UNIVERSITY OF MISSOURI    COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

F. B. MUMFORD, *Director*

# GROWTH AND DEVELOPMENT

*With Special Reference to Domestic Animals*

## XLIII.  Diurnal Metabolic and Activity Rhythms.

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# FOREWORD

The special investigation on growth and development is a cooperative enterprise in which the departments of Animal Husbandry, Dairy Husbandry, Agricultural Chemistry, and Poultry Husbandry have each contributed a substantial part. The parts for the investigation in the beginning were inaugurated by a committee including A. C. Ragsdale, E. A. Trowbridge, H. L. Kempster, A. G. Hogan, F. B. Mumford. Samuel Brody served as Chairman of this committee and has been chiefly responsible for the execution of the plans, interpretation of results and the preparation of the publications resulting from this enterprise.

The investigation has been made possible through a grant by the Herman Frasch Foundation, now represented by Dr. F. J. Sievers.

F. B. MUMFORD
*Director Agricultural Experiment Station*

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## ABSTRACT

In some species vision is best in day light, in other in night light. The diurnal vision differences result in conditioned diurnal sleep and activity rhythms which are reflected in diurnal metabolic rhythms. In the rat the metabolic peak is at night (usually before midnight) and the trough in day (usually before noon). The metabolic difference between peak and trough is of the order of 25%-30%, which is at least equal to the metabolic difference between fast and feeding, and which explains certain apparent anomalies in metabolic studies. The diurnal metabolic rhythm in the rat is not extinguished by a month of continuous light, or by continuous feeding (1/8 of the day's maintenance ration at 3-hour intervals) or by a combination of continuous light and fast; but it is extinguished by a week of continuous light together with continuous feeding. In addition to the diurnal rhythm there are shorter and longer rhythms associated with various physiologic processes which complicate investigation of factors influencing metabolic rates.

# Diurnal Metabolic and Activity Rhythms

VIRGIL V. HERRING AND SAMUEL BRODY

We became interested in the problem of diurnal rhythm when we obtained some rather unexpected results in a study of heat production following feeding. The anomalous result could only be ex-
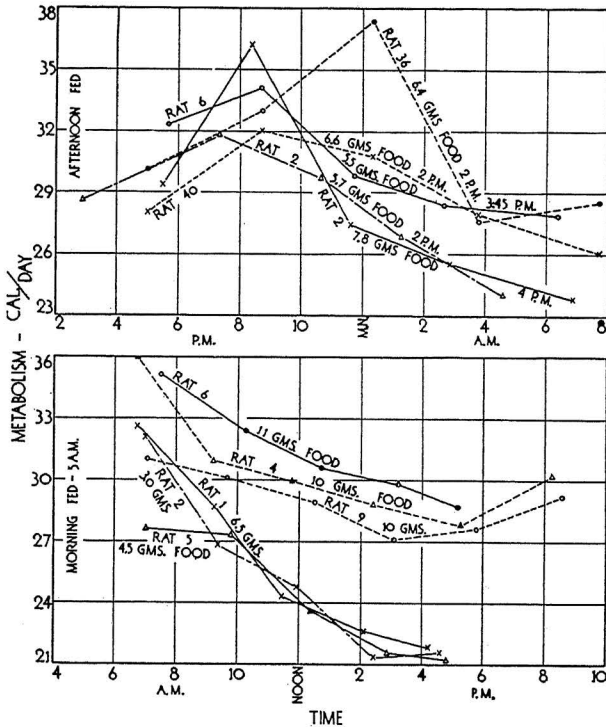


Chart A.—The apparent difference in heat production in rats following morning and afternoon feeding, as found by Kenneth Kaufman and John Riggs, undergraduate students in this institution, which suggested the present research on the diurnal rhythm in energy metabolism.

plained by assuming the presence of a diurnal change in metabolism exceeding the thermogenic effect of food. (See Chart A.) This led to a review of the literature on diurnal rhythms, and to the performance of a series of experiments on this problem. The object of this bulletin is to summarize some of the results of this work very briefly.

## I. REVIEW OF LITERATURE

**1. Body Temperature.**—One of the widely investigated diurnal periodicities concerns body temperature. In man, body temperature is at a minimum (36.5° C) between 2 and 5 in the morning, and at a maximum (37.5° C) between 2 and 5 in the afternoon. Gibson[1] and Osborne[2] found in their travels that the body temperature rhythm follows the local light rhythm. The obvious conclusion is that the body temperature rhythm is not an "inherent" characteristic but is dependent directly or indirectly on the sun rhythm.

Burckard, Dontcheff and Kayser[3] in their excellent review of the literature on the problem, cite Chossat (1843) who observed that the midnight temperature of the pigeon (41.5°C) is 0.7°C below the noon temperature (42.2°C), and the night respiration rate (32 R.P.M.) is 4 below the noon rate (36 R.P.M.). Chossat attributed the thermal and respiratory rhythm to a nervous rhythm, which is in turn associated with the sleep rhythm; which is in turn, one might add, a function of the position of the sun.

**2. Social Conditioning.**—Kleitman[4] has some quotable remarks on social conditioning in his 1929 review.

"The cycle of day and night serves to develop what Pavlov calls a natural conditioned reflex. Darkness makes for poor vision and discourages movements. This leads to inactivity and relaxation, and sleep follows. Repeating the performance a great many times results in the establishment of a conditioned reflex of a temporal character—relaxation at a certain time.

"This is the theoretical history of diurnal sleep. Practically we are born into a social organization where diurnal sleep is the universally accepted mode of sleeping. . . . . The first habit that a mother tries to develop in a baby is that of an unbroken nights' sleep. As he gets older other functions develop a periodicity that coincides with the enforced sleep periodicity. For instance a temperature curve develops with a minimum at night, and produces a disinclination to night activity. Even the modest tear apparatus stops its function at bedtime procuring dry eyes favoring their closure. And so on."

The control of the diurnal rhythm by the sun, through the hereditary photoreceptor mechanisms, thus appears to be supplemented by an elaborate social conditioning process. Richter[5] found that in newborn rats the activity is not related to time of day. By the age of 13 days, periodicity of activity appears, and becomes clear cut by the age of 17 days due presumably to "social conditioning."

[1]Gibson, R. B., The effect of transposition of the daily routine on the rhythm of temperature variation. Am. J. Med. Sc., *129*, 1048, 1905.
[2]Osborne, W. A., Body temperature and periodicity. Proceedings in J. Physiol., *36*, p. XXIX, 1908.
[3]Burckard, E., Dontcheff, L., and Kayser, C., Le rythme nycthéméral chez le pigeon, Ann. Physiologie, *9*, 303, 1933.
[4]Kleitman, N., Sleep. Physiol. Rev., *9*, 624, 1929.
[5]Richter, C. P., A behavioristic study of the activity of the rat. Comp. Psychol. Monographs, *1*, 55, 1922; Animal behavior and internal drives, Quar. Rev. Biol., *2*, 307, 1927.

