ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS
Vol. 284, No. 2, February 1, pp. 332–337, 1991

Effect of Aging on the Activity of the Phosphate Carrier and on the Lipid Composition in Rat Liver Mitochondria

Giuseppe Paradies[1] and Francesca Maria Ruggiero
Department of Biochemistry and Molecular Biology and CNR Unit for the Study of Mitochondria and Bioenergetics, Bari, Italy

Received June 15, 1990, and in revised form September 14, 1990

The effect of aging on the activity of the phosphate carrier and on the lipid composition in rat liver mitochondria has been investigated. It was found that the rate of phosphate transport in mitochondria from aged rats (28 months old) is significantly reduced (around 40%) compared to that obtained in mitochondria from young control rats (5 months old). Kinetic analysis of the phosphate transport indicates that only the V_{max} of this process is affected, while there is no change in the K_m values. The lower activity of the phosphate carrier in mitochondria from aged rats is also documented by swelling experiments. The age-related decrement in the activity of the phosphate carrier was found not to be due neither to a change in the endogenous content of phosphate nor to a change in the transmembrane ΔpH value. Inhibitor titrations with mersalyl provide no evidence for a lower content of functional phosphate translocase in mitochondria from aged rats. There is no difference either in the respiratory control ratios or in the ADP/O ratios between mitochondria from young and aged animals. The hepatic mitochondrial lipid composition is altered significantly in aged rats: the total cholesterol increases (31%), the phospholipids decrease (12%), and the cholesterol/phospholipid molar ratio increases (44%). Among the phospholipids cardiolipin shows the greatest alteration (30% decrease with age). Alterations were also found in the pattern of fatty acids. The age-related decrement in the activity of the phosphate carrier appears to be dependent on changes in the lipid domain surrounding the carrier protein molecule in the mitochondrial membrane.

Aging is a biological phenomenon characterized by impairment of various aspects of cell functions. The molecular mechanism of age-dependent deterioration of cellular processes is still unclear. At the mitochondrial level aging is known to cause changes in biochemical pathways involved in energy metabolism (for review see Ref. (1)). These changes have been related to molecular and functional alterations in the properties of biological membranes. A crucial point in the regulation of mitochondrial energy metabolism is represented by the transport of anionic substrates across the mitochondrial membranes (2). Changes in membrane permeability properties appear to occur quite widely with aging (3–8). Age-dependent decrements in the activity of several mitochondrial anion carrier proteins, such as acylcarnitine–carnitine translocation (3), adenine nucleotide translocation (4, 5), Ca^{2+} transport (6), and very recently pyruvate transport (7), have been reported.

The synthesis of ATP during oxidative phosphorylation requires the uptake of ADP and phosphate from the cytosol into the mitochondrial matrix. The translocation of phosphate across the inner mitochondrial membrane is mediated by a specific transporting system (for reviews see Refs. (2, 8)). The phosphate carrier has been purified to homogeneity from bovine and rat liver mitochondria and its activity reconstituted in artificial membranes such as liposomes (9, 10). Lipids have a strong influence on the activity of the reconstituted phosphate carrier (11, 12). Cardiolipin, in particular, was found to influence the activity of the isolated protein in liposomes (13).

Changes in membrane lipid content, lipid composition, and lipid–protein interactions do occur with aging in several tissues (14–18). These changes have been considered, at least in part, to be responsible for the changes in the activity of certain anion carrier proteins (4, 5, 7).

The present experiments were designed to compare the activity of the phosphate carrier and the lipid composition in mitochondria isolated from liver of young control (5 months old) and aged (28 months old) male Fisher rats. The results demonstrate an age-related decrement in the activity of the phosphate carrier which appears to be dependent on changes in the lipid environment of the membrane in which the carrier protein is embedded.

[1] To whom correspondence should be addressed at Dipartimento di Biochimica e Biologia Molecolare, Università degli Studi di Bari, via Amendola 165/A, 70126 BARI, Italy.

0003-9861/91 $3.00

MATERIALS AND METHODS

Chemicals. The radioactive [^{32}P]orthophosphate, $^{3}H_2O$, and [U-^{14}C]sucrose were obtained from the Radiochemical Centre, Amersham, England. 5,5-Dimethyl-[^{14}C]oxazolidine-2,4-dione was obtained from New England Nuclear. All other reagents were of reagent grade purity and were purchased from Sigma.

