Other examples of enhanced ^{14}C-glycine incorporation into urate occurring with nearly normal values for urate production have been observed both in patients with primary gout and also in patients with psoriasis (*9*), and, although this might possibly be related to a reduction in the size or turnover rate of the glycine pools, the exact metabolic explanation for this phenomenon has not been elucidated. Mother J has a considerably elevated production rate, but maintains an only moderate increase in her urate pool and serum urate concentration by a high urinary urate excretion and efficient extrarenal disposal. Mother L, with a high renal clearance of urate and normal renal function, maintains a normal serum urate by excreting almost all of her produced urate by the kidney each day. Her ability to excrete such a high proportion of her produced uric acid in each 24 hours is supported by the finding that she is able to excrete a similarly high proportion of the administered ^{15}N-urate within 7 days. Perhaps appropriate studies may yet enable some abnormality of urate metabolism to be detected in all such heterozygotes.

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References

1. M. Lesch and W. L. Nyhan, *Amer. J. Med.* **36**, 561 (1964); D. Hoefnagel, E. D. Andrew, N. G. Mireault, W. O. Berndt, *N. Engl. J. Med* **273**, 130 (1965); D. S. Newcombe, S. L. Shapiro, G. L. Sheppard, F. E. Dreifuss, *J. Amer. Med. Ass.* **198**, 315 (1966).
2. J. E. Seegmiller, F. M. Rosenbloom, W. N. Kelley, *Science* **155**, 1682 (1967).
3. B. R. Migeon, V. M. D. Kaloustian, W. L. Nyhan, W. J. Young, B. Childs, *ibid.* **160**, 425 (1968); F. M. Rosenbloom, W. N. Kelley, J. F. Henderson, J. E. Seegmiller, *Lancet* **1967-II**, 305 (1967).
4. W. N. Kelley, *Fed. Proc.* **27**, 1047 (1968).
5. ———, M. L. Greene, F. M. Rosenbloom, J. F. Henderson, J. E. Seegmiller, *Ann. Intern. Med.* **70**, 155 (1969).
6. W. L. Nyhan, *Fed. Proc.* **27**, 1092 (1968).
7. B. T. Emmerson and J. B. Wyngaarden, in preparation.
8. S. Jakovcic and L. B. Sorensen, *Arthritis Rheum.* **10**, 129 (1967).
9. J. B. Wyngaarden, *J. Clin. Invest.* **36**, 1508 (1957); J. E. Seegmiller, A. I. Grayzel, L. Laster, L. Liddle, *ibid.* **40**, 1304 (1961); A. Z. Eisen and J. E. Seegmiller, *ibid.*, p. 1486.
10. E. Beutler, Ed., *Hereditary Disorders of Erythrocyte Metabolism* (Grune and Stratton, New York, 1968), p. 114.
11. L. Liddle, J. E. Seegmiller, L. Laster, *J. Lab. Clin. Med.* **54**, 903 (1959); K. Brøchner-Mortensen, S. Cobb, B. S. Rose, in *The Epidemiology of Chronic Rheumatism* (Blackwell, Oxford, 1963), vol. 1, p. 295.

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Peroxidation of Subcellular Organelles: Formation of Lipofuscinlike Fluorescent Pigments

Abstract. *Lipid peroxidation of subcellular organelles gives fluorescent products which have fluorescence and excitation spectra similar to those of lipofuscin pigments. Fluorescence and excitation spectra and total fluorescence in the 460-nanometer region are useful for qualitative identification and quantitative measurement of the Schiff base product, a molecular damage site of lipid peroxidation which develops during some aging processes.*

Lipofuscin pigments accumulate in animal tissues, especially the brain and heart, as a function of age. The pigments have characteristic fluorescence spectra with a maximum at 470 nm when excited at 365 nm. Formation of the pigments appears to involve peroxidation of polyunsaturated lipids of subcellular membranes (*1*). Malonaldehyde, a major product of peroxidation of polyunsaturated lipids, reacts with primary amino groups of amino acids and proteins in a cross-linking reaction (*2*). The Schiff base product, RN=CH–CH=CH–NH–R, has fluorescent maximums in the 450- to 470-nm region and excitation maximums in the 360- to 390-nm region. Fluorescent products are also produced when malonaldehyde undergoes similar reactions with amino groups of nucleic acids and their bases and with phospholipids. Thus a number of properties identify this cross-link as a chromophoric molecular damage site of lipofuscin pigments.

