medium and on nearly solid substrates. The mantle end indeed represents the advancing head of a 'borer', around which the jelly is only beginning to liquify, whereas the animal's head and arms trailed behind have their cilia beating in a medium which is already relatively less viscous.

A more detailed analysis of this highly effective ciliary apparatus is actually in progress. The study of its structure and of its development in the embryos of different decapod cephalopods may throw new light on the evolution of the functional relationships between special embryonic structures and protective devices produced by the adult.

1 The assistance of Mrs D. Guillaumin and Mrs M. André (Service de Microscopie électronique à balayage, 105 Boulevard Raspail, Paris) in making the scanning electron micrographs is gratefully acknowledged. I thank Dr Peter Boyle (Zoology Department, University of Aberdeen) for his critical reading of the manuscript and for his valuable suggestions.

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## Alteration in thermal stability of ribosomes from *Drosophila melanogaster* with age

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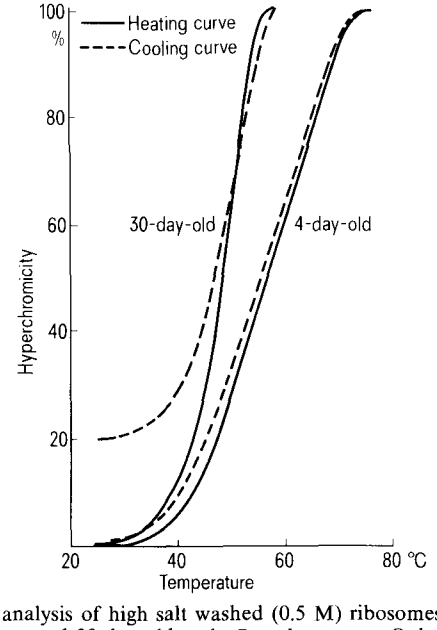
*Summary.* Thermal analysis of high salt (0.5 M) washed ribosomal monomers from young and old male *Drosophila melanogaster* revealed an 8 °C downshift in the mean temperature of denaturation (Tm). Moreover, there was observed a marked loss in the ability of ribosomes extracted from older flies to reassociate upon cooling. These observations suggest that age-dependent alterations in the structural integrity of the rRNA-r protein complex could, at least in part, be responsible for the diminished capacity for protein synthesis in this species with advancing age.

A diminished capacity for protein synthesis with advancing age has been well documented in various mammalian systems[2-7] including man[8]. Similarly, age-dependent decreases in net protein synthesis have been demonstrated in higher insects[9-11]. However, the mechanism(s) underlying this widespread correlate of the aging process have not been fully elucidated. Much of the current research in this area has been directed toward possible age-dependent alterations in the various regulatory factors associated with the ribosome[12,13]. And although the ribosome has been suggested as a possible site where changes could effect the quantity and/or fidelity of protein synthesis[14-17], little experimental information is available as to what changes occur within the ribosome with age. A rather general finding with advancing age is a decrease in the amount of polysomes with a concomitant increase in 80S monomers[18,19]. These observations could be a reflection of any number of alterations including decreased availability of mRNA, quantitative and/or qualitative changes in the ribosomes, or changes in levels of fidelity of the various accessory factors necessary for the initiation of protein synthesis. Previous studies from this laboratory have demonstrated a 23% reduction in the amount of extractable ribosomes from older *Drosophila* males as well as an increase in the amount of protein dissociated from the high salt washed ribosomes in the presence of increasing concentrations of KCl, some 5-fold at 2.0 M KCl[20]. The current studies were undertaken in an attempt to further characterize and identify the molecular lesions(s) responsible for the previously observed physicochemical alterations in ribosomal structure with advancing age.

*Materials and methods.* Ribosomes from young (4-day) and old (30-day) male *D. melanogaster* (Sevelen strain) were isolated as previously described[20,21]. Males of this strain demonstrate 50% and 90% mortalities of 29 and 40 days respectively, when reared and maintained as previously described[22]. Only ribosomal preparations with an $OD_{260}/OD_{280}$ ratio between 1.70 and 1.90 were employed for thermal analysis. Melting profiles were obtained on a Beckman Acta CIII UV spectrophotometer equipped with a Braun Melsungen Thermomix 1480 and Thermograd 1491 regulated temperature bath. The slope-time rise of the temperature as monitored by a platinum thermo-electrode was maintained at 1 °C/min over the operating temperature range of 25–85 °C. The ribosomal monomers were melted in 1 ml Teflon stoppered cuvettes in degassed, deionized DD $H_2O$ at an initial concentration of 0.5 $OD_{260}$/ml. When no further increase in hyperchromicity was observed for




Thermal analysis of high salt washed (0.5 M) ribosomes extracted from 4-day- and 30-day-old male *D. melanogaster*. Only ribosomal preparations with $OD_{260}/OD_{280}$ ratios between 1.8 and 1.9 were employed in these studies. The graph above represents the mean change in hyperchromicity at 260 nm/°C of melts obtained on at least 6 separate preparations for each age. Thermal analysis of ribosomes was carried out in degassed, deionized DD $H_2O$ at a rate of 1 °C/min in jacketed Teflon stopper cuvettes.

