ilarly, after the administration of adenine-^{14}C the conversion to guanine-containing moieties of nucleic acids does not involve predominantly guanine *per se*, and therefore the coadministration of AIC did not produce a significant effect on the incorporation pattern of adenine into nucleic acids.

SUMMARY

In investigations in which guanine-^{14}C was injected into mice, the relative specific activities of the guanine fractions isolated from liver nucleic acids were increased approximately five-fold when 4-amino-5-imidazolecarboxamide (AIC) was co-administered. It was concluded that AIC inhibited guanase, thus blocking the catabolism of guanine. The potentiation of 8-azaguanine actions by AIC previously reported is apparently caused by inhibition of the same enzyme. No significant effect of AIC on the metabolism of adenine-^{14}C was observed.

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ATPASE ACTIVITY OF RAT LIVER MITOCHONDRIA

H. G. KLEMPERER

Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Oxford (England)

The rate at which mitochondria hydrolyse adenosine triphosphate (ATP) is known to be variable. Thus mitochondrial ATPase may be inactive, *e.g.* in mitochondria prepared in sucrose (KIELLEY AND KIELLEY[1]; LARDY AND WELLMAN[2]; POTTER, SIEKEVITZ AND SIMONSON[3]). On the other hand mitochondria prepared in a saline medium can hydrolyse ATP at a rate which is approximately balanced by the rate of ATP formation by oxidative phosphorylation. Under these conditions the rate of incorporation of radioactive inorganic phosphate (^{32}P) into ATP measures both the rate of oxidative phosphorylation (KREBS, RUFFO, JOHNSON, EGGLESTON AND HEMS[4]) and the rate of ATPase activity. ATPase activity may also be increased by extraneous agents *e.g.* by DNP (HUNTER[5]). This paper is concerned with the activity of mitochondrial ATPase under various conditions and with its relation to oxidative phosphorylation.

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EXPERIMENTAL

Special chemicals

Sodium adenosine triphosphate (ATP) solution was made from barium "ATP" prepared according to LEPAGE[6]; it contained approximately 20% adenosine diphosphate. A $4 \cdot 10^{-3}$ *M* solution of 2:4 dinitrophenol (DNP) (British Drug Houses, Ltd.) was prepared by dissolving 0.4 mmoles in 0.8 ml 2 *N* NaOH, adding 95 ml 0.9% (w/v) KCl, neutralizing with *N* KOH, and making up to 100 ml with 0.9% KCl. Solutions of sodium L-thyroxine (Glaxo Laboratories Ltd., Greenford, Middlesex) stabilized by the presence of sheep serum were prepared as described by KLEMPERER[7], except that the concentrations of NaOH and HCl and the final concentration of serum were all higher when solutions stronger than $4 \cdot 10^{-4}$ *M* were prepared. "Serum medium" prepared in the same way but without thyroxine was used as a control and to prepare dilutions of the test solution.

In order to obtain approximately similar final concentrations of phosphate and magnesium in all experiments the "phosphate medium" which was used for suspending mitochondria and preparing solutions of L-thyroxine had the composition (by vol.) 95.33% (w/v) 0.9% KCl, 2.33% 0.1 *M* $MgCl_2$, 2.33% 0.1 *M* sodium phosphate buffer pH 7.4 in experiments involving L-thyroxine, and 93.33% KCl, 3.33% $MgCl_2$, 3.33% sodium phosphate buffer in other experiments.

Mitochondria

These were prepared as described by KLEMPERER[7] except that 7.5 g rat liver were homogenized with 0.25 *M* sucrose, and that the mitochondria were in all cases twice washed with 10–15 times their own volume of phosphate medium before they were finally suspended in 50 ml phosphate medium.

"Aged" mitochondria were prepared by shaking the suspension in phosphate medium for 20 min at 37.4° in a conical flask, with air as the gas phase. This caused a decrease in dry weight and a release of phosphate from the mitochondria.

