T8. The other media gave only exceptionally comparable results.

Zusammenfassung. Lymphozyten des chinesischen Hamsters (*Cricetulus griseus*) wurden in verschiedenen Media mit verschiedenen Zusätzen gezüchtet. Die beste Mitosenausbeute wurde erhalten mit dem Medium R.P.M.I. 1640, bei einem Zusatz von 10% fötalem Kalbsserum und 6 µl/ml Phytohämagglutinin.

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Free Amino Acids in the Haemolymph of *Eoperipatus weldoni* (Onychophora)

Arthropods belonging to the class Onychophora are of special zoological interest in that they are considered to be the most primitive terrestrial arthropods closely related to the myriapodan-insectan stem[1]. A characteristic feature of Insecta and Myriapoda is the presence, in their haemolymph, of free amino acids in very high concentrations, ranging from 219 mg to 2340 mg per 100 ml[2,3]. It will therefore be of interest to find out if the haemolymph of Onychophora also has a similar feature.

The species investigated was *Eoperipatus weldoni*, collected from Templer's Park, Malaysia, as given by SUNDARA RAJULU and SINGH[4]. They were collected, brought to the laboratory and maintained as described elsewhere[5].

The haemolymph was obtained from living animals by the method described by SUNDARA RAJULU[6]. The sample of haemolymph obtained from each animal was kept separately and centrifuged at 5°C for 15 min at 2000 *g*/min to separate haemocytes. The clear supernatant was collected and deproteinized following the method of STEIN and MOORE[7]. The Folin method, as given in the publication of HAWK et al.[8], was adopted for quantitative estimation of total free amino content. For quantitative estimation of individual free amino acids, samples of 1 to 2 ml of the deproteinized haemolymph were analyzed in an automatic amino acid analyzer[9]. Quantitation was based on the ninhydrin colouring intensity of the effluent from ion-exchange columns. The absorbancy of the colour developed was estimated at 570 and 440 nm in a spectrophotometer. The peaks on the recorded curve were integrated for loads varying from 0.1 to 3.0 µmoles for each amino acid.

From the results recorded in Table I, it is evident that *Eoperipatus weldoni* has an average of 261.67 mg of free amino acids per 100 ml of haemolymph, the minimum being 254 mg/100 ml and the maximum being 271 mg/100 ml. This value is much higher than the values reported for crustaceans[10] and arachnids[11], but comparable to that

Table I. Concentration of total free amino acids in the haemolymph of *Eoperipatus weldoni* in mg/100 ml

No. of specimen	Free amino acids
1	263
2	271
3	254
4	259
5	268
6	255
	Average 261.67

Table II. Free amino acids in the haemolymph of *Eoperipatus weldoni* in mg/100 ml

No.	Amino acids	Quantity
1	Alanine	7.9 ± 1.6
2	Aspartic acid	15.1 ± 1.9
3	Arginine	2.2 ± 0.4
4	Cystine	3.1 ± 0.3
5	Glutamic acid	18.7 ± 0.8
6	Glycine	56.6 ± 5.2
7	Histidine	23.2 ± 1.8
8	Isoleucine	3.9 ± 0.2
9	Leucine	12.4 ± 2.1
10	Lysine	8.2 ± 0.6
11	Methionine	4.7 ± 1.2
12	Phenylalanine	13.3 ± 1.6
13	Proline	42.1 ± 3.3
14	Serine	9.2 ± 1.1
15	Threonine	11.5 ± 1.8
16	Tyrosine	18.4 ± 1.7
17	Valine	9.8 ± 0.7
		Total 260.3 ± 11.8

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for myriapods such as *Spirostreptus asthenes* and *Ethmostigmus spinosus*[3]. It is also interesting to note that the value is closer to that of primitive hemimetabolus insects such as *Carausius morosus*[12], but is only $^1/_2$ to $^1/_3$ those of the holometabolus insects like the honey bee and silk worm[12,13].

The results of quantitative estimations of individual free amino acids are presented in Table II. The sample analysed was a mixture of deproteinized haemolymph from 6 individuals. It may be noted that there are 17 amino acids of which glycine, glutamic acid, histidine, proline and tyrosine are found in high concentrations as in myriapods[3]. A noteworthy feature is the presence of cystine as a free amino acid in the haemolymph of *Eoperipatus weldoni*, a feature characteristic of Myriapoda[3].

These results would substantiate the suggestion of TIEGS and MANTON[1] that Onychophora are more closely related to Myriapoda and Insecta than to the other groups of Arthropoda.

Free amino acids in the haemolymph of *Eoperipatus weldoni* were determined by the methods of Folin and Spackman. Total concentrations of free amino acids were comparable to those of the myriapods and primitive hemimetabolus insects. Seventeen amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, thereonine, tyrosine and valine) were present in high concentrations.

Zusammenfassung. Mit der Methodik nach Folin und Spackmann wurden die Freien Aminosäuren in der Hämolymphe von *Eoperipatus weldoni* festgestellt und in ihrer Gesamtkonzentration mit den Aminosäuren der Myriapoden und primitiven hemimetabolischen Insekten vergleichbar gefunden.

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Influence of the Oestrous Cycle on the Nucleic Acid and Protein Content of the Rat Pineal Gland

Biochemical composition and activity of the rodent pineal gland appear to be influenced by the same neuroendocrine mechanisms which control the oestrous cycle; however, few investigations have been made to confirm this postulation. It has been shown that the phospholipid content[1] and HIOMT activity[2] of the pineal are at their highest during dioestrus and lowest at prooestrus-oestrus, pointing towards maximal activity of the pineal when ovarian function is slackest. However, QUAY[3,4] reported an opposite trend, significantly decreased pineal content of serotonin and 5-hydroxyindole acetic acid and an insignificant reduction in melatonin, at dioestrus, and higher levels during prooestrus and oestrus. Oophorectomy, which produces features resembling dioestrus, brings about an increase in weight but not in HIOMT activity of the pineal of adult rats, whereas in the immature it gives rise to elevated HIOMT activity of the pineal but no significant increase in its weight[2,5,6]. Pregnancy[7] or treatment with oestradiol[2,5] have been reported to cause a decrease in pineal weight and activity but experiments carried out in our laboratory produced the opposite effect[8].

In the present study, an attempt has been made to elucidate in what way oestrus influences the basic metabolism of the pineal gland by measuring the pineal levels of RNA, DNA and protein during the 4 phases of the oestrus cycle.

Materials and methods. Adult female rats of the Hebrew University 'Sabra' strain weighing 140-160 g each were used. They were kept 5 to a cage in controlled lighting (12 h light commencing at 07.00 h) and at a constant temperature of 23 ± 1°C. For a minimum of 2 oestrous

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Rat pineal nucleic acids and total protein during oestrous cycle (μg/pineal ± S.D.)

Pineal component	Prooestrus	Oestrus	Metoestrus	Dioestrus
RNA (Orcinol method)	4.6 ± 1.3	4.5 ± 1.3	4.5 ± 0.9	5.0 ± 1.0
DNA	4.1 ± 0.9	4.1 ± 0.9	4.2 ± 0.9	4.0 ± 0.8
Protein	120.6 ± 18.6	114.0 ± 14.6	120.1 ± 22.9	124.2 ± 22.9
Total body weight (g)	184 ± 7.6	183 ± 10.9	182 ± 11.3	182 ± 11.2

Each figure represents 20 determinations.