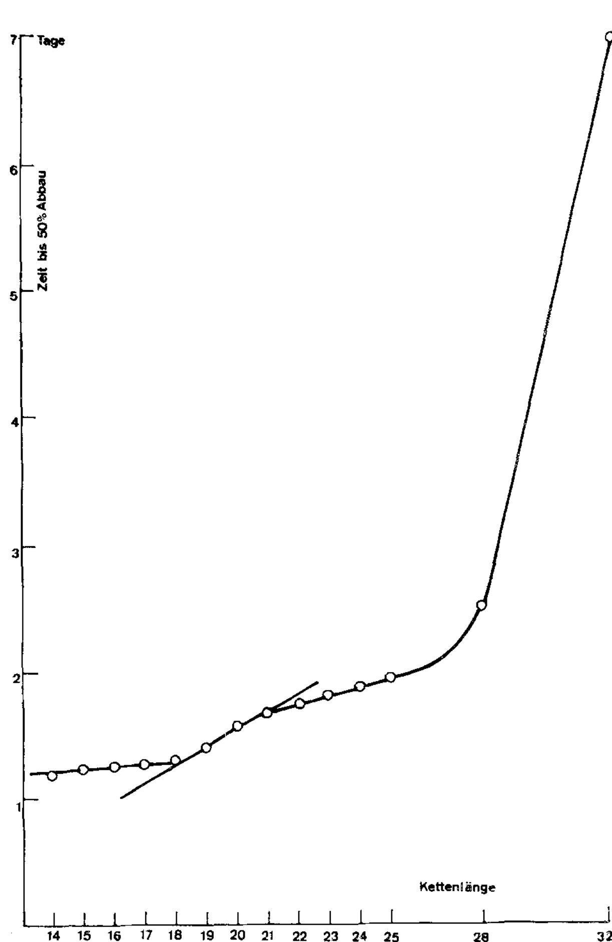

Fig. 2. Halbwertszeit (Zeitdauer bis 50%-Abbau) verschiedener n-Alkane in einem homogenen Gemisch bei Inkubation mit *A. elegans*. Abszisse: Länge des Kohlenstoffskelets; Ordinate: Halbwertszeit in Tagen.

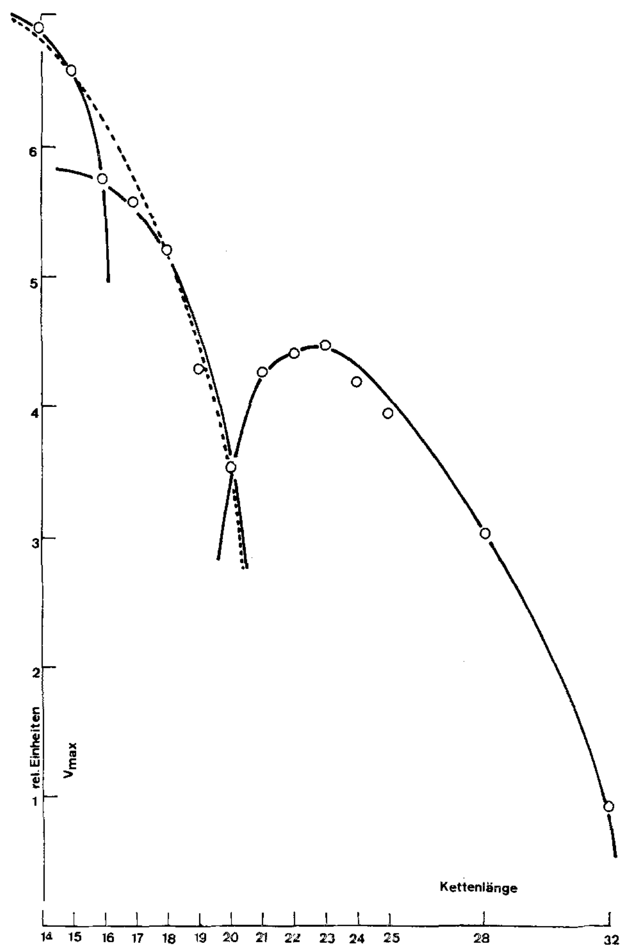

Fig. 3. Maximale Abbaugeschwindigkeit von n-Alkanen aus einem homogenen Gemisch durch *A. elegans* als Funktion der Kettenlänge. Abszisse: Länge des Kohlenstoffskelettes. Ordinate: eine Einheit entspricht 5×10^{-5} g/h.

