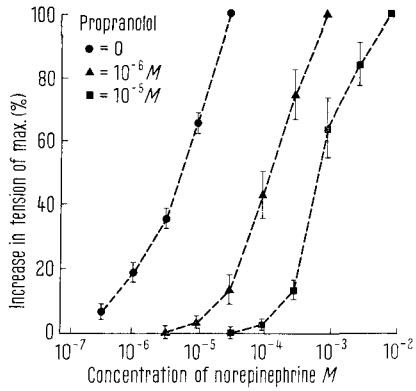
effect of propranolol on myocardial contractile force. As shown in the Figure, each dose-response curve of norepinephrine on myocardial contractile force is shifted progressively to the right, essentially in parallel with each other, as the dose of propranolol increases. This family of dose-response curves appears to satisfy the concept of competitive antagonism between two pharmacological agents, which has been advocated by ARIENS et al.[10] and by SCHILD[11]. Hence, one could conclude that pharmacological competitive antagonism does exist between norepinephrine and propranolol[4,6,12].



Summary of average effect of graded doses of norepinephrine on myocardial contractile force in the guinea-pig ventricular strips. Mean ± S.E.

Zusammenfassung. Der Einfluss von Propranolol, einem neuen β-adrenergischen Blockierungsmittel, auf die Wirkung von Noradrenalin auf die Myokardkontraction wurde an isolierten Meerschweinchenventrikelstreifen studiert. Dabei wurden Indizien für einen kompetitiven Antagonismus beigebracht.

J. NAKANO and M. SAKATO

Department of Pharmacology, University of Oklahoma School of Medicine, Oklahoma City (USA),
September 24, 1965.

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Persisting Circadian Rhythm in Hepatic Glycogen of Mice during Inanition and Dehydration[1]

CHOSSAT's early data[2] demonstrate that significant circadian periodicity persists in certain birds until the day of their death from starvation and dehydration: statistically significant within-day differences in cloacal temperature of birds completely deprived of food and water as well as in controls (feeding and drinking ad libitum) can be computed from his data published in 1843 (Tables 60 and 68 in [2])[3]. More recently, circadian rhythms in rectal temperature, pinnal mitosis, corticosterone content of serum and adrenal, as well as in pituitary adrenocorticotropic activity, were found to persist in inbred Bagg albino (C) mice deprived of food and water[4,5]. Interest in the question of how long certain other biochemical rhythms demonstrably persist in a mammal deprived of food and water was renewed by problems arising in the construction of a programmed feeding device in preparation for a biosatellite study on rhythms.

Apart from this background, liver glycogen was chosen as a variable for study, since for this function the timing of feeding is still generally regarded as an essential condition of a rhythm. This is done in keeping with a role assigned to nutritional factors already by BERNARD[6] and in line with a report by HIGGINS et al.[7], the pioneering investigations on this subject by ÅGREN et al.[8] notwithstanding. Contrary to the conclusion of ÅGREN et al., the fact that hepatic glycogen drops to very low levels during starvation is misinterpreted for a lack of rhythm. This status quo is not surprising since, with few exceptions, most curves published by students of rhythms more recently on liver glycogen show multiple turning points[9] – a circumstance arising from the failure to 'isolate' a circadian rhythm from other superimposed frequencies (such as the ultradian changes) and 'noise'.

Against this background, the data of the Figure provide a clear view of the persisting circadian rhythm in several groups of animals. Three groups of mice were standardized for periodicity analysis, as described elsewhere[10], but two of these groups were on a modified schedule only in that either all food or all food and water as well was removed 16 h before the starting time of the serially independent sampling here employed.

766 C female mice were singly housed in three separate rooms under conditions of 12 h light (0600 to 1800) alternating with darkness; they were standardized on this regimen for seven days prior to sampling and during the sampling span. Food and water were available ad libitum