Similar activity studies were reported by Richter on rabbits, kittens, guinea pigs, and chicks, and by Stier[6] on mice.

The diurnal rhythm in activity, feeding, and body temperature must naturally be associated with a diurnal periodicity in energy metabolism. Chossat (1843) credits Prout with the discovery of the diurnal rhythms in $CO_2$ production in homeotherms. Sonden and Tigerstedt[7] and others demonstrated the partial independence of the diurnal temperature and metabolic rhythm from food intake, since the rhythm persists for some time during fast.

Lusk (his textbook, p. 116) quotes from Johansson the following table on the diurnal rhythm of $CO_2$ production in man:

|  | Night $CO_2$ | Day $CO_2$ | Author |
|---|---|---|---|
| Complete muscular rest | 100 | 105 | Johansson |
| Ordinary rest in bed | 100 | 110 | ,, |
| Ordinary life (no hard work) | 100 | 142 | Tigerstedt |
| ,, ,, ,, ,, ,, | 100 | 128 | Pettenkofer & Voit |
| ,, ,, ,, ,, ,, | 100 | 147 | Tigerstedt |

The diurnal variations tend to disappear with complete muscular rest. At the same time the body temperature tends to decline (perhaps by 0.6°C).

**3. Inversion of the Diurnal Rhythm.**—The older experimental investigations on the diurnal rhythm consisted largely in attempts to invert the rhythm. The data on humans are less consistent than on lower animals. Thus Benedict[8] failed to obtain inversion of the diurnal rhythm. Toulouse and Pieron[9] on the other hand report an inversion, but their data are not convincing. Lindhard[10] reported inversion of body temperature in night laborers. Völker[11] found not an inversion but a shift in the diurnal curves of the respiratory exchange and urinary excretion during an eight-day period. Gessler[12] thought that the normal diurnal rhythm was present in Johansson's and Lindhard's men, but masked by secondary factors. If space permitted, we might cite other reports on humans with similar results. The lack of agreement is due to the fact that different investigators

[6]Stier, T. J. B., Spontaneous activity of mice. J. Gen. Psychol., 4, 67, 1930.
[7]Sonden, K., and Tigerstedt, R., Skand. Arch. Physiol., 6, 136, 1895.
[8]Benedict, F. G., and Snell, J. F. Körpertemperatur-Schwankungen mit besonderer Rücksicht auf den Einfluss welchen die Umkehrung der täglichen Lebensweise beim Menschen ausübt. Pflügers Arch. ges Physiol., 90, 33, 1902; Benedict, F. G., Influence of the inversion of the daily routine: the temperature of night workers. Am. J. Physiol., 11, 145, 1904.
[9]Toulouse, E. F., and Pieron, H., Le Méchanisme de l'inversion chez l'homme du rythme nycthéméral de la température. J. Physiol. et path. gén., 9, 245, 1907.
[10]Lindhart, cit. from Deighton, Physiol. Rev., 13, 427, 1933.
[11]Völker, H., Über die tages periodischen Schwankungen einiger Lebensvorgänge des Menschen. Pflügers Arch. ges Physiol., 215, 43, 1926.
[12]Gessler, H., Die täglichen Schwankungen der Körpertemperatur, Pflügers Arch. ges. Physiol., 207, 390, 1925.

employed different methods. The apparent conclusions would vary with the time intervals between measurements. These were shorter and the results therefore more reliable on the subjects of Benedict than of Toulouse and Pieron. The activity controls were much better for Benedict's than for Toulouse's and Pieron's subjects. There is, of course, always the possibility that failure to invert the diurnal body rhythm is due to failure to completely invert the diurnal environmental rhythm (not only the light but also the auditory rhythm) for a period long enough to abolish the diurnal cortical and muscular reflexes implanted during the period preceding the experiment. A complete inversion is difficult in man because it is difficult to isolate him psychically and intellectually from the social rhythm of the environment even if he can be isolated from the light rhythm.

The results on animals on the contrary show that the diurnal light rhythm is a decisive factor conditioning the diurnal body rhythm. Galbraith and Simpson[13] reported an inversion in body temperature in monkeys. Hilden and Stenback[14] reported that night lighting tended to shift the diurnal body-temperature rhythm in the pheasant, fowl, and pigeon. Fifteen days were required to complete the inversion in male pigeons.

The persistence of the diurnal rhythm in spite of prolonged rest and fast stimulated speculations on cosmic influences other than the sun, including cosmic rays and ionized air[15]. Deighton[16] observed a diurnal rhythm in metabolism of swine screened from changes in electrical conductivity.

4. **Metabolic Rhythm in Pigeons.**—Benedict and Riddle[17] reported that muscular activity of pigeons in dark chambers is less at night than in daytime. After excluding periods showing muscular activity, the energy metabolism during the day is of the order of 15% above that at night (ring doves 3047 Cal./day during night, 3621 Cal./day during day; common pigeons 5716 Cal./day during night, 6165 Cal./day during day; common pigeons in a 10-bird apparatus 6116 Cal./day during night, 7134 Cal./day during day).

[13]Galbraith, J. J., and Simpson, S., Conditions influencing the diurnal wave in the temperature of the monkey. J. Physiol., *30*, Proc. Physiol. Soc. XX, 1903. See also by same authors Temp. variations in nocturnal and other birds. Id., *30*, XIX, 1903.
[14]Hilden, A., and Stenback, K. S., Zur kenntnis der Tagesschwankungen der Körpertemperatur bei den Vogeln. Skand. Arch. Physiol., *34*, 382, 1916.
[15]Völker, H., Uber die tagesperiodischen Schwankungen einiger Lebensvorgange des Menschen. Pflügers. Arch. ges. Physiol., *215*, 43, 1926.
[16]Deighton, T., Physical factors in body temperature maintenance and heat elimination. Physiol. Rev., *13*, 427, 1933.
[17]Benedict, F. G., Riddle, O., The measurement of the heat production in pigeons. J. Nut., *1*, 475, 1929.

The most extensive investigation on the diurnal metabolic rhythm in animals was conducted on pigeons by Kayser and his pupils[18]. These investigators found a diurnal difference in cloacal temperature of 1°C and a difference in "basal metabolism" of the order of 15% to 17% (maxima between 11 in the morning and 3 in the afternoon; minima 6 to 10 in the evening). However, great individual variations were observed in the maximum-minimum range. Kayser and his associates attributed the diurnal variation of heat production in the pigeon mainly to the diurnal variation in tonicity of posture especially of the pectoral muscles; which in turn is caused reflexively by the diurnal light rhythm which excites the nerves controlling muscle-tone.