Incubation procedure

All experiments were duplicated and were performed in conical Warburg flasks or Thunberg tubes with hollow stoppers, shaken at 20° in a constant temperature bath. The gas phase was air or N_2 except in vessels which were first made anaerobic and then aerobic by gassing with O_2. Side arms of Warburg flasks and stoppers of Thunberg tubes contained 0.45 ml 0.027 *M* sodium ATP (except in some expts. in Table III) and 0.05 ml $KH_2{}^{32}PO_4$ solution. The following components were measured into the main compartments of Warburg flasks or into Thunberg tubes standing on ice: 0.5 ml 0.5 *M* sodium succinate, 1 ml solution of DNP or L-thyroxine, and 2 ml suspension of mitochondria (approx. 14 mg dry wt of mitochondria). In control flasks corresponding solutions without DNP or L-thyroxine were substituted for solutions of the test substances. Flasks without substrate contained 0.5 ml 0.5 *M* NaCl in place of sodium succinate. To obtain zero time values the additions were made to 0.5 ml 30% (w/v) trichloracetic acid in test tubes. Mitochondria remained in contact with L-thyroxine for 35 min at 0° before incubation at 20°.

Flasks for anaerobic incubation were gassed with N_2 for 2 min with yellow phosphorus in the centre well. After gassing, manometers were placed on a rack in such a way that the flasks were immersed in a trough of ice. To achieve complete anaerobiosis these flasks were shaken for 10 min at 20° before tipping in ATP from the side arm. Where the effect of this treatment on oxidative phosphorylation was investigated the flasks were cooled to 0°, the yellow phosphorus was removed and the flasks gassed with O_2 for 0.5 min.

Thunberg tubes were evacuated with a water pump while being shaken until the contents effervesced. They were then filled with O_2-free N_2 and the whole process was repeated three times within a period of 4 minutes. The tubes were finally closed when they were gas-filled. Where the effect of this treatment on oxidative phosphorylation was investigated the tubes were finally filled with O_2.

The contents of the side arm were mixed with the other material in the vessel just before insertion in the bath at 20° (except where flasks were gassed with N_2). Aerobic phosphate exchange of ATP was measured for 2.5 min and net breakdown of ATP for periods varying from 3 to 5 minutes. Incubation was stopped by transferring the vessel to ice and immediately adding 0.5 ml 30% (w/v) trichloracetic acid. The supernatants were stored frozen at —20°.

The O_2 uptake was measured in Warburg manometers after a 10 min equilibration period. The flask contents were similar to those previously described, but contained in addition 0.2 ml 2 *N* NaOH and filter paper in the centre well.

Phosphate estimation and measurement of radioactivity

Phosphates were separated as bands by paper chromatography as described by BARTLEY[8] with the formic acid-*iso*propyl ether solvent of HANES AND ISHERWOOD[9].

The bands corresponding to the total adenosine phosphates and to inorganic phosphate were

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cut out and wet-ashed with the ashing fluid of HANES AND ISHERWOOD[9]. After dilution with water, samples were withdrawn and analysed for phosphate by the method of BERENBLUM AND CHAIN[10], or counted in a beta-counter tube, liquid type M6 (20th Century Electronics).

Wet and dry weights of mitochondria

For wet weight determinations (Table VII) mitochondria were centrifuged at 0° from 3.5 ml medium of the same composition as that used in the main compartment of Warburg flasks. The centrifuge was accelerated to 24,000 *g* in 15–20 sec and the motor switched off after 4 minutes. The supernatant was decanted and the tube drained and dried inside with filter paper before weighing. The tubes were then heated in an oven at 110° for 24 h for measuring the dry weight of the mitochondria. In other experiments, mitochondria from a measured volume of suspension were separated and dried in the same way.

Expression and calculation of results

All results are average values for duplicate vessels. The rate of net ATP breakdown is expressed as μmoles increase in phosphate/min of incubation. The radioactivity incorporated into adenosine phosphates is expressed as the percentage of the total radioactivity found in adenosine phosphates plus inorganic phosphate. This gives an accurate measure of the rate of phosphorylation for very short periods of incubation; it is useful as an approximate measure also for longer periods when exchange rates cannot be calculated because no steady state exists.

