affinity for cytochrome P_{450}. This enzyme–substrate interaction evidently also lies at the basis of the inductive action of diethylnicotinamide.

The results suggest that repeated administration of nikethamide in clinical practice can lead to weakening of its intrinsic pharmacotherapeutic action and also to a change in the therapeutic effect and toxicity of substances in whose metabolism the mono-oxygenase system of the liver participates.

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LIVER MITOCHONDRIAL DNA TURNOVER IN RATS OF DIFFERENT AGES

A. Ya. Litoshenko

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One of the principal characteristics of aging is a decrease in the intensity of energy metabolism [1, 7]. This decrease may be due to functional insufficiency of the mitochondria. The number of mitochondria in the liver of old animals has been found to decrease, while at the same time the increase in size, so that the ratio of the area of the mitochondrial membranes expressed per unit area of cell or per unit area of mitochondrion falls [14], as also does the activity of several mitochondrial enzymes [15]. These changes lead to a decrease in the adenosine phosphate pool and a decrease in the ATP content and ATP/ADP ratio in the liver of old rats [3]. A search for the causes of these changes in energy metabolism led the writer to postulate age changes in biogenesis of mitochondria. In particular, it was shown that although the rates of synthesis and breakdown of mitochondrial proteins, coded by the nuclear genome, remain unchanged during aging, the rates of these same processes as regards proteins coded by the mitochondrial genome fall in old age [2, 4]. The rate of replication of mitochondrial DNA (mtDNA) in the liver of old rats also decreases [12].

Since cellular homeostasis is maintained by coordination between synthesis and breakdown of macromolecules, the aim of this investigation was to determine the velocity constants of mtDNA turnover in the liver of rats of different ages. Since the mitochondrial population is heterogeneous as regards its sedimentation behavior, morphology, and function [4, 10, 11, 13], fractions of heavy and light mitochondria also were investigated.

EXPERIMENTAL METHOD

Mature (7 months) and old (28 months) female Wistar rats received an intraperitoneal injection of ^{3}H-methylthymidine (USSR origin) in a dose of 400 μCi/100 g body weight. The animals

Laboratory of Molecular Biology, Institute of Gerontology, Academy of Medical Sciences of the USSR, Kiev.

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TABLE 1. Breakdown Constants of Rat Liver mtDNA at Different Ages (M ± m)

Mitochondrial fraction	Age of animals, months	Regression coefficient, log SR · day^{-1}	Half-life, days	P
Total population	7	—0,0337±0,0017	8,9±0,4	<0,001
	28	—0,0235±0,0023	12,6±1,3	
Heavy	7	—0,0299±0,0018	10,0±0,6	
	28	—0,0238±0,0025	12,6±1,3	<0,05
Light	7	—0,0532±0,0056	5,6±0,6	
	28	—0,0338±0,0029	8,9±0,8	<0,01

were decapitated on the 8th, 15th, and 22nd days after injection of the label. The liver (3 g), washed with cold physiological saline, was put through a press and homogenized in 20 ml of isolation medium (0.25 M sucrose, 0.01 M Tris-HCl, 0.001 M EDTA, pH 7.4) in a Potter's homogenizer (glass—Teflon). Nuclei and undestroyed cells were removed (600g, 10 min) and fractions of heavy (6500g, 10 min) and light (10,000g, 10 min) mitochondria [11] were separated by differential centrifugation. Each fraction was washed twice and resuspended in 3 ml of isolation medium without EDTA. Pancreatic DNAase and $MgCl_2$ were added to the suspension to final concentrations of 100 μg/ml and 0.005 M, respectively. The samples were incubated for 30 min at 37°C. To 0.1 ml of suspension 1 ml of 10% TCA was added. After precipitate formation the samples were filtered through type HA Millipore filters (USA) with a pore diameter of 0.45 μ and their radioactivity was measured on an Intertechnique SL-30 spectrometer (France) in ZhS-106 toluene scintillator. mtDNA was determined quantitatively [8] in an aliquot of mitochondrial suspension. Specific radioactivity (SR) was expressed in cpm/μg phosphorus of mtDNA. From values of SR the half-life and coefficient of regression of mtDNA were calculated [5, 6]. What were in fact determined were the apparent half-life and regression coefficient of mtDNA, for possible reutilization of breakdown products cannot be ruled out.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that during aging the turnover rate of liver mtDNA decreases. The age-dependent decrease in the rate of breakdown of liver mtDNA may reflect either the total decrease in the rate of metabolism of all mtDNA molecules in old age or the nonrenewability (and nonreplicability) of only some of these molecules.

