cyclization to yield the final lactones via intermediate cations. Inspite of many valuable reports[6] on cyclization of unconjugated polyenoic acids containing isolated carbon-carbon double bonds, only a few cases involving a conjugated dienoic acid system were described so far[7], where α, β-unsaturated γ- or δ-lactone was obtained as a product among various reaction products. It is now disclosed from our cyclization studies that the title compound undergoes double-cyclization in sulfuric acid to afford the saturated γ- and δ-lactones almost exclusively. Thus, our finding offers an important insight into the chemistry of conjugated polyenoic acids, including vitamin A acid, toward acids, and it does not seem absurd to expect that our double-cyclization mode may exist in nature.

Zusammenfassung. Es wird eine neuartige Doppel-Zyklisierungs-Reaktion an einer konjugierten Polyencarbonsäure beschrieben.

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Dark Induced Increase in Pineal Serotonin N-Acetyltransferase Activity: A Refractory Period

Rat pineal serotonin N-acetyltransferase (NATase) activity exhibits a 30–70-fold daily change when animals are kept in a 24-h light-dark cycle (LD 14:10)[1,2]. Once this rhythm was discovered, the next logical step was to study the regulation of the rhythm by examining the points of transition, dark-to-light and light-to-dark. The dark-to-light transition has previously been studied[3]; this transition produces a rapid decrease in NATase activity that is mediated by the eyes in rats. In the present report we discuss our experiments with the light-to-dark transition. We have examined 1. the importance of the timing of the light-to-dark transition, and 2. the effect of the absence of the expected light-to-dark transition.

Female Osborne-Mendel rats (NIH strain, 200 g) which had been reared from birth in a light cycle (LD 14:10) were used. We subjected groups of rats (N = 4 or 5) to light or to 3 or 6 h of darkness during their normal light-time or during their expected dark-time (Figure). To test whether the pineal system was capable of response, we injected physiological saline or a drug, DL-isoproterenol (15 mg/kg in saline), s.c. into the rats at

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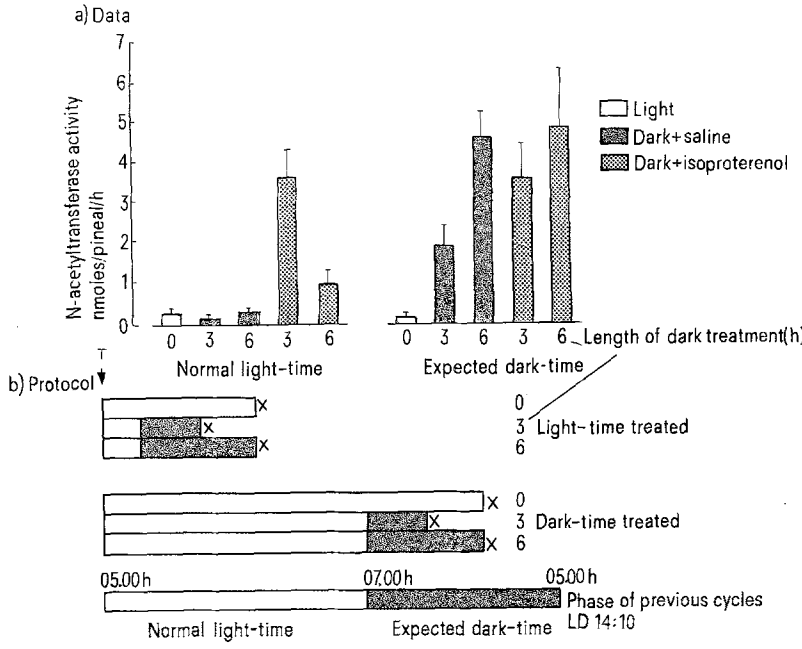

a) Data and b) protocol for NATase activity measured in pineal glands from rats which had been subjected to experimental light, dark, and isoproterenol treatments during either the normal light-time or the expected dark-time of their previous lighting regimes. In b) the lighting regime (LD14:10) in which the animals had been kept from birth is indicated by the bottom bar. Rats were treated during the normal light-time or during the expected dark-time with light, dark, saline injections, and/or drug injections. Each treatment is shown from the normal 'dawn' or dark-to-light transition at 05.00 h (T) to the time the animals were killed (X). Injections, saline or isoproterenol in saline, were given only to the dark treated animals; the injections were given just prior to the light-to-dark transition. The results of the N-acetyltransferase activity measurements are shown in a). Dark did not stimulate enzyme activity during the light-time but did stimulate NATase activity during the dark-time. High enzyme activity was not seen in the presence of light. The drug, isoproterenol, stimulated enzyme activity during either the normal light-time or during the expected dark-time. NATase activity is normally high in the dark-time and low in the light-time in rats killed at time points in an LD14:10 light cycle[1] (+ 1 standard error).

the beginning of their experimental dark period. Isoproterenol is known to increase NATase activity in rats kept in light by acting directly on their pineal glands[4]. The animals were stunned at the end of their experimental periods and then decapitated. Pineal glands were immediately dissected out and frozen on dry ice. Later the pineal glands were thawed and assayed for NATase activity[1,2,5].

