

# EFFECT OF PROGESTERONE ON MITOCHONDRIAL ADENOSINETRIPHOSPHATASE\*

BY RUTH WADE AND HOWARD W. JONES, JR.

(From the Department of Gynecology, The Johns Hopkins University School of Medicine, Baltimore, Maryland)

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It has previously been reported that progesterone accelerates the rate of hydrolysis of ATP<sup>1</sup> by aqueous rat liver homogenates (1). The experimental data suggested that progesterone acts directly on an enzyme or coenzyme involved in ATPase activity. This action has now been compared with the effect of DNP on the ATPase of isotonically prepared mitochondria of rat liver, and the substrate and steroid specificity have been further investigated.

## Methods

Mitochondria were prepared from livers of 21 to 24 day-old male rats by the method of Schneider and Hogeboom (2). They were washed once in cold, isotonic sucrose, recentrifuged, and suspended in 1 ml. of 0.25 M sucrose per 50 mg. of fresh liver. The ATPase activity was measured at 31°, pH 7.4, in a medium containing 6  $\mu$ moles of ATP, 2 per cent  $\gamma$ -globulin, 30  $\mu$ moles of Tris buffer, 100  $\mu$ moles of KCl, 5  $\mu$ moles of MgCl<sub>2</sub>, 0.025 ml. of aqueous progesterone solvent,<sup>2</sup> and mitochondria from 25 mg. of rat liver. The final volume was 2.5 ml. The reactions were stopped with 5 per cent trichloroacetic acid, and, after centrifugation, 1 ml. of the supernatant fluid was taken for inorganic phosphate determination by the method of Fiske and Subbarow (3).

Sacktor has found that, in the absence of a supplementary protein in the incubation medium, degenerative changes to fly wing mitochondria began almost immediately and lysis was complete within 30 minutes of incubation (4). By the addition of an inactive protein, the physical state

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<sup>1</sup> The following abbreviations are used: ATP, adenosine triphosphate, ADP, adenosine diphosphate, AMP, adenosine monophosphate, ATPase, adenosinetriphosphatase, DNP, 2,4-dinitrophenol, Tris, tris(hydroxymethyl)aminomethane.

<sup>2</sup> Supplied by E. R. Squibb and Sons, New York, in collaboration with Schering-Kahlbaum, A. G., Berlin, West Germany. The composition of the solvent is as follows: progesterone 20 mg., ethyl urea 440 mg., ethyl urethane 255 mg., and propylene glycol 200 mg. These ingredients are dissolved in distilled water with a final volume of 1 ml.

of the mitochondria may be preserved for a longer period of time. In our experiments it was found that 2 per cent  $\gamma$ -globulin was a very satisfactory protein for this purpose. Also, when  $\gamma$ -globulin is added, the progesterone becomes much more soluble. Control tubes contained the aqueous solvent without progesterone.

### Results

*ATPase of Fresh and Aged Mitochondria*—In previous work with aged rat liver homogenates prepared in water from frozen tissue, the ATPase activity was found to be constant with each preparation (1). Under these

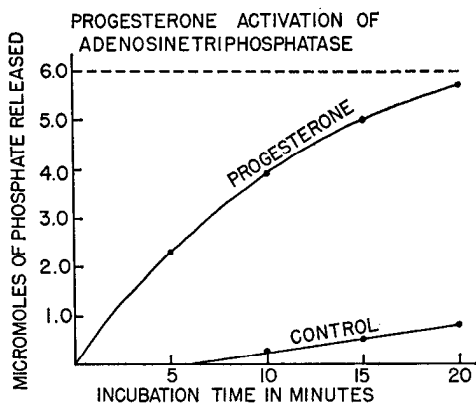


FIG. 1. The incubation medium contained 6  $\mu$ moles of ATP, 2 per cent  $\gamma$ -globulin, 30  $\mu$ moles of Tris buffer, 100  $\mu$ moles of KCl, 5  $\mu$ moles of  $MgCl_2$ , 0.025 ml. of aqueous progesterone solvent, and mitochondria from 25 mg. of rat liver. The final volume was 2.5 ml., pH 7.4, temperature 31°. Progesterone concentration was  $6.4 \times 10^{-4}$  M.

conditions it was found that the acceleration by progesterone of the reaction was proportional to the hormone concentration between  $2 \times 10^{-4}$  M and  $6 \times 10^{-4}$  M progesterone. With  $6 \times 10^{-4}$  M progesterone, the greatest concentration which could be kept in solution, there was a 65 per cent increase in enzyme activity over the control.

On the other hand, in fresh, isotonicity prepared mitochondria, it has been observed that ATPase is "latent" and exhibits no activity during the first 10 minutes of incubation time (5). With the addition of  $6 \times 10^{-4}$  M progesterone to the reaction mixture, the ATPase activity is released immediately and is greatly enhanced (Fig. 1). The degree of acceleration is much greater in fresh mitochondria than in aged preparations and is a function of the state of "latency." However, even with aged mitochondria, as previously reported (exposed to a hypotonic condition, frozen, and thawed to break mitochondrial membranes) (1), progesterone will still

produce an acceleration. This would seem to indicate that progesterone is not activating ATPase by affecting membrane permeability.