Animals. Male Fisher rats of 5 or 28 months (25% survivorship) were used throughout these studies. They were fed *ad libitum,* until killed, with a basal diet consisting of 25% protein, 4.3% lipid, 59.7% carbohydrate (of which 7.1% was cellulose), and a salt and vitamin mixture.

Rat liver mitochondria were prepared by differential centrifugation of liver homogenates essentially as described previously (19). Mitochondria were resuspended in 0.25 M sucrose and stored in ice. The yield of mitochondrial protein (per gram liver wet wt) within each group of animals was consistent, suggesting minimal variation in the preparation of the mitochondrial fractions.

The standard medium used in the measurements of phosphate transport, respiratory activity, and ΔpH measurements usually contained 100 mM sucrose, 50 mM KCl, 20 mM Tris–HCl, 1 mM $MgCl_2$, and 0.5 mM EDTA.

Mitochondrial phosphate transport. The transport of phosphate into mitochondria was measured at 0°C by the "inhibitor stop method" essentially as described in (20) using mersalyl as inhibitor. Mitochondria corresponding to 2 mg of protein/ml were preincubated in a reaction medium that contained in 1 ml of the reaction medium described above, 1 mM *n*-butylmalonate (to inhibit dicarboxylate carrier which is also able to transport phosphate) and 2 μg/ml rotenone. The final pH was 7.4. After a period of equilibration of 2 min, phosphate transport was initiated by adding radioactive phosphate and stopped after time *t* by rapid addition of 0.2 mM mersalyl. The tubes were rapidly centrifuged at 15,000*g* for 2 min. The pellets were washed several times in 0.25 mM sucrose and dissolved in $HClO_4$ (15%, w/v). The vials were then recentrifuged at 15,000*g* for 2 min in a refrigerated microcentrifuge. Solubilized mitochondria were transferred to 10 ml scintillation liquid and then the radioactivity was counted in a scintillation counter. Phosphate transport was considered as the difference between noninhibited and mersalyl-treated samples (in the latter case mersalyl was added in the preincubation phase 2 min before radioactive phosphate).

Phosphate transport was also measured as phosphate–[^{32}P]phosphate exchange. In these experiments mitochondria from both young control and aged rats were first preloaded with cold phosphate as follows. Aliquots of mitochondria (40–50 mg of protein) were incubated at 0°C in 20 ml of the buffer containing 100 mM sucrose, 50 mM KCl, 20 mM Tris–HCl, 1 mM EDTA, 3 μg/ml rotenone, and 2 mM cold phosphate. The final pH was 7.4. After a period of equilibration of 5 min, mitochondria were centrifuged at 12,000*g* for 10 min. The mitochondrial pellet was resuspended in 0.25 mM sucrose. The rate of phosphate–[^{32}P]phosphate exchange was then measured as described above.

Mitochondrial swelling. Mitochondrial osmotic volume changes were measured by the apparent absorbance changes at 540 nm with a spectrophotometer linked to a suitable recorder. The reactions were carried out at 25°C in 3 ml of the appropriate isoosmotic medium as indicated in the legends to the figures.

Transmembrane ΔpH measurements. The transmembrane ΔpH values in mitochondria were determined essentially as described in (21).

Mitochondrial respiration. Reactions were carried out in 1 ml water-jacketed closed chamber with magnetic stirring, at 25°C. O_2 uptake was measured polarographically with a Clark oxygen electrode connected to a suitable recorder.

High-pressure liquid chromatography analysis of lipids. Phospholipids, fatty acids, and cholesterol were analyzed by HPLC, using a Beckman 344 gradient liquid chromatograph. Extraction and analysis of phospholipids, fatty acids, and cholesterol were carried out essentially as described in Ref. (22).

Determination of phosphate and protein. The endogenous level of phosphate was determined chemically (23) in perchloric acid extracts. Protein concentration was measured by the usual biuret method, using serum albumin as standard.

Statistical analysis. Results are expressed as mean values ± standard error. Statistical significances were determined by the Student *t* test.

RESULTS

Figure 1 illustrates the results of six different experiments on the time course of phosphate transport by hepatic mitochondria isolated from young control and senescent rats. At 0°C and external phosphate concentration of 2 mM, phosphate uptake was linear for the first 10–15 s. Both the rate and the final extent of phosphate uptake by mitochondria from aged rats were significantly lower than those obtained with mitochondria from control rats.

