Nocturnal Changes in Oxidative Activities of Rat Liver Mitochondria

Abstract. *Male rats kept on an artificial day-night cycle were active at night and rested during the day. The rate of succinate oxidation in liver mitochondria from these animals was 40 percent greater at night. Significant nocturnal increases in the ratio of phosphate to oxygen were obtained with citrate, α-ketoglutarate, and malate.*

Several lines of evidence suggest that the normal biochemical systems of an organism can vary periodically. For example, there are rhythms in DNA and RNA syntheses in mouse liver (*1*) and in protein and niacin syntheses in plant tissue culture (*2*). Other investigators have reported that liver mitochondria which are isolated from rodents subjected to exercise (*3*), hypothermia (*4*), hibernation (*4, 5*), high altitude (*6*), and thyroidectomy (*7*) exhibit changed oxidative activities. Such experiments demonstrate that (i) gross variations in the physiological state of the living animal are reflected in changes in a subcellular biochemical system, and (ii) that such changes survive the procedure for preparing and washing isolated mitochondria. Since rats are physically active at night and inactive by day (*8*), we have investigated periodic variations in oxidation rates and ATP (*9*) production in rat-liver mitochondria. In related studies, Glick and Bronk (*3*) have reported that oxidative changes are elicited by exercising the animals during the day. It is thus important to distinguish between those nocturnal metabolic changes related to exercise and those independent of exercise.

Table 1. Rates of succinate oxidation and P/O ratios in mitochondria prepared at noon and midnight. The conditions were: 200 μmole of sucrose; 20 μmole of phosphate, *p*H 7.0; 10 μmole of $MgCl_2$; 5 μmole of succinate; 0.5 μmole of ADP; 2 ml total volume; temperature 30°C; 0.21 to 0.39 mg of mitochondrial nitrogen per incubation. Each value reported is the mean of observations with four animals. Percentage changes occurring at night are given in parentheses.

Time	Oxidation rate (μatom O per min per mg N)	P/O ratio (μmole ADP per μatom O)
Noon	0.752	1.91
Midnight	1.064 (+41)*	1.90 (−1)

* $p < 0.01$

Male Wistar rats were kept in pairs, matched according to weight, and were permitted food and water as desired for at least 1 week prior to being killed. They were maintained on a cycle of 12 hours in light (day) and 12 hours in dark (night), with the light period commencing at about 6:00 A.M. In some cases both rats were placed in an activity cage for several days, and kymograph recordings from the cage demonstrated that the animals were active only at night. When the animals were killed, they weighed between 157 and 262 g. In each pair one rat served as a control and was sacrificed during the day, while the other rat was sacrificed at night exactly 12 hours later. Liver mitochondria were prepared by the method of Kielley and Kielley (*10*). Oxygen uptake was measured polarigraphically with the Clark oxygen electrode. Ratios of phosphate to oxygen (P/O) were obtained by the technique of Chance and Williams (*11*). Mitochondrial nitrogen was determined by a modified micro-Kjeldahl method.

The results (Table 1) show that the rates of succinate oxidation in mitochondria from animals killed at midnight were significantly higher than in mitochondria from animals sacrificed at noon. The P/O ratios were similar for both groups, indicating that the apparent rate of ATP production increased to the same extent as the rate of oxygen uptake.

A study with other substrates is shown in Table 2. Four pairs of animals were used, but members of each pair were sacrificed according to the following schedule: 9:00 A.M. and P.M.; 11:00 A.M. and P.M.; 1:00 P.M. and A.M.; 3:00 P.M. and A.M. Again nocturnal increases in the rates of oxygen uptake were observed, but statistical significance was noted only for succinate oxidation. The P/O ratios, however, were significantly increased at night with all substrates except succinate.

The differences between preparations of nighttime and daytime mitochondria may be compared with the differences between mitochondria from exercised and nonexercised rats. In both nighttime rats (see Table 2) and exercised rats (*3*) the percentage increases in the rates of oxidation of those substrates with NAD-linked dehydrogenases were highest for malate, intermediate for citrate, and lowest for α-ketoglutarate. This suggests that one of the biochemical mechanisms responsible for the nocturnal changes may be similar to that operating in exercised animals, in which an increase in NAD apparently activates the rate-limiting dehydrogenase steps (*12*). The finding that the elevated rates of oxidation at night were not as high nor statistically significant, for the number of animals used, as those from rats exercised by day was not unexpected. When rats are killed at night, they may by chance be eating or temporarily resting between exercise periods. However, when animals are forced to exercise continuously for 3 hours in a motor-driven activity cage, they are subjected to physical demands much greater than those normally occurring at night, and they are always sacrificed at a peak of exercise.

Table 2. Oxidation rates and P/O ratios with various substrates in mitochondria prepared at intervals throughout the day and night. The conditions were the same as in Table 1, except for the following: 10 μmole of either α-ketoglutarate, citrate, or malate when used; 1.0 μmole of ADP; 0.66 to 0.76 mg of nitrogen for each incubation. Each value reported is the mean of observations with four animals. Percentage changes occurring at night are given in parentheses.