VAN DER LINDEN und THIJSSE[13]: C_2 bis C_5; C_6 bis C_{12}; und C_{12} bis C_{20} müsste demnach ein neues System zugefügt werden: C_{21} bis C_{32}. Diese Aussage wird erhärtet, wenn die maximale Abbaugeschwindigkeit (V_{max}) der einzelnen Paraffine errechnet und grafisch dargestellt wird (Figur 3).

Im Gang befindliche Versuche sollen zeigen, ob durch kinetische Betrachtungen ein Paraffinaufnahme- und/oder Paraffinoxidationssystem für Kettenlängen der festen n-Alkane zwingend bewiesen werden kann.

Summary. Actinomucor elegans (CBS 104 29) is shown to be a paraffin-oxidizer. The usual fermenter-method for the biological degradation of solid alkanes proved to be impracticable. A culture system was developed to analyse the kinetics of solid paraffin degradation. Degradation of the paraffins by *A. elegans* up to a chain length of C_{32} was observed.

M. EGLI und H. WANNER[14]

Institut für allgemeine Botanik der Universität, Künstlergasse 16, CH-8006 Zürich (Schweiz),
15. August 1973.

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Effects of Heat, Urea and Dimethylsulfoxide on Ribosomal RNA of the Honey Bee (*Apis mellifera* L.)

The thermolability of the large ribosomal-RNA (rRNA) has been described in some insects[1-4]. These reports have demonstrated that the heat treatment of the large rRNA determines its conversion to a product with sedimentation coefficient similar to that of the small rRNA.

We found that the 26 S rRNA of the honey bee is also thermolabile. In addition, we observed that the thermal conversion of the 26 S rRNA to an 18 S product is always accompanied by the release of a low molecular RNA. In order to verify whether this phenomenon is due to the rupture of hydrogen bonds, the effects of urea and dimethylsulfoxide on the large rRNA of *Apis mellifera* were investigated.

Material and methods. All experiments were carried out with 15-day-old pupae of honey bees reared under natural conditions. The pupae were taken from worker cells

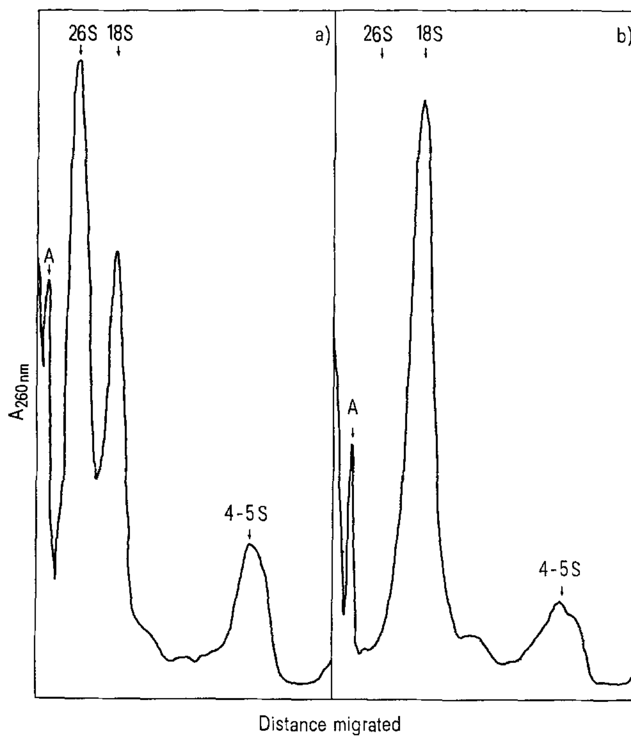

Fig. 1. Effect of heat on RNA extracted by the cold-phenol method. Samples of unheated (a) and heated (b) RNA were analyzed by electrophoresis on a 2.5% polyacrylamide gel for 40 min at 4 mA/gel.