The dependence on substrate concentration of the rate of phosphate uptake by mitochondria from young and aged rats was studied at 0°C by changing the concentration of externally added [^{32}P]phosphate. The results from a typical experiment (see Fig. (2)) show that the concentration dependence of the phosphate uptake by both these two types of mitochondria reveals hyperbolic saturation characteristics. It can be noted, however, that while the affinity of the phosphate anion for its carrier remained almost the same, the maximal velocity of the phosphate uptake was markedly decreased in mitochondria from aged rats compared to that obtained in mitochondria from young control rats. Very similar results were obtained when mitochondria from both control and aged rats were

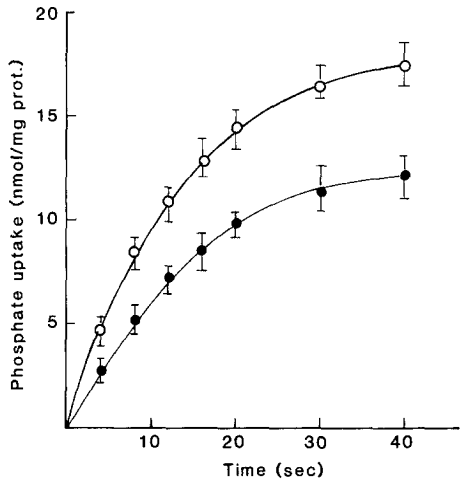

FIG. 1. Time course of phosphate uptake by liver mitochondria from young and aged rats. Phosphate uptake was measured as described under Materials and Methods. Mitochondria (2 mg of protein/ml) were preincubated in the standard reaction medium at pH 7.4 and *T* 0°C. After 2 min of preincubation 2 mM radioactive phosphate was added. The reaction was stopped at the times indicated by adding 0.2 mM mersalyl. The data represent the results of one of six experiments which gave similar results. (○) Mitochondria from young rats; (●) mitochondria from aged rats.

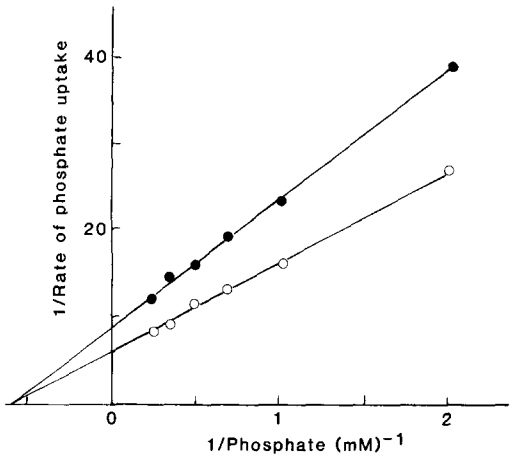

FIG. 2. Double-reciprocal plots of phosphate uptake by liver mitochondria from young and aged rats. The rate of phosphate uptake was measured as described under Materials and Methods and in the legend to Fig. 1. Mitochondria (2 mg of protein/ml) were added to the standard reaction medium. After 2 min of preincubation, radioactive phosphate was added at the concentrations indicated and 6 s later, 0.2 mM mersalyl was added to stop the transport reaction. The rate of phosphate uptake is expressed as μmol × min × mg protein^{-1}. (○) Mitochondria from young rats; (●) mitochondria from aged rats.

first preloaded with 2 mM cold phosphate and then the activity of the phosphate carrier was measured as rates of phosphate–[^{32}P]exchange (results not reported).

The statistical analysis of the kinetic parameters of the phosphate uptake in liver mitochondria from young and aged rats, obtained from six different experiments, gave the following results: K_m 1.78 ± 0.19 and 1.71 ± 0.21 mM and V_{max} 174 ± 22 and 115 ± 14 nmol/min/mg of protein in mitochondria from young control and aged rats, respectively. It should be noted that the observed values for the kinetic parameters of the phosphate transport in mitochondria from young control rats are in good agreement with previous data reported in the literature (24–26).