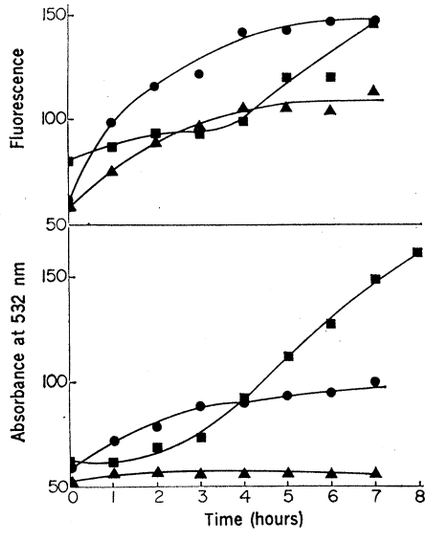

Fig. 1. Fluorescence at 460 nm (top) and thiobarbituric acid reactants (bottom) in the oxidation products of lysosomes (triangles), mitochondria (circles), and microsomes (squares). Fluorescence is given in units per milligram of protein per milliliter, and thiobarbituric acid reactants in absorbance at 532 nm per milligram of protein per milliliter.

The damaging effects of lipid peroxidation are well documented for mitochondria (*3*), microsomes (*4*), and lysosomes (*5*). Lipid peroxidation in vivo is a basic deteriorative reaction in vitamin E deficiencies (*6*), in hepatotoxic mechanisms (*7*), in cellular mechanisms of oxygen toxicity (*8*), and in cellular mechanisms of aging processes (*9*). Fluorescent lipofuscin pigments have been observed in many pathological processes (*10*), especially in vitamin E deficiencies and aging processes, where their deposition has been correlated with lipid peroxidation dam-

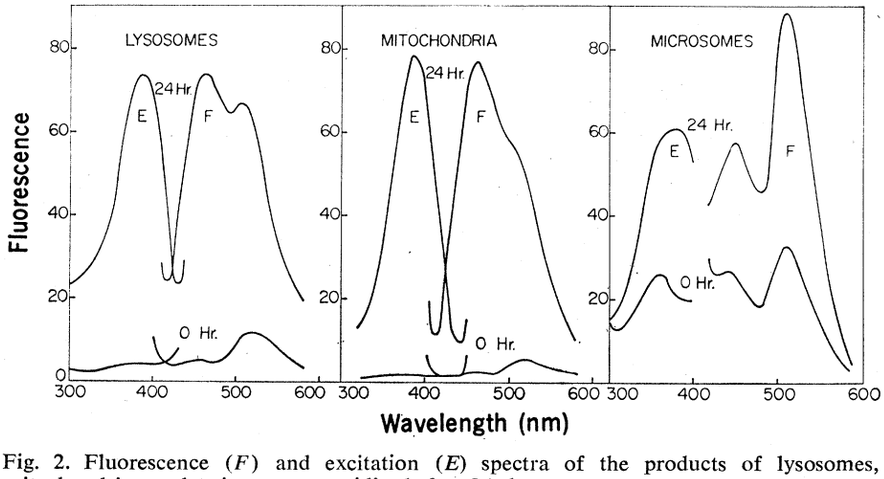

Fig. 2. Fluorescence (*F*) and excitation (*E*) spectra of the products of lysosomes, mitochondria, and microsomes oxidized for 24 hours.

age. Lipid peroxidation aging processes may be a universal disease whose chemical deteriorative effects might be slowed by increased intake of antioxidants (*11*).

We have studied the fluorescent characteristics of products formed in lipid peroxidation reactions of subcellular organelles in vitro. Rat liver mitochondria, microsomes, and lysosomes, prepared according to the method of Ragab *et al.* (*12*), were suspended in 0.15*M* NaCl. Lipid peroxidation reactions proceeded when these suspended organelles were shaken in oxygen at 37°C (*5*). At intervals, the suspensions were centrifuged at 12,000*g* for 10 minutes, and the supernatants were analyzed. Fluorescence and excitation spectra and thiobarbituric acid reactions (*13*) were obtained for all samples. Fluorescence was measured (Fig. 1) with an Aminco-Bowman spectrophotofluorometer which was standardized with quinine sulfate.

In addition to the increases in the fluorescence and excitation spectra (Fig. 2), characteristic of the Schiff base product, an increase is found in flavin fluorescence in the 515-nm region as a result of enzymic hydrolysis of flavin-adenine dinucleotide (*14*). Lysosomes show an increase in fluorescence at 460 nm as a function of oxidation time, while the free thiobarbituric acid reactants remain constant. When oxidations were extended to 24 hours, the intensity of the fluorescence at 460 nm was greatly increased, with only a small increase in the flavin peak. Among the organelles, mitochondria gave fluorescence and excitation spectra most similar to those of age (lipofuscin) pigment and showed concurrent increases of fluorescence at 460 nm and thiobarbituric acid reactants as a function of oxidation. Microsomes showed an increase in fluorescence and thiobarbituric acid reactants beginning at 3 hours. Although there was a definite fluorescence peak for the Schiff base product, the fluorescence spectrum of microsomes was dominated by increased flavin fluorescence. For all three organelles the production of the chromophore fluorescent at 460 nm was inhibited approximately 75 percent by an antioxidant (0.02 percent propyl gallate).

