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.

cycles vaginal smears were taken daily beween 9 and 11.00 h and only those rats manifesting 2 consecutive 4-day oestrous cycles were included in the study. Each day vaginal smears were taken from 6 to 8 rats at each stage of the oestrous cycle and examined. The animals were then sacrificed by neck fracture at approximately 11.00 h, weighed, their pineal glands removed, rinsed and 2 glands (from rats at the same oestrous stage) homogenized under cooling in 1 ml distilled water. An 0.2 ml aliquot of the suspension was withdrawn for determination of protein and the remainder used for nucleic acids. Protein was determined by the method of LOWRY et al.[9]. The homogenate was washed in order to eliminate any free nucleotids and sugars. RNA was separated from DNA as described by SCHMIDT and TANNHAUSER[10]. RNA in the supernate was determined directly by reading its absorption at 260 nm and again by CERIOTTI's orcinol method[11]. DNA was eluated from the sediment by incubation with 1 *M* perchloric acid at 80°C for 30 min and estimated by the indole procedure of CERIOTTI[12].

Results. As can be seen in the Table, there were no significant changes in pineal levels of protein and nucleic acids during the entire oestrous cycle. However, a clear and consistent tendency to high protein and RNA values at dioestrus and low ones at oestrus is evident, the levels at prooestrus and metoestrus being intermediate. Correlation between RNA values obtained by direct reading and those by CERIOTTI's colorimetric method was good, although those obtained by the latter procedure were slightly lower. They were also lower than the values obtained by us in the pineal on previous occasions, as here an additional treatment of the homogenate has been introduced to remove any free nucleotids. DNA levels remained fairly constant throughout the oestrous cycle.

Discussion. Our results indicate possible fluctuations in the metabolism of pineal protein linked to the oestrous cycle. However, though consistent throughout the study, the differences between the levels of pineal RNA and protein encountered at the various phases of oestrus were not sufficiently drastic to reach significance and should be confirmed by a more sensitive radioactive method of determination.

Increased metabolic activity of protein at dioestrus is in agreement with the findings of ZWEENS[1] and WURTMAN[2] who reported higher phospholipid and HIOMT contents of the pineal and a gain, although an insignificant one, in its weight during the dioestrous phase of the cycle.

It is interesting that the specific activity of RNA in hypothalamus and pituitary was low at dioestrus and high at oestrus[13,14], the opposite of that in the pineal gland. These converse hypothalamic-hypophysial and pineal undulations may be related to the cycles of gonadotrophins and gonadal steroids, increased circulating levels of which could be stimulating pituitary and inhibiting pineal metabolism. The link between oestrus and changes in pineal metabolism may be either through primary hypophysial influence[2,6], or primary gonadal effect mediated by the hypothalamic-hypophysial axis. The possibility also exists that gonadal function regulates independently both pituitary and pineal activity, since hypophysectomy had no influence on oestradiol-induced reduction of pineal adenyl cyclase activity[15]. Moreover, administration of oestradiol abolishes the increased HIOMT activity produced by oophorectomy[6]. Thus, it can also be supposed that ovarian steroids, which are released cyclically and control vaginal cytology, act directly on the pineal and decrease its metabolic activity. It appears, therefore, that both ovary and anterior pituitary could be involved in modifying pineal cyclic activity during the oestrous cycle within the framework of a complicated feedback system.

Zusammenfassung. Gesamteiweiss und RNS der Zirbeldrüse steigen während der Dioestrusphase bei Ratten an. DNS bleibt während des ganzen Cyclus konstant.

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Sexual Dimorphism in Contraction Properties and Fibre Pattern of the Flexor Carpi Radialis Muscle of the Frog (*Rana temporaria* L.)

Sex hormones play a paramount role in the regulation of size and function of secondary sexual organs and sexual behaviour, involving presumably genetically controlled formation of specific receptor mechanisms[1] in target organs, including cross striated muscles. The resulting androgen-dependency differs, however, in different muscles and varying degrees of sexual dimorphism of muscles may be observed[2]. Some are absolutely endrogen-dependent, e.g. the levator ani muscle of the rat, which undergoes complete perinatal involution in the female rat[3], others are relatively androgen-dependent, e.g. the temporal muscle of the guinea-pig, which exists in both sexes but differs in weight, fibre size[4] and enzyme pattern[5].

A further example of a relatively androgen-dependent muscle is the flexor carpi radialis of the frog, associated with the 'clasping reflex' of the male during the mating act. The sexual dimorphism of this muscle has been known for a long time[6], the 'male' muscle being larger and consisting of larger diameter fibres than the 'female'[7]. Muscle fibre size shows seasonal variations, it is largest at the mating time (March–April) and smallest in summer, when progressive decrease of androgen levels can be assumed. Correspondingly, there is a decrease of muscle fibre size after castration and an increase after testosterone administration[7].

It appeared of interest to study the sexual dimorphism of this muscle also with respect to its contraction properties and histochemical fibre patternt, especially that of ATPase activity, which is closely related to speed of contraction[8].