The difference in the activity of the phosphate carrier in hepatic mitochondria from aged rats with respect to young control rats is further documented by swelling experiments. Nonrespiring mitochondria swell when suspended in isoosmotic ammonium phosphate due to the entrance of NH_3 and $H_2PO_4^-$ transported into mitochondria with a proton by the phosphate carrier (27). A typical experiment reported in Fig. 3 shows that mitochondria from young and aged rats underwent large-amplitude swelling when suspended in an isoosmotic solution of NH_4PO_4. These results were compared with the rate of swelling of the same mitochondrial preparations when suspended in ammonium acetate. The following facts can be noted: (i) both the rate and the final extent of the swelling in NH_4-phosphate were markedly decreased in mitochondria from aged rats compared to those obtained from young control rats; mersalyl, a powerful inhibitor of the phosphate carrier, completely prevented mitochondrial swelling; (ii) the rate of swelling in ammonium salt of acetate, which is known to freely permeate the mitochondrial membrane as undissociated acid (28), was much higher than the rate of swelling in NH_4-phosphate; (iii) no change in either the rate or the final extent of the swelling in NH_4-acetate was observed between these two types of mitochondria.

The major driving force for the net uptake of phosphate by mitochondria is represented by the transmembrane ΔpH gradient (29, 30). Thus, the reduced activity of the phosphate carrier in mitochondria from aged rats could be related to a decrease in the transmembrane ΔpH value. However, no substantial changes in the transmembrane ΔpH values were observed in mitochondria from either young control or aged rats, the values being 0.84 ± 0.09 and 0.82 ± 0.09 (means ± SE for five separate experiments), respectively.

The decreased activity of the phosphate carrier in mitochondria from aged rats could also be due to a lower content of functional translocase enzyme. To assess this, inhibitor titrations of phosphate transport with mersalyl were carried out in mitochondria from both young control and aged rats. Mersalyl is a powerful inhibitor of the phosphate transport in mitochondria (31). Thus, the minimal amount of this inhibitor required to obtain maximal inhibition of the phosphate transport should give an indirect measure of the amount of the phosphate translocase in mitochondria. Total inhibition of the phosphate transport was achieved by approximately the same amount of mersalyl in both mitochondrial preparations

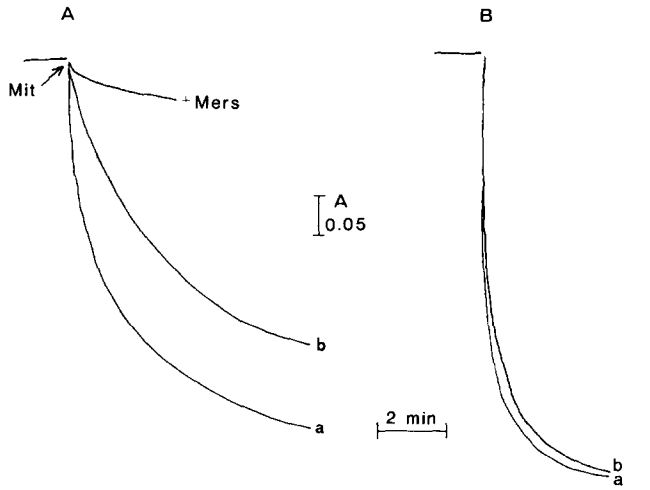

FIG. 3. Swelling of liver mitochondria from young and aged rats suspended in isoosmotic solution of ammonium phosphate (A) or ammonium acetate (B). Mitochondrial swelling was monitored as described under Materials and Methods. Mitochondria (0.7 mg of protein) were suspended in 3-ml solution of 125 mM NH_4PO_4 (A) or 125 mM NH_4-acetate (B) containing in addition 5 mM Hepes, 0.5 mM EDTA and, only in (A), 6 μg of rotenone. pH 7.4; T 25°C. When present, mersalyl was 0.2 mM. The reaction was initiated by the addition of mitochondria to the medium. Trace (a) mitochondria from young rats; trace (b) mitochondria from aged rats.

