The Effect of Light and Darkness on Lactic Acid Content of the Pineal Gland (34495)

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A general reduction in the metabolic processes of the pineal gland has been shown to occur in rats exposed to continuous light. A decrease in its weight (1), serotonin (2), melatonin (3), lipid (4), and glycogen content (5), and the activity of a number of its enzymes, was found (5–8).

In a study carried out recently by us (9) it has been shown that continuous light inhibits the physiological parenchymal hypertrophy of the pineal which occurs during the first 10 weeks of life, supposedly by inhibiting ribosomal RNA synthesis of the cells. Reduced protein and enzyme synthesis evidently causes a decrease in the metabolic activity of the cells.

In the present work we have investigated whether there may be any concurrent changes in the main metabolic pathway supplying energy in the pineal for cellular synthesis. Such a study seemed of special interest in view of the findings of Bostelmann (8), which suggest that exposure to continuous light causes a decrease in the activity of pineal oxidative enzymes, whereas darkness has the reverse effect.

A change in energy production may result from either depressed protein metabolism, found to occur upon exposure to continuous light (9), or, be brought about directly, through interference with a specific energy pathway, which could consequently cause a reduction in protein synthesis (10).

The cellular redox state can be used for measuring tissue hypoxia which would produce an accumulation of lactate (11). We are reporting the pineal lactate concentration of maturing female rats exposed to the same conditions as those which caused a decrease in RNA and protein synthesis (9). This would indicate whether the decreased protein and enzyme synthesis is connected with a change in cell metabolism and energy formation. Moreover, information may be obtained as to whether any change in cell metabolism and energy formation is a consequence of impaired RNA-protein synthesis found to occur in the pinealocytes, or is brought about directly through interference with a specific energy pathway, the resulting energy deficiency causing a decrease in protein-synthesizing ability.

Materials and Methods. Female rats of the Hebrew University's "Sabra" strain, weighing 35–45 g each, were weaned when 21 days old, housed six to a cage at a constant temperature of 24 ± 1°, and fed *ad libitum*. The animals were divided into three groups: one was exposed to constant artificial light; another kept in total darkness; and a third, serving as control, was subjected to alternating light and darkness, the light being switched on at 7:30 AM and off at 7:30 PM each day. The light was provided by overhead fluorescent tubes (40 W "daylight"). In the darkroom faint red light was used when feeding the animals.

After 10, 20, and 30 days of exposure to the different conditions of light, *i.e.*, on Days 31, 41, and 51 of life, the animals were killed by neck fracture, weighed, and their pineal glands removed and instantly frozen by liquid nitrogen. Six frozen pineals from the rats in each cage were pooled in a glass test tube and homogenized under cooling in 0.4 ml of bidistilled water. An aliquot of 0.04 ml of the suspension was withdrawn for protein determination, and the remainder, after dilution with percholoric acid to an optimal concentration of 0.6 *M* (approximately 0.4 ml 1.2 *M*), was used to determine the lactic acid. The suspension was centrifuged at 4500 rpm

TABLE I. Effect of Light and Darkness on Pineal Lactic Acid Content.[a]

Light exposure	Lactic acid (μM/pineal gland ± SE of the mean)		
	Days of exposure		
	10	20	30
Alternating light (control)	(27) 12.3 ± 0.71	(34) 12.8 ± 0.41	(19) 12.3 ± 1.29
Continuous light	(30) 13.4 ± 0.60	(35) 16.9 ± 1.05[b]	(20) 18.4 ± 0.79[b]
Continuous darkness	(27) 14.7 ± 1.09	(34) 12.9 ± 0.50	(20) 12.0 ± 1.03

[a] Six pineals were pooled for one assay. The number of assays comprised in each result is shown in parentheses. The animals were exposed to the different conditions of light for 10, 20, and 30 days respectively, *i.e.*, killed and examined when 31, 41, and 51 days old.

[b] $p < .001$.

for 20 min, and the deproteinized fluid was divided into two equal aliquots. After neutralization the lactic acid content was determined in each aliquot by the method of Hohorst (12). Measurement was made in a final volume of 3 ml at 340 mμ using a Zeiss spectrophotometer. All enzymes and coenzymes used were obtained from C. F. Boehringer (Mannheim).

The method of Lowry *et al.* (13) was employed for protein determination, using a sample of 0.04 ml of the native suspension.

A full experiment with controls comprised nine cages of six rats each (three cages from each group) and was carried out in the course of 1 day. The experiments were repeated until a total of at least 120 animals from each group had been examined for each of the respective periods of exposure, *i.e.*, 10, 20, and 30 days.

Results. The results of the lactic acid determination in the pineals of rats exposed to continuous light and darkness are summarized in Table I. A 10-day exposure to continuous light, although indicating a trend toward increased pineal lactic acid levels, did not produce significant change. Exposure of the animals to continuous light for 20 days, however, resulted in a considerable increase (about 26%) in their pineal lactic acid content ($p < .001$), when compared with the control group kept under conditions of alternating light and darkness. By prolonging the exposure to continuous light to 30 days an even further increase in the pineal lactic acid level was produced, reaching a concentration of 18.4 μM per pineal, against a 12.3 μM physiological level in the controls ($p < .001$).

No change occurred throughout the duration of the experiment in the pineal lactic acid levels of either the control animals or those exposed to continuous darkness (Table I).