Kayser was led to the pectoral-muscle tone explanation of the diurnal metabolic rhythm by the following considerations. He carried out his investigation on the diurnal metabolic rhythm in the pigeon at three widely differing temperatures, 29°C, 17°C, and 2.5°C. The day metabolism was in all cases about 15% above the night metabolism in spite of the fact that the metabolism at 2.5° was nearly double (1.8 times) that at 29°C, and in spite of the fact that there was no difference in recordable muscular movements. Kayser's reasoning ran somewhat as follows: Chemical thermoregulation was investigated by noting the influence of environment temperature (2.5°C, 17°C, 29°C) on the diurnal rhythm. No substantial difference was found; therefore, there was *no diurnal difference in chemical thermoregulation*. Physical thermoregulation was investigated by noting the diurnal difference in extrarenal water excretion. No difference was found; therefore, there was *no diurnal difference in physical thermoregulation*. Since both physical and chemical thermoregulation are independent of the diurnal rhythm, the relation between physical and chemical regulation is likewise independent of the diurnal rhythm, and it was consequently concluded *that it is not possible to demonstrate the existence of a diurnal rhythm in subcortical excitability*. The diurnal metabolic rhythm was therefore attributed to a diurnal muscle-tonus rhythm caused by the diurnal excitation (mainly visual) rhythm. The importance of postural tonus was further demonstrated by denervation of a large muscular mass which caused the diurnal rhythm to disappear. The data on the $H_2O/O_2$ ratio indicate that the temperature change was not due

[18]Burckard, E., Dontcheff, L., and Kayser, C., Le rythme nycthéméral chez le pigeon. Ann. Physiol., 9, 303, 1933; Dontcheff, L., Kayser, C., and Reiss, P., Le rythme nycthéméral de la production de chaleur chez le pigeon ses rapports avec l'excitabilite des centres thermo-regulateurs. Id., 1, 1185, 1935. Attention may be here called to a paper on the diurnal rhythm in the fowl by Bacq, Q. M., Sur l'existence d'un rythme nycthéméral de metabolisme chez le coq. Ann. Physiol. et physicochim. biol., 5, 497, 1929, and note by Terroine, E. F., De l'existence d'un rythme nycthéméral de metabolisme chez les oiseaux. Id., 5, 842, 1929.

to peripheral vasodilation accompanying sleep. Removal of the cerebral hemispheres reduced the general metabolic level by about 16%, but did not, in all cases, abolish the diurnal rhythm. This demonstrates that visual excitation is not the only factor in the diurnal metabolic rhythm. Keeping normal pigeons in complete darkness, resulted in the gradual disappearance of the rhythm, and it completely disappeared in two months. No difficulty was found in inverting the diurnal rhythm in pigeons by inverting the normal light rhythm, indicating that while visual excitation is not the only factor, it is a major one.

**5. Stomach Rhythms.**—One of the confusing factors in the evaluation of the influence of the sun rhythm on the diurnal physiological rhythm consists in the presence of other rhythms, for example stomach-movement rhythms, difficultly separable from the diurnal rhythm. Many locomotor and heart activities seem to have their "center" in the stomach. There is a large literature on "hunger contractions" and muscular activities in humans and in lower animals[19].

**6. Polyphasic Rhythms.**—Szymanski[20] made important contributions to this problem of inherent, "spontaneous", "endogenous", or "autogenous" rhythms as found in newborn animals prior to their conditioning to the environmental rhythm. His investigations on various species led him to conclude that some species are *polyphasic*, that is exhibit several periods of activity and rest during the day, while others are *monophasic*, that is, exhibit one prolonged period of activity and another of rest during each day. Rabbits are said to be polyphasic, having 16 to 21 periods of activity alternating with rest; albino rats have 10 periods of activity concentrated, as in other nocturnal animals, at night; canaries are monophasic, active during day and quiet during night; young dogs exhibited 8-13 periods of activity at night; cats exhibited 2/3 of their activity during the day, and 1/3 during the earlier part of night; human infants are polyphasic, human adults, monophasic, and human aged resemble infants in being polyphasic. There are obvious difficulties in differentiating between such inherent phasic rhythms and merely spurious movements.

[19]See *inter alia* Carlson, A. J., The control of hunger in health and disease, Chicago, 1916; Elliot and Bousfield, Psychol. Review, *43*, 94, 1936, reviewed the literature on interrelations of periodicities of hunger contractions; Hellebrandt, F. A., et al., nocturnal and diurnal variations in the acidity of the spontaneous secretion of gastric juice. Am. J. Dig. Dis. and Nutrition, *3*, 477, 1936; Kleitman, N., Cooperman, N. R., and Mullin, F. J., Is there a continuous curve of the depth of sleep? Proceedings in Am. J. Physiol., *116*, 1936; Richter, loc. cit., *2*, 307, 1927; Boldyreff, W., Quart. J. Exp. Physiol., *10*, 175, 1916-17.

[20]Szymanski, J. S., Die Verteilung der Ruhe-und Activitäts perioden bei weissen Ratten and Tanzmäusen, Arch. ges. Physiol., *171*, 324, 1918.

**7. Rhythmic Muscular Activity in the Rat.**—Richter[21] reported that a diurnal rhythmic activity of the fasting rat persisted in the absence of light cycles. During the early period of fast, the total activity was greater than during five previous feeding days at noon. But this initial high activity and rhythmicity rapidly declined with prolongation of fast. Horst, Mendel, and Benedict[22] concluded that "our experimental evidence is so obscured by the uncontrollable muscular activity of the rats that the question as to whether there is a diurnal variation in metabolism irrespective of the night period remains unsolved." The metabolism was found to be lowest between 10 a. m. and 4 p. m. because activity is low, much of the time having been spent in sleep. The metabolism was not increased during this period by darkness, possibly because of the persistence of the daily habits.

**8. Rhythmic Nervous Sensibility in Humans.**—There are a series of interesting reports on diurnal variations in nervous irritability in man. The importance of the influence of nervous irritability on metabolism and body temperature may be inferred from the facts that about half of the peripheral nerves supply the skeletal muscles, that about half of the body mass is muscle and that the contractions of skeletal muscle increase proportionately to nervous irritability[23]. Lombard[24] observed a diurnal rhythm of irritability as measured by the height of the knee jerk during the day.

