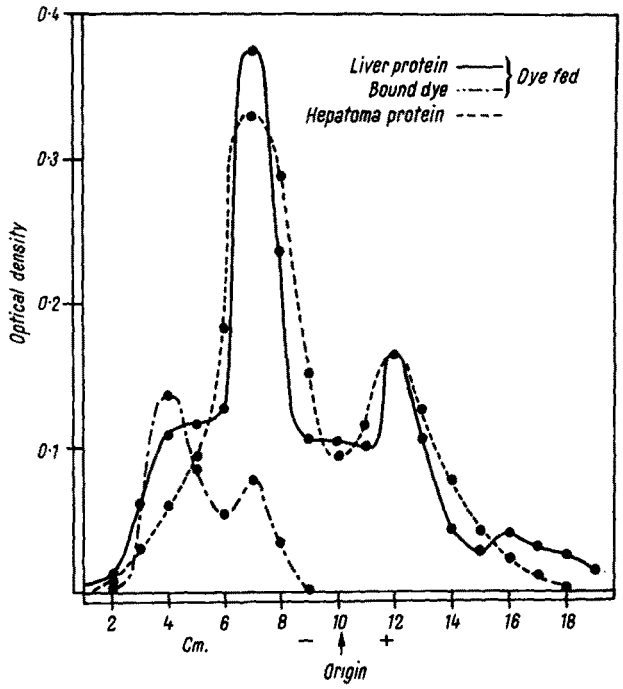
Studies on the Dye-Binding Fraction of Soluble Liver Proteins from Rats Fed Aminoazo Dyes[1]

E. C. MILLER and J. A. MILLER[2] observed, when carcinogenic azo dyes are fed to rats, the dye is bound by covalence to certain proteins in the liver, prior to the appearance of tumors. Two-third to three-fourth of the cytoplasmic bound dye is attached to a particular electrophoretic fraction, which has been called the 'h' proteins by SOROF *et al.*[3]. Since this 'h' fraction is considerably lowered in hepatomas induced with azo dyes[3], the idea was advanced by E. C. MILLER and J. A. MILLER[4] that the 'h' protein may play a role in the carcinogenic process. A brief account is given of various studies on this 'h' fraction, isolated in mg amounts by electrophoresis on starch, and of a comparative study of the electrophoretic patterns of rat liver and hepatoma supernatant fluids.

The Figure shows the typical distribution pattern of the supernatant fluid from the liver of rats fed for 2 weeks 3'-Methyl-*p*-dimethylaminoazobenzene (3'-Me DAB). About two-third of the dye is associated with the first shoulder from the left on the protein pattern, the 'h' fraction. It is interesting, that the electrophoretic patterns of the soluble protein and of the bound dye, obtained with the relatively non-carcinogenic isomer 2-Me DAB, (2 weeks), have shown an overall similarity to those of the carcinogenic 3'-Me DAB (Figure). The supernatant fluid, obtained from rats fed the same semi-synthetic diet, but containing no dye, gave also essentially the same pattern. However, the 'h' fraction and the bound dye were absent in the supernatant fluid of hepatoma induced with 3'-Me DAB (Figure). The isolation of mg amounts of 'h' fraction was carried out in similar conditions, but $2 \times 5 \times 31$ cm blocks were used and 1500 mg samples of lyophilizate; time 21–24 h. After dialysis and lyophilization of the extracts of the corresponding segments, the yield was 5–10 mg 'h' protein per block.



Electrophoretic patterns on starch block of liver and hepatoma supernatant fluid of rats fed 0·06% 3'-Me DAB in a riboflavin low semi-synthetic diet. 150 mg samples of lyophilizate in sucrose were used, containing 20% protein. The parallel, horizontal blocks were $1 \times 2 \times 31$ cm. The electrophoresis was carried out in a 0·02 *M* pH 7·4 barbital buffer, containing also 0·055 KCl/l, at 225 V, with 10–12 mA per block, during 12–16 h. The protein distribution has been followed by a modified Folin reaction and the bound dye pattern by measuring the optical densities at 525 mμ in formic acid, on the extracts of the segments of the blocks.

Amino acid composition. Paper chromatography of the hydrolyzates of both the normal and 3'-Me DAB containing 'h' fractions, by the concurrent use of the methods of MCFARREN[5] and CLAYTON and STRONG[6], has shown no qualitative differences in amino acid composition. The chromatograms showed for both high proportion of leucin, alanine, valine, lysine, and probably arginine. Cystine and cysteine were determined quantitatively after NAKAMURA and BINKLEY[7]. The percentage of tyrosine and tryptophane was measured by U.V. absorption after BEAVEN and HOLIDAY[8]. The following amino acids have been identified: aspartic and glutamic acids; serine; glycine; threonine (traces); alanine; cysteine (normal: 0·18%, dye containing: 0·22%); cystine (normal: 0·21%, dye containing: 0·15%); arginine: lysine; proline; histidine; tryptophan (normal: 0·95%, dye containing: 0·94%); tyrosine (normal: 3·24%, dye containing: 4%); methionine; valine; leucine; phenylalanine. The cystine + cyst-

[1] This investigation was supported by the Institutional Grant 71 from the American Cancer Society and the Grant C-355 of the National Cancer Institute, Public Health Service to Drs. J. A. MILLER and E. C. MILLER, to whom we are greatly indebted.

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