Les hormones éprouvées sont efficaces à des doses très faibles (100 nM/ml d'un milieu avec sérum) comme pouvaient le laisser prévoir les résultats récents sur la prostate de rat[6,7]. Avec la testostérone, la réponse est beaucoup plus importante aux doses de 100 nM/ml qu'à fortes doses. Les images de l'épithélium sécréteur sont très voisines de celles observées chez les animaux en activité sexuelle. Nous ne pensons pas que l'augmentation des temps de culture suffise à expliquer l'amélioration importante des résultats enregistrés avec la testostérone bien que l'on sache par ailleurs que la reprise d'activité de cet organe au cours du cycle sexuel s'étale sur plusieurs mois. Il est vraisemblable que la testostérone manifestait un effet toxique à des doses élevées qui n'empêchait pas l'augmentation du volume cellulaire mais freinait la sécrétion. Sous réserve d'une étude quantitative ultérieure, cette réponse nous parait même meilleure qu'avec les différents métabolites testés jusqu'à présent à fortes doses[3]. D'autrepart, si une bonne stimulation a été observée avec la 5 α-DHT à la dose de 100 nM/ml, la réponse n'est pas meilleure qu'avec des doses de 20 μM/ml.

Ces résultats apportent une lumière nouvelle au modèle du mécanisme d'action des androgènes dans l'épididyme de lézard[3]. À moins de pouvoir montrer ultérieurement que la dose de 100 nM/ml de 5 α-DHT est elle même trop élevée, le rôle joué par ce métabolite nous parait devoir être réévalué.

Summary. In contrast to earlier results obtained with hight doses of testosterone and 5 α-DHT given for short periods (10 days), very low doses of testosterone are more potent than 5 α-DHT in stimulating regressed epididymis cultivated for 23 days.

ANNIE GIGON

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Free Amino Acids in the Haemolymph of the Last Instar Nymph of the Dragonfly, *Orthetrum chrysis* (Selys) (Odonata: Libellulidae) I. Effects of Centrifugation and Starvation

While much work has been done in higher groups of insects, there is little information about the amino acids in the haemolymph of the dragonflies. The present investigation has been undertaken to determine the free amino acid composition of the last instar nymph of the dragonfly, *Orthetrum chrysis* (Selys). In this paper, the effects of centrifugation as a stress factor, and of starvation on amino acid composition of haemolymph are discussed.

The amino acids were analyzed by thin layer chromatography (TLC) as described elsewhere[1]. The procedure was repeated several times to confirm the results.

The analysis of the haemolymph of the last instar nymph of the dragonfly, *Orthetrum chrysis* reveals the presence of 15 amino acids (Table). They are: ornithine, histidine, lysine, aspartic acid, serine, arginine, threonine, alanine, proline, tyrosine, tryptophan, valine, isoleucine and phenylalanine. After 10 min of centrifugation, the haemolymph shows the presence of 20 amino acids. Besides 15 amino acids found in the haemolymph of normal nymphs, the 5 additional amino acids are glutamic acid, α-amino-*n*-butyric acid, methionine, cystine and leucine.

After starvation for 4 days, there are only 17 amino acids. They are; ornithine, histidine, lysine, aspartic acid, serine, arginine, glycine, threonine, alanine, proline, tyrosine, tryptophan, valine, isoleucine, leucine, phenylalanine and glutamic acid. The additional amino acids observed are glutamic acid and leucine. They were not observed in the haemolymph of the normal nymphs. Moreover, in the starved nymphs, these amino acids are more prominent.

The composition of free amino acids in haemolymph depends upon the nature of the food, physiological activities and ecological conditions[2–8]. In the haemolymph of the last instar nymph of the dragonfly, *Orthetrum chrysis*, under normal conditions, 15 amino acids are present. 5 of these, lysine, histidine, serine, tryptophan and proline, occur in greater concentration as compared to other amino acids, and this state persists throughout the intermolt period of the last instar nymph. GILMOUR[3] also noted the presence in greater concentration of the same amino acids and believed that they play an active role in molting and metabolism. Most of the other free amino acids do not show significant changes in their concentration.

After centrifugation, 5 amino acids, glutamic acid, methionine, α-amino-*n*-butyric acid, cystine and leucine, appear, in addition to 15 amino acids found in the haemolymph of the normal insects. The appearance of these additional 5 amino acids in the centrifuged insects and their non-detection under normal conditions suggest their active utilization in the metabolic process.