Kleitman, Titelbaum, and Feivson[25] reported that the reaction time of normal man to lights and sounds is lowest in the morning when body temperature is lowest, increases with increasing body temperature in the afternoon, then declines to a morning minimum. It appears that 3 to 6 a. m., when the body temperature in man is at a minimum, irritability is likewise at a minimum. Early morning is also the time when it is most difficult to keep awake in experiments on sleeplessness. Only continuous movements keep the individual awake during these hours. Likewise skills of coordination are at a minimum during these early hours. It is generally known that energy metabolism, the tempo or "rate of living", is associated with body temperature, and it is not surprising that nervous irritability and conductivity should also be associated with body temperature.

[21]Richter, l. c., 1922.
[22]Horst, K., Mendel, L. B., and Benedict, F. G., The metabolism of the albino rat during prolonged fasting at two different environmental temperatures. J. Nut., 3, 177, 1930.
[23]Jacobson, E., Variations in muscular tonus. Am. J. Physiol.. 113, 71, 1935.
[24]Lombard, cited from W. M. Howell, Text Book of Physiol. 1930 (160).
[25]Kleitman, N., Titelbaum, S., and Feiveson, P., Diurnal variation in reaction time and its relation to body temperature. Am. J. Physiol., 113, 82, 1935.

**9. Rhythm in Liver Function.**—There is an interesting chapter in the literature on liver function rhythm, which may explain some anomalous respiratory quotient values. Forsgren[26] reported that in the rabbit glycogen storage is at a maximum and bile formation is at a minimum during the night. Agren, Wilander, and Jorpes[27] confirmed Forsgren on rabbits, and extended this conclusion to rats and mice. Higgins[28] reported that in nocturnal animals, the night time when the animals naturally feed, is the assimilatory phase when the glycogen is deposited. The glycogen deposition at this time tends to be counteracted, however, by physical activity thereby resulting on occasions in a bimodal curve of glycogen storage.

There appears to be a diurnal rhythm in blood sugar level and adrenalin level which tends to vary inversely with the liver glycogen[29] level. Möllerstrom[30] found a definite blood sugar rhythm in diabetics, the sugar falling during certain hours to a level below fasting normal.

**10. Diurnal Rhythm in Milk Production.**—A practically important but theoretically unexplained difference is known to exist in the composition of morning and evening dairy-cow milk. The following chart (Fig. 1) modified from Bartlett[31] shows the difference in
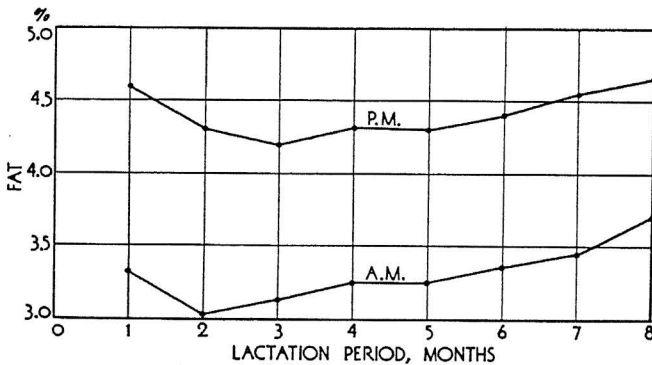


**CHART LEGENDS**

Fig. 1.—Diurnal rhythm in milk composition as indicated by the difference in fat percentage in morning (A. M.) and evening (P. M.) milk during the lactation period.

[26]Forsgren, H., Über die Rhythmik der Leberfunktion des Stoffwechsels und des Schlaffes. Stockholm, 1935. See also Skand. Arch. Physiol., *53*, 137, 1928; *55*, 144, 1929; Acta Med. Scand., *73*, 213, 1930.
[27]Ågren, C., Wilander, O., and Jorpes, E., Cyclic changes in the glycogen content of the liver and muscles of rats and mice. Biochem. J., *25*, 777, 1931.
[28]Higgins, G. M., Berkson, J., and Flock, E., The diurnal cycle of the liver. Am. J. Physiol., *102*, 673, 1932, and *105*, 177, 1935.
[29]Euler, U. S., Holmquist, A., Plugers Arch. ges. Physiol., *234*, 210, 1934.
[30]Möllerstrom, J., Periodicity in the carbohydrate metabolism. Acta Med. Scand., Suppl. *50*, 250, 1937.
[31]Bartlett, S., Studies in milk secretion based on the variations and yields of milk and butterfat produced at morning and evening milkings. J. Agric. Sc., *19*, 38, 1929.

fat percentage in morning and evening milk throughout the course of lactation. There is also a difference in amount of milk yield at morning and evening milking. The morning yield tends to be higher while its fat and other solid percentages tend to be lower than the evening yield.

At the time of this writing the Wisconsin Agricultural Experiment Station reported (Wis. Bull. 439, Dec. 1937, p. 76) that the morning milk is richer in bacteria than the evening, and the evening milk is richer in chlorine and catalase than the morning (the higher evening chlorine and catalase might be the aftermath of the higher morning bacterial metabolism).

**11. Rhythm in Blood Composition.**—In connection with the diurnal rhythm in milk flow, it is interesting to note a few illustrations in blood composition rhythms, as there is of course an interrelation between milk and blood composition. Allcroft[32] found a diurnal trend in blood sugar. The trend was rather confused because of the sensitiveness of the blood sugar level to psychic disturbances involved in securing the blood. (In lactating cows the blood sugar level was a high of 70 mg. sugar per 100 cc. blood at noon, and a low of 58 mg. at 9:30 a. m. There was no such trend in non-lactating cows or sheep.)

Diurnal rhythmicity for blood was also reported on sugar by Holmgren[33] and Möllerstrom[34], non-protein nitrogen by Forsgren[35], serum calcium by Ehrstrom[36], red corpuscles and hemoglobin by Short[37], water and minerals by Norn[38].

It was already noted that Higgins found a glycogen rhythm.

Holmquist[39] observed an adrenaline peak during the day when body temperature is highest, and a blood-calcium peak during the night when body temperature is lowest. In the nocturnal rabbit, the opposite was observed, adrenalin and body temperature peak at night and calcium peak during the day. We need not discuss the diurnal rhythm of the well-known alkaline tide and urine composition.