The value given for μmoles ATP is calculated on the assumption that all adenosine phosphate was ATP, and labile phosphate was taken as two thirds of the total phosphorus of adenosine phosphates. Table headings give the amount of ATP found after 2.5 min incubation of the control in the presence of air and succinate. The rate of ATP hydrolysis under steady state conditions, equivalent to the phosphorylation rate (μmoles P of ATP exchanged/min), was calculated by the method of KREBS *et al.*[4] from μmoles phosphate and ATP in the flasks after 2.5 min incubation, assuming that these amounts remained constant during the time of incubation.

Swelling of the mitochondria is given by the decrease in the "% dry weight" (= dry wt as percentage of wet wt).

Accuracy of procedures

Duplicate phosphate estimations agreed within 3%. When mitochondria were incubated with 10 μmoles phosphate in the absence of ATP for the same times and under the same conditions as were used for measuring net ATP breakdown, there was no measurabe release of phosphate. In the presence of ATP, therefore, an increase of phosphate was due to a net ATP breakdown. This rate of net ATP breakdown could be compared with the rate of ATP hydrolysis under steady state conditions, since ATPase was saturated with substrate in both instances. This was so because the same initial concentration of ATP was present in each case, and the net ATP breakdown was linear with time under the experimental conditions.

With 0.0625 *M* succinate as the substrate only the one-step oxidation to fumarate took place (WHITTAM, BARTLEY AND WEBER[11]). Therefore phosphorylation quotients (equivalents of organic phosphate formed/atoms of oxygen absorbed) of at least 2 indicated normal phosphorylation. Oxidative phosphorylation in the control was measured for 2.5 min only, since the incorporation of ^{32}P into ATP may have been limited by the rate at which myokinase transfers the terminal P of ATP to the β position, and in calculating the phosphate exchange the error due to this limiting factor increases with the period of incubation (WHITTAM *et al.*[11]).

In many experiments phosphorylation of adenosine phosphate present in the ATP led to some decrease of the initial phosphate concentration before a steady state was attained. However, calculations based on the assumption of a steady state, and on data from systems where the initial phosphate concentrations changed slightly were not necessarily erroneous. This is shown by the fact that the rate of oxidative phosphorylation appeared to be the same whether calculated from data for the initial period during which there was net ATP synthesis or from data for a later period during which the concentration remained constant (Table III).

RESULTS

Net aerobic ATP breakdown in absence of added substrate

Mitochondria incubated aerobically in the presence of succinate maintained an approximately constant concentration of ATP. Without succinate there was a slow release of phosphate from ATP, accompanied by a small oxygen uptake and incorporation of ^{32}P into ATP, indicating some oxidative phosphorylation due to the respiratory ac-

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tivity that remained in these mitochondria even after two washings. (The phosphorylation quotient without added substrate was about 3.) This phosphorylation diminished the net ATP breakdown, so that a true value for ATPase activity could not be obtained under these conditions. This was borne out by the increased rate of net ATP breakdown under anaerobic conditions or aerobically with DNP.

TABLE I

NET ATP BREAKDOWN IN THE ABSENCE OF ADDED SUBSTRATE

Warburg flasks contained $KH_2{}^{32}PO_4$, 11 μmoles ATP, 14 mg dry wt mitochondria, and either 0.0625 *M* sodium succinate or 0.0625 *M* NaCl. Phosphorylation quotient for flasks with succinate was 2. Initially all flasks contained 10.5 μmoles inorganic phosphate.

		Incubation time (min)	*Phosphate (μmoles)*		*Radioactivity of ATP as % of total radioactivity*	*O_2 uptake (μl)*		
			final	*change/min*		*10–20 min*	*20–30 min*	*30–40 min*
Succinate, air		2.5	10.0	—0.2	16.9	46	46	46
No substrate	air	10	12	0.2	23.7	10	9	7
	anaerobic	5	14	0.7	5.7	—	—	—
	DNP, air	3	15.5	1.7	0.7	—	—	—

ATP breakdown in presence of succinate

Table II shows data of an experiment in which the formation of inorganic phosphate from ATP was measured in the presence of succinate. At the same time the rate of incorporation of ^{32}P into ATP was measured. From the latter the rate of aerobic hydrolysis of ATP under steady state conditions was calculated to be 0.95 μmoles/min. The rate of net ATP breakdown anaerobically was 30% less than this.