[1] Work supported by the Elsa U. Pardee Foundation, the National Aeronautics and Space Administration of the USA (NsG-517) and the US Public Health Service (5-K6-GM-13).
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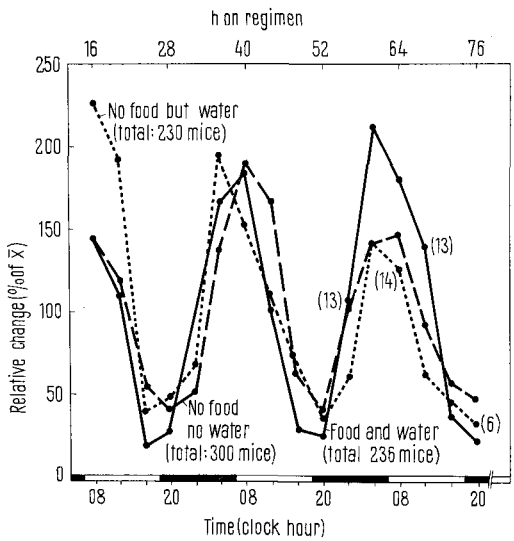
to all animals until 16 h prior to the first sampling, when food was removed from the cages in one room while food and water were both removed from the cages in another room. The animals in the third room were checked (to provide a similar disturbance), but had food and water available ad libitum throughout the whole sampling span.

Subgroups of approximately 15 mice from each of the three groups were sacrificed at 4 h intervals. The liver was immediately removed, frozen, and the glycogen concentration determined by the method of KEMP and KITS VAN HEIJNINGEN[11].

In the animals with food and water ad libitum, the liver glycogen concentration varied predictably as a function of time, from mean crest values of about 8.0 g glycogen/100 g tissue wet weight to trough values as low as 0.36 g. The starving and thirsting as well as the starving animals showed on inspection of time plots similar circadian rhythms that were roughly comparable also with the rhythm in the fully-fed and fully-watered mice, as can be seen from the relative values shown in the Figure – although the absolute liver glycogen levels in starving groups of animals ranged from a mean value of 0.356 g glycogen/100 g tissue wet weight at the circadian crest time to 0.049 g at the time of trough.



Chossat phenomenon in hepatic glycogen of the mouse (see text). Liver glycogen concentration in C female mice, 4.5 months of age (15 mice per point, unless otherwise noted in parantheses).

At the extremely low levels of liver glycogen in the starved and dehydrated mouse, the circadian rhythm persists and its amplitude during the first day of dehydration and/or starvation does not compare unfavorably with that in controls. Apart from the practical interest in these results related to a biosatellite survey of biochemical and other rhythms, the Figure demonstrates for yet another function what might be called, in honor of a pioneer in this field, a CHOSSAT[12] phenomenon[13].

Zusammenfassung. Eine Circadianrhythmik des Mäuseleberglycogens lässt sich durch Standardisierung anderer biologischer Frequenzen und «Lärm» isolieren. Dies gelingt auch nach Entzug von Futter oder Futter und Wasser. Persistente Rhythmen mit grosser Amplitude kennzeichnen eine Reihe physiologischer Funktionen nach Futter- und Flüssigkeitsentzug und können als Chossatphänomene bezeichnet werden.

E. HAUS and F. HALBERG

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Hexamethylentetramine-Silver Reaction for Lysosomes

In the course of a study (DE MARTINO et al.[1]) about the feasibility of the application of the hexamethylentetramine-silver technique in order to stain various cytoplasmic structures, silver-stained granules were observed in the parenchymal cells of the liver and in those of the intestinal epithelium. Similar granules were also stained in the proximal convoluted tubules of the kidney. This study was performed to assay the specificity of the reaction and to define the identity of the stained granules.

Kidney, liver and small intestine of adult newts (*Triturus cristatus* Laur.) were used in this study. Four different fixatives were used: Sanfelice, Bouin, Carnoy and acetic sublimate. 5 μ paraffin sections, after oxidation with 10% H_5IO_6 (15–20 min), were exposed to silver hexamethylentetramine (5–12 h). The silver solution was prepared according to the methods of JONES[2] and MARINOZZI[3] for the basal membranes, as modified by DE MARTINO et al.[1]. Other sections from the same block were oxidized with 10% $NaIO_4$ before silver impregnation. Frozen sections, 8–10 μ thick, from formol-calcium fixed tissues, were incubated for acid phosphatase activity to demonstrate the presence of lysosomes by the GOMORI[4] procedure.

In the liver, many silver-stained granules were localized around the bile canaliculi. Similar granules were also stained in the proximal convoluted tubules of the kidney and in the supranuclear region in the intestinal epithelial cells. These granules matched in size and distribution the lyosomes demonstrated by the Gomori technique. However, in the intestinal cells, these granules were revealed by the silver reaction only when the material had been fixed in Sanfelice or in Bouin.

The results obtained in our study confirm and extend the observations of other authors. LILLIE[5] described

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