Cystine is detectable only in centrifuged samples, though in minute quantity, which confirms the observations of other workers[9–12].

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Only 17 amino acids are spotted out from the haemolymph of starved nymphs. These amino acids appear in higher concentration than the control nymphs, suggesting that under normal conditions they are actively utilized in protein synthesis or in other metamorphic or metabolic processes, while during the starvation they are stored in the haemolymph as there is total or partial blockage of protein synthesis, metabolism and metamorphosis[8].

Amino acids	Concentration*		
	Control nymphs	Centrifuged nymphs	Starved nymphs
Ornithine	+	+++	+++
Histidine	++	+	+++
Lysine	++	+	+++
Aspartic acid	+	+	+
Serine	++	+	+++
Arginine	+	++	+++
Glycine	+	+++	+++
Threonine	+	+++	++
Alanine	+	+	++
Proline	+++	++	+++
Tyrosine	+	+	++
Tryptophan	++	+	++
Valine	+	+	+++
Isoleucine	+	+	++
Leucine	−	+	++
Phenylalanine	+	+	+
Glutamic acid	−	+++	++
Methionine	−	+	−
α-Amino-*n*-butyric acid	−	+	−
Cystine	−	+	−

*Concentration represented by the following: − negative; + low; ++ moderate; +++ high.

THAKARE and TEMBHARE[13] observed sudden release of neurosecretory material in haemolymph from the cerebral neurosecretory cells and the corpora cardiaca during stress, and its accumulation in the neurosecretory cells during starvation in the last instar nymph of *Orthetrum chrysis*. In the present study, an appearance of additional amino acids during stress and an accumulation of several amino acids in starvation have been tentatively observed. Thus there appears to be some functional correlation between the release of cerebral neurosecretory material and the metabolism of amino acids.

Summary. The free amino acids were determined by thin layer chromatography in the haemolymph of the last instar nymph of the dragonfly, *Orthetrum chrysis* (Selys) during normal, centrifugation and starvation periods and a functional correlationship between the cerebral neurosecretory material and amino acid metabolism has been suggested.

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PRO EXPERIMENTIS

A Cell Culture Substrate Obtained from Heat-Fused Collagen

The use of reconstituted rat tail collagen as a substrate for tissue explants or for certain types of cells is now a common practice. Recently, coverslips coated with dried collagen were accidentally included with glassware sterilized in a hot-air oven at 160 °C. The resulting clear film appeared to offer some advantages as a multipurpose substrate and further investigations were undertaken.

Materials and methods. Collagen was prepared by the BORNSTEIN modification[1] of the original method of EHRMANN and GEY[2], except that the entire process, including dialysis for 24 or 48 h, was carried through without interruption and aliquots of collagen solution were stored in sterile refrigerated containers. Coverslips placed inside petri dishes were coated with a thin film of liquid applied with a glass rod, and immediately dried for 24 h in a 37 °C oven[3]. The petri dishes were then wrapped in foil and placed in a hot-air sterilizing oven at 160 °C for 2 h. After cooling, they were stored at room temperature until required.

A drop of medium, dispersed with a glass rod to form a moist film, was placed on the surface of the substrate before explants were placed in position. The latter were left for several hours with minimal medium in order to attach them firmly.

When specific concentrations of dissociated cells were required, flat-ground glass rings were placed on the fused collagen films and standardized cell suspensions were seeded into them. To ensure that no liquid or cells escaped, the outer rim of each ring was usually sealed to the coated coverslip with hot wax but, with adequate care in handling, leakage could usually be avoided even without waxing. For electron microscopy, thicker layers of substrate, easily removed later from the coverslip, were obtained by placing small (10 mm diam.) glass rings on glass coverslips and filling the wells to varying depths with liquid collagen. This required extended drying time at 37 °C. The subsequent dry-heat sterilizing was carried out as usual with the rings in place. Glutaraldehyde and osmium fixation was carried out before the rings were removed.

Results and discussion. Heat fusion of dried collagen produced a transparent, even layer of unknown chemical composition, in which no evidence of structure has so far been demonstrated. A negative result was obtained with PAS staining. The substrate was produced entirely by dry heat.

Phase-contrast photography of cells through a film of fused substrate produced pictures of excellent clarity. Time-lapse cinematography indicated that the substrate provided an adequate locomotory surface for embryonic

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