TABLE I  
*Relation of Progesterone Concentration to Adenosinetriphosphatase Activity*

Progesterone concentration	Phosphate liberation
<i>molarity</i>	<i>μmoles</i>
None	0.8
$2 \times 10^{-5}$	1.2
$4 \times 10^{-5}$	1.5
$1 \times 10^{-4}$	2.4
$2 \times 10^{-4}$	3.7
$4 \times 10^{-4}$	4.8
$6 \times 10^{-4}$	5.4

The incubation medium contained 6  $\mu$ moles of ATP, 2 per cent  $\gamma$ -globulin, 30  $\mu$ moles of Tris buffer, 100  $\mu$ moles of KCl, 5  $\mu$ moles of  $MgCl_2$ , 0.025 ml. of aqueous progesterone solvent, and mitochondria from 25 mg. of rat liver. The final volume was 2.5 ml., pH 7.4, and the temperature 31°; incubation time 20 minutes.

TABLE II  
*Comparison of Progesterone with DNP As ATPase Activator*

Experiment No.	Conditions	Phosphate liberation	Experiment No.	Conditions	Phosphate liberation
		<i>μmoles</i>			<i>μmoles</i>
1	Fresh mitochondria	1.06	3	Fresh mitochondria,	0.96
	“ “	3.27		propylene glycol	
	progesterone			Fresh mitochondria,	2.96
	Fresh mitochondria,	2.60		progesterone	
	DNP			Fresh mitochondria,	1.06
2	Fresh mitochondria,	0.57	4	DNP	
	KF			Aged mitochondria	4.30
	Fresh mitochondria,	0.87		“ “	5.63
	progesterone			progesterone	
	Fresh mitochondria,	0.85		Aged mitochondria,	4.45
	DNP			DNP	

All the tubes contained 30  $\mu$ moles of Tris buffer, 100  $\mu$ moles of KCl, 5  $\mu$ moles of  $MgCl_2$ , 6  $\mu$ moles of ATP, 2 per cent  $\gamma$ -globulin, 0.025 ml. of progesterone solvent, and mitochondria from 15 mg. of rat liver. The final volume was 2.5 ml. and the incubation time 20 minutes at 31°. Final concentration of progesterone was  $6 \times 10^{-4}$  M, of DNP  $9 \times 10^{-5}$  M, of KF  $6 \times 10^{-3}$  M, and of propylene glycol 4 per cent.

As shown in Fig. 1, the rate of the progesterone-stimulated hydrolysis of ATP decreases with time. This may be explained by the accumulation of ADP which inhibits ATPase (6) as well as by decrease in substrate concentration.

With isotonic mitochondria, the activating effect of progesterone is detectable at much smaller concentrations of the hormone than it is with the aged preparations previously studied. According to the data in Table I, the ATPase activity is proportional to the progesterone concentration between  $2 \times 10^{-5}$  M and  $6 \times 10^{-4}$  M progesterone. Greater concentrations could not be kept in solution in the incubation medium.

*Specificity of Progesterone*—The effect of progesterone on the enzymatic hydrolysis of some other phosphorylated intermediates was tested by replacing ATP with equimolar concentrations of AMP, ADP, and  $\beta$ -glycerophosphate. Progesterone had no effect on the rate of phosphate liberation from any of these substrates in the presence of freshly prepared mitochondria.

The effect of other steroid hormones on ATPase activity of mitochondria was investigated by substituting them in equimolar concentrations for progesterone in the incubation medium. Cholesterol, estradiol, testosterone,  $17\alpha$ -hydroxyprogesterone, and pregnanediol dissolved in the progesterone solvent had no effect in either fresh or aged mitochondrial preparations.

*Progesterone and DNP*—In Table II are recorded the results of various experiments comparing progesterone with DNP as an ATPase activator in both fresh and “aged” mitochondria. One major difference was found; although both progesterone and DNP activate ATPase in fresh mitochondria, progesterone is a much more effective activator than DNP in “aged” preparations. Also, if an incubation medium containing 17 per cent propylene glycol is used to dissolve the progesterone instead of  $\gamma$ -globulin and an aqueous solvent, progesterone greatly accelerates ATPase activity, while DNP has no effect.

In fresh, isotonic mitochondria, the effects of progesterone and DNP are similar. If fluoride is used to inhibit ATPase activity, it also inhibits the effects of progesterone and DNP. Also, if ADP is used as an inhibitor of ATPase, the effects of progesterone and DNP are proportionally inhibited.

#### SUMMARY

1. Progesterone accelerates the rate of hydrolysis of adenosine triphosphate in fresh rat liver mitochondria.
2. The increase in adenosinetriphosphatase activity is proportional to the progesterone concentration between  $2 \times 10^{-5}$  M and  $6 \times 10^{-4}$  M.
3. Progesterone does not activate the release of phosphate from adenosine monophosphate, adenosine diphosphate, or glycerophosphate.
4. Estradiol, testosterone, pregnanediol, or  $17\alpha$ -hydroxyprogesterone has no effect on mitochondrial adenosinetriphosphatase activity in this system.

5. A comparison is made of the activation of adenosinetriphosphatase by progesterone and 2,4-dinitrophenol.

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