Changes in 80S Ribosomes from *Drosophila melanogaster* with Age

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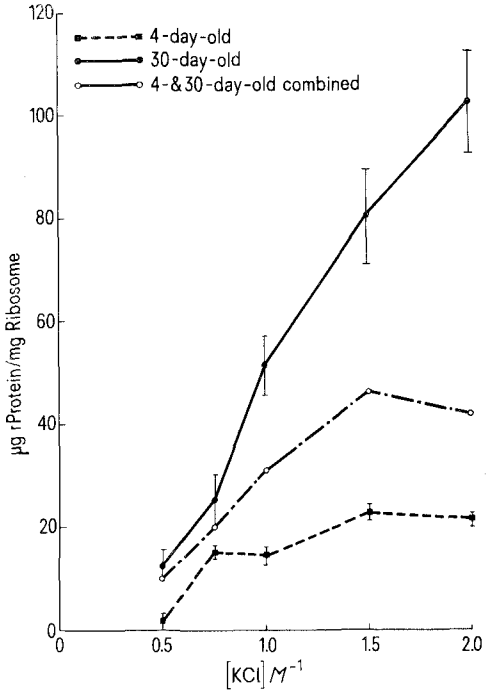
Summary. A 23% reduction in the amount of 80S ribosomes extracted from young and old male *Drosophila melanogaster* on a per g wet weight basis was demonstrated. Furthermore, a significant alteration in the structural integrity of the ribosomal RNA-protein complex from older flies as demonstrated by the dissociation of protein from the 80S particle in the presence of varying concentrations of KCl was observed.

Aging in insects is characterized by a number of morphological, physiological, and biochemical changes[3]. With regard to the latter, and although some controversy has existed, it is generally agreed that insect tissues, particularly those of dipteran species, undergo a decreased capacity for net protein synthesis with advancing age[4,5]. Wattiaux et al.[6] have suggested, based on *in vivo* actinomycin-D studies with *Drosophila melanogaster*, a progressive decrease in the stability of mRNA with age. However, the mechanisms underlying the diminishing capacity for protein synthesis have not been fully elucidated. In the present study, ribosomes extracted from *D. melanogaster* were examined for possible alterations which might affect the overall protein-synthesizing capacity of the almost exclusively postmitotic adult insectean cells with age.



Amount of protein dissociated from ribosomes extracted from 4-day-, 30-day- and 4- and 30-day-old male *Drosophila melanogaster* in the presence of various concentrations of KCl. Ribosomes (3–6 mg) were suspended in 0.01 *M tris*-HCl, pH 7.6, containing 0.5 m*M* $MgCl_2$, 20 m*M* 2-mercaptoethonal and the appropriate KCl concentration (0.5, 0.75, 1.0, 1.5, or 2.0 *M*) and extracted for 30 min at 0 °C. The suspensions were then layered on to a 2 *M* sucrose cushion containing an equivalent KCl concentration without Mg^{2+} ions and centrifuged at 180,000 *g* for 13 h at 2 °C. The upper layer, which contained only those proteins (split proteins) dissociated from the ribosomes, was carefully removed, dialyzed against 0.01 *N* HCl containing 7 *M* urea (2×, 1:1000 v/v), then 0.01 *N* HCl (4×, 1:5000 v/v) and the protein concentration determined. All values, with the exception of the control (4- and 30-day-samples extracted together), represent the average of 3–5 determinations ± S. D.

Material and methods. Ribosomes were isolated from young (4-day) and old (30-day) male *D. melanogaster* (Sevelen) according to methods described by Lambertsson[7]. Males of this strain demonstrate a 50% mortality of 29 days, when reared and maintained as previously described[8]. Subsequently, the isolated 80S ribosomes were subjected to various concentrations of KCl, and the amount of protein (split proteins) dissociated from the ribosomes was determined according to methods described by Delaunay et al.[9].