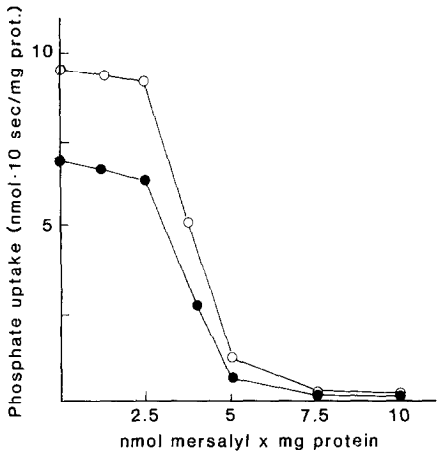

FIG. 4. Mersalyl inhibition of phosphate transport in mitochondria from young and aged rats. Experimental conditions as in Fig. 1. Mitochondrial protein was 2 mg/ml. Mersalyl was added, at concentrations indicated, during the preincubation phase. The data represent the results of one of five experiments which gave similar results. (○), Mitochondria from control rats; (●) mitochondria from aged rats.

from young control and aged rats (see Fig. 4). This suggests that aging is without effect on the amount of functional phosphate translocase in mitochondria.

Respiratory activities of hepatic mitochondria from young control and aged rats are summarized in Table I. No differences in the ADP/O ratios, respiratory control ratios, or State 3 and State 4 of respiration were found between mitochondria from old and young control rats in respiratory studies supported by succinate. This result is in accordance with the findings of several authors (5, 32) but at variance with others (33, 34).

The effect of aging on rat liver mitochondrial phospholipid and total cholesterol content is shown in Table II. From this table, total cholesterol can be seen to increase by 31% and inversely, phospholipids decrease by 12% in mitochondria from aged rats. Consequently, the inverse relationship between the change in the total cholesterol and phospholipid content observed in these organelles caused a 44% increase in the total cholesterol/phospholipid molar ratio.

To evaluate whether the phospholipid composition of hepatic mitochondria was altered in aged animals, the major phospholipid species were quantitated following separation by high-performance liquid chromatography (22). The results of these analyses are given in Table III. The most marked change was the aging-dependent decrease (approximately 30%) of negatively charged cardiolipin.

Chemical changes in the mitochondrial membrane lipid composition were further investigated by analyzing the fatty acids composition in these two types of mitochondrial membranes (see Table IV). Alterations of fatty acids distribution were observed in mitochondrial membrane from aged rats. In particular, a significant increase in di-

TABLE I

Respiratory Activities of Hepatic Mitochondria from Young and Aged Rats

Animals	Oxidative activity (ngatom O/min per mg protein) State 3	State 4	Respiratory control ratio	ADP/O
Young	173 ± 15	28 ± 2.5	6.17 ± 0.63	1.87 ± 0.11
Aged	166 ± 14	27 ± 2.7	6.15 ± 0.55	1.84 ± 0.12

Note. The succinate-dependent oxygen consumption was measured polarographically in a medium containing the standard reagents, described under Materials and Methods, and in addition 5 mM P_i. Mitochondrial protein was 1.5 mg/ml. When a steady state of oxygen consumption was obtained, 3 mM succinate was added. One minute later respiration was stimulated by the addition of 0.3 mM ADP. Each value represents the means ± SEM obtained for five experiments.

TABLE II

Cholesterol and Phospholipid Content in Rat Liver Mitochondria from Young and Aged Rats

	Young	Aged
Cholesterol	14.7 ± 1.5	19.2 ± 1.9[a]
Phospholipids	168.4 ± 10.2	148.2 ± 11.0[b]
Ratio cholesterol/phospholipid	0.09 ± 0.01	0.13 ± 0.01[a]

Note. For cholesterol and phospholipid extraction and characterization, see Materials and Methods. Each value represents the mean ± SEM obtained for five experiments with two rats each. Cholesterol is expressed as nmol/mg protein and phospholipids as nmol lipid P_i/mg protein.

[a] $P < 0.01$ vs young.

[b] $P < 0.02$ vs young.

TABLE III

Phospholipid Composition in Rat Liver Mitochondria as Determined by HPLC

Phospholipid	Distribution (mol %) Young	Aged
Cardiolipin	13.5 ± 1.0	9.5 ± 0.9[a]
Phosphatidylethanolamine	28.4 ± 1.4	24.5 ± 1.5[b]
Phosphatidylinositol	6.2 ± 0.9	6.3 ± 1.0
Phosphatidylserine	2.4 ± 0.5	2.9 ± 0.4
Phosphatidylcholine	49.5 ± 1.9	56.8 ± 2.0[a]

Note. For phospholipid extraction and analysis, see Materials and Methods. Each value represents the mean ± SEM obtained for five experiments with two rats each.

