1–2 μm or about half of lip-width. Amphidial apertures oval, 3 μm wide, situated at 6–9 μm or 2–3 lip-widths from anterior end of body. Nerve ring at 117–127 μm from anterior end of body. Oesophagus 255–310 μm long, basal part occupying 25–29% of its length. Oesophago-intestinal junction 4–5 μm long. Rectum 9–12 μm or more than one anal body-width long. Female reproductive system mono-prodelphic, 186–205 μm long. Vulva transverse, vagina thick-walled, inclined anteriorly, 7–9 μm or 0·5–0·6 vulval body-width long. Tail elongate, conoid, 90–117 μm or 10–17 anal body-widths long. Vulva-anus distance 279–355 μm or 2·5–3·0 times the tail length.

Male : Not found.

Remarks

The present specimens conform with the description and dimensions of the species as given by Andrássy[1] except that they have a smaller and slender body, anteriorly situated amphidial apertures and vulva ($L = 1·14–1·16$ mm; $a = 54–59$; distance of amphid from anterior end = 15 μm; $V = 65–66$ according to Andrássy).

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1. Andrássy, I., *Opusc. Zool. Budapest*, 1977, **14** (1), 3.

LIPOFUSCIN ACCUMULATION AND LIPID PEROXIDATION IN RAT MYOCARDIUM AS A FUNCTION OF AGE

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Lipofuscin accumulation in the myocardium of 12½-, 25-, 50-, 75- and 100-week-old rats was investigated spectrophotofluorometrically by chloroform-methanol extraction method. Since heart is an organ that is particularly sensitive to lipid peroxidation, malonaldehyde (an end product of lipid peroxidation) production was also studied. A dramatic age-related increase in the lipofuscin concentration was observed. Lipid peroxidation rate also increased as a function of age. It is suggested that lipofuscin is formed by peroxidative destruction of polyunsaturated lipids of the subcellular membranes and their consequent co-polymerization with other biological molecules. Lipofuscin accumulation as a function of age is considered as a strong evidence of the occurrence of lipid peroxidation process in cells *in vivo* and it is suggested that there is a positive correlation between aging, lipofuscin and lipid peroxidation. The mode of formation and the possible functional significance of lipofuscin in the myocardium are discussed.

Introduction

Accumulation of age pigments or lipofuscin is one of the most prominent age associated cytological alterations in a variety of long-lived post-mitotic cells including the myocardial fibres[1]. The nature of the relationship between the lipofuscin granules and the process of aging, however, remains obscure[2]. Lipofuscin accumulation has been linked to the aging process because of a striking correlation found between the degree of accumulation and the actual age[3]. A major reason for the paucity of information on the functional significance of lipofuscin appears to be the lack of techniques for the quantitative determination of lipofuscin.

The origin of lipofuscin is not certain, but, it is generally thought to arise from the oxidative polymerization of unsaturated lipids and to co-exist in combination with the components of the lysosomes[4]. Evidence suggests that lipofuscin granules may originate from the peroxidation of unsaturated fatty acids, resulting in carbonyl compounds such as malonaldehyde[5]. These compounds may cross link with biological molecules, resulting in cross-polymers[5], that may be hydrolytically undigestible by lysosome and accumulate intracellularly as tertiary lysosomes or residual bodies[6].

In non-dividing cells like the myocardial cells, the indigestible substances can be observed without the evidence of exocytic elimination[7]. Cellular dysfunction in these cells may affect the metabolism in such a way as to cause a mechanical disruption of the cellular organization and lead to the eventual death of the cell[8].

The objective of the present study is to investigate the accumulation of lipofuscin and the lipid peroxidation potential of the myocardium as a function of age.

Materials and Methods

Male albino rats of 12½-, 25-, 50-, 75- and 100-week-old were used for the present study. The rats were decapitated and the myocardium was excised and washed in 0·9% saline to remove the blood clots.