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References and Notes

1. H. Hyden and B. Lindstrom, *Discuss. Faraday Soc.* **9**, 436 (1950); D. D. Hendley, A. S. Mildvan, M. C. Reporter, B. L. Strehler, *J. Gerontol.* **18**, 144 (1963).
2. K. S. Chio and A. L. Tappel, *Biochemistry* **8**, 2821, (1969); *ibid.*, p. 2827.
3. A. K. Schneider, E. E. Smith, F. E. Hunter, Jr., *ibid.* **3**, 1470 (1964).
4. J. D. Robinson, *Arch. Biochem. Biophys.* **112**, 170 (1965).
5. I. D. Desai, P. L. Sawant, A. L. Tappel, *Biochim. Biophys. Acta* **86**, 277 (1964).
6. A. L. Tappel, *Fed. Proc.* **24**, 73 (1965).
7. T. F. Slater, *Nature* **209**, 36 (1966); R. O. Recknagel, *Pharmacol. Rev.* **19**, 145 (1967).
8. N. Haugaard, *Physiol. Rev.* **48**, 311 (1968).
9. A. A. Barber and F. Bernheim, *Advan. Gerontol. Res.* **2**, 355 (1967); L. Packer, D. W. Deamer, R. L. Heath, *ibid.*, p. 77.
10. M. Wolman, in *Handbuch der Histochemie* (Fischer, Stuttgart, 1964), vol. 5, p. 96; W. S. Hartroft and E. A. Porta, in *Present Knowledge in Nutrition* (Nutrition Foundation, New York, 1967), p. 28.
11. A. L. Tappel, *Geriatrics* **23**, 97 (1968); D. Harman, *J. Gerontol.* **23**, 476 (1968).
12. H. Ragab, C. Beck, C. Dillard, A. L. Tappel, *Biochim. Biophys. Acta* **148**, 501 (1967).
13. I. D. Desai and A. L. Tappel, *J. Lipid Res.* **4**, 204 (1963).
14. M. H. Ragab, R. Brightwell, A. L. Tappel, *Arch. Biochem. Biophys.* **123**, 179 (1968).
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Temporal Coordination of DNA Replication with Enzyme Synthesis in Diploid and Heteroploid Cells

Abstract. *The rate of DNA synthesis in the S phase of growth of synchronized diploid Chinese hamster cells shows two maximums, while in heteroploid hamster cells the DNA replication rate is constant. In diploid cells a reciprocal relationship exists between maximum DNA synthetic rates and maximum lactate dyhydrogenase and thymidine kinase enzyme levels. Enzyme activity in heteroploid cells increases continuously through the cell cycle with no evidence of oscillations. It seems possible that these differences in molecular organization may accompany or precede the transition to heteroploidy.*

Oscillatory changes in the intracellular concentration of large and small molecules occur in both prokaryotic and eukaryotic cells. Some can be induced by altered nutrient levels, and involve transient perturbations in the concentration of metabolites (*1*), while others are revealed only by the establishment of a synchronous cell population and may involve fluctuations in levels of metabolites (*2*) or macromolecules (*3, 4*). Macromolecular periodicities show a great deal of regularity and occur at precise times in each cell cycle. Because of this stability they can be accorded an important function in the cellular timekeeping process.

DNA replication in metazoan cells is an intermittent process whose onset and termination are used to subdivide the cell cycle (*5*). Genetic and cytological studies have given additional significance to the temporal order of DNA replication, since certain chromosomes and chromosome segments, usually exemplified by the sex chromosomes in somatic cells of mammals, are distinctly and repeatedly the last to replicate their DNA in the S phase of growth (*6*). The correlation of late DNA replication with sex chromatin, and the many demonstrations that heteropyknosis and late-replicating DNA are associated with gene inactivation, suggest that time of replication might be a rather permanent and general method in a hierarchy of regulatory mechanisms, insuring that unwanted gene products will not be made (*7*). It seems evident that replication patterns are a manifestation of nuclear differentiation, since they can vary from tissue to tissue and have a distinct onset during embryogenesis (*8*).

In rapidly dividing mammalian cells the temporal coordination of DNA synthesis with RNA and protein synthesis is clearly expressed. Both RNA and protein synthesis show gene dosage effects or stimulated synthesis following DNA replication (*9–11*). In pursuing this effect Klevecz and Ruddle observed that lactate dehydrogenase and glucose-6-phosphate dehydrogenase levels oscillated through the cell cycle of a Chinese hamster diploid strain (*4*), and subsequently I observed that these oscillations represented changing amounts of enzyme protein (*11*). In this report I show that the rate of DNA synthesis is constant through the S phase of growth of heteroploid (*12*) hamster cells, but intermittent in diploid cells. A reciprocal relationship exists between maximum rates of DNA synthesis and maximum enzyme levels in diploid but not in heteroploid cells, and I suggest that this is a general distinction between diploid and heteroploid cells.

The following Chinese hamster cells were used: the diploid line Don (CCL-16); a pseudodiploid strain (CS134) derived from diploid Don; a cell line