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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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

Effect of α-bromopalmitate on rat glucose tolerance

Treatment	Dose		Mean blood sugar (mg/100 ml ± SE) Time after glucose (min)						
	(i.p.)	N	0	30	60	90	120	150	180
Control (4)[a]			89 ± 4	158 ± 5	135 ± 3	133 ± 9	155 ± 12	118 ± 4	118 ± 6
α-bromo palmitate	(500 mg/kg)	(4)	92 ± 5	114 ± 13[b]	105 ± 15[b]	104 ± 25	94 ± 29[b]	77 ± 23[b]	61 ± 23[b]
Control (4)			71 ± 8	147 ± 7	132 ± 5	128 ± 6	114 ± 6	105 ± 5	105 ± 11
α-bromo palmitate	(100 mg/kg)	(4)	78 ± 8	132 ± 4	115 ± 5[a]	107 ± 2[a]	98 ± 2[a]	89 ± 6[a]	94 ± 6
Control (4)			74 ± 8	125 ± 9	125 ± 4	115 ± 3	99 ± 6	94 ± 6	88 ± 4
α-bromo palmitate	(50 mg/kg)	(4)	76 ± 3	95 ± 6[b]	110 ± 8[b]	100 ± 6[b]	99 ± 3	78 ± 6	82 ± 1
Control (5)			88 ± 8	125 ± 7	117 ± 11	112 ± 4	99 ± 4	95 ± 6	96 ± 5
α-bromo palmitate	(25 mg/kg)	(5)	91 ± 2	112 ± 7	101 ± 5[b]	99 ± 6	80 ± 5[b]	85 ± 4	82 ± 6
Controls (4)			83 ± 9	175 ± 15	160 ± 7	140 ± 7	124 ± 10	114 ± 9	94 ± 12
α-bromo palmitate	(10 mg/kg)	(3)	99 ± 11	185 ± 17	177 ± 13	140 ± 7	140 ± 15	133 ± 27	94 ± 12
	(1 mg/kg)	(3)	83 ± 2	160 ± 11	151 ± 1	136 ± 5	122 ± 2	109 ± 11	99 ± 7

[a] Number in parenthesis is the number of animals per group.
[b] Significance determined by Student's t-test $p < 0.05$, all other differences were not significant.

fatty acid oxidation was suppressed. From these facts it seemed reasonable to expect that the fatty acid analogue, α-bromopalmitate might also increase glucose tolerance. Therefore, using glucose tolerance tests, the present study explores the effect of α-bromopalmitate on the tolerance of the intact rat to glucose.

Methods. Male Sprague-Dawley rats (Charles River Laboratories) of 200–230 g body wt. were used throughout. Before experiments, all animals were kept at 30–31 °C and fed ad libitum standard Wayne Chows. α-Bromopalmitic acid was purchased from K & K Laboratories, Inc. (Planview, N.Y.). Purity was checked by thin-layer chromatography on Silica gel G plates using 1:1 acetone-95% ethanol and 9:1 chloroform: acetone solvent systems. Spots were located by charring with 50% H_2SO_4. α-Bromopalmitic acid gave only a single spot in both systems and was used without further purification.

The animals were fasted for 24 h before administration of glucose ('O' time) having access to water ad libitum. Blood samples (0.1 ml) were obtained from the tail without anesthesia at 0, 30, 60, 90, 120, 150 and 180 min after oral administration of 1 g glucose/kg body wt. Specimens of blood were immediately deproteinized with $Ba(OH)_2$ and $ZnSO_4$ and glucose levels determined with glucose oxidase (Glucostat®, Worthington Biochem. Corp. USA). α-Bromopalmitic acid dissolved in 0.002 N NaOH was administered i.p. in a volume of 0.5 ml at 30 min before 'O' time. Vehicle was injected as a control.

Results and discussion. The results of the effect of α-bromopalmitate on the carbohydrate tolerance of normal intact rats is shown in the Table. α-Bromopalmitate caused a significant increase in glucose tolerance at 500, 100, 50 and 25 mg/kg, i.p. It did not affect glucose tolerance at 10 or 1 mg/kg, i.p.

A number of normal physiological and pathophysiological states characterized by high plasma free fatty acid levels have also been found to be associated with glucose intolerance. For example, diabetes mellitus[5], acromegaly[6], obesity[7], and starvation[8] have all been associated with glucose intolerance. RANDLE et al.[9,10] have proposed that increased levels of free fatty acids and ketones bring about a switch from carbohydrate to lipid metabolism and that the augmented lipid metabolism inhibits glucose oxidation in muscle and impairs the responsiveness of muscle to insulin. The present study demonstrates that fasted rats, utilizing mainly fatty acids for energy have an increased tolerance to orally administered glucose when treated with an inhibitor of fatty acid oxidation (α-bromopalmitate). α-Bromo fatty acids may thus be a useful tool for establishing the role of fatty acid oxidation in metabolic disturbances in experimental diabetes and other diseases.

Zusammenfassung. Bei Hunger-Ratten, denen vorwiegend Fettsäure als Energiespender gegeben wurde, wird eine erhöhte Toleranz gegen per os eingegebene Glukose während der Behandlung mit der die Fettsäureoxydation hemmenden α-Brompalminsäure gefunden. Bei Dosierungen von 25–500 mg/kg. i.p. verursachte α-Brompalminsäure eine signifikante Erhöhung der Glukose-Toleranz.

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