Analysis of the fluorescent products was performed according to Fletcher *et al.*[9] on 200 mg of the tissue after homogenization and extraction with 2 : 1 chloroform-methanol mixture. The solvent to tissue ratio was 20 : 1 (V : W). Spectrophotofluorometric measurements were made with a Perkin Elmer MPF–44 fluorescence spectrophotometer. The excitation maximum was 365 nm and the emission maximum was 445 nm. The spectrofluorometer was standardized each time with a fresh solution of quinine sulphate. The instrument was calibrated to read 100 units for 1 μg of quinine sulphate/ml of 0·1 N sulphuric acid.

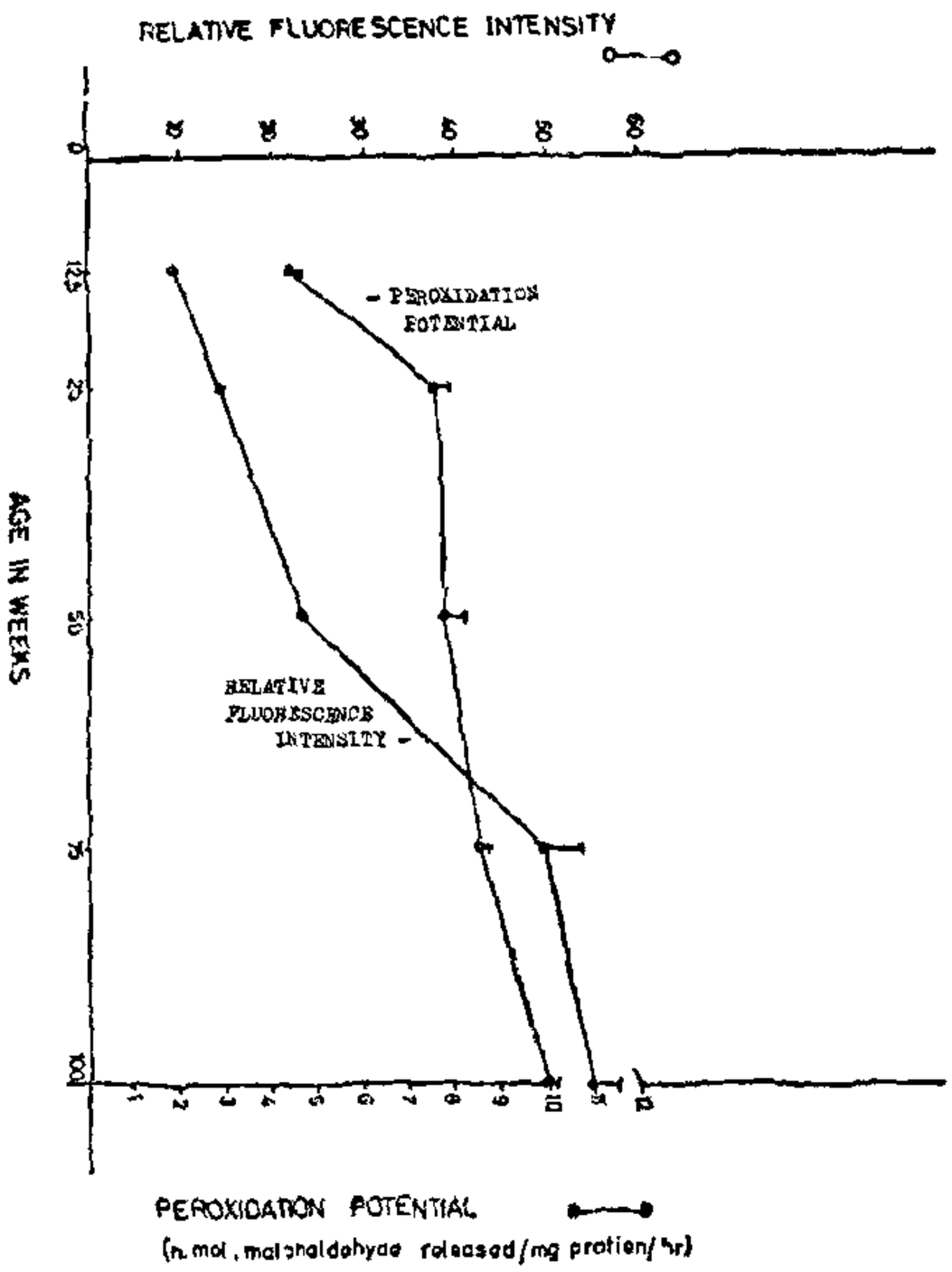

FIG. 1

Lipid peroxide formation was determined by the Thiobarbituric acid (TBA) test according to Wilbur *et al.*[10]. The tissue was homogenized in 0·05 M phosphate buffer pH 7·8 and centrifuged in an IEC refrigerated centrifuge for 30 minutes at 6,000 RPM. 1 ml of the supernatant was incubated at 37° C for 1 hour and at the end of this period the reaction was stopped by the addition of 1 ml of 35% trichloroacetic acid (TCA) and 2 ml of 0·75% aqueous TBA. The contents were mixed well and the tubes were kept in boiling water bath for 15 minutes, with occasional shaking. After cooling the tubes to room temperature, 2ml of 70% TCA was added and the contents of the tubes were thoroughly mixed. Turbid lecithins were dissolved by the addition of 3 ml of chloroform. After centrifugation for 15 minutes at 2,500 RPM the optical density of the clear pink supernatant was read in a colorimeter at 532 nm.

Results and Discussion

Age-related changes in the myocardial lipofuscin and lipid peroxidation rate are given in Fig. 1. There is remarkable age-related increase in the lipofuscin content of the myocardium. It appears that the *lipofuscinogenesis* in the rat myocardium starts quite early in life. Appreciable amount of lipofuscin is detected by 12½ weeks. The percentage increase the myocardial lipofuscin content in 25-, 50-, 75-, and 100-week-old rats is 56, 151, 428 and 484% respectively. A similar, though less dramatic, age-dependent increase in the lipid peroxidative potential was observed.

