As a result of the biphasic nature of the kinetic curve, the light sum of chemiluminescence of the neutrophils under the influence of two activators during different time intervals was nonadditive. During the first 30 min of activation of the neutrophils the effect of the combined action of TNF-α and the ionophore, in the presence of luminol or lucigenin, was synergic, whereas during 3 h of incubation it was less than additive (Table 1). Thus the integrative parameter (the light sum of chemiluminescence), without allowing for the kinetics of the process, gives no idea about synergism of action of activators. From this point of view data on the synergic action of PMA and the ionophore on O_2 production by neutrophils in the course of 10 min [3] cannot be extended to later times of activation of the cells.

The appearance of the early chemiluminescence response under the influence of TNF-α or PMA, jointly with ionophore A23187 (Figs. 2 and 3), and also depression of neutrophil activation by the preparation H7, a specific inhibitor of protein kinase C [5], can be taken as evidence that the latter is involved in activation of neutrophils by tumor necrosis factor. Consequently, the cause of the effect of synergic action of the Ca^{2+} ionophore and TNF-α on chemiluminescence observed in these experiments may be stimulation of different pathways of activation of NADPH-oxidase of the neutrophils by them: an increase in the intracellular calcium concentration and activation of protein kinases.

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AGE DIFFERENCES IN THE EFFECT OF OSMOTIC PRESSURE ON RESTORATION OF RAT LIVER MITOCHONDRIA

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During aging changes take place in the properties of the mitochondria, and they are most marked in relation to the structural state of a membrane: the "osmotic" strength of the outer mitochondrial membrane is reduced in the liver of male rats, and sensitivity of these organelles to freezing and thawing is altered [2, 3]. It has been shown that after short-term hypotonic shock, the respiratory control (RC) of the liver mitochondria is reduced by a greater degree in old rats than in young animals [4]. Data also have been obtained to show that mitochondria and tissues of old animals contain less water than those of young, a fact attributable to age changes in the structural state of the membranes [11]. This fact may perhaps be of physiological importance know that the osmotic pressure of the medium has a significant influence on respiration and oxidative phosphoryla-

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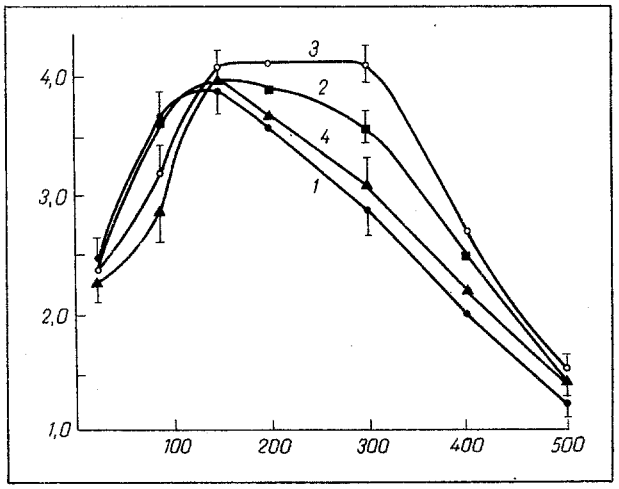

Fig. 1. Effect of osmotic pressure on RC of rat liver mitochondria. Abscissa, sucrose concentration in incubation medium (in mM); ordinate, values of RC. 1) 1 month, 2) 3 months, 3) 12 months, 4) 24 months.

tion of the mitochondria [1, 5, 6, 9]. Meanwhile the role of the osmotic factor in the change in functional state of the mitochondria with age has not been studied. The aim of this investigation was to study dependence of respiration and RC of liver mitochondria from rats of different ages on the osmotic pressure of the incubation medium.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats aged 1, 3, 12, and 24 months. Liver mitochondria were isolated by differential centrifugation with one washing. The residue of mitochondria was suspended in 0.3 M sucrose with 10 mM Tris-HCl, pH 7.4. Mitochondrial respiration was determined polarographically at a temperature of +25°C. The incubation medium contained one of the following concentrations of sucrose: 25, 90, 150, 300, 400, or 500 mM plus KCl — 10 mM KH_2PO_4 — 3 mM $MgSO_4$ — 3 mM; pH 7.4. The following ingredients were added in succession to the polarographic cell: rotenone $1 \cdot 10^{-6}$ M, succinate 4 mM, and mitochondria (1-1.5 mg protein/ml incubation medium), ADP 400 μM, or 2,4-dinitrophenol (DNP) in the optimal concentration, which for media containing 25, 150, 300, and 500 mM sucrose, was 150, 120, 60, and 30 μM DNP respectively. Protein was determined by the method of Lowry et al. in Miller's modification [7].

