Effect of the feeding pattern on the *in vitro* oxidation of palmitate-1-C^{14} by rat liver slices

Experimental series	Feeding pattern	Diet[a]	Fasting prior sacrifice to (h)	$C^{14}O_2$ as percentage of activity added ± S.E.	P^c
I	Intermittent starvation	S	0	8.95 ± 0.61 (6)[b]	<0.001
	Continuous underfeeding	S	0	3.91 ± 0.33 (6)	
	Feeding *ad libitum*	S	0	3,78 ± 0.27 (6)	
II	Intermittent starvation	S	24	10.59 ± 0.53 (6)	<0.001
	Continuous underfeeding	S	24	2.75 ± 0.23 (5)	
	Feeding *ad libitum*	S	24	2.60 ± 0.25 (6)	
III	Intermittent starvation	S	48	11.21 ± 0.74 (6)	<0.01
	Feeding *ad libitum*	S	48	5.48 ± 0.40 (6)	
IV	Intermittent starvation	C	0	4.65 ± 0.29 (5)	<0.01
	Feeding *ad libitum*	C	0	2.90 ± 0.22 (5)	
	Intermittent starvation	HF	0	12.11 ± 1.13 (5)	<0.01
	Feeding *ad libitum*	HF	0	5.34 ± 0.52 (5)	
V	Intermittent starvation	C	16	6.46 ± 0.67 (5)	<0.01
	Feeding *ad libitum*	C	16	3.18 ± 0.74 (5)	
	Intermittent starvation	HF	16	12.18 ± 1.19 (5)	<0.001
	Feeding *ad libitum*	HF	16	4.76 ± 0.53 (5)	

[a] S = standard laboratory diet; C = control diet; HF = high-fat diet.
[b] Number of animals.
[c] Statistical significance of the difference between the intermittently starving group and the comparable *ad libitum* and continuously underfed group respectively. Differences between the *ad libitum* fed and continuously underfed groups are not significant.

The results obtained provide evidence that in addition to an increased formation of glycogen[7] and body fat[2], which are also found in other similar dietary patterns characterized by larger and infrequent meals[8], the feeding pattern used in our experiments also leads to an increased ability of the organism to oxidize available nutrients, including fatty acids. This metabolic change is further moderated by the composition of the diet, i.e. the predominant substrate available for tissue oxidation.

Zusammenfassung. Intermittierendes Hungern führt bei Albinoratten zu einer 2–3fachen Erhöhung der *in vitro* Oxydation von Palmitat-1-C^{14} bei Leberschnitten. Dies im Vergleich zu *ad libitum* gefütterten oder kontinuierlich unterernährten Tieren.

R. Petrásek, P. Fábry, and R. Poledne

Physiological Department of the Institute of Human Nutrition, Prague-Krč (Czechoslovakia), January 23, 1964.

[7] P. Fábry, Physiol. bohemoslov. *4*, 33 (1955).
[8] J. Tepperman and H. M. Tepperman, Am. J. Physiol. *193*, 55 (1958). – C. Cohn and D. Joseph, Am. J. Physiol. *196*, 965 (1959). – G. Hollifield and W. Parson, J. clin. Invest. *41*, 245 (1962).

Sleep Induced by the Administration of Melatonin (5-Methoxy-N-acetyltryptamine) to the Hypothalamus in Unrestrained Cats

The relatively high level of melatonin in the pineal gland of the mammalian brain[1] suggests that besides its inhibitory action on gonadal function[2] it may also play the role of a modulator substance within the central tryptaminoceptive structures postulated by Brodie and Shore[3]. The recent finding that it is capable of preventing thyroid hyperplasia caused by methylthiouracil[4] also suggests such a possibility.