The study of Munnel and Getty[11] on the age pigments in dog myocardium confirms the notion held by Strehler[1]. Munnel and Getty counted the number of granules in dog myocardia of different ages and found that the number of granules increased progressively with age. Earliest pigmentation was observed by six months. The rate of pigment deposition in the dog was 5·5 times greater than the human myocardium and this was approximately the ratio of life span between the two species. Ulissova[12] is of the opinion that the deposition of the pigment in heart is related to the aging process *per se*. He observed that the rate of pigment deposition increased till the age of 12 years in humans and at old age the rate of pigment deposition (and not the amount of pigment in cells) progressively declined.

Lipofuscin is generally regarded as the indigestible residue of the lysosomal enzymatic activity[13]. How the cellular organelles and constituents become indigestible can be explained by the process of lipid peroxidation, which produces malonaldehyde—a good tissue fixative. Malonaldehyde has 2 reactive sites and thus can cross-link separate molecules. Random covalent binding with cross-linking in organelles would make potential substrates unavailable for the degradative enzymes of the lysosomes. The association of lysosomal membranes and enzymes with lipofuscin granules therefore can be interpreted as a frustrated attempt to recycle the components of the organelles that are damaged by peroxidation.

Lipofuscin, the hallmark of aging, appears to indicate inadequate protection from free radicals that are induced metabolically. If nuclear damage results from the reaction with malonaldehyde, the subsequent impairment of protein synthesis would depress replacement of contractile proteins. The limitation of cellular response is a way explaining the reduced capacity of the heart in older person to adapt to an increased work load.

The relation of lipofuscin to decline in cardiac function requires interpretation. Tappel[14] has developed a model by which he estimated the free radical activity of the cell from the content of lipofuscin determined by induced fluorescence. Approximations of this kind reveal that rat myocardium may have as much as 0·6 μmole free radical activity per gram tissue.

Lipofuscin may not hinder myocardial function but the free radical reactions associated with this pigment formation could affect the nearby nucleus. Brooks and Kalmerth[15] observed decreased growth and earlier death of cultured fibroblasts that were exposed to malonaldehyde at 25 μg/ml.

Unfortunately the damage to myocardium incurred from peroxidation is cumulative and irreversible. Therefore the accumulation of lipofuscin with age can be considered as a strong evidence for the occurrence of lipid peroxidation process in cells *in vivo*.

