normotensive Wistar rats. The increases in the enzyme activities of both adrenals were about 40% and 30%, respectively. In contrast, the activities of monoamine oxidase and catechol *O*-methyltransferase in the adrenals of SH-rats did not change significantly as compared with those of normotensive Wistar rats (Table II).

These results showed that, among the enzymes involved in the biosynthesis of catecholamines, enzyme activities of DOPA decarboxylase and phenylethanolamine N-methyltransferase were only slightly increased, as compared with those of tyrosine hydroxylase and dopamine β-hydroxylase, which had been shown to increase about 1.9-fold and 1.8-fold, respectively[1]. The enzyme activities of monoamine oxidase and catechol *O*-methyltransferase did not change.

The results indicate that the increase in the activities of tyrosine hydroxylase and dopamine β-hydroxylase in the adrenal glands of SH-rats are specific, and that both enzymes may be easily induced[8].

Table II. The activities of monoamine oxidase and catechol *O*-methyltransferase in the adrenals of spontaneously hypertensive rats

Enzyme activity	Normotensive Wistar rats	Spontaneously hypertensive rats
Monoamine oxidase		
nmoles/min/both adrenals ± S.E.M.	1.51 ± 0.07	1.48 ± 0.10
nmoles/min/mg protein ± S.E.M.	0.25 ± 0.01	0.22 ± 0.01[a]
Catechol *O*-methyltransferase		
pmoles/min/both adrenals ± S.E.M.	42.6 ± 2.2	40.0 ± 3.5
pmoles/min/mg protein ± S.E.M.	6.99 ± 0.40	5.99 ± 0.32[b]

The result is the average for 6 animals. [a] $p < 0.20$. [b] $p < 0.10$.

Zusammenfassung. Es wird gezeigt, dass Dopa-Decarboxylase und Phenylethanolamin-N-methyl-transferase bei spontan hypertonischen Ratten im Vergleich zu normotonen Kontrollen leicht erhöht sind; hingegen wurde keine Veränderung der Catecholamin-abbauenden Enzyme festgestellt.

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On the Nature of Biological Clocks?

There has been much speculation on the fundamental cellular mechanism underlying Biological Clocks. Various mathematical models have been proposed[1,2] and physiological oscillations have been cited to explain their working[3,4].

Light, dark cold (and even pressure) are all agencies that readily phase Biological Clocks[5]. Moreover, rhythms may be extinguished under continuous application of the agency, to be reinstated in a fresh phase when it is removed[6,7]. A notable general observation made by BUNNING[5] is that the physiological effect of cold during a light period coincides with that produced by dark. Current theories of Circadian rhythms do not however, admit a resolution of this paradox. A possible general explanation is however implicit in some recent work of the present author, who has shown that in a wide variety of cells and tissues, ATP levels vary inversely with temperature[8–12], and decrease under illumination[10–12]. Both such effects have been demonstrated in the leaves of *Chenopodium rubrum* and *Phaseolus vulgaris*[10], in *Dictyostelium* myxamoebae[11] and also in chick embryos in ovo[12].

All the phasing agencies described have the common effect of altering cytoplasmic viscosity[13]; and reference to the literature[13,14] establishes that the increases in ATP level described above in dark or cold correspond to decrease of cytoplasmic viscosity, a predictable correlation knowing the effect of microinjected ATP[15].

On this basis therefore, the paradoxical common effect of dark and cold applied during illumination in phasing rhythmic phenomena is readily explicable, for whereas the effect of cold is to raise ATP, its level in the dark is also higher than that under illumination. The effect of pressure in phasing clocks[5,6] may be on this basis also, for LANDAU[16] has also described pressure-induced increases in ATP level.

[1] C. S. PITTENDRIGH and V. E. BRUCE, in *Rhythmic and Synthetic Processes in Growth* (Ed. D. RUDNICK; Princeton University Press 1957), p. 75.
[2] R. WEVER, in *Circadian Clocks* (Ed. J. ASHOFF; North-Holland, Amsterdam 1965), p. 47.
[3] J. W. HASTINGS and A. KEYNAN, in *Circadian Clocks* (Ed. J. ASHOFF; North Holland, Amsterdam 1965), p. 167.
[4] J. W. HASTINGS and V. C. BODE, Ann. N.Y. Acad. Sci. *117*, 876 (1962).
[5] E. BUNNING, *The Physiological Clock* (Longmans/Springer-Verlag, London, New York 1967).
[6] E. J. NAYLOR, J. exp. Biol. *40*, 669 (1963).
[7] J. W. HASTINGS and B. M. SWEENEY, Biol. Bull. *115*, 440 (1958).
[8] P. C. T. JONES, J. Cell Physiol. *73*, 37 (1969).
[9] P. C. T. JONES, Cytobios *1 B*, 65 (1969).
[10] P. C. T. JONES, J. exp. Bot. *21*, 58 (1970).
[11] P. C. T. JONES, Cytobios *6*, 89 (1970).
[12] P. C. T. JONES, Comp. Biochem. Physiol. *36*, 87 (1970).
[13] H. V. HEILBRUNN, *Dynamics of Living Protoplasm* (Academic Press, London, New York).
[14] H. VIRGIN, Physiol. Plant. *4*, 255 (1951).
[15] R. J. GOLDACRE and I. J. LORCH, Nature, Lond. *166*, 497 (1950).
[16] J. V. LANDAU, Expl Cell Research. *32*, 538 (1961).