5 min, the samples were cooled at the same rate as heating and the OD changes continuously monitored.

*Results and discussion.* Significant age-dependent alterations in the thermal profiles of ribosomes were observed. As shown in the figure and the table there is an 8 °C decrease in the mean temperature of thermal denaturation between ribosomes extracted from young versus old flies. There is also a marked and reproducible inability of the ribosomes from older animals to reassociate upon cooling. Thermal analysis of ribosomes co-extracted from young and old animals exhibit melting and cooling profiles which one would expect based on the arithmetic mean of young and old samples alone (data not shown). The observed alterations are therefore apparently not an artifact of the extraction procedures nor due to age-related increases in ribonuclease or protease activities but rather must reflect an age-dependent alteration in the structural integrity of the rRNA-r protein complex. Ribosomal proteins have been reported to turn over with a half-life of approximately 10 days in adult *Drosophila*[23]. Thus, the observed changes could reflect altered synthetic processes in either ribosomal proteins or presumably rRNA as both synthetic processes are apparently closely coordinated within eukaryotic cells[24,25].

Wallach and Gershon[18] reported a similar decrease in the Tm's of ribosomal monomers from aging nematodes. These authors also observed a 5-6 °C earlier commencement of the hyperchromic effect in melts of ribosomes from older animals which compares well with our results.

These results collaborate and extend earlier studies on ribosomal monomers from young and old *Drosophila* which indicated a loss in the structural integrity of the rRNA-r protein complex with advancing age. The age-dependent alterations in the structural stability of ribosomes are apparently not due to changes in the ribosomal proteins as analysis by 2-dimensional polyacrylamide gel electrophoresis of the total ribosomal proteins from young and old flies revealed no major quantitative or qualitative differences[26]. It is therefore suspected that the primary lesion(s) responsible for the altered physicochemical stability of the ribosomal monomers resides within the rRNA. Indeed, preliminary analysis of the melting profiles of the rRNA from young and old animals supports this hypothesis[27]. In conclusion, it is suggested that the observed loss of structural integrity within the ribosomal complex may contribute to the diminished capacity for net protein synthesis reported for this species[9] with advancing age.

Thermal analysis of ribosomes from young and old male *Drosophila*

| | 4-day-old | 30-day-old |
|---|---|---|
| Purity $OD_{260}/OD_{280}$ | 1.88±0.06 (N=15) | 1.87±0.08 (N=18) |
| Mean temperature of thermal denaturation | 55.17±2.60 (N=9) | 47.45±3.20 (N=6)** |
| %Δ Absolute hyperchromicity | 15.21±2.40 (N=7) | 15.44±2.30 (N=6) |
| % Renaturation | 99.00±2.50 (N=7) | 79.30±9.00 (N=5)* |

Mean ±SD (N=sample size); probability: Student's t-test; *$p < 0.01$, **$p < 0.001$.

1 Acknowledgments. We thank Dr R. Christian for critical reading of the manuscript and Ms P.C. Snelling for her skillful preparation of the manuscript. This work was supported by Drexel Research Scholar Award to G.T.B. and the Paul Glenn Foundation for Medical Research.

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## Dependence on the lipophilicity of maleimide derivatives in their inhibitory action upon chemotaxis in neutrophils[1]

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*Summary.* Modification of polymorphonuclear neutrophils by a series of maleimide derivatives with various degrees of lipophilicity and hydrophilicity indicated that hydrophilic reagents had little effect on chemotaxis, whereas the degree of the inhibitory effect of lipophilic reagents on the chemotaxis was parallel to the degree of their lipophilicity.

Parachloromercuribenzoate (PCMB) can penetrate the cell membrane while parachloromercuribenzenesulfonate (PCMBS) may penetrate the membrane at a much slower rate than PCMB due to its marked hydrophilicity, reacting only with sulfhydryl groups on the outer surface of the lipid membrane of red blood cells[2,3]. N-ethylmaleimide (NEM) is also said to be a rapidly penetrating sulfhydryl reagent although it is very water-soluble. Recent observations on the effect of NEM and PCMBS on the functions of human polymorphonuclear neutrophils (PMNs)[4] were of interest