Table II shows that the quantity of lactic acid, when calculated per microgram of pineal protein is significantly increased after a

TABLE II. Effect of Light and Darkness on Pineal Lactic Acid Content Expressed per Pineal Protein Concentration.[a]

Light exposure	Lactic acid (μM/μg protein/pineal gland ± SE of the mean)		
	Days of exposure		
	10	20	30
Alternating light (control)	0.140 ± 0.009	0.114 ± 0.006	0.113 ± 0.013
Continuous light	0.186 ± 0.010[c]	0.183 ± 0.016[b]	0.186 ± 0.010[b]
Continuous darkness	0.162 ± 0.014	0.115 ± 0.005	0.106 ± 0.010

[a] For details (*e.g.*, number of assays) see Table I.

[b] $p < .001$.

[c] $p < .002$.

10-day exposure to continuous light ($p <$ .002). This is due to reduced protein levels which occur after 10 days' exposure to continuous light (9). The difference between the pineal lactic acid/protein ratios of the control animals and of those exposed to continuous light was further enhanced by extending the experiment to 20 and 30 days (Table II).

The ratio of pineal lactic acid to its protein in the animals exposed to continuous light remained constant throughout the experimental period, notwithstanding the rise in lactic acid levels (Table II). This is explained by the fact that there is a physiological development of the pineal protein throughout the entire 30-day experimental period, the result being that, in spite of the inhibition of pineal protein formation in animals exposed to continuous light, there is still an increase in the absolute level of the protein (9). Moreover, the pineal lactic acid content will increase according to the protein-synthesizing activity allowed by the conditions of continuous light.

There was no difference between the lactic acid/protein ratios of the animals exposed to continuous darkness and those of the controls (Table II).

Discussion. Following our previous findings that exposure of rats to continuous light brought about a significant inhibition in pineal protein synthesis, we postulated that continuous light causes a decrease in the metabolic activity of the cells. Reduced cellular metabolism will result in decreased oxygen supply to the interior of living cells. If the supply of oxygen is insufficient to meet current metabolic tissue demands, the cellular oxidation-reduction systems must shift toward a reduced state. When the oxidative potential in the hypoxic cell has diminished to the level of that of the metabolic systems, the DPN-coupled (*i.e.*, lactic dehydrogenase) system will be one of the first to become reversed.

$$\text{Pyruvate} - \text{DPNH}_2 \overset{\text{LDH}}{\rightleftarrows} \text{Lactate} - \text{DPN}$$

The end product of reduction in the LDH system is lactate, which has no other function in metabolism. Endogenous lactate does not participate in any other equilibrium or enter any other reaction which would be affected by its accumulation in the cell. It would, therefore, be expected that any alteration in oxygen supply would be reflected in lactate changes. A low supply of oxygen to tissues will result in an increase in lactic acid production. Glycogen-glucose breakdown proceeds via pyruvate to lactate which accumulates in the cell. Hence in all probability, pyruvate oxidation is depressed owing to a decline in the cytochromes in the tissues which may be accounted for by the slower rate of energy utilization observed in the pineals of rats during anoxia. Indeed, such a hypoxia-like state has been shown to exist in the pineals of rats exposed to continuous light.

This agrees with the findings of Quay (5), who established a significantly low respiration in pineals of rats exposed to continuous light, and Bostelmann (8), who found a strong diminution in the activity of several pineal oxidative enzymes, *i.e.*, cytochrome *c*-oxidase, succinic dehydrogenase, NADH oxidase, and others. Quay's finding of a considerable reduction in pineal glycogen content (58% in 12 weeks) (5) also concurs with our postulation of the existence of a hypoxic or hypoxia-like condition in the pineals of young female rats kept under continuous light. This status seems to be a consequence of the decreased synthesis of protein by the pineal which has been observed in rats exposed to continuous light and most probably results from depression of the cytochrome oxidase in the tissues through slower rates of energy utilization.

Definite proof that the suppressed oxidative cell processes are a consequence of depressed RNA and protein metabolism is the fact that the changes in protein content occur prior to those in the oxidative pathway. A significant decrease in the pineal protein content was already evident after 10 days' exposure to continuous light, whereas the increase in lactic acid was not significant until Day 20. These findings seem to have settled the dispute between the stands of Bostelmann (8) and Quay (5) as to whether the

oxidative cell suppression is a primary or secondary effect of light.

As in the case of protein, continuous darkness was found to have no effect on the lactic acid levels of the pineal throughout the duration of the experiment. It is interesting to note, however, that Bostelmann (8), using histochemical procedures, reported that when maturing rats were exposed to continuous darkness for as long as 11 weeks an increase in pineal oxidative metabolism could be observed.

The parallel effects of light and darkness on the pineal lactic acid and nucleic acid-protein metabolisms observed in our two studies, reaffirm the interrelationship between the changes occurring in the two metabolic processes upon exposure of rats to continuous light.

Summary. The pineal lactic acid content was measured in maturing female rats kept under conditions of continuous light, continuous darkness, and alternating light and darkness (controls) from the day of weaning (at 21 days of age) for 10, 20, and 30 days.

A significant increase in the pineal lactate levels of the animals kept in continuous light was observed after 20 and 30 days when compared with those of the controls. When calculated per microgram of pineal protein, the increase in pineal lactate was already significant after 10 days' exposure to continuous light.

The high lactic acid level indicates suppression of oxidative cell processes, which appears to be a consequence of the depressed protein metabolism found to occur earlier in animals exposed to continuous light.

No change took place throughout the duration of the experiment in the pineal lactate levels of the animals exposed to continuous darkness, this finding paralleling that obtained on the metabolism of pineal RNA and protein.

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Received Oct. 9, 1969. P.S.E.B.M., 1970, Vol. 133.