EXPERIMENTAL RESULTS

Dependence of RC of the liver mitochondria from rats of different ages on the sucrose concentration in the incubation medium is shown in Fig. 1. Most experimental material was obtained with sucrose concentrations of 25, 90, 150, 300, and 500 mM. To determine the precise character of the curves, two measurements were made for each sucrose concentration of 200 and 400 mM. As the results show, three conclusions can be drawn regarding the age changes in RC depending on the composition of the incubation medium: 1) that RC falls with age of the rats (the region of 90 mM sucrose); 2) that no appreciable changes in RC take place (region of 150 mM sucrose), and 3) that RC increases toward middle age and then decreases in old rats (region of 300 mM sucrose). Probably one cause of the contradictory nature of the data in the literature on age changes in the degree of energy coupling in mitochondria is some difference in the composition of the incubation media used.

The greater decrease in RC of the mitochondria of old rats on the transition from medium with 150 mM sucrose to medium with 90 mM sucrose is in agreement with data on the higher sensitivity of the liver mitochondria of old rats to hypotonic shock (40-52 milliosmoles/liter) for 20 sec, followed by addition of sucrose to 0.25 M [4].

Another conclusion which follows from the data in Fig. 1 is that liver mitochondria of middle-aged rats preserve high values of respiratory control over a wider range of osmotic pressures than mitochondria of younger and older animals. The decrease of RC in rats aged 1 and 24 months with an increase in the sucrose concentration in the incubation medium from 150 to 300 mM is connected with inhibition of respiration in state 3 (V_3), and not with an increase in the rate of respiration in

TABLE 1. Velocity of Oxygen Consumption by Liver Mitochondria in Chance's Metabolic States 4 and 3 in Media With Different Sucrose Concentrations (M ± m; n = 4-7)

Metabolic state	Age, months	Sucrose concentration, mM				
		25	90	150	300	500
4	1	36,2±1,2*	27,0±1,7	27,6±0,8	27,6±0,8	27,8±1,0
	3	40,2±2,3*	26,4±0,8	25,5±1,0	24,2±1,5	20,1±0,7
	12	35,8±1,9*	26,3±2,1	23,7±0,9	24,9±1,1	23,6±1,5
	24	34,6±1,1*	27,9±1,6	24,1±0,8	25,4±1,1	26,1±1,4
3	1	96,4±6,5	102,2±3,7	108,9±4,9	80,2±7,0**	34,3±1,9**
	3	96,0±7,5	97,7±1,8	102,3±5,3	89,2±6,1	28,4±1,9**
	12	86,0±2,1**	90,1±7,4	99,0±3,4	101,6±3,8	37,0±2,4**
	24	80,3±2,3**	82,2±5,3**	97,7±2,8	80,2±4,7**	36,3±1,9**
		$p_{1-24}<0,05$	$p_{1-24}<0,02$		$p_{1-12}=0,02$ $p_{12-24}<0,005$	$p_{3-12}<0,02$

Legend. Velocity of oxygen consumption in nanoatoms O/mg protein/min. *p < 0.05 compared with variants with 90, 150, 300, and 500 mM sucrose; **p < 0.05 compared with variant with 150 mM sucrose.

