Dependence of Aminonucleoside-Inhibition of Kidney Mitochondrial Adenosine Triphosphatase on Activation and Structural State of Mitochondria.* (28577)

PAUL BARTLETT, TAKESHI TSUJIMOTO† AND HEIDI SCHAEFER
Edsel B. Ford Institute for Medical Research, Henry Ford Hospital, Detroit, Mich.

Previous communications from our laboratory(1,2) have reported adenosine triphosphatase (ATPase) activity of normal rat kidney mitochondria, and of partially purified submitochondrial fractions prepared therefrom, to be inhibited *in vitro* by the nephrotogenic aminonucleoside, 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine. While this effect was readily demonstrable in experiments with intact mitochondria assayed in the presence of Mg^{++} and Ca^{++}, no significant difference was noted in similarly activated ATPase of mitochondria prepared from kidneys of normal and aminonucleoside nephrotic rats. Reported herein are more detailed studies of the *in vitro* effects of aminonucleoside on kidney mitochondrial ATPase assayed in the presence of a wide variety of activating agents, as well as in the absence of such agents.

Methods. Rat kidney mitochondria were prepared essentially as described by Weinbach(3). Special precautions, such as the use of deionized 0.25 M sucrose in homogenization of the tissue and in subsequent washing of the mitochondrial pellet, were, however, taken to avoid preactivation of the kidney mitochondrial ATPase. ATP, deionized by passing freshly prepared solution of the sodium salt of ATP over Amberlite 120, H^+ form, followed by neutralization to pH 7 with cold 2-amino-2-methyl-1,3-propanediol, was used in the assay.

Details such as the composition of the reaction mixtures are presented along with the data in the Tables. All incubations were conducted at 30°C for a period of 12 minutes unless otherwise specified. Reaction mixtures were deproteinized with perchloric acid, and inorganic phosphate determinations conducted essentially as described by Fiske-Subbarow (4).

Results and discussion. Experiments, summarized in Tables I and II respectively, clearly demonstrate inhibitory effects of aminonucleoside on both dinitrophenol (DNP) and calcium-ion activated ATPase of rat kidney mitochondria. The concentration of Ca^{++} was that required for maximum activation of kidney mitochondrial ATPase, assayed as indicated.

It is of course a well-known experimental fact that mitochondrial ATPase is also activated by ageing. Table III summarizes ex-

TABLE I. *In vitro* Effects of Aminonucleoside on DNP Activated ATPase of Normal Rat Kidney Mitochondria.*

Aminonucleoside concentration, mM	ATPase activity, μM P_i/mg mitochondria N	% activity
None	11.13	100
.5	10.35	93
1.0	10.42	93.6
2.5	9.19	82.5
5.0	8.08	72.5
10.0	7.04	63.2
20.0	6.72	60.4

* Assay: Tris-acetate buffer, pH 7.4, .05 M; DNP, 30 μM; ATP, 5 mM; sucrose, .25 M; mitochondria, 80 mg equivalent.

TABLE II. *In vitro* Effects of Aminonucleoside on Calcium Activated ATPase of Normal Rat Kidney Mitochondria.*

Aminonucleoside concentration, mM	ATPase activity, μM P_i/mg mitochondria N	% activity
None	13.54	100
.5	12.79	94.5
1.0	12.68	93.5
2.5	12.15	89.7
5.0	12.20	90.0
10.0	11.67	85.2
20.0	10.33	76.3

* Assay: Tris-acetate buffer, pH 7.4, .05 M; Ca^{++}, 5 mM; ATP, 5 mM; sucrose, .25 M; mitochondria, 80 mg equivalent.

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† On leave-of-absence from Dept. of Physiology, Wakayama Medical College, Wakayama, Japan.

TABLE III. Effects of Aminonucleoside on ATPase Activity of Fresh and Aged Normal Rat Kidney Mitochondria.*

Preincubation period, min	Activity, μM P_i/mg mitochondria N: No aminonucleoside	5 mM aminonucleoside
0	.56	2.27
5	2.61	2.86
10	2.20	2.29
20	2.27	2.07
30	2.52	2.37
40	3.39	2.22
60	2.86	2.47

* Assay: Tris-acetate buffer, pH 7.4, .05 M; ATP, 4.37 mM; sucrose, .25 M; mitochondria, 83 mg equivalent.

periments in which *in vitro* effects of aminonucleoside on the ATPase activity of rat kidney mitochondria, aged by preincubation at 30°C for varying periods of time, were investigated. Of special interest is the finding that ATPase activity of freshly prepared rat kidney mitochondria, assayed in the absence of exogenously added activating agents, is actually stimulated by 5 mM aminonucleoside. This effect, still apparent following a 5-minute period of ageing, virtually disappears after 10 minutes, and indeed, following longer periods of ageing, is reversed. Further study of the activating effect of aminonucleoside on rat kidney mitochondrial ATPase not only confirms this effect but also reveals what appears to be a polyphasic response of ATPase to varying aminonucleoside concentrations. Thus in Table IV, maximal activation of ATPase activity is noted at an aminonucleoside concentration of 0.5 mM, followed by progressive reduction as the concentration of aminonucleoside is increased to 5 mM, and, finally another phase of increased activity as the aminonucleoside concentration is increased to 10 and 20 mM.