It may be noted incidentally that in the sparrow at least, there is a diurnal rhythm in spermatogenesis. Active spermatogenesis in

[32]Allcroft, W. M., Diurnal variations in the blood sugar level of the lactating cow. Bioc. J., *27*, 1827, 1933.
[33]Holmgren, H., Acta Med. Scand. Suppl., *59*, 104, 1935.
[34]Möllerstrom, J., Periodicity in carbohydrate metabolism. Acta Med. Scand. Suppl., *50*, 250. 1932; Acta Med. Scand. Suppl., *59*, 145, 1934.
[35]Forsgren, E., Acta Med. Scand., *73*, 213, 1930.
[36]Ehrstrom, M. C., Ueber Serumcalcium-Tageskurven. Acta Med. Scand. Suppl., *59*, 97, 1934.
[37]Short, J. J., J. Lab. Clin. Med., *20*, 708, 1935.
[38]Norn, M., Scand. Arch. Physiol., *55*, 184, 1929.
[39]Holmquist. A. G., Tägliche cyclischeschwan kungen in Calciumgehalt des Blutes bei Menschen und Kaninchen. Z. ges. exper. Med., *93*, 370, 1934.

this species was reported by Riley[40] to be confined to early morning, 2 a.m. to 4 a.m. when body temperature is at a minimum. It appears that there is a 7° body-temperature range in the sparrow, from 109.8° F during light of day and 103.1° F during the early morning.

Recently[41] a diurnal rhythm has been reported to exist in the retina.

**12. Rhythms in Brain Potentials.**—Finally we might note the extreme sensitivity to light and the periodicity of the pattern of brain potentials[42]. The intensity of these brain potentials is an index of the state of excitation. Some of these patterns are spontaneous, autogenous, or endogenous and others are exogenous caused by sensory impulses, reflexes, and humoral agents, especially by light. This literature has an important bearing on our problem by indicating its complexity. There are many types of physiologic rhythms: life-cycle rhythm, annual rhythm, lunar rhythm, diurnal rhythm. Many types of inherent or spontaneous rhythms which have not been definitely explained are perhaps associated with the autogenous activities of the digestive, circulatory, respiratory, excretory and endocrine systems. The intimate interrelations between the various rhythms increase the interpretative difficulties. *A metabolic difference may be the result of a diurnal or another type of activity rhythm rather than of the factor under investigation.*

[40]Riley, G. M., Factors controlling the diurnal spermatogenic cycle of the male sparrow. Anat. Rec., 64, supp. 41, 1936.
[41]Welsh, J. H., and Osborn, C. M., Diurnal changes in the retina of the catfish. J. Comp. neurology, 1937.
[42]Davis, H., Davis, P. A., Loomis, A. L., Harvey, E. H., and Hobart, C., Human Brain potentials during the onset of sleep. J. Neurophysiology, 1, No. 1, Jan., 1938. Davis, H., Some aspects of the electrical activity of the cerebral cortex. Cold Spring Harbor Symposia on quantitative biology, 4, 285, 1934. Also papers in the same volume by H. H. Jasper and H. Hoagland; Travis, L. E., and Milison, R. L., Brain potentials from the rat, J. Genetic Psych., 49, 405, 1936; Gerard, R. W., Metabolism and excitation, Cold Spring Harbor symposia on quantitative biology, 4, 194, 1936.

## II. THE LABORATORY RESEARCH ON DIURNAL METABOLIC RHYTHMS

1. **Summary of Results, Aims and Methods.**—Photographs of the apparatus employed for measuring the metabolism and muscular activity of the rats are shown respectively in Figs. 2 and 3. The results are shown in graphic form in Figs. 4 to 7.
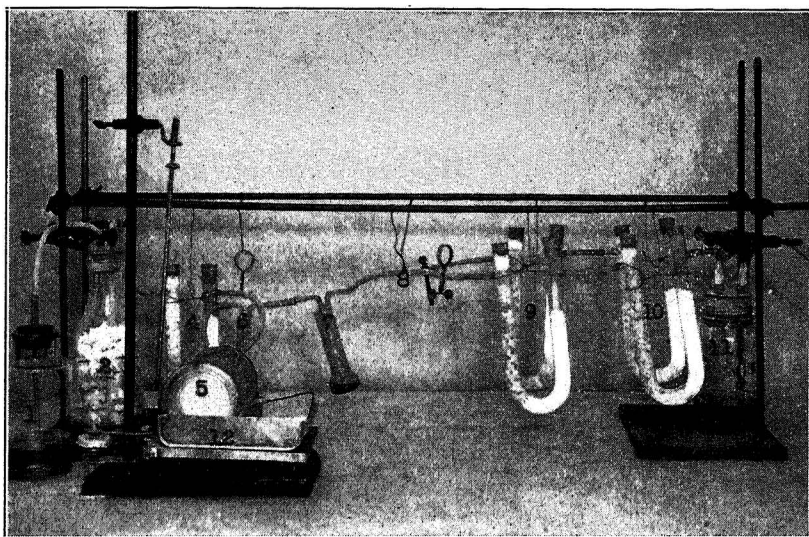


Fig. 2.—The metabolism apparatus used. A. *Absorbers for incoming air*: 1. Conc. $H_2SO_4$, water absorber; 2. Shells of NaOH, $CO_2$ absorber; 3. Conc. $H_2SO_4$, water absorber behind 2; 4. Check tube for $H_2O$ and $CO_2$; 5. Metabolism chamber, a No. 5 wide-mouth Kerr Mason jar supported on light tin holder 12. A ½ inch mesh hardware cloth inside the chamber holds the rat 1 inch above the jar wall. The air-inlet copper tube is soldered at the top of the two-piece lid, and extends to the rear of the jar. The short outlet tube is soldered at the bottom of the lid. B. *Absorbers for outgoing air*: 6. Conc. $H_2SO_4$ in Bowen potash absorber for absorbing outgoing moisture; 7. Same as 6, check for 6; 8. A glass Y and pinch-clamp which permits alternate use of duplicate pairs of absorbers; 9. $CO_3$ absorber; 10. $CO_2$ check tube; 11. A moisture trap to protect absorbers 9 and 10 when not in use. The air leaving trap 11, differs from air entering chamber 5, by having a little less $O_2$ which was consumed by the rat. The increase in weight of system 5 to 10 inclusive represents this amount of $O_2$ retained by the rat. The increase in weight of 6 and 7 represents water exhaled and the increase in weight of 9 and 10 represents $CO_2$ exhaled.