TABLE II

ATP BREAKDOWN IN PRESENCE OF SUCCINATE

Vessels contained 0.0625 *M* sodium succinate and 13.5 mg dry wt mitochondria. All vessels except the control were made anaerobic, but some were subsequently incubated in O_2. The aerobic control after 2.5 min incubation contained 11 μmoles ATP and gave an exchange rate of 0.95 μmoles phosphate/min (phosphorylation quotient = 2.4.) Initially all vessels contained 10.1 μmoles inorganic phosphate.

		Final gas phase	*Incubation time (min)*	*Phosphate (μmoles)*		*Radioactivity of ATP as % of total radioactivity*
				final	*change/min*	
Control		air	2.5	9.4	—0.3	21.0
Anaerobic procedure	Warburg flask	N_2	5	13.1	0.6	2.7
		O_2	2.5	9.7	—0.2	20.0
	Thunberg tube	N_2	5	13.1	0.6	3.5
		O_2	2.5	9.8	—0.1	20.0

A slow incorporation of ^{32}P into ATP occurred anaerobically (5–10% of the aerobic rate). This can hardly account for the difference of 30% between the aerobic and anaerobic ATPase activities. The anaerobic ^{32}P incorporation was not due to any residual O_2 since it occurred even after repeated gassing with O_2-free N_2 in anaerobic Thunberg tubes, and other experiments showed that it was not affected by the presence of 0.02 *M* potassium cyanide. The diminished anaerobic ATPase activity was not

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a consequence of any irreversible enzyme destruction under the anaerobic conditions. This is shown by the finding (Table II) that on readmission of O_2 the level of ATP was maintained and ^{32}P was incorporated at the same rate as in the control.

Since a small net synthesis of ATP generally occurred in the first few minutes of aerobic incubation (due to phosphorylation of adenosine diphosphate), an experiment was carried out (Table III) in which the net anaerobic ATP breakdown rate was compared with the rate of ATP hydrolysis under the more exactly steady state conditions found over the 10–12.5 min period of aerobic incubation. The results confirm that the anaerobic rate of ATP breakdown (0.5 μmoles/min) was about 2/3 of that occurring aerobically (0.73 μmoles/min).

TABLE III

RATE OF ATP HYDROLYSIS UNDER STEADY STATE CONDITIONS AND THE ANAEROBIC NET ATP BREAKDOWN RATE

Flasks contained 0.0625 *M* succinate and 12.5 mg dry wt mitochondria. Phosphate exchange in aerobic flasks was measured over the periods 0–2.5 and 10–12.5 min by tipping in $KH_2{}^{32}PO_4$ at 0 and 10 min. The phosphorylation quotient in both cases was 2. Initially all flasks contained 10.3 μmoles inorganic phosphate.

Gas phase	*Incubation time (min)*	*Phosphate (μmoles)*		*Labile P of ATP (μmoles)*	*^{32}P exchange*		
		final	*change/min*		*Interval (min)*	*Radioactivity of ATP as % of total radioactivity*	*P exchanged/min (μmoles)*
Air	2.5	9.2	—0.4	22.4	0–2.5	17.1	0.72
	10	8.8					
	12.5	8.6	—0.1	23.2	10–12.5	18.5	0.73
N_2	5	12.9	0.5	—	0–5	2.4	—

Effects of DNP and L-thyroxine on ATPase

DNP at 10^{-4} *M* inhibited phosphorylation almost 100% (Table IV) and the rate of the net aerobic ATP breakdown was 30% greater than the rate of ATP hydrolysis under steady state conditions in the absence of DNP. The net ATP breakdown in the presence of 10^{-4} *M* DNP was therefore not merely due to uncoupling but also to a 30%