A natural extension to this argument is to suggest that Biological Clocks operate as a result of cyclical fluctuations in intracellular ATP (and of concomitant changes in other nucleoside phosphates). There is other evidence moreover, to suggest that such ATP variations are a central feature of Biological Clocks. Some chloroplasts (which are known to contain an actomyosin-like protein[17]) show a circadian rhythm of expansion in the dark and contraction under illumination[18,19] a phenomenon only explicable on the basis suggested above.

That the free-running rhythm may be quenched by cold (as in *Carcinus maenas*[6] or in *Gonyulax polyhedra*[7]) is also understandable, for if the metabolic variable reaches and is maintained at its maximal level, no cyclical variation is then possible until a reduction on its mean level occurs.

It was decided further to test this general proposition. In man, and other mammals, the most obvious natural rhythm is that of sleep, which is correlated with others such as that of mitotic activity[20]. It was decided therefore to determine if alternations in ATP level can be correlated with the natural rhythm of sleep and wakefulness.

Alert or sleeping Golden Hamsters (*Mesocricetus auratus*) were killed by stunning with an air pistol pellet. The head was then instantly decapitated and dropped into liquid nitrogen before removal of the frozen brain; and the liver excized and dropped into liquid nitrogen in a chilled mortar. Both were separately ground to a fine powder, triturated and homogenized when cold with 0.6 *M* perchloric acid for extraction of the nucleoside phosphates, and after centrifuging at 4 °C, the clear supernatants were used for ATP estimation, the pellet being used as the basis of a dry weight estimation. ATP levels were then determined by the method of ADAM[21] using an UV-test kit (Boehringer und Söhne, Mannheim, Germany) and expressed on a dry weight basis. Further descriptions of this method have been given elsewhere[8,9,12]. A summary of the results is given in the Table.

Intracellular ATP levels in the brain and liver of alert and sleeping golden hamsters (nmoles/mg dry weight)

a) Liver	
Alert	Sleeping
5.34 ± 0.77	7.87 ± 0.45
b) Brain	
Alert	Sleeping
8.07 ± 1.78	13.70 ± 3.21

It will be seen that ATP levels in both Hamster brain and liver rise significantly with sleep, and clearly indicate a diurnal rhythm of ATP level. Moreover, ATP levels are also known to be high in hibernating animals and under anaesthesia[22,23]. It is therefore interesting to reflect that BULLOUGH[21,25] has observed a diurnal rhythm of mitotic maxima to be present in natural sleep, and to be induced under barbiturate anaesthesia[20,24]. Much evidence has moreover been presented to suggest a relationship between mitotic incidence and ATP[25,26]. PLESNER[27], moreover, has shown a rhythm of ATP level in synchronized cultures of *Tetrahymena pyriformis* which correlates with the rhythm of cell division.

Preliminary evidence of similar circadian variations in ATP level has also been detected in cockroaches and in the shore crab, *Carcinus meanas*, and will be published in due course.

It is therefore proposed that the primary underlying rhythm of Biological Clocks may be one of ATP level (and of variations in the corresponding di- and monophosphates). The experimental results cited above lend support to this proposition, which also affords a ready explanation of the phasing effects of parameters of state, and of the means of their transduction.

Zusammenfassung. Feststellung, dass schlafende Goldhamster einen höheren ATP-Gehalt in Gehirn und Leber besitzen als wache Tiere und dass Zeitgeber, wie Licht und Temperatur, sowohl Zellviskosität als auch ATP-Niveau verändern.

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[17] L. PACKER, in *Biochemistry of Chloroplasts* (Ed. J. W. GOODWIN; 1966), vol. 1, p. 233.
[18] G. SENN, Z. Bot. *11*, 81 (1919).
[19] J. T. HOPKINS, in *Light as an Ecological Factor* (Eds. R. BAMBRIDGE, G. C. EVANS and O. RACKHAM; Blackwell, London 1966), p. 355.
[20] W. S. BULLOUGH, Proc. R. Soc. B *135*, 212 (1948).
[21] H. ADAM, in *Methods of Enzymatic Analysis* (Ed. H. V. BERGMEYER; Academic Press, London, New York 1963), p. 539.
[22] M. L. ZIMNY and R. GREGORY, Am. J. Physiol. *195*, 230 (1958).
[23] L. BUCHEL and H. MCILWAIN, Br. J. Pharmac. *5*, 465 (1950).
[24] W. S. BULLOUGH, Proc. R. Soc. B *135*, 233 (1948).
[25] E. GUTTES and S. GUTTES, Science *129*, 143 (1959).
[26] D. EPEL, J. Cell Biol. *17*, 315 (1963).
[27] P. PLESNER, C. r. Trav. Lab. Carlsberg. *34*, 1 (1964).
[28] I am grateful for the assistance of Mrs. KAREN GOWING, and of Mr. PHILIP LLOYD during the Hamster experiments described above.

Liver and Uterine Lipid Metabolism: a Comparative Study[1]

AIZAWA and MUELLER[2] were among the first of several workers[3–6] to study the effects of estrogens on uterine lipid metabolism by using castrated animals treated with exogenous hormones. Only a few persons have studied uterine lipid metabolism in normal untreated animals[7–9]. Since the above studies were performed to elucidate either hormonal actions or normal uterine function, either untreated animals or time was used as the control reference. The following study was performed to evaluate uterine lipid metabolism in terms of a different reference, i.e. liver tissue from the same rat.

Two to three normal adult female rats (Holtzman Company) were sacrificed by cranial fracture during each stage of the estrous cycle. (For this paper, the data are not evaluated as functions of time.) Uteri and livers were removed and blotted dry with filter paper. The uteri were slit longitudinally; the livers were sliced into strips which approximated, as nearly as possible, the