The metabolic level at any moment is of course the sum of: (1) "basal" or irreducible metabolism, plus (2) the metabolism of muscular activity and muscle tonus, plus (3) the heat increment of feeding (specific dynamite action). As the rat is nocturnal, his muscular activity, muscle tonus, feeding etc. tend to be high during the night when his vision is best and he is therefore most widely awake, and low during the day when his vision is poorest and he is therefore least alert. We may then expect the charts to show high metabolic rates during the night, and low metabolic rates during the day, just as

Kayser found the opposite to be the case in the pigeon. The diurnal changes in muscular activity, muscle tonus, food consumption, and related activities are reflected in rising and declining time curves of metabolism somewhat similar in shape to the diurnal rising and declining curves of light, but, for the nocturnal rat, in the opposite direction.
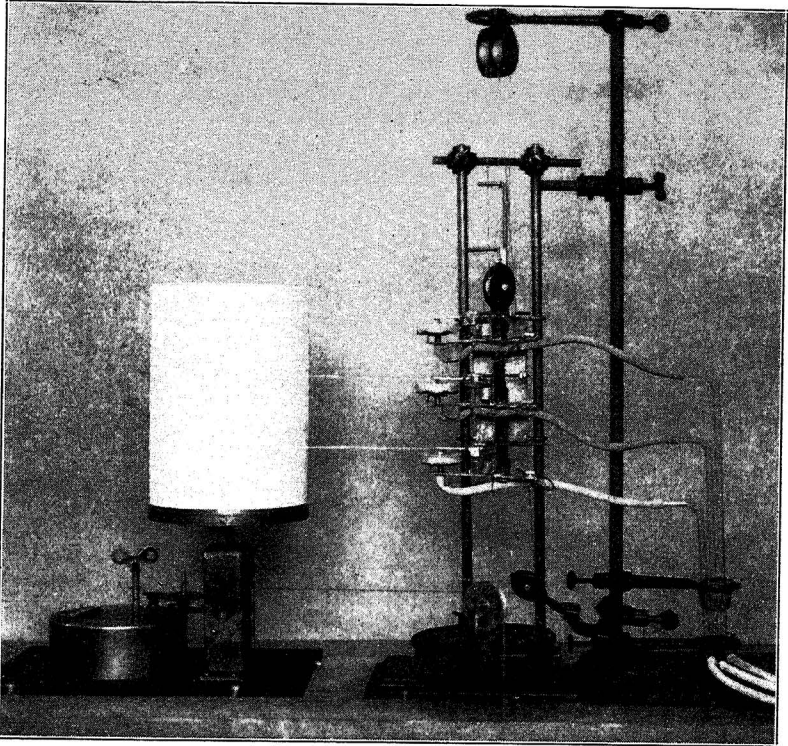


Fig. 3. The activity recorder. The movement of the rat in 5 (see Fig. 2) vibrates platform 12, thus changing the air volume in the Marey capsules actuating the capillary glass fountain pens which record some rat movements by vertical lines on the kymograph.

As the weights of animals vary, their metabolism also varies. The metabolic differences due to body size differences may be eliminated by representing metabolic data in terms not of simple Calories, or of Calories per unit simple body weight, but in terms of Calories per $(weight)^{0.73}$, because the 0.73 power of body weight represents "physiologic weight", or "metabolic weight", or "active mass",[43]

[43]Cf., Brody, S., Relativity of physiologic time and physiologic weight. GROWTH, *1*, 60, 1937.

and the metabolism per unit of "metabolic weight" is consequently more comparable in animals of different size than metabolism per unit of simple body weight.
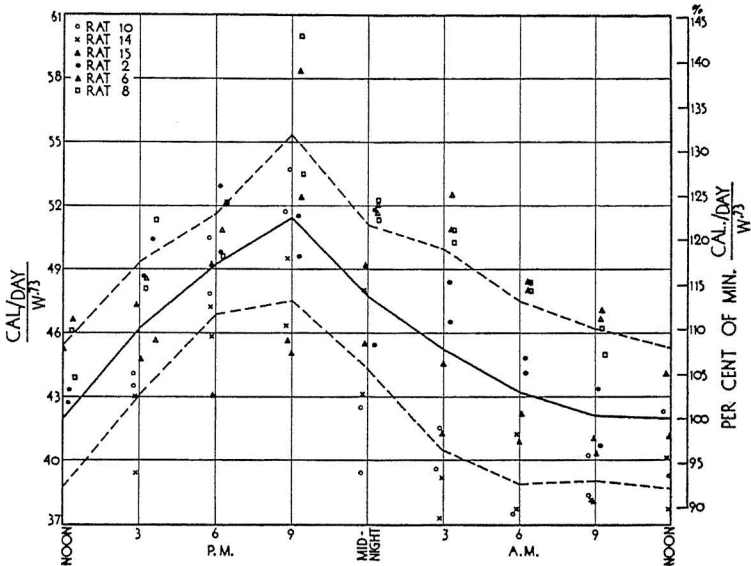


Fig. 4.—Diurnal metabolic rhythm in normal white rats. The continuous line represents the average of the data for the 6 rats. The area between the broken curves includes ⅔ of the data.

Employing these methods, the metabolism shows a daily rhythm, with the peak at night (before midnight) and trough during the day (before noon). Attempts to extinguish this diurnal rhythm by one month of constant illumination were not successful. Attempts to extinguish this rhythm under constant illumination combined with fasting for a week were likewise unsuccessful. Fasting reduced the metabolic levels of day and night, but the diurnal *rhythm* remained. If, however, a *constant illumination was combined with feeding the maintenance ration in 8 equal portions at 3-hour intervals, the diurnal rhythm was extinguished in about a week. These in brief are the results of this investigation which we shall proceed to illustrate graphically.*

a. *Diet:* Certain peculiarities of the employed methods are due to the fact that the research was originally undertaken to evaluate the energy cost of pregnancy and lactation in the rat. The aim was later changed to a study of diurnal variation in metabolism because, as previously noted, we suspected that our anomalous results were due to such a superimposed diurnal rhythm.

In the study of the energy cost of pregnancy and lactation, it was desired to feed a complete diet which is rapidly digested so that the postabsorptive condition might be quickly reached and the "basal metabolism" of the pregnant or lactating rats compared with the controls without unduly prolonging the periods which might interfere with normal lactation between feedings. We could not think of a better diet than dry milk supplemented with minerals and vitamins. The diet consisted of 94% of "cremora A" dried whole milk (which contained 30.13% fat); 5% dried brewers yeast, 1% wheat germ oil. To one part by weight of this mixture was added one part of the following solution[44]: $FeSO_4 \cdot 7 H_2O$, 5.00 gm; $CuSO_4 \cdot 5 H_2O$, 0.63 gm; $MnSO_4 \cdot 4 H_2O$, 0.82 gm; KI, 0.04 gm; distilled water, 2.5 liters; conc. HCl, 3.0 cc. The resulting paste was fed from small beakers attached by loop of wire to the side of round individual cages. Food consumption records were kept.

The animals were housed at a room temperature of 25° to 29° C, and their metabolism measured at 28 ± 0.5° C. The shades of the room were kept drawn, and the animals were under constant electric-light illumination.

*b. Apparatus and computations:* The energy metabolism was measured by the Haldane set-up shown in Fig. 2, explained in the legend.