The rates of synthesis and breakdown of mtDNA molecules in the mature animal were coordinated so that the number of mitochondrial genomes in the cell remained constant [9]. This equilibrium between velocities of opposite processes is probably observed in the old animal also, as shown by the fact that the mtDNA content in the liver does not change with age. For instance, the mtDNA content (in μg phosphorus/g tissue) in the adult rat liver was as follows (M ± m): whole population of mitochondria 3.68 ± 0.67, heavy mitochondria 2.99 ± 0.53, light mitochondria 0.70 ± 0.16; the corresponding figures for old animals were 3.36 ± 0.64, 2.66 ± 0.48, and 0.70 ± 0.19. **One result of the decrease in the rate of breakdown of mtDNA during aging may be delayed elimination of defective mtDNA molecules. The fall in the rate of breakdown, accompanied by a parallel decline in the rate of mtDNA synthesis in the liver [12], although enabling the number of mitochondrial genomes to be maintained at the level in the mature organism, will lead to slowing** of biogenesis of **the mitochondria and to a reduction in energy metabolism during aging.**

Since the mitochondrial population is heterogeneous and consists of fractions of light and heavy mitochondria it might be supposed that the age-dependent decrease in the rate of mtDNA breakdown is due to redistribution of the mitochondrial fractions toward an increase in the proportion of heavy, more slowly renewed, mitochondria. In fact, this does not take place and the decrease in the rate of mtDNA breakdown is observed in both mitochondrial fractions in the liver of old rats. The gradient of the regression line for SR of mtDNA (Fig. 1), characterizing the rate of its breakdown, irrespective of the animals' age, is greater in the fraction of light mitochondria than in the fraction of heavy mitochondria and in the whole mitochondrial population. Incidentally, the similar turnover rates of mtDNA of heavy mitochondria and of the total mitochondrial population can be explained on the grounds that mtDNA of

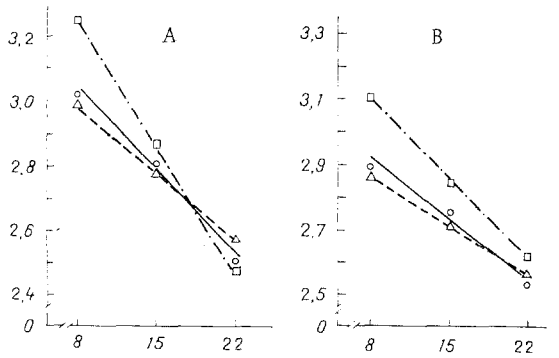

Fig. 1. mtDNA turnover in liver of mature (A) and old (B) rats. Abscissa, days after injection of label; ordinate, log. of specific radioactivity of mtDNA. Circles — total population of mitochondria; triangles — heavy, squares — light mitochondria.

this fraction accounts for about 80% of the total liver mtDNA in both mature and old rats. Since light and heavy mitochondria are forms which change from one into the other during mitochondrial biogenesis, which is a kinetic process [10, 13], the decrease in the mtDNA turnover rate in them is evidence of overall slowing of mitochondrial biogenesis during aging. On the basis of the results described above, and also results of the study of rates of mtDNA synthesis and of synthesis and breakdown of mitochondrial proteins [2, 4, 12], it can be tentatively suggested that the age-dependent decrease in energy metabolism is due to slowing of mitochondrial biogenesis during aging.

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