TABLE IV. *In vitro* Effects of Aminonucleoside on ATPase Activity of Fresh Normal Rat Kidney Mitochondria.*

Aminonucleoside concentration, mM	ATPase activity, μM P_i/mg mitochondria N	% activity
None	2.30	100
.5	3.00	130
1.0	2.84	123
2.5	2.66	115
5.0	2.44	105
10.0	2.89	125
20.0	3.25	141

* Assay: Tris-acetate buffer, pH 7.4, .05 M; ATP, 5 mM; sucrose, .25 M; mitochondria, 80 mg equivalent. Mitochondria washed one time.

Effects of aminonucleoside on fresh and aged normal rat kidney mitochondrial ATPase activated by Mg^{++}, alone or in combination with Na^+ and K^+, were also investigated. Data are summarized in Table V. The striking similarity of the effects of 5 mM aminonucleoside on Mg^{++}-activated ATPase to those for the same lot of mitochondria assayed in the absence of exogenous activating ions (Table III) are most interesting. Thus, for example, Mg^{++}-activated ATPase activity of normal rat kidney mitochondria, aged for periods as long as 10 minutes at 30°C, appears to be enhanced in the presence of 5 mM aminonucleoside, while that of the same lot of mitochondria aged 20 to 60 minutes is inhibited. *A priori* it thus seems tempting to speculate that kidney mitochondrial ATPase, assayed in the absence of exogenous activating ions, may be identical with Mg^{++}-activated ATPase, there being sufficient endogenous Mg^{++} present in the mitochondria to provide partial activation. In the case of the multiple-ion-activated ATPase (*i.e.*, $Mg^{++} + Na^+ + K^+$ activated), on the other hand, the effects, if any, during the 10-minute ageing period appear to be largely inhibitory, as they clearly are on the mitochondria aged for 20 to 60 minute periods.

TABLE V. Effects of Aminonucleoside on Cation-Activated ATPase Activity of Fresh and Aged Normal Rat Kidney Mitochondria.*

Preincubation period, min	ATPase activity, μM P_i/mg mitochondria N: Mg^{++} activated — Aminonucleoside: None	5 mM	$Mg^{++} + Na^+ + K^+$ — Aminonucleoside: None	5 mM
0	5.79	5.86	10.49	9.68
5	6.79	7.11	10.41	10.39
10	7.26	8.24	11.12	11.05
20	8.11	7.94	12.61	10.68
30	9.75	9.71	12.78	11.76
40	11.32	10.10	13.32	12.25
60	11.54	10.05	12.64	11.32

* Assay: Tris-acetate buffer, pH 7.4, .05 M; ATP, 4.37 mM; sucrose, .25 M; Mg^{++}, 6 mM; Na^+, 100 mM; K^+, 15 mM; mitochondria, 83 mg equivalent.

From the results of the studies summarized in Tables I-V of this paper and those previously reported, indicating aminonucleoside-inhibition of partially purified submitochondrial preparations of ATPase(2), it now seems quite evident that the inhibitory effects of aminonucleoside are dependent upon activation and structural state of the mitochondria. Not so clear, however, is the significance of the inhibitory effect, particularly in view of aminonucleoside-activation of the ATPase of freshly prepared kidney mitochondria, assayed in the absence of commonly added activating agents, or even in the presence of Mg^{++} when mitochondrial ageing is for periods of less than 10 minutes. From these studies, however, fairly well-defined requirements for aminonucleoside activation and inhibition have emerged. Thus aminonucleoside-inhibition of normal rat kidney mitochondrial ATPase is readily demonstrable with mitochondrial preparations in which latent ATPase has been released either by ageing, disruption of the mitochondria, or the presence of activating agents. Generally speaking, mitochondria subjected to such procedures become loosely coupled or even completely uncoupled in respect to phosphorylating capability. Activating effects of aminonucleoside, on the other hand, appear to be more readily demonstrable with freshly prepared mitochondria, which exhibit relatively low ATPase activity and are tightly coupled in respect to phosphorylating capability. Since the latter might therefore be considered to more nearly represent the normal state, effects of aminonucleoside thereon are perhaps of greater physiological significance.

Summary. 1. *In vitro* effects of aminonucleoside on ATPase activity of normal rat kidney mitochondria, assayed in the presence and in the absence of a wide variety of activating agents, have been studied. 2. Results clearly indicate dependence of aminonucleoside inhibition of rat kidney mitochondrial ATPase on activation and structural state of the mitochondria. 3. ATPase activity of freshly prepared normal rat kidney mitochondria, assayed in the absence of commonly added exogenous activating agents, is stimulated by aminonucleoside. The latter is more readily demonstrable with kidney mitochondrial preparations showing relatively low initial ATPase activity and appears to be polyphasic in response to increasing aminonucleoside concentration.

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Some Biochemical and Serologic Properties of the Pneumococcal C Polysaccharide.* (28578)

MARIA O. HORNUNG† AND GERALD S. BERENSON
(Introduced by M. F. Shaffer)
Department of Microbiology, Tulane University School of Medicine, and Department of Medicine, Louisiana State University School of Medicine, New Orleans, La.

The appearance of C-reactive protein (CRP) in serum of humans is one of the acute phase phenomena that occur in response to many forms of stress. Although CRP is antigenically distinctive and can be detected conveniently by a specific antise-

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† N.I.H. post-doctoral fellow, Inst. of Allergy and Infect. Dis.