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 $0.6M$ 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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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

Carbon 14 concentrations

	No. rats	Total lipid	Total neutral lipid	Phospholipid	Triglyceride	Sterol ester	Free fatty acid
Liver	14	1160.9 (204.2)	1141.7 (176.2)	115.9 (25.0)	102.1 (24.0)	93.2 (51.7)	898.5 (167.4)
Uterus	14	413.0 (69.6)	348.6 (70.7)	71.6 (21.3)	43.8 (11.5)	18.6 (5.8)	227.8 (39.7)
P-value		0.0018*	0.0003*	0.1889	0.0379*	0.1635	0.0006*

Carbon 14 concentrations are expressed as cpm/mg wet weight. Duplicate determinations were performed on each specimen. Each value represents the average from 14 tissues and is presented with the standard error in brackets. As indicated by the asterisks, liver tissues incorporated significantly more ^{14}C into the total lipid, total neutral lipid, triglyceride and free fatty acid fractions than did uterine tissue.

length, width, and depth of the opened uterus from the same rat. The tissues were weighed and placed into 10 ml of Hanks' solution and 0.01 mC of acetate-1-^{14}C and incubated for 2 h under 95% O_2, 5% CO_2 at 37 °C in a shaking metabolic incubator.

Following incubation, enzymatic processes were stopped by quick freezing and water was removed by lyophilization. The lipids were extracted with repeated washes of chloroform-methanol[9]. The total lipid extract was defined by changing the solvent system to chloroform and filtering out precipitated materials as described elsewhere[9,10]. Samples of the total lipid fraction were removed and ^{14}C concentrations were measured using standard techniques and a Packard Tri-Carb liquid scintillation spectrometer.

Neutral lipids were separated from the phospholipids by acetone precipitation with $MgCl_2$ at 4 °C followed by filtration[10]. Both fractions were recovered and the ^{14}C concentration in each was measured.

Neutral lipids were separated into classes by thin-layer chromatography using silica Gel G and developing with 7% ethyl ether in petroleum ether (v/v). The various lipid classes were collected and their ^{14}C concentrations measured. Duplicate analyses were performed on each sample. All ^{14}C concentrations were corrected for volume changes, background activity, and machine fluctuations and expressed as cpm/mg wet weight.

Liver tissue incorporated significantly more acetate into the total lipid fraction than did uterine tissue ($p < 0.002$). The total lipid ^{14}C concentrations averaged 1161 and 413 cpm/mg for liver and uterine tissues, respectively (Table). As reflected by the standard errors, incorporation also varied more in liver tissue.

No tissue differences in ^{14}C incorporation into phospholipids existed ($p > 0.1$). The total neutral lipid fractions of liver contained more ^{14}C than did those of uteri ($p < 0.001$). The increased incorporation by liver could be partially accounted for by the rapid rate of fatty acid synthesis. The ^{14}C concentration in free fatty acids from liver was 4 times greater than the ^{14}C concentration from uterine tissue ($p < 0.001$). Esterification of fatty acids to sterols proceeded at similar rates in both tissues, and no differences between them were detected in sterol ester ^{14}C concentrations ($p > 0.1$). Liver tissue incorporated more ^{14}C into triglycerides than did uterine tissue ($p < 0.05$). Even though liver tissue incorporated ^{14}C into fatty acids 4 times faster than did uterine tissue, the formation of triglycerides by liver was only 2.4 times faster. Hence, when considered as a function of fatty acid synthesis, the uterine tissue esterified fatty acids into triglycerides more quickly than did liver tissue.

For a detailed account of lipid biosynthesis, the reader is referred to the excellent review articles by FRANTZ[11], WAKIL[12], and SHAPIRO[13].

Except for phospholipids and sterol esters, liver tissue incorporated more ^{14}C into the various lipids than did uterine tissue from the same rat. This relation between the tissues was not dependent upon time in the estrous cycle; 2–3 samples of each tissue were incubated for each phase of the cycle, yet in only 2 unrelated instances did the total ^{14}C incorporation by uterine tissue exceed that by liver.

Uterine tissue esterified newly synthesized fatty acids into triglycerides faster than comparable liver tissue. This finding supports other studies which have demonstrated that the rat uterus preferentially synthesizes and accumulates triglycerides during the post-copulatory period as a prelude to nidation[8,9].

Zusammenfassung. In-vitro-Inkubation von Leber- und Uterusgewebe der Ratte in Azetat-1-C^{14} ergab bei beiden Geweben Inkorporation gleicher Mengen von C^{14} pro mg Frischgewicht in Phospholipoide und in Estercholesterin. Die Leber synthetisiert wohl rascher Fettsäuren, vermag sie aber weniger schnell in die Triglyceride einzubauen als der Uterus.

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