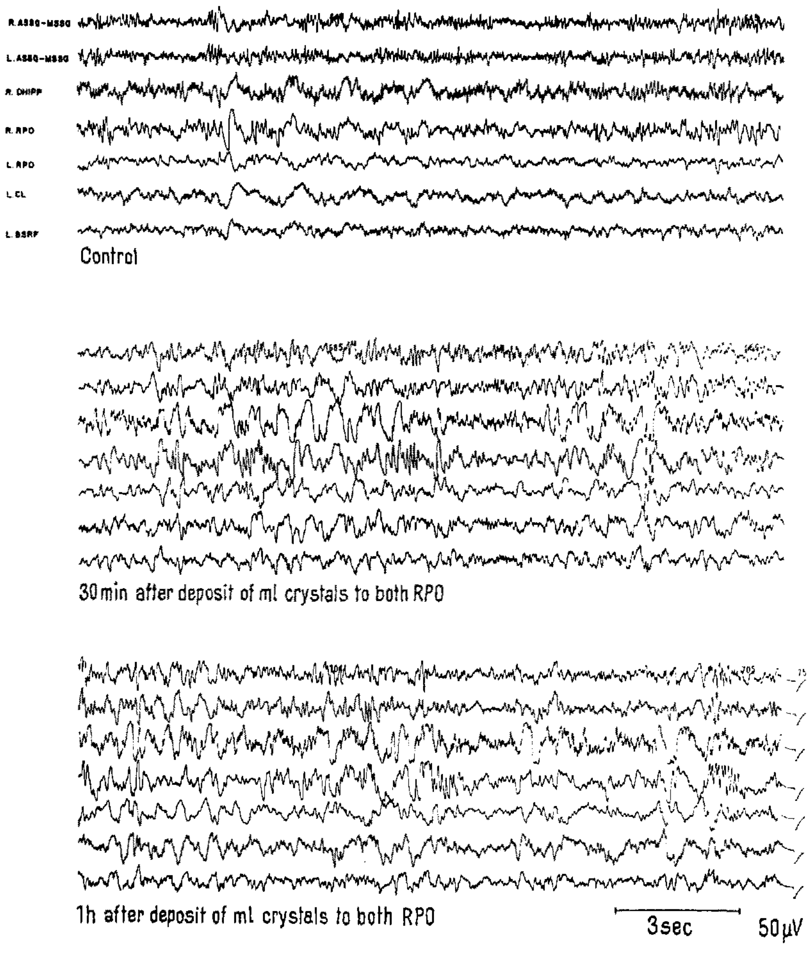
In the present study, carried out upon 11 adult cats, micro-amounts (15–30 μg) of crystalline melatonin (used as free base) where administered directly through chronically implanted stainless steel cannulae into three subcortical structures according to Jasper, Ajmone-Marsan coordinates: preoptic region (F 14.5 to 15; L 2.5 to 4; H −3 to −4), nucleus centralis medialis (F 9; L 0.0; H 0.0) and to the brain stem reticular formation (F 2 to 3.5; L 3 to 4; H −2 to −2.5). The general behavior of the animals was observed in a relatively sound-proof box and EEG recordings made simultaneously. After 3–5

[1] A. B. Lerner and J. D. Case, Fed. Proc. *19*, 590 (1960).
[2] R. J. Wurtman, J. Axelrod, and Elizabeth Chu, Science *141*, 277 (1963).
[3] B. B. Brodie and P. A. Shore, Ann. N.Y. Acad. Sci. *66*, 631 (1957).
[4] L. Baschieri, F. de Luca, L. Cramarossa, C. de Martino, A. Oliverio, and M. Negri, Exper. *19*, 15 (1963).

experiments repeated on each animal at 6–8 day intervals the brains were fixed in formalin and the sites of deep electrodes and of cannulae checked histologically.

Results. The most striking effect was observed after bilateral administration of melatonin to both preoptic regions. The action usually appeared after 15–30 min, lasted about 2–3 h and then gradually subsided during the next 2 h. During the peak action an obvious synchronization in cortical leads and a remarkable increase of amplitude and slowing of electrical activity in subcortical structures were observed (Figure). Previously alert animals showed loss of interest in the immediate environment, did not react to acoustic stimuli, curled up in a corner of the cage and went to sleep. Simultaneously, the respiration and heart rate decreased by about 20–30% of the mean initial value. Though not so obvious, the administration of melatonin to the nucleus centralis medialis induced qualitatively similar behavioral and EEG changes. No consistent effects, however, were obtained following the implantation of melatonin in the brain stem reticular formation. In control experiments similar amounts of crystalline glucose administered to the investigated brain areas caused no effects which might be comparable with those produced by the administration of melatonin to the preoptic region.

Discussion. As has been reported previously, bilateral administration of serotonin creatinin sulfate (15–30 µg) to the preoptic region induced sleep and a trophotropic syndrome[5,6] (using the terminology coined by HESS[7]), in contrast to the effects of noradrenaline bitartrate which in the same dose and in the same animal produced a typical ergotropic syndrome with concomitant EEG changes[5,6]. We believe that these effects depend on the presence of specific and highly localized receptor sites, since the same amount of noradrenaline when placed more rostrally (F 16) produced sleep or drowsiness[8], and when implanted in the nucleus centralis medialis, caused consistently alternating periods of high voltage, slow wave activity and 'activated sleep patterns' characterized by low voltage, fast cortical activity and hippocampal rhythmic θ-waves associated with deep behavioral sleep[5,6].



Representative EEG record from an unanesthetized cat: before, and 30 and 60 min after the administration of melatonin (15 µg per cannula) to the left and right preoptic region. Note the high amplitude θ-waves in the right dorsal hippocampus (R. DHIPP), right and left preoptic region (R. RPO, L. RPO) and left Nucleus centralis lateralis (L. CL.). The remaining abbreviations: ASSG = anterior suprasylvian gyrus, MSSG = medial suprasylvian gyrus, BSRF = brain stem reticular formation.