TABLE 2. Velocity of Oxygen Consumption by Liver Mitochondria in Uncoupled State and of RC_u in Media With Different Sucrose Concentrations

Parameter	Age, months	Sucrose concentration, mM			
		25	150	300	500
V_{3u}	1	130,6±5,8*	168,0±8,4	95,5±1,4*	59,2±4,6*
RC_u	12	120,4±6,0*	160,1±3,2	94,6±0,6*	62,4±5,6*
	1	3,8±0,1*	5,7±0,3	3,5±0,1*	2,2±0,1*
	12	3,7±0,1*	5,8±0,2	3,7±0,1*	2,6±0,2*

Legend. Velocity of oxygen consumption in nanoatoms O/mg protein/min.
*p < 0.05 compared with variant with 150 mM sucrose.

Chance's stage 4 (Table 1). It is a very significant fact that the respiration of the mitochondria of 12-month-old rats (V_{3u}), uncoupled by DNP, unlike V_3, changes depending on osmotic pressure in the same way as respiration of rats aged 1 month (Table 2). The coefficient of stimulation of respiration by the uncoupler also shows similar changes (RC_u).

One cause of the reduction of respiration in the region of hypertonic incubation media may be an increase in viscosity of the matrix and slowing of diffusion of metabolites [5, 8]. Diffusion of ATP^{4-} and ADP^{3-} in the matrix may perhaps be the bottleneck. Within the scope of this hypothesis, another feature distinguishing osmotic behavior of V_3 and RC of the mitochondria of 12-month-old rats could also be explained assuming more intimate structural coupling of the adenine-nucleotide carrier in the membrane with the H^+-ATP-synthetase complex [12]. It has also been recently shown that activity of the adenine nucleotide carrier is maximal in the absence of osmotic pressure difference on the two sides of the membrane, and it falls sharply if there is a change of pressure in hypo- or hypertonic directions.

Age differences in the effect of osmotic pressure on respiration and on the value of RC of rat liver mitochondria thus exist and are perhaps connected with transport of adenine nucleotides through the membrane and (or) the degree of condensation of the matrix.

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CHANGES IN GUANYLATE CYCLASE ACTIVITY OF HUMAN PLATELETS IN ADP-INDUCED AGGREGATION

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The role of the cyclic GMP (cGMP) system in the regulation of platelet aggregation and disaggregation in mammals has aroused increased interest during the last few decades. Whereas it was previously held that cGMP mediates initiation of aggregation [11], subsequent studies have suggested that the cGMP system exercises negative control over platelet aggregation and transmits the signal for their disaggregation [5, 9-11, 14]. It is assumed in this case that the process of platelet aggregation itself can induce activation of the cGMP system, which exerts its regulatory action by a "negative feedback" principle. Experimental data so far available in support of this view are based mainly on two groups of facts: 1) guanylate cyclase (GC) activators, which raise the cGMP level in platelets, exhibit an antiaggregative action [5, 11, 14]; 2) during aggregation, the cAMP concentration in platelets is increased [7, 10]. Under these circumstances, no information is available on the state of GC in aggregating platelets or on the effect of the aggregation process on GC activity.

We showed previously that activity of GC and its sensitivity to the NO-containing activator sodium nitroprusside (SNP) are depressed in platelets with increased ability to aggregate [2, 3]. This observation assumes a functional connection between the aggregation process and GC. In the investigation described below a more detailed study was made of changes in activity of GC and its ability to undergo activation in human platelets during their aggregation. Platelet aggregation was induced by ADP in concentrations enabling only the reversible phase of aggregation to be recorded.

EXPERIMENTAL METHOD

Blood was taken from donors before breakfast, from the cubital vein, and a 3.8% solution of trisodium citrate was used as the anticoagulant, in the ratio of 1:9 by volume with blood. The blood was centrifuged for 10 min at 450g, and the resulting platelet-rich plasma was diluted to the concentration of $2.5 \cdot 10^8$ platelets/ml, necessary to study reversible aggregation [3]. Platelet-deprived plasma was obtained by centrifugation of platelet-rich plasma for 30 min at 650g. ADP was used as the inducer of aggregation and the course of aggregation was monitored by measuring the increase in the transmittance of light (540 nm) in the test plasma during continuous mixing (1000 rpm) [3].

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