TABLE IV

ACTION OF DPN ON OXIDATIVE PHOSPHORYLATION AND ATP BREAKDOWN

Vessels contained 0.0625 *M* succinate and 15 mg dry wt mitochondria. The control contained 10.7 μmoles ATP after 2.5 min incubation and gave an exchange rate of 1.06 μmoles phosphate/min (phosphorylation quotient = 2.1.) All other vessels were incubated for 3 min. The anaerobic expt. under N_2 was carried out in Thunberg tubes. Initially all vessels contained 9 μmoles inorganic phosphate

DNP × 10^{-5} M	*gas phase*	*Phosphate (μmoles)*		*Radioactivity of ATP as % of total radioactivity*
		final	*change/min*	
0	air	8.5	—0.2	25.5
3		11.6	0.9	2.8
5		12.4	1.1	0.5
10		13.1	1.4	0.3
10	N_2	13.1	1.4	0.3

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TABLE V

ACTION OF L-THYROXINE ON OXIDATIVE PHOSPHORYLATION AND ATP BREAKDOWN

Flasks contained 13.5 mg dry wt mitochondria, 0.0625 *M* succinate, and 0.1 ml serum added with the L-thyroxine or control solutions. Mitochondria were in contact with L-thyroxine for 35 min at 0° before incubation. The control without thyroxine contained 11.8 μmoles ATP after 2.5 min incubation and gave an exchange rate of 0.76 μmoles phosphate/min (phosphorylation quotient = 2.1). All other flasks were incubated for 4 min. Initially all flasks contained 9.1 μmoles inorganic phosphate.

L-thyroxine $\times 10^{-5}$ *M*	*Phosphate (μmoles)*		*Radioactivity of ATP as % of total radioactivity*
	final	*change/min*	
0	8.9	—0.1	18.4
5	9.9	0.2	15.5
10	13.1	1.0	0.6
30	11.5	0.6	0.4

increase of the aerobic ATPase activity. In other experiments 10^{-3} *M* DNP caused the same net ATP breakdown rate as 10^{-4} *M* DNP. At $5 \cdot 10^{-5}$ *M* DNP almost completely inhibited phosphorylation without increasing the rate of ATP hydrolysis. Therefore at this concentration DNP uncoupled oxidative phosphorylation without activating ATPase. In the presence of DNP the net ATP breakdown and ^{32}P incorporation into ATP were the same aerobically and anaerobically. Thus DNP inhibited the anaerobic ^{32}P incorporation into ATP recorded in Table II.

L-thyroxine had effects similar to those of DNP (Table V); 10^{-4} *M* L-thyroxine almost completely inhibited oxidative phosphorylation and activated ATPase. However $3 \cdot 10^{-4}$ *M* L-thyroxine inhibited ATPase.

Effect of ageing on ATPase

In the presence of succinate aged mitochondria showed 50% of the oxygen uptake of fresh mitochondria but oxidative phosphorylation was uncoupled, as indicated by the

TABLE VI

NET ATP BREAKDOWN BY AGED MITOCHONDRIA AND THE ACTION OF DNP AND L-THYROXINE

Flasks contained 0.0625 *M* succinate, 15.5 mg dry wt fresh mitochondria (or equivalent wt aged mitochondria) and 0.04 ml serum. The control with fresh mitochondria contained 11.8 μmoles ATP after 2.5 min incubation and gave an exchange rate of 1.07 μmoles phosphate/min (phosphorylation quotient = 2.4). All other flasks were incubated for 3 min. Mitochondria remained in contact with L-thyroxine for 35 min at 0° before incubation. Aged mitochondria were prepared by shaking the suspension for 20 min at 37.4° in air. Final concentration of DNP and L-thyroxine was 10^{-4} *M*. Initial phosphate was 10.3 μmoles in flasks containing fresh mitochondria and 11.2 μmoles in flasks containing aged mitochondria owing to the release of phosphate during ageing.