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1. Strehler, B. L., Mark, D. D., Mildvan, S. A and Gee, M. V., *Journal of Gerontology*, 1959, **14**, 430.
2. Brizzee, K. R., Kaack, B. and Klara, P., In : *Neurobiology of Aging*, Plenum Press, New York, 1975, p. 463.
3. Bondareff, W., In *Advances in Gerontological Research*, Academic Press, New York, 1964, p. 1.
4. Brizzee, K. R., Ordy, J. M. and Kaack, B., *Journal of Gerontology*, 1974, **4**, 366.
5. Chio, K. S., and Tappel, A. L., *Biochemistry*, 1969, **8**, 2821.
6. Siakotos, A. N. and Armstrong, D., In *Neurobiology of Aging*, Plenum Press, New York, 1975, p. 369.
7. Brunk, U., *Experimental Cell Research*, 1973, **79**, 15.
8. Mann, D. M. A. and Yates, P. O., *Brain*, 1974, **97**, 481.
9. Fletcher, B. L., Dillard, C. J. and Tappel, A. L., *Anal. Biochem.*, 1973, **52**, 1.
10. Wilbur, K. M., Bernheim, F. and Shapiro, O. W., *Archives of Biochemistry*, 1949, **24**, 305.
11. Munnel, J. F. and Getty, R., *Journal of Gerontology*, 1968, **23**, 154.
12. Ulissova, T. N., *Bulletin of Experimental and Biological Medicine, U.S.S.R.*, 1957, **43**, 635.
13. Bloom, W. and Fawcett, D. W., In : *A Text Book of Histology*, W. B. Saunders and Co., Philadelphia, 1975, p. 50.
14. Tappel, A. L., *Annals of New York Academy of Sciences*, 1972, **203**, 12.
15. Brooks, B. R. and Kalmerth, O. L., *European Journal of Biochemistry*, 1968, **5**, 178.

COMPARATIVE EVALUATION OF METHYL PARATHION TOXICITY TO SOME SELECTED FRESHWATER ORGANISMS

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METHYL parathion is one of the organophosphate pesticides extensively used in agricultural operations. The present study concerns a comparative evaluation of methyl parathion toxicity to different test species of aquatic ecosystem. Since an extensive research programme has been undertaken on the fish, *Cyprinus carpio* the effect of body weight of the fish on the toxicity of pesticide has also been studied.

The test animals except the leeches were fed daily and acclimatised to laboratory conditions for one week before exposure to pesticide. Lethal concentrations (LC 50/24 hrs and LC 50/ 8 hr) of methyl parathion [Technical grade, 95% W/V, Bayer (India) Ltd.] were computed by static bioassay using the probit method[1], for the freshwater organisms such as the teleost *Cyprinus carpio*, the leech *Poecilobdella granulosa*, the pond snail *Pila globosa*, the freshwater mussel *Lamellidens marginalis* and the freshwater field crab *Oziotelphusa senex senex*. The animals were not fed during the exposure period. A stock solution of 100 mg/ml of methyl parathion was prepared in organic solvent, 2-methoxy ethanol and appropriate amounts were taken from the stock solution to prepare various concentrations of the chemical in water. Variables such as dissolved oxygen content (6·8–7·2 ml/l), pH (7·1–7·3) and temperature (28–30°C) have been controlled. Five replicates were maintained with 10 animals per treatment.

It is evident from Table I that crab and leech showed least tolerance to methyl parathion. Fish showed a fairly good resistance while the molluscs exhibited very good tolerance. In the case of molluscs, it was observed that immediately after the exposure to pesticide, they have completely withdrawn into the shell. The tight closing of the shell for 10–12 hours restricted the free flow of pesticide medium over the soft parts during that period. Copious secretion of

TABLE I

Comparative evaluation of methyl parathion toxicity to some representative freshwater organisms

Species	Weight range (g)	LC_{50}/24 hr mg/l	LC_{50}/48 hr mg/l
Leech :			
Poecilobdella granulosa	1–3	5	4
Crab :			
Oziotelphusa senex senex	25–30	3	1
Fresh water mussel :			
Lamellidens marginalis	35–40	50	40
Apple snail :			
Pila globosa	15–20	40	30
Common carp :			
Cyprinus carpio	30–40	15	12