[a] $P < 0.001$ vs young.

[b] $P < 0.01$ vs young.

TABLE IV

Pattern of Fatty Acids in Rat Liver Mitochondria as Determined by HPLC

Fatty acid	Distribution (mol %)	
	Young	Aged
16:0	21.6 ± 1.3	17.7 ± 1.5[a]
16:1	7.9 ± 0.7	11.5 ± 0.9[b]
18:0	14.4 ± 1.4	18.8 ± 1.1[a]
18:1	8.6 ± 1.0	7.8 ± 0.8
18:2	20.6 ± 1.5	15.8 ± 1.3[a]
20:3	0.7 ± 0.3	1.7 ± 0.5[b]
20:4	25.7 ± 1.5	26.3 ± 1.6
22:6	0.5 ± 0.1	0.4 ± 0.1
UI	165.6 ± 3.2	163.6 ± 2.8
20:4/18:2	1.25 ± 0.04	1.66 ± 0.05[a]

Note. Extraction and analysis of fatty acids were carried out as described under Materials and Methods. Each value represents the mean ± SEM obtained for five experiments with two rats each. The unsaturation index (UI) is defined as Σmol% of each fatty acid × number of double bonds of the same fatty acid.

[a] $P < 0.001$ vs young.

[b] $P < 0.01$ vs young.

homo-γ-linolenic (20:3) and palmitoleic (16:1) and a decrease in linoleic acid (18:2) occurred with aging. The unsaturation index did not change significantly with aging.

DISCUSSION

The results presented in this paper demonstrate that the activity of the phosphate carrier in hepatic mitochondria from aged rats is markedly decreased compared to that obtained in mitochondria from young control rats. The transport of phosphate in mitochondria is driven by the transmembrane ΔpH. Thus, a decrease in the mitochondrial pH gradient could, in principle, account for the reduced activity of the phosphate carrier in mitochondria from aged rats. However, the swelling experiments reported in Fig. 3 indicate that while the transport of phosphate in mitochondria is reduced with aging, that of acetate, an anion which moves across the mitochondrial membrane as function of ΔpH (28) independent of phosphate carrier, is not affected. In addition, no change in the transmembrane ΔpH values was observed in mitochondria from young control and aged rats. This minimizes the possibility that the reduced activity of the phosphate carrier in mitochondria from aged rats is simply due to a decrease in the transmembrane ΔpH.

The lower activity of the phosphate carrier in mitochondria from aged rats could also be due to a lower size of the internal pool of exchangeable phosphate. However, no difference in the amount of endogenous phosphate was detected in the two preparations of mitochondria (12.4 ± 1.8 and 11.9 ± 1.7 nmol/mg of protein in mitochondria from young control and aged rats, respectively).

A lower content of phosphate translocase enzyme could also be responsible for the lower velocity of the phosphate transport in mitochondria from aged rats. Inhibitory studies (see Fig. 4) provide no evidence of a decreased mitochondrial content of functional enzyme.

Deterioration of the inner mitochondrial membranes with aging could represent another factor responsible for the decreased activity of the phosphate carrier. However, neither the respiratory control ratios nor the ADP/O ratios were altered in these two types of mitochondria. This indicates that aging affects the activity of the phosphate carrier, without affecting the intactness of the mitochondrial membrane and the efficiency of the mitochondrial respiratory functions.

The kinetic analysis of the phosphate carrier indicates that only the maximal velocity of the phosphate transport is changed while no alteration in the affinity of the phosphate for its carrier in mitochondria from both young control and aged rats occurs. These data provide evidence that the change in the activity of the phosphate carrier in hepatic mitochondria from aged rats does not reflect a general change in the carrier protein molecule.

The change in the activity of the mitochondrial phosphate carrier with aging can also be due to a modification of the lipid surrounding of the phosphate carrier in the membrane leading to an altered carrier protein–lipid interaction. There are many examples showing that the carrier-mediated transport processes in mitochondria are dependent on the composition and on the physicochemical state of membrane lipids and hence on the membrane fluidity (4, 7, 21, 35, 36). Among the factors shown to affect membrane fluidity are cholesterol/phospholipid molar ratio, phospholipid composition, degree of fatty acid unsaturation, and lipid/protein ratio. The analysis of the mitochondrial membrane lipids reveals substantial changes in the lipid composition in young control and aged rats. The total cholesterol and the cholesterol/phospholipid molar ratio were both significantly increased in the mitochondrial membrane from aged rats. These changes are associated with a loss of fluidity of the membrane which, in turn, would lead to a hindered orientation and/or to a lower mobility of the phosphate carrier molecule in the membrane.

