

du glucose et des acides gras libres<sup>10, 11</sup>. Ces faits soulignent le type insulinaire des effets précoces de GH, l'insuline, étant hypophosphatémiant<sup>12</sup> et favorisant la pénétration des phosphates dans les cellules<sup>13</sup>.

La rétention rénale des Pi, précoce et persistante, que nous observons démontre d'une part que l'hypophosphatémie induite par GH est indépendante de toute variation de l'élimination urinaire des Pi et d'autre part que la GH exerce un effet rénal ne nécessitant pas la répétition du traitement, bien qu'une seule injection de GH ne modifie pas le transport maximum des phosphates chez le chien pour CORVILAIN<sup>9</sup>. L'absence de facteur hormonal tendant à élever rapidement le taux sérique des Pi (dans les mêmes conditions expérimentales que celles décrites ici, une seule injection d'iodothyronine ne modifie pas la phosphatémie — observations personnelles) confirme que le maintien de la concentration cellulaire en phosphore des tissus mous est l'élément primordial<sup>14</sup>. Les Pi plasmatiques représentent une forme disponible de transport vers le stock des Pi cellulaires<sup>15</sup> qui participent au pool des groupements phosphorylés. Cette conception de la régulation des phosphates peut expliquer l'effet hypophosphatémiant précoce de plusieurs hormones, ACTH, adrénaline, insuline, glucagon, et de GH en particulier, qui activent

le métabolisme cellulaire sans être directement impliquées dans le métabolisme phosphocalcique.

L'intervention de GH dans le métabolisme phosphaté ayant pour résultat global une épargne de l'ion phosphate, l'hormone s'oppose sur ce point à la parathormone. L'hypophosphatémie précoce jointe à la diminution de la phosphaturie correspondent à une captation cellulaire accrue de l'anion puisque dans les mêmes conditions l'hormone ne touche pas le métabolisme calcique.

En conclusion GH ne peut être présentée comme le facteur tendant à élever la phosphatémie de façon rapide, tout en intervenant précocement dans le métabolisme des phosphates<sup>16</sup>.

<sup>10</sup> A. VÉZINHET, J. CHARRIER et L. DAUZIER, *Ann. Biol. anim. Biochim. Biophys.* 12, 431 (1972).

<sup>11</sup> N. I. SWISLOCKI et C. M. SZEGO, *Endocrinology* 76, 665 (1965).

<sup>12</sup> S. NATELSON, J. B. PINCUS et G. RANAZZI, *Clin. Chem.* 9, 31 (1963).

<sup>13</sup> J. SACKS et F. M. SINEX, *Am. J. Physiol.* 175, 353 (1953).

<sup>14</sup> H. COPP, in *Phosphate et Métabolisme phosphocalcique* (Ed. D. J. HICO, L'expansion scientifique française, Paris 1971), p. 111.

<sup>15</sup> R. W. MARSHALL et B. E. C. NORDIN, in *Phosphate et Métabolisme phosphocalcique* (Ed. D. J. HICO, L'expansion scientifique française, Paris 1971), p. 127.

<sup>16</sup> Remerciements. L'hormone de croissance utilisée dans cette expérimentation nous a été gracieusement fournie par le NIAMD.

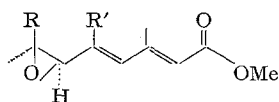
## Juvenile Hormone Active Principle in *Attacus atlas* L.<sup>1</sup>

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**Summary.** The ether extract of *Attacus atlas* L., subjected to an isotope dilution method for the determination of known insect juvenile hormones, revealed the presence of an active substance which has a similar chromatographic behaviour with the known JH-3, methyl (2E, 6E)-10, 11-epoxy-3,7,11-trimethyl-2, 6-dodecadienoate.

The ether extracts of *Attacus atlas* L. were found to have morphogenetic and ovicidal activity on corn borer, *Ostrinia furnacalis* GUENÉE<sup>2</sup>. To determine whether this activity corresponds to one of the three known juvenile hormones (JH 1-3) or not, the ether extract was subjected to an isotope dilution method, which allows the qualitative and quantitative determination of all naturally occurring insect juvenile hormones<sup>3</sup> as yet known. The



JH 1: R = R' = ethyl

JH-2: R = ethyl, R' = methyl

JH-3: R = R' = methyl

biological activity of the ether extract and fractions from all consecutive purification steps were determined, using the *Galleria* wax test<sup>4</sup>. The results, expressed in *Galleria* units (GU) as well as the quantitative biological estimates of juvenile hormone aliquots, were obtained using the standard values of 1.6 and 70 pg JH-1 and JH-3, respectively for a GU.

The lipid extract of 100 *A. atlas*<sup>5</sup> moths (11.9 g) was found to be highly biologically active (total 134,357 GU; Table). Its specific activity of 88 µg/GU is comparable to that of *Platysamia cecropia* (35 µg/GU)<sup>6</sup>. After each of

the 3 first purification steps, the main biological activity was found in the fraction containing JH radioactive material. Likewise, a certain amount of bioactivity was also found in non-radioactive fractions. This activity, however, is attributed to the loss of material which naturally occurs in this procedure, substantiated with the radioactive yield of 51%.

After the column chromatography procedure, a first TLC purification employing plates of 0.5 and 0.25 mm thickness, consecutively, was carried out. The bioactive fraction corresponding to JH-1 to JH-3 was found to contain 46,689 GU. The loss of intrinsic activity is similar to the 51% yield of added radioactive JH mentioned above<sup>7</sup>. The rest of the TLC fractions was also bioassayed, but was found inactive.