Time	Oxidation rate (μatom O per min per mg N)	P/O ratio (μmole ADP per μatom O)
	Succinate	
Day	2.326	1.79
Night	3.224 (+39)*	1.72 (−4)
	Malate	
Day	0.247	1.62
Night	0.346 (+40)	2.06 (+27)*
	Citrate	
Day	0.468	1.99
Night	0.592 (+26)	2.54 (+28)*
	α-Ketoglutarate	
Day	0.899	2.38
Night	0.986 (+10)	2.81 (+18)*

* $p < 0.05$

The nocturnal enhancement of the rate of succinate oxidation is probably not the result of exercise alone. The relatively high increase of 40 percent was not observed with mitochondria from exercised animals, in which the percentage increase with succinate was among the lowest of all the substrates tested (*3*). Since succinic dehydrogenase is linked to the electron-transport chain by way of flavoprotein instead of pyridine nucleotide, control of succinate oxidation may differ from that

of other substrates. It may be noted that Nielson and Klitgaard (*13*) have suggested the existence of a biological rhythm for succinate oxidation in tissue homogenates from starved rats.

There are no changes in P/O ratios in mitochondria from artificially exercised animals (*3*), so that nocturnal increases in these ratios seem to be part of a rhythm independent of physical activity. The fact that the P/O ratio for succinate alone was unaffected at night implies that the regulation of P/O ratios for the other substrates occurs at the site of phosphorylation associated with NAD. Perhaps adenosine triphosphatase activity, in which an intermediate of oxidative phosphorylation is destroyed, is relatively low at night; ATP production might accelerate. At daybreak large concentrations of ATP or an ATP derivative would either stimulate the enzyme activity directly or inactivate an inhibitor of this enzyme; P/O ratios would fall. By evening, the utilization of excess ATP for other purposes would result in diminished adenosine triphosphatase activity. It should be emphasized that other intracellular feedback systems might regulate P/O ratios. In addition, the hormonal output of animals at night presumably differs from that during the day (*14*), so that there may be many complicating extracellular influences on intracellular rhythms.

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Following and Imprinting: Effects of Light and Social Experience

Abstract. *Ninety-six Vantress broiler chicks were used in two studies of the effects of light and social experience on the "following" response. In addition, the animals used in one study were later tested to determine the amount of imprinting during following. The difference between the behavior of these animals and animals maintained in isolation and in darkness prior to exposure to a model, indicate that variability in treatment can influence the following behavior, and that following in itself cannot be equated with imprinting.*

The variability in techniques and procedures used in the study of imprinting has increased greatly with the large number of experimenters now working on the same subject. This would not be a problem if there were not the tendency to relate the results of one study to those of another without taking these differences in procedure into consideration.

The maintenance of the animals prior to the initial exposure to a model is one of the areas of variability in imprinting procedures. Animals have been kept isolated in the dark (*1–3*), isolated in the light (*4, 5*), and maintained in groups in the light up to the time of imprinting (*6*). The two short studies in this report were designed to show the effects of light and group experience on the "following" response in an effort to indicate the impossibility of making direct comparisons between the results of two individual experimenters when each has used a different procedure. While this report deals only with the variable of how the animals are treated before imprinting, it would be expected that differences in behavior would be found where different procedures have been used regarding the use of sound, speed of model, and species of animal.

In the first study, 56 Vantress broiler chicks hatched in this laboratory were divided into four groups of 12, 14, 14, and 16. Within 2 hours after hatching in the darkroom, all chicks were removed from the incubator and placed individually in cardboard boxes measuring 11.5 by 11.5 by 14 cm. The chicks remained in these boxes until used experimentally.

One group of 12 chicks and another of 14 chicks were exposed to a model at 16 hours after hatching, and one group of 14 and one group of 16 chicks were exposed to the same model at 48 hours. The control groups ($N = 14$) were given no experience prior to imprinting. The animals in the experimental groups ($N = 12$, $N = 16$) were treated in exactly the same way except that they were placed individually in a wooden isolation box, 10 by 25.5 by 30.5 cm, for 2 hours before imprinting, with a 100-watt bulb suspended over the box.

All animals were run in the Hess imprinting apparatus, described in detail elsewhere (*7*). The model used was a blue ball (Ostwaldpa 14) 20 cm in diameter. A speaker inside the ball, connected to a conventional tape recorder, provided a continuous and rhythmic call: "Come-chick-chick-chick." The model moved 30.5 cm in 6 seconds and was stationary for 12 seconds, making one turn around the 3-m runway in 3 minutes.

The experimental animals were returned to their cardboard boxes for transportation to the imprinting room. With the room in darkness the chick was eased onto the runway next to the model. The experimenter took his place behind the control panel where the chicks could be observed through a one-way screen. The lights and sound of the apparatus were turned on. The model remained stationary for 10 minutes, then made four turns around the runway. The control animals were run in the same way. Table 1 shows the mean distance followed by each of the four groups. The effect of the light experience on the chicks at 16 hours was slight. There was also little difference between the groups given light experience at 16 hours and at 48 hours. There was, however, significantly more following by the animals given light experience at 48 hours than by the controls at 48 hours ($p < .05$, Mann-Whitney U-Test) (*8*). There was also significantly more following by the 16-hour-old controls than by the 48-hour-old controls ($p < .05$, Mann-Whitney U-Test).

In the second study, four groups of ten Vantress broiler chicks were used. One control group was imprinted at