Exposure to darkness increased NATase activity only during the expected dark-time. In contrast with this result, light prevented the increase in enzyme activity during the normal dark-time which is consistent with previous reports of light suppression of the activity of the enzyme[1]. Injections of isoproterenol during either the normal light-time or the expected dark-time caused a marked increase in enzyme activity at 3 h. There was a difference in the response to isoproterenol treatment at 6 h which depended on the expected lighting. This difference could be due to darkness alone because there was no difference between the dark treated group and the isoproterenol group at this time.

Results similar to ours have been reported by QUAY[6] who examined the responses of rat pineal serotonin to changes in lighting conditions. He found that light would prevent the nocturnal drop in pineal serotonin, and that the drop in serotonin could be stimulated by darkness falling in a 4-h time span near start of the expected dark-time. The similarity of our NATase results to the serotonin results of QUAY is not surprising because circadian changes in rat pineal serotonin appear to be regulated by changes in pineal serotonin NATase activity[1]. Responses similar to those we obtained with NATase have been reported by DEGUCHI and AXELROD[4]. Our experiments differ from theirs in that we studied the effect of isoproterenol in the same experiment, we kept isoproterenol-treated rats in the dark, our dark-time and light-time treatments were identical, and we used serotonin as a substrate for NATase.

We conclude that there is a sensitive period during which dark can stimulate NATase activity. This period coincides roughly with the expected dark-time. Conversely, there is a 'refractory' period coincident with the normal light-time during which dark evokes no response. However, the mechanism for this refractory period probably is not located in the pineal gland because isoproterenol was effective during the light-time and dark-time. This drug acts directly on the pineal gland; it is effective in animals with denervated pineal glands[4] and in organ culture[7]. The mechanism for the refractory period probably lies more centrally in the nervous system for the following reasons: The rhythm in NATase relies upon intact adrenergic innervation from the superior cervical ganglia to the pineal gland and neural input to the ganglia[8]; and recent studies indicate that lesions of the central nervous system (specifically in the medial forefrain bundle and in the suprachiasmatic nucleus) also block the rhythm in NATase which means that the driving input to the superior cervical ganglion may pass through these structures and perhaps originate in one of them[9].

The rhythm in NATase activity is clearly endogenous in origin because it persists in blinded rats or in rats kept in constant darkness (DD)[1]. It follows from this that in the absence of light the pineal gland periodically receives stimulatory signals from the central controlling system. The results of the experiment presented here are in agreement with the above conclusion because they show that darkness stimulates the pineal gland only when the central nervous system is receptive to stimulation. Therefore, the dark-induced increase in enzyme activity depends on the 'coincidence' of the 'external' darkness with a photosensitive phase of the endogenous system regulating pineal NATase. These data are consistent with the 'external coincidence model' of PITTENDRIGH and MINIS[10] which is an elaboration of the BÜNNING hypothesis for circadian sensitivity in photoperiodic responses[11,12]. The hypothesis states that the important factor in photoperiodic responses is when light strikes an organism relative to its endogenous cirdian rhythm rather than, within limits, the length of the light stimulus.

Zusammenfassung. Nachweis, dass Ratten, aus normaler Lichtperiode abrupt in Dunkelheit versetzt, keine Zunahme der NATase-Aktivität im Pinealorgan zeigen und dass Isoproterenol-Injektion die Zunahme der NATase auslöst.

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[14] Support was provided to S. B. by NIH Postdoctoral Fellowship No. 1 FO2 HD 52858-01.
[15] We thank W. LANGEBARTEL and M. VELTRUM.

Hypoglycemic Activity of α-Bromopalmitate in Rats

The depression of glucose utilization of muscle and the stimulation of gluconeogenesis by the liver observed in conditions of high lipid mobilization have been demonstrated to be caused by the products of fatty acid oxidation[1,2]. BURGES et al[3] demonstrated in vivo and in vitro that the fatty acid analogue, α-bromopalmitate, could inhibit fatty acid oxidation and secondarily result in increased oxidation of glucose. In this preliminary report, he mentioned that α-bromopalmitate could lower blood glucose. Subsequently, RANDLE[4] demonstrated that the resistance of heart muscle from diabetic rats to insulin in vitro could be reversed by prior perfusion with α-bromostearate. This α-bromo fatty acid increased glucose uptake, glycolysis and glucose oxidation while