Mitochondria	*Uncoupling agent*	*Phosphate (μmoles)*		*Radioactivity of ATP as % of total radioactivity*
		final	*change/min*	
Fresh	—	9.4	—0.4	24.4
	L-thyroxine	14.1	1.3	1.1
	DNP	14.7	1.5	0.3
Aged	—	15.3	1.4	0.4
	L-thyroxine	15.3	1.4	0.1
	DNP	18.4	2.4	0.1

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reduced rate of ^{32}P incorporation into ATP (Table VI). The rate of net ATP breakdown after ageing was greater than the rate of hydrolysis under steady state conditions in fresh mitochondria, and about the same as the rate of net breakdown in the presence of 10^{-4} *M* L-thyroxine or 10^{-4} *M* DNP.

No action of L-thyroxine on ATPase was detectable in aged mitochondria whilst DNP increased their ATPase activity. This suggests that the effects of ageing and L-thyroxine on ATPase are similar but differ from the action of DNP.

TABLE VII

SWELLING OF MITOCHONDRIA UNDER CONDITIONS LEADING TO ACTIVATION OF ATPASE

Centrifuge tubes at 0° contained 2 ml mitochondrial suspension (dry wt, fresh = 13.9 mg, aged = 11.7 mg), 0.5 ml 0.5 *M* sodium succinate and 1 ml $4 \cdot 10^{-4}$ *M* solution DNP or L-thyroxine, or a control solution. Tubes with L-thyroxine contained 0.04 ml serum and were allowed to stand for 35 min at 0°. Other tubes contained no serum and stood for 5 min at 0°. At the end of these times all tubes were centrifuged at 24,000 *g* for 4 min. The supernatants were decanted and the tubes drained and dried with filter paper before weighing.

Mitochondria	*Uncoupling agent*	*Wet wt (mg)*	*% Dry wt*
Fresh	—	68.3	20.4
	DNP	68.5	20.3
	DNP*	68.7	20.2
	L-thyroxine	82.5	16.9
Aged	—	76	15.4

* Incubated for 0.5 min at 20° before centrifugation.

Swelling of mitochondria after procedures that activate ATPase

The dry weight of mitochondria expressed as a percentage of wet weight was used as a measure of mitochondrial swelling. Both ageing and 35 min contact with L-thyroxine at 0° caused swelling (Table VII), but mitochondria did not swell in the presence of DNP at 0°, nor after 30 seconds incubation with DNP at 20°. This again indicates that ageing acts in the same way as L-thyroxine, but not as DNP.

DISCUSSION

According to HUNTER[5], KIELLEY AND KIELLEY[1], LARDY AND WELLMAN[2], and POTTER *et al.*[3], mitochondria prepared in 0.25 *M* sucrose have little or no ATPase activity, but DNP considerably increases this activity. The present results confirm that mitochondria prepared in a medium consisting mainly of 0.9% KCl differ from "sucrose mitochondria" in showing appreciable ATPase activity in the fresh state. Stimulation of this ATPase by DNP was relatively slight (30%), and $5 \cdot 10^{-5}$ *M* DNP uncoupled oxidative phosphorylation without activating ATPase. That uncoupling and ATPase activation are not necessarily associated has already been shown by WITTER, NEWCOMB AND STOTZ[12], who found that under certain conditions DNP uncoupled oxidative phosphorylation but did not increase the rate of anaerobic ATP breakdown.

The main new finding is the observation that the ATPase activity of "KCl mitochondria" is about 30% lower anaerobically than aerobically, but in the presence

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of 10^{-4} *M* DNP ATPase activity is the same aerobically as anaerobically. DNP also inhibits the slow anaerobic exchange between inorganic phosphate and ATP. A similar inhibition is known to occur in "sucrose mitochondria", in which however this anaerobic exchange is relatively rapid (BOYER, FALCONE AND HARRISON[13]; SWANSON[14]).