Results and discussion. A substantial reduction in the amount of 80S ribosomal material extracted from young and old flies on a per g wet weight basis, 2.46 ± 0.3 mg ($N = 5$) vs. 1.90 ± 0.2 mg ($N = 8$) ($p = 0.01$, Student's *t*-test) respectively, was observed. This difference, on a per fly basis, would be magnified as in males of this strain a loss in body weight with advancing age has been reported[10]. Under conditions of increased salt concentration, protein could be dissociated from the ribosomes of both the young and old animals; however, the amount washed off the ribosomes from the older animals was significantly higher at all KCl concentrations tested. Indeed, as shown in the figure, there was observed a 5fold increase in the amount of protein removed from the ribosomes of the 30-day-old flies as compared with those of the 4-day-old flies at the highest KCl concentration (2.0 *M*) employed.

As there were no differences in the purity of the ribosomal preparations as judged by optical density readings at 260/280, i.e., O.D. 260/280 of 1.88 ± 0.08 ($N = 5$) vs. 1.86 ± 0.09 ($N = 8$), for young and old preparations, respectively, and as the results are apparently not an artifact of the extraction methods (see figure), the ob-

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[3] M. Rockstein and J. Miquel, in *The Physiology of Insecta*, 2nd ed., vol. 1 (Ed. M. Rockstein; Academic Press, New York 1973), p. 371.

[4] P. S. Chen, in *Molecular Genetic Mechanisms in Development and Aging* (Eds M. Rockstein and G. T. Baker; Academic Press, New York 1972), p. 199.

[5] G. T. Baker, Gerontologia *22*, 334 (1976).

[6] J. M. Wattiaux, M. Libion-Mannaert and J. Delcour, Gerontologia *17*, 289 (1971).

[7] A. G. Lambertsson, Molec. gen. genet. *128*, 241 (1974).

[8] G. T. Baker, Gerontologia *21*, 203 (1975).

[9] J. Delaunay, F. Crensot and G. Schapira, Biochimie *56*, 189 (1974).

[10] G. T. Baker, Exp. geront. *10*, 231 (1975).

servations must reflect an alteration in the integrity of the protein-rRNA complex. Further study is required in order to determine whether these changes are the result of a loss in the complementary fidelity of the ribosomal protein for the rRNA, or vice versa or both, through any number of mechanisms which would alter the strength of ionic bonding within the complex. However, an analysis of the 80S ribosomal proteins (56 basic and 11 acidic) by 2 dimensional polyacrylamide electrophoresis revealed no major detectable quantitative or qualitative differences between the ribosomal proteins of the 4-day- and 30-day-old flies[11].

With regard to the decreased content of ribosomes observed in older flies, a selective loss of genes coding for ribosomal RNA has been reported in postmitotic tissues of the dog with age[12]. This is, however, apparently not a contributing factor in the present observations as no significant differences in the saturation values of DNA/RNA hybridization in RNA excess with ribosomal ^{128}I-rRNA from adult flies or ^{3}H-rRNA from a *Drosophila* cell culture ribosomes were detectable between 4 and 30 days of age (MÖRMANN, BAKER and HENNIG, unpublished). Recently, there was observed a 29% decrease in the amount of extractable DNA from the brain of 30-day-old flies, as well as histological evidence of cellular deterioration in brain tissue with age which could, at least in part, account for the loss of ribosomal material[13].

Studies on the in vitro translational ability of ribosomes from *Drosophila* with age have not yet been completed; however, it is more than likely that such pronounced changes in the integrity of the ribosomal protein-RNA complex would not be without deleterious effect. Thus it is suggested that the quantitative loss as well as the alterations in structural stability in ribosomes from older flies may be contributing factors in the age-dependent decreases in net protein synthesis reported for *D. melanogaster*[14] as well as for other dipteran species[15, 16].

[11] T. SCHMIDT and G. T. BAKER, (in preparation).
[12] R. JOHNSON and B. L. STREHLER, Nature *240*, 412 (1972).
[13] G. T. BAKER and W. HENNIG, (in preparation).
[14] P. A. BAUMANN and P. S. CHEN, Rev. suisse zool. *75*, 1051 (1968).
[15] L. LEVENBOOK and I. KRISHNA, J. insect. physiol. *17*, 9 (1971).
[16] M. ROCKSTEIN and G. T. BAKER, Mech. age dev. *3*, 271 (1974).