The air leaving the Haldane system through the moisture trap differs from that entering the metabolism chamber only by having a small amount of oxygen removed by the rat. This removed oxygen is determined by the increase in weight of the system 5 to 10 in Fig. 2.

The air ventilation through the system was about 1 liter per minute. This was regulated by a water aspirator which created a small vacuum (7 inches of water) and maintained constant by a water-column negative pressure regulator. This regulator maintains the rate of air flow very constant.

The metabolism calculations were carried out from the $CO_2$ production and the respiratory quotients (R. Q.):

$$R. Q. = \frac{gm. \ CO_2}{gm. \ O_2} \div \frac{mol. \ wt. \ CO_2 \ (44)}{mol. \ wt. \ O_2 \ (32)}$$

$$R. Q. = \frac{gm. \ CO_2}{gm. \ O_2} \times 0.727$$

Example: During a 240-minute period a 238-gram rat produced 1.208 gm. $CO_2$ and 1,220 gm. $O_2$. R. Q. $= \frac{1.208}{1.220} \times 0.727 = 0.720$. At this R. Q., 1 gm. $CO_2 = 3.33$ Cal. The heat production was then $1.208 \times 3.33 = 4.023$ Cal. in 240 minutes, or $4.023 \times \frac{1440}{240} = 24.14$ Cal. per day, or $24.14 \times \frac{1000}{238} = 101.4$ Cal. per Kg. per day.

The chamber and rat weighed about 1000 grams. This was weighed on a 5-Kg. balance of 0.5 Mg. sensitivity. The $CO_2$ absorbers were weighed on a 200-gram balance to 1 mg. The error in determining $CO_2$ is then $2/1208 \times 100 = 0.17\%$. Allowing a 3 mg. error in weighing the chamber and water absorbers, the maximum error for $O_2$ determination would be $\frac{2+3}{1220} \times 100 = 0.41\%$. The error in the R. Q. is then $0.17 + 0.41 = 0.58\%$.

As shell soda does not absorb $CO_2$ readily when dry, a little water is added occasionally to maintain efficient absorption. The efficiency of the shell soda is gauged by the weight of the check tube.

[44]Cf., Daniels, A. L., and Everson, G. S., The relation of manganese to congenital debility. J. Nut., 9, 191, 1935.

After removal of the reagents, the washed U-tubes are dried overnight at 110° - 120° C. (If a film of moisture is present in the glass wool partition, shell soda reacts with the magnesium perchlorate and sets free perchloric acid, which oxidizes the cotton filter and cork stopper of outlet side tubes, as indicated by blackening of the cotton filter, and loss in weight of check tube.)
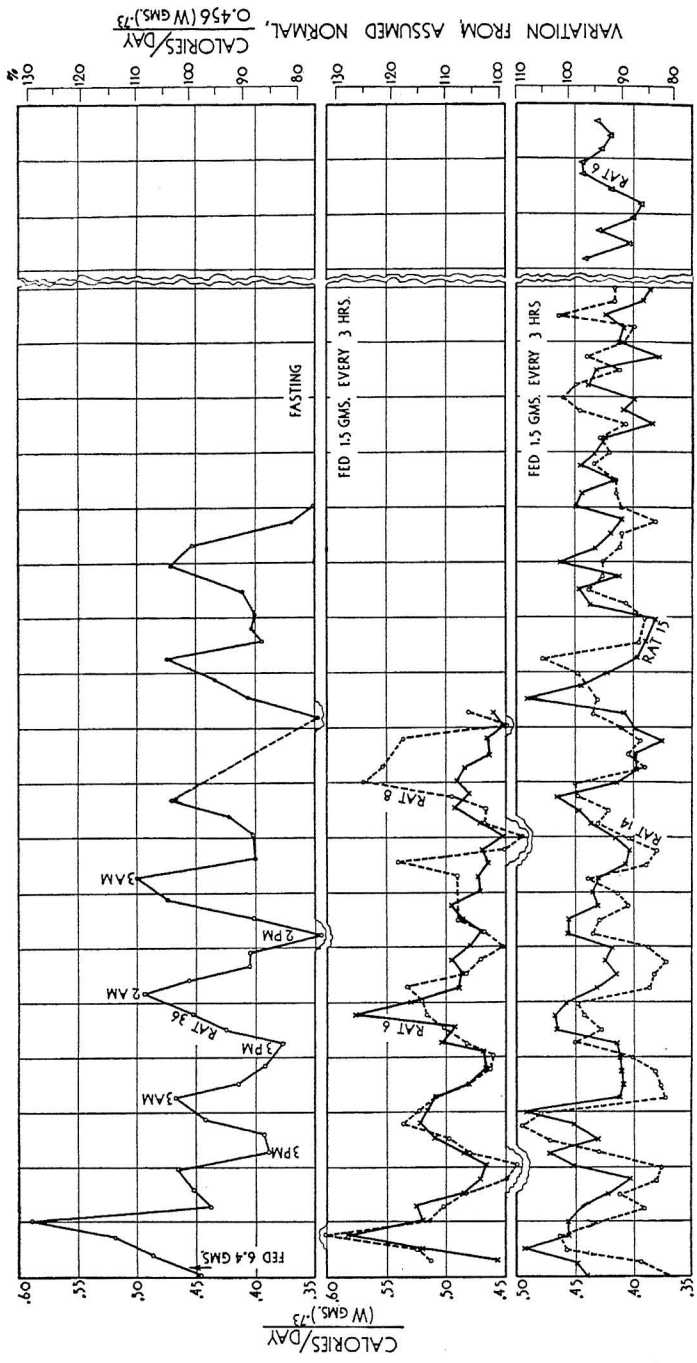
The activity of the animals was recorded by a set-up part of which is shown in Fig. 3. It consists of two units connected by glass and rubber tubing. The "sending unit" numbered 12 in Fig. 2, consists of a platform on a spring support which holds the metabolism chamber. The platform moves with the movements of the rat. These movements are transmitted from the Marey capsule in the sending unit (Fig. 2) to the Marey capsule in the recording unit (Fig. 3). These movements are recorded on the kymograph drum by vertical marks from the glass capillary fountain pens. The activity is stated as per cent of time active, by multiplying the horizontal distance of vertical marks by 100, and dividing by the total horizontal distance. This method for estimating activity is in fair agreement with planimeter measurements of the inked areas.

The kymograph drum turns by gravity: a weight just sufficient to overcome friction is suspended from a string wound around the axle; the clock is the governor of the kymograph drum which thus makes a revolution in 30 minutes. The recording pens are automatically lowered ½ inch during each drum revolution. The lowering of the pens is accomplished by suspending the pen unit from a string that passes through a 2: 1 ratio of pulleys and is wound around the ⅜ inch axle of the kymograph drum. As the drum turns the string unwinds and lowers the recording pens.