Thyroxine (10^{-4} *M*) and DNP (10^{-4} *M*) both activated ATPase in fresh "KCl mitochondria", but differences between these two substances appeared at higher concentrations. Thyroxine at $3 \cdot 10^{-4}$ *M* inhibited ATPase, whilst DNP up to 10^{-3} *M* did not. Since mitochondrial ATPase is known to require magnesium ions (SWANSON[15]; NOVIKOFF, HECHT, PODBER AND RYAN[16]; KIELLEY AND KIELLEY[17]), its inhibition by thyroxine may be explained in the same way as the inhibition of creatine phosphokinase by thyroxine (ASKONAS[18]), which has been taken to be due to a combination of thyroxine with magnesium (LARDY AND MALEY[19]).

Thyroxine also differed from DNP in failing to stimulate ATPase that had already been activated by ageing the mitochondria. Both ageing and thyroxine caused swelling of the mitochondria, whereas DNP did not. The association of ATPase activation with mitochondrial swelling has already been noted by DIANZANI[20] in mitochondria suspended in hypotonic solutions. In certain cases ATPase activation may be accounted for by an altered permeability of the mitochondria which makes ATPase more accessible to its substrate. This could explain why thyroxine failed to activate the ATPase of aged mitochondria. DNP in contrast probably had a more direct effect on ATPase, resembling its action both in the case of the ATPase prepared from mitochondria (LARDY AND WELLMAN[2]), and in the case of myosin (CHAPPELL AND PERRY[21]; GREVILLE AND NEEDHAM[22]).

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SUMMARY

1. ATPase and oxidative phosphorylation were studied in rat liver mitochondria prepared in a medium consisting chiefly of 0.9% KCl. The aerobic rate of ATP hydrolysis was calculated from the rate of ^{32}P incorporation into ATP under steady state conditions of oxidative phosphorylation. This was compared with the rate of net ATP breakdown under various conditions.

2. In the presence of succinate the anaerobic net ATP breakdown rate was 30% less than the aerobic hydrolysis rate as calculated from the rate of incorporation of ^{32}P into ATP.

3. The net ATP breakdown rate in presence of 10^{-4} *M* DNP was 30% greater than the rate of ATP hydrolysis as calculated from the rate of ^{32}P incorporation under steady state conditions in the absence of DNP. However, the latter was the same as the rate of net ATP breakdown in the presence of $5 \cdot 10^{-5}$ *M* DNP, which shows that at this concentration DNP uncouples without activating ATPase.

4. The action of 10^{-4} *M* L-thyroxine resembled that of 10^{-4} *M* DNP, but the rate of net ATP breakdown in presence of $3 \cdot 10^{-4}$ *M* L-thyroxine was less than the rate of ATP hydrolysis under steady state conditions.

5. DNP increased the rate of net ATP breakdown by aged mitochondria, but L-thyroxine had no effect. Unlike DNP, both ageing and L-thyroxine caused swelling of the mitochondria. DNP and L-thyroxine therefore seem to activate ATPase in different ways.

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A COLORIMETRIC METHOD FOR THE DETERMINATION OF THIOSULFATE

BO SÖRBO*

Laboratoire de Chimie biologique de la Faculté des Sciences, Paris (France)

In a recent study[1] on the formation of thiosulfate in biological systems, the classical iodometric method for the determination of this compound was attempted. However, it was found inapplicable to some of these systems, as in these cases a sharp end-point could not be obtained, due to interfering reducing compounds. Other existing methods for the determination of thiosulfate appeared too laborious and time-consuming[2,3]. When looking for a better method the possibility of converting thiosulfate to thiocyanate and determining the latter colorimetrically with ferric ions, was considered. The conversion of thiosulfate to thiocyanate can be accomplished by heating with cyanide for 30 minutes on a steam bath[4], but it has now been found that this conversion occurs much more rapidly and at room temperature in the presence of cupric ions. The present communication presents a simple colorimetric method for the determination of thiosulfate, based on this procedure.

* Fellow of the Rockefeller Foundation. Present address: Biochemical Department, Medical Nobel Institute, Stockholm, Sweden.

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