Light Stimulated 'Shunt-Metabolism' Succinate-α-Ketoglutarate-Isocitrate Cycle and Accumulation of Citric Acid in *Aspergillus niger*

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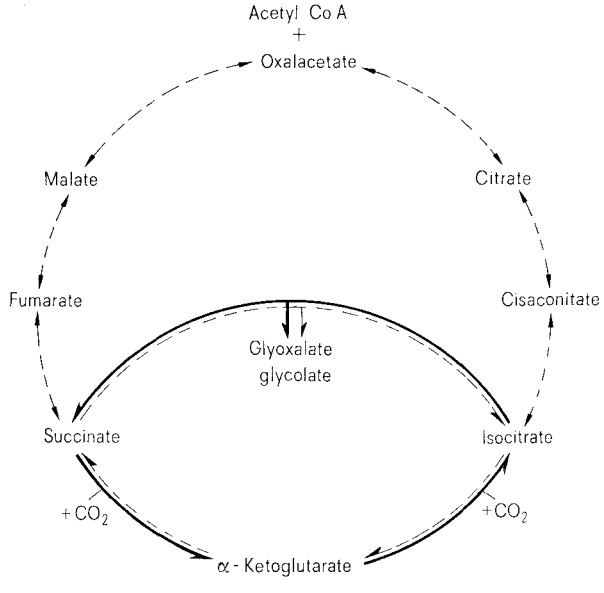
Summary. Studies with light of the visible range had shown that light plays a significant role in the biosynthesis and accumulation of citric acid in *Aspergillus niger*. Accumulation of ^{14}C-labelled carbon atoms in α-ketoglutaric, isocitric, succinic and glycolic acids in the cultures grown under illumination suggest a probable 'shunt-metabolism' leading to the succinate-α-ketoglutarate-isocitrate (SKI) cycle. This shunt metabolism minimizes the accumulation of citric acid in cultures due to depletion of intermediates.

Influence of light of the visible range on mycelial growth (HUSKINS and WESTON[2]; CANTINO and HORENSTEIN[3]), spore germination (HUTCHINSON and ASHTON[4]) and other phenomena (NEERGAARD and NEWHALL[5]) associated with the metabolism of fungal organisms was reported earlier. The present investigation was taken up, since little had been reported as yet on the effect of light on fermentation of citric acid by *Aspergillus niger*.



SKI-cycle proposed by CANTINO and HORENSTEIN[3].

Materials and methods. Aspergillus niger 6N3 isolated from the soil of Naihati, West Bengal, India, was grown in 100 ml capacity Erlenmeyer flasks containing 25 ml of SHU and JOHNSON's medium[6] at 30 °C under light, dark and alternate light and dark conditions. Control cultures were kept in an incubator at 30 °C. On the 7th day of incubation 1-^{14}C sodium acetate having an activity of 20 μCi each was added to the flasks and incubated for 1 h. Organic acids accumulated in the culture filtrate were extracted in ether and separated by the thin layer chromatographic methods of MEYERS and KEN-YEN-HUANG[7]. Radioactive acidic spots appearing on the autoradiograms were compared with the known acids. For quantitative estimation of the acids accumulated, cpm/ml of the radioactive spots were taken in a Beckman

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[2] R. H. HUSKINS and W. H. WESTON, JR., Am. J. Bot. *37*, 739 (1950).
[3] E. C. CANTINO and E. A. HORENSTEIN, Mycologia *48*, 777 (1956).
[4] A. H. HUTCHINSON and M. R. ASHTON, Can. J. Res. *3*, 187 (1930).
[5] P. NEERGAARD and A. G. NEWHALL, Phytopathology *41*, 1021 (1951).
[6] P. SHU and M. J. JOHNSON, Ind. Eng. Chem. *40*, 1202 (1948).
[7] W. E. MEYERS and KEN-YEN-HUANG, Analyt. Biochem. *17*, 210 (1966).