[5] N. YAMAGUCHI, T. J. MARCZYNSKI, and G. M. LING, EEG clin. Neurophysiol. *15*, 154 (1963).

[6] N. YAMAGUCHI, G. M. LING, and T. J. MARCZYNSKI, Rec. Adv. Biol. Psychiat., in press (1964).

[7] W. R. HESS, *Das Zwischenhirn, Syndrome, Lokalisationen, Funktionen* (Benno Schwabe, Basel 1954).

[8] T. J. MARCZYNSKI, C. D. CLEMENTE, and M. B. STERMAN, unpublished results (1962).

Confrontation of the above-mentioned results[9] with the present experiments seems to lend further support to the hypothesis that within the preoptic region two different autonomic systems overlap which might be represented on one side by catecholamine and, on the other side, by tryptamine receptor sites. It is suggested that melatonin, among other factors, may also play a neurohumoral role in modulating the state of wakefulness and sleep[10].

Zusammenfassung. An nicht narkotisierten Katzen wurde die Wirkung einer direkten lokalen chemischen Reizung subkortikaler Strukturen durch Melatonin untersucht. Diese Substanz, appliziert im Bereich der Area praeoptica, löste trophotropische Effekte und Schlaf aus.

T. J. Marczynski, N. Yamaguchi, G. M. Ling, and L. Grodzinska

Department of Pharmacology, Medical School, Krakow (Poland) and Brain Research Institute, University of California, Los Angeles (USA), February 17, 1964.

[9] T. J. Marczynski, N. Yamaguchi, and G. M. Ling, Pharmacologist *4*, No. 2 (1962).

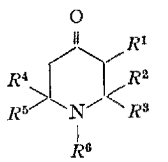
[10] *Acknowledgment.* We wish to thank Drs. J. V. Supniewski and H. W. Magoun for helpful suggestions, and Dr. S. Misztal for the synthesis of melatonin. Part of this work was carried out during the tenure of the Rockefeller Foundation fellowship by one of us (T. J. M.).

Ketal Formation in 4-Piperidones

Acid-catalysed hemiketal and ketal formation in alcoholic solutions of homocyclic ketones has recently been demonstrated by physical methods[1,2]. We and others find that certain heterocyclic ketones, in the presence of ethanol and acid, give either ketone or ethylketal salts (see Table). Ketal formation, where it occurs, is induced by merely treating the basic ketone with a slight excess of ethanolic hydrogen chloride at room temperature and allowing the hydrochloride salt to separate. The results of the Table may be interpreted in terms of (a) ring size and (b) generation of new 1,2-*cis* or 1,3-diaxial interactions, i.e. by the same factors that influence hemiketal formation in homocyclic ketones[2].

The results with the 4-piperidones (Ia–d) and the five and seven ring ketones (VII–X) are consistent with Brown's I-strain concept[3]. Carbonyl addition in 4-piperidones relieves strain inherent in a six membered ring containing one trigonal carbon atom and leads to an ideally staggered conformation, not attainable in the five and seven ring analogues (VII–X). If the 4-piperidone be substituted in the α-position (axial or equatorial), or the β-position (axial), ketal formation results in unfavourable

Action of ethanol-acid on some 4-piperidones and related ketones



Compound	R^1	R^2	R^3	R^4	R^5	R^6	Product isolated	Reference
I a	H	H	H	H	H	H	ketal HCl	7
b	H	H	H	H	H	*n*-Bu	ketal HCl	8
c	H	H	H	H	H	Bz	ketal HCl	8
d	H	H	H	H	H	$(CH_2)_2Ph$	ketal HCl	9
II a	Me	H	H	H	H	Me	ketone HCl	10
b	Me	H	H	H	H	$(CH_2)_2Ph$	ketone picrate	9
c	Et	H	H	H	H	$(CH_2)_2Ph$	ketone picrate	9
d	*n*-Pr	H	H	H	H	$(CH_2)_2Ph$	ketone picrate	9
III	H	Me	H	H	H	$(CH_2)_2Ph$	ketal HCl	5
IV	H	Me	H	Me	H	$(CH_2)_2Ph$	ketal HCl	5,11
V	H	Me	H	Me	H	H	ketal HCl	5
VI	H	Me	Me	Me	Me	H	ketone HCl	11
VII	N-benzylazacycloheptan-4-one						ketone HCl	12
VIII	N-(2-phenethyl)azacycloheptan-4-one						ketone HCl	12
IX	N-benzyl-3-pyrrolidone						ketone HCl	12
X	N-(2-phenethyl)-3-pyrrolidone						ketone HCl	12