In addition to this general effect, the change in the membrane lipid composition may have more specific effects on the activity of the phosphate carrier. It has been reported that cardiolipin, a phospholipid which is concentrated on the matrix side of the inner mitochondrial membrane, is specifically required for the reconstitution of the isolated phosphate carrier activity in artificial membranes such as liposomes (11, 13). Furthermore, the transport of phosphate has been shown to be sensitive to the antitumoral agent doxorubicin, which is known to form specific complexes with cardiolipin (37, 38). Thus, it appears that cardiolipin provides the lipid environment necessary for the activity of the phosphate carrier in the

mitochondrial membrane. Specific cardiolipin requirement has been demonstrated for other mitochondrial protein and enzymes (39–42). As shown in Table III, the level of cardiolipin is markedly reduced (around 30%) in the mitochondrial membrane from aged rats. Assuming that the cardiolipin requirement for the activity of the phosphate carrier in intact mitochondria is the same as in the liposomes, it can be proposed that the lower activity of the phosphate carrier in hepatic mitochondria from aged rats can be ascribed, in addition to a general alteration of the membrane lipid composition leading to a loss in the membrane fluidity, to a more localized change in the lipid microenvironment (specifically to a lower content of cardiolipin) surrounding the carrier molecule in the membrane.

The transport of phosphate may be involved in regulating the supply of phosphate from the cytosol to the mitochondrial matrix for the reactions of oxidative phosphorylation. In addition, the transport of phosphate is closely linked to that of ADP and Ca^{2+} and it is an obligatory step for the uptake of some Krebs-cycle intermediates such as malate, citrate, and oxoglutarate (43–46). Thus, the lower activity of the phosphate carrier in hepatic mitochondria from aged rats can account, at least in part, for the decrement in energy metabolism typical of senescent animals.

ACKNOWLEDGMENTS

We acknowledge the excellent technical assistance of Mr. L. Gargano and Drs. P. DiNoi and F. Cafagna. This work was supported by the Consiglio Nazionale delle Ricerche (P. F. "Invecchiamento") and by Ministero Pubblica Istruzione (60%).