and the RNA was extracted by the cold and hot-phenol methods[2,5]. The cold-phenol procedure was performed according to GREENBERG[2], except that the extraction medium contained 0.5% sodium dodecyl sulphate and 0.1% bentonite. The concentration of RNA was estimated on the basis of UV-absorption and 1 unit of $A_{260\,nm}$ was equivalent to 40 µg of RNA/ml[6]. The $A_{260\,nm}/A_{280\,nm}$ ratio was about 1.9–2.0, indicating a high degree of purity[7] of our RNA preparations. The 26S rRNA was purified by zonal centrifugation as reported by GIORGINI and DE LUCCA[8]. The heat treatment was done at 60 °C for 3 min in 0.02 M *Tris*-HCl buffer, pH 7.6, containing 0.01 M NaCl and 0.001 M EDTA. The treatments with 8 M urea and 80% dimethylsulfoxide were performed as described previously[8]. Electrophoresis on polyacrylamide gel was carried out according to LOENING[9]. After fixation, the gels were scanned at 260 nm in a Beckman spectrophotometer, model Acta III.

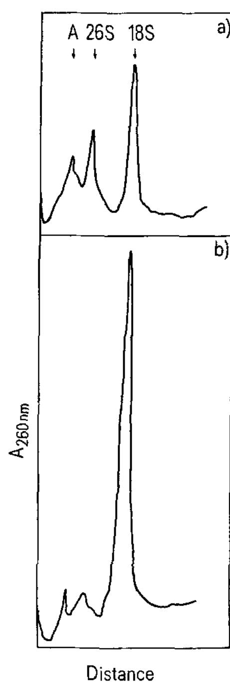

Fig. 2. Electrophoresis of RNA extracted by the hot-phenol method on a 2.5% polyacrylamide gel. The extraction was performed using a salt concentration of 0.1 M (a) and 0.05 M NaCl (b). RNA samples were run for 60 min at 4 mA/gel.

Results. The electrophoretic profile of the RNA extracted by the cold-phenol method is shown in Figure 1a. The values of 26 S and 18 S, for the large and small rRNA respectively, were calculated previously[10]. Another type of RNA (peak A) was also observed with electrophoretic mobility lower than that of 26 S rRNA. When the RNA obtained by the cold-phenol procedure was heated at 60 °C for 3 min, a complete loss of the UV-absorbance peak corresponding to the 26 S rRNA was observed (Figure 1b). Moreover, there was a increase of the UV-absorbance in the region of the 18 S rRNA. A similar result was obtained when the heating was performed in the presence of 0.1%

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sodium dodecyl sulphate. On the other hand, the peak A was not sensitive to the heat treatment (Figures 1a and b).

The thermal conversion of the 26S rRNA to an 18S component was also noted during the hot-phenol extraction (Figures 2a and b). This conversion was quantitatively lower when the extraction buffer contained 0.1 *M* NaCl.

Figure 3b shows that thermal conversion was accompanied by the release of a '6S' RNA species. The heat treatment of purified 26S rRNA also releases this low molecular weight RNA. Another type of low molecular weight RNA (peak B) was found both in unheated and heated samples (Figures 3a and b), which was also described in the brain of the honey bee[11].

All results reported here with the heat treatment were also obtained with 8 *M* urea and 80% dimethylsulfoxide treatments.