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<sup>2</sup> P. PAGUIA, Master's Thesis, University of the Philippines at Los Baños, Philippines.

<sup>3</sup> K.-H. TRAUTMANN, A. SCHULER, M. SUCHÝ and H.-K. WIPF, *Z. Naturforsch.* 29, 161 (1974).

<sup>4</sup> J. D. DE WILDE, G. B. STAAL, C. A. D. DE KORT, A. DE LOOF and G. BAARD, *Proc. K. med. Akad. Wet. Ser. C* 77, 321 (1968).

<sup>5</sup> Courtesy of Dr. BELEN MORALLO-REYES, Asst. Prof., Dept. of Entomology College of Agriculture, University of the Philippines at Los Baños.

<sup>6</sup> K.-H. TRAUTMANN and P. MASNER, unpublished results.

<sup>7</sup> H. ROELLER and J. S. BJERKE, *Life Sci.* 4, 1617 (1965).

Biological activity of the juvenile hormone active principle in *Attacus atlas* L.

Fractions	Total <i>Galleria</i> Units (GU)
Ether extract	134,357
First TLC	46,689
JH-1/JH-2/JH-3 corresponding fractions	46,689
Rest of the TLC plates	0
Final TLC	61,855
Fractions 1 and 2	0
Fraction 3	3,333
Fraction 4	7,692
Fraction 5	50,000
Fraction 6	830
Fractions 7 and 8	0

The bioactive material from this first TLC work-up was further separated into 8 very narrow bands by a final TLC in order to localize the active principle more accurately. The total recovered bioactivity of 61,855 GU is close to that found in the first TLC work-up (Table). The detailed analysis of each fraction revealed the highest activity of 80.8% in fraction 5 (TLC  $R_f = 0.49$ ). An activity of 12.4% was detected in fraction 4 ( $R_f = 0.52$ ), 5.4% in fraction 3 ( $R_f = 0.55$ ) and traces of activity corresponding to 1.3% were found in fraction 6 ( $R_f = 0.47$ ). Thus, the main active ingredient of *A. atlas* was localized in fractions 4 and 5 (total of 93.2% activity), which co-chromatographs with JH-3 ( $R_f = 0.51$ ). The low activity observed in fraction 3 (5.4%), on the other hand, behaved similarly to the internal standard JH-1 isomer mixture ( $R_f = 0.56$ ). The remaining fractions were devoid of any bioactivity.

The bioactivity containing fractions were subjected to further chemical analysis. The detection limit of the high

resolution glass capillary GC system used in this study is approximately 1.5 ng per cm peak height, for all 3 hormones. The total volume of the GC solution was 25  $\mu$ l in hexane. With an injection volume of 1  $\mu$ l, approximately 40 ng of each JH must be present in the 25  $\mu$ l hexane solution in order to obtain a 1 cm peak height. Based on the JH equivalent of 70 pg for JH-3, an amount of 538 and 3,500 ng equivalent was estimated to be present in fractions 4 and 5, respectively. Although the computed JH equivalent amount was far above the GC detection limit, no peak corresponding to the known JH-3 was detected in either fraction. Based on the JH equivalent of 1.6 pg for JH-1, a 5 ng equivalent only was biologically quantified in fraction 3, an amount which is below the GC detection limit. Considering the estimate of JH presence by means of the *Galleria* bioassay as semi-quantitative due to its unavoidable error, as well as the given GC detection limit, the result presented as far as JH-1 and JH-2 is concerned cannot be conclusive. However, it can be stated from the GC results that the possible content of JH-1 and JH-2 in *A. atlas* cannot exceed the amount of 0.1 ng per g fresh weight.

Based on the results presented, it may be concluded that the ether extracts of *A. atlas* investigated in this study do not contain JH-1, JH-2 or JH-3 at a concentration corresponding to more than 0.1 ng per g fresh weight. The remarkable biological activity which co-chromatographs with JH-3 in the TLC systems employed, and which corresponds to a concentration of JH-3 far above the detection limit of the chemical analysis, could not be characterized chemically. Further investigations will be necessary to identify the JH-active principle in *Attacus atlas* L.

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## STUDIORUM PROGRESSUS

### Transparency in Organisms

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**Summary.** The occurrence in animal phyla of species having a relatively transparent body is noted and measurements of the transmittance of medusae made in a spectrophotometer are reported, but the approximate nature of the results obtained with a commercial instrument and the importance of the correct physical design of the measuring apparatus are emphasized. The application to invertebrates of the structural explanation of the predominant transmission of incident light by the vertebrate cornea is discussed and the role of other factors considered. Destructive interference of the scattered rays, sufficient to account for the transparency of the cornea, has been shown not to demand a completely regular arrangement of collagen fibres. The small diameter and regularity of the fibrillar components in the muscles of *Sagitta* may be adequate to account for their transparency.

**Introduction.** Light which is incident upon living organisms can be absorbed, scattered, reflected or transmitted. The visibility of an organism to an observer depends upon which fate befalls the incident light or which fate predominates. Absorption is enhanced by the presence of pigment contained in cells or tissues and its importance in nature needs no emphasis. Scattering in a medium results from inhomogeneities which, in organisms, may include the presence of fibrils, particles and intracellular membranes separating regions of different composition and hence of different refractive index. As an extreme case reflexion or light may occur at crystalline surfaces in certain cells e.g. those underlying fish scales

and those in the tapetum of the eyes of nocturnal mammals. Transmission, resulting in transparency, occurs among planktonic organisms and in specific regions in many animals especially in the dioptric apparatus of the eye and may be regarded as a special condition in that it is dependent upon the reduction or elimination of light scattered by the inhomogeneities.

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