REFERENCES

1. Hansford, R. G. (1983) *Biochim. Biophys. Acta* **726,** 41–80.
2. LaNoue, K. F., and Schoolwerth, A. C. (1979) *Annu. Rev. Biochem.* **48,** 871–922.
3. Hansford, R. G. (1978) *Biochem. J.* **170,** 285–295.
4. Nohl, H., and Kramer, R. (1980) *Mech. Ageing Dev.* **14,** 137–144.
5. Kim, J. H., Woldgiorgis, G., Elson, C. E., and Shrago, E. (1988) *Mech. Ageing Dev.* **46,** 263–277.
6. Hansford, R. G., and Castro, F. (1982) *Mech. Ageing Dev.* **19,** 5–13.
7. Paradies, G., and Ruggiero F. M. (1990) *Biochim. Biophys. Acta* **1016,** 207–212.
8. Fonyo, A. (1979) *Pharmacol. Ther.* **7,** 627–645.
9. Wohlrab, H., Collins, A., and Costello, D. (1984) *Biochemistry* **23,** 1057–1064.
10. Wohlrab, H. (1986) *Biochim. Biophys. Acta* **853,** 115–134.
11. Kadenbach, B., Mende, P., Kolbe, H. V. J., Stipani, I., and Palmieri, F. (1982) *FEBS Lett.* **139,** 109–112.
12. Kramer, R., and Palmieri, F. (1989) *Biochim. Biophys. Acta* **974,** 1–23.
13. Mende, P., Kolbe, V. J., Kadenbach, B., Stipani, I., and Palmieri, F. (1982) *Eur. J. Biochem.* **128,** 91–95.
14. Grinna, L. S. (1977) *Mech. Ageing Dev.* **6,** 197–205.
15. Vorbeck, M. L., Martin, A. M., Long, J. W., Smith, J. M., and Orr, R. R. (1982) *Arch. Biochem. Biophys.* **217,** 351–361.
16. Hegner, D. (1980) *Mech. Ageing Dev.* **14,** 101–118.
17. Lewin, M. B., and Timiras, P. S. (1984) *Mech. Ageing Dev.* **24,** 343–351.
18. Yechiel, E., and Barenholz, Y. (1985) *J. Biol. Chem.* **260,** 9123–9131.
19. Paradies, G., and Papa, S. (1977) *Biochim. Biophys. Acta* **462,** 333–346.
20. Palmieri, F., and Klingemberg, M. (1979) *in* Methods in Enzymology (Fleischer, S., and Packer, L., Eds.), Vol. **56,** pp. 279–301, Academic Press, San Diego, CA.
21. Paradies, G., and Ruggiero, F. M. (1989) *Arch. Biochem. Biophys.* **269,** 595–602.
22. Ruggiero, F. M., Landriscina, C., Gnoni, G. V., and Quagliariello, E. (1984) *Lipids* **19,** 171–178.
23. Wahler, B. E., and Wollemberg, A. (1958) *Biochem. Z.* **329,** 508–520.
24. Fonyo, A. (1978) *J. Bioenerg. Biomembr.* **10,** 171–194.
25. Coty, W. A., and Pedersen, O. (1974) *J. Biol. Chem.* **249,** 2593–2598.
26. Ligeti, E., Brandolin, G., Dupont, Y., and Vignais, P. V. (1984) *Biochemistry* **24,** 4423–4424.
27. Mitchell, P., and Moyle, J. (1969) *Eur. J. Biochem.* **9,** 149–155.
28. Papa, S., and Paradies, G. (1974) *Eur. J. Biochem.* **49,** 265–274.
29. Hoek, J. B., Lofrumento, N. E., Meyer, A. J., and Tager, J. M. (1971) *Biochim. Biophys. Acta* **226,** 297–308.
30. McGivan, J. D., and Klingemberg, M. (1971) *Eur. J. Biochem.* **20,** 392–399.
31. Fonyo, A., and Vignais, P. V. (1980) *J. Bioenerg. Biomembr.* **12,** 137–149.
32. Mertens-Strijthagen, J., and De Schryver, C. (1989) *Arch. Biochem. Biophys.* **274,** 37–46.
33. Weindruch, R., Cheung, M. K., Verity, M. A., and Walford, R. L. (1980) *Mech. Ageing Dev.* **14,** 375–392.
34. Nohl, H., Breuninger, V., and Hegner, D. (1978) *Eur. J. Biochem.* **90,** 385–390.
35. Paradies, G., and Ruggiero, F. M. (1990) *Arch. Biochem. Biophys.* **278,** 425–430.
36. Mak, I. T., Shrago, E., and Elson, C. E. (1983) *Biochim. Biophys. Acta.* **722,** 302–309.
37. Cheneval, D., Muller, M., and Carafoli, E. (1983) *FEBS Lett.* **159,** 123–126.
38. Goormaghtigh, E., Chatelain, P., Caspers, J., and Ruysschaert, J. N. (1980) *Biochim. Biophys. Acta* **597,** 1–14.
39. Ernster, L., Sandri, G., Hundal, T., Carlsson, C., and Nordenbrand, K. (1977) *in* Structure and Function of Energy-Transducing Membranes (Van Dam, K., and Van Gelder, B. F., Eds.), pp. 209–222, Elsevier, Amsterdam/New York.
40. Robinson, N. C., Stry, F., and Talbert, L. (1980) *Biochemistry* **19,** 3656–3661.
41. Fry, M., and Green, D. E. (1980) *Biochem. Biophys. Res. Commun.* **93,** 1238–1246.
42. Fry, M., and Green, D. E. (1981) *J. Biol. Chem.* **256,** 2874–2880.
43. McGivan, J. D., and Klingemberg, M. (1971) *Eur. J. Biochem.* **20,** 392–399.
44. Lehninger, A. L. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 1520–1524.
45. Coty, W. A., and Pedersen, P. L. (1975) *Mol. Cell. Biochem.* **9,** 109–124.
46. Reed, K. C., and Bygrave, F. L. (1975) *Eur. J. Biochem.* **55,** 497–504.