### 2. Diurnal Metabolic Rhythm in the Rat and its Extinguishment.

—Fig. 4 shows the composite diurnal metabolic rhythm of 6 white rats picked at random from the animal room. Metabolism was, under the given conditions, at a maximum between 9 p.m. and midnight and minimum during the morning, noon, and early afternoon.

The curve in Fig. 4 represents the diurnal cycle of a ''normal'' rat. Now let us attempt to break his normal diurnal light and feeding habits by: (1) placing him under continuous (24-hour a day) light, and (2) feed his ration in equal parts every 3 hours day and night, or not feed him at all. Fig. 5 shows the effect of such procedures on metabolism. First, let us observe the fasting rat (top curve) under continuous light. The first day he reached a maximum metabolism at midnight which is, roughly, 50% above the minimum of the following day. In the following days of fasting the diurnal rhythm continued but at lower level. Both minima and maxima declined, but there is still a day to night difference of, roughly, 25%. The conclusion is that fasting under continuous light does not break up the diurnal metabolic rhythm.

Another important practical conclusion is that during the first 24-hour interval after feeding the 50% difference between the maximum and minimum, which might be attributed to the feed intake, in-
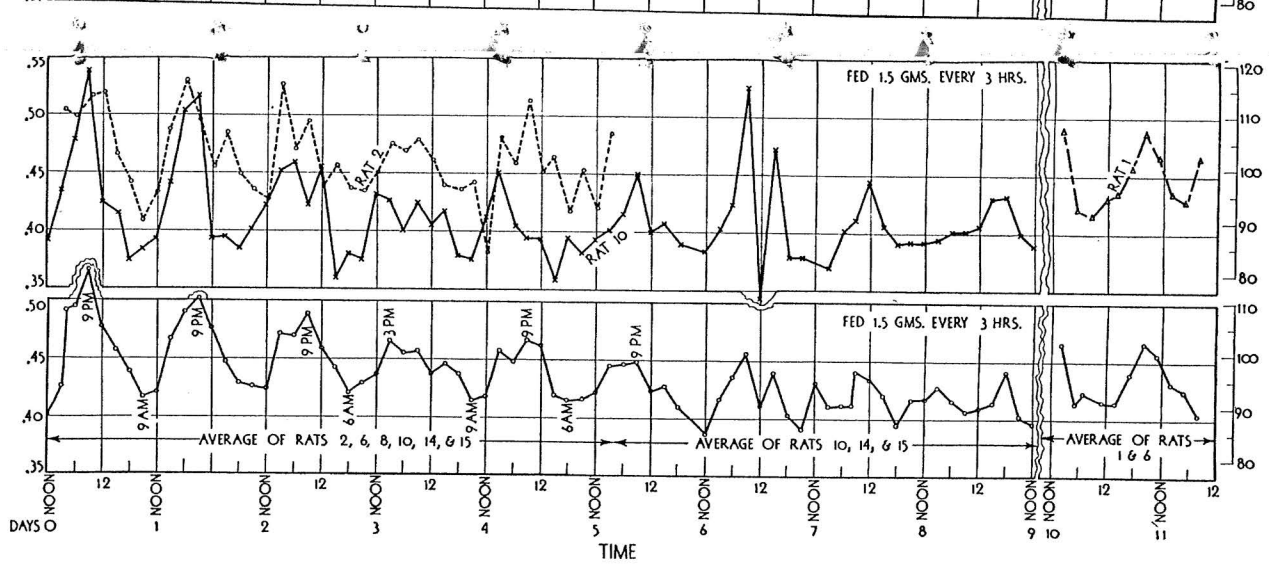
Fig. 5.—Diurnal metabolic rhythms of white rats under continuous light of constant intensity when (a) fasting (upper curve) and (b) when consuming ⅛ of the maintenance ration at 3-hour intervals throughout day and night for over a week (bottom curve represents average of 6 rats, middle curves represent individual rats). Note that the rhythm is maintained in the fasting rat, but is extinguished in the continuous-feeding rats, so that only residual "autogenous" rhythms remain.

cluded a 25% difference due to the diurnal effect, as indicated in the following table:

| 24-hour Period of fast | Metabolism | | | | Percentage Difference |
| | Maximum | | Minimum | | |
| | Cal./day | Time | Cal./day | Time | |
| --- | --- | --- | --- | --- | --- |
| 1 | 37.4 | Midnight | 24.4 | 3 p.m. | 53.3 |
| 2 | 30.7 | 9 p.m. | 23.2 | 3 p.m. | 32.3 |
| 3 | 30.2 | Midnight | 24.1 | 3 p.m. | 25.3 |
| 4 | 30.2 | 3 a.m. | 24.0 | Noon | 25.8 |

The midnight measurement during the 1st day of fast includes the feeding effect of the last meal (6.4 grams) fed at 3 p.m. The midnight peak thus represents a summation of: (1) diurnal effect; (2) feed-intake effect. The diurnal effect is shown in the above table to be of the order of 25% (differences between maximum and minimum during 3d and 4th days fast). The maximum 50% difference shown during the first 24-hour period is thus to the extent of one-half due to the feed-intake effect, and one-half to the diurnal effect. The diurnally changing metabolic base line is in this case apparently equal to the metabolic response of the food.

In one trial, one group of rats was fed a 10-gram meal at 4 a.m. which was followed not by a rise but a decline in metabolism until noon (8 hours); another group of rats was fed a 10-gram meal at 4 p.m. which was followed by a steady increase in metabolism reaching a 20% to 40% peak increment in 5 to 7 hours, then declined to a minimum in 16 hours.

It is evident from the above[45] and from *a priori* considerations that there is an algebraic summation of the effects of food-intake and diurnal rhythm. If the food is given on the ascending slope (after 3 p.m.) of the diurnal rhythm, the two effects reinforce each other with a resulting abnormally high metabolism; when food is given on the descending slope of the diurnal rhythm (after 3 to 5 a.m.), the heat increment of feeding is apparently below normal. All this is obvious. Let us then return to Fig. 5.

We next examine the rats fed 1/8 of the daily ration at 3-hour intervals and kept under continuous light. By such feeding the diurnal metabolic rhythm is not complicated by the heat increment of feeding. The curve at the bottom represents the average of 6 rats, while the second, third, and fourth series of curves represent individual curves. Fig. 5 clearly shows that this method of 3-hourly feeding combined with continuous lighting extinguishes the diurnal

[45]And other extensive data in Mr. Herring's M. A. thesis in the University of Missouri Library.

metabolic rhythm in about a week, whereas continuous lighting but ad libitum feeding failed to extinguish it in 30 days.