Discussion and conclusions. Our results indicate that the 26S rRNA of *Apis mellifera* is unstable to brief heat treatment. The thermal conversion of the large rRNA observed in the honey bee was similar to that reported in other insects[1,2,4]. However, the release of a low molecular weight RNA ('6S') during the thermal conversion was observed in the honey bee only. This finding in *Apis mellifera* supports the idea that this RNA species is characteristic of the eukariotic cells[15,16]. It has been demonstrated that a similar low molecular weight RNA is hydrogen-bonded to the large rRNA of eukaryotes[8,12–15]. Since the conversion of 26S rRNA to an 18S component and the release of '6S' RNA species were induced by urea and dimethylsulfoxide, it was concluded that this phenomenon in *Apis mellifera* results also of the rupture of hydrogen bonds[1,4,12,16,17]. This conclusion is supported by the fact that the degree of the thermal conversion was dependent of the salt concentration[18]. Moreover, the action of nucleases was ruled out by the addition of sodium dodecyl sulphate to the buffer used for heat treatment.

Finally, our results indicated that the peak A (Figures 1 and 2) is not heat sensitive, which constitutes strong evidence that it represents the precursor of rRNA[1,4]. This is in accordance with several reports[1,2,4,12,17] which have been postulated that large rRNA of animal cells has 'hidden breaks' which appear during processing of the precursor of rRNA.

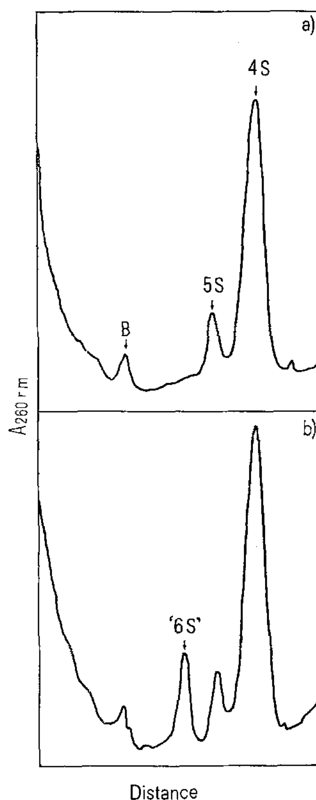

Fig. 3. Electrophoresis of RNA extracted by the cold-phenol method on a 7.5% polyacrylamide gel. Samples of unheated (a) and heated (b) RNA were run for 100 min at 5 mA/gel.

Résumé. Nous avons trouvé que l'ARN ribosomique 26S de pupe d'*Apis mellifera* est thermosensible. Les résultats montrent que, par un bref traitement thermique cet ARN se transforme en un ARN dont le coefficient de sédimentation est égal à celui de l'ARN ribosomique 18S. En même temps, un ARN de faible poids moléculaire ('6S') est libéré. Ces résultats ont été obtenus autant sur l'ARN total que sur l'ARN 26S purifié. Les effets de l'urée ,du diméthylsulfoxide et de la concentration saline ont aussi été étudiés. Il est probable que ces phénomènes proviennent de la rupture de liaisons d'hydrogène.

F. L. De Lucca[19], J. F. Giorgini[20] and A. Calabrese

Departamento de Bioquímica, Faculdade de Medicina da Universidade de São Paulo, 14.100 Ribeirão Preto, S.P. (Brasil), 12 June 1973.

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Existe-t'il une action particulière des différentes longueurs d'onde du spectre visible sur la physiologie des chrysalides et prénymphes de *Pieris brassicae* conditionnées à la diapause?

Depuis plusieurs années nous nous sommes attachés à mettre en évidence le rôle photorécepteur du pigment tégumentaire vert (ptérobiline) chez la chenille de *Pieris brassicae*[1,2].

Nous avons ainsi montré que les chenilles élevées sous le maximum d'absorption de ce pigment (630–670 nm) ne donnent jamais naissance à des chrysalides en diapause quelle que soit la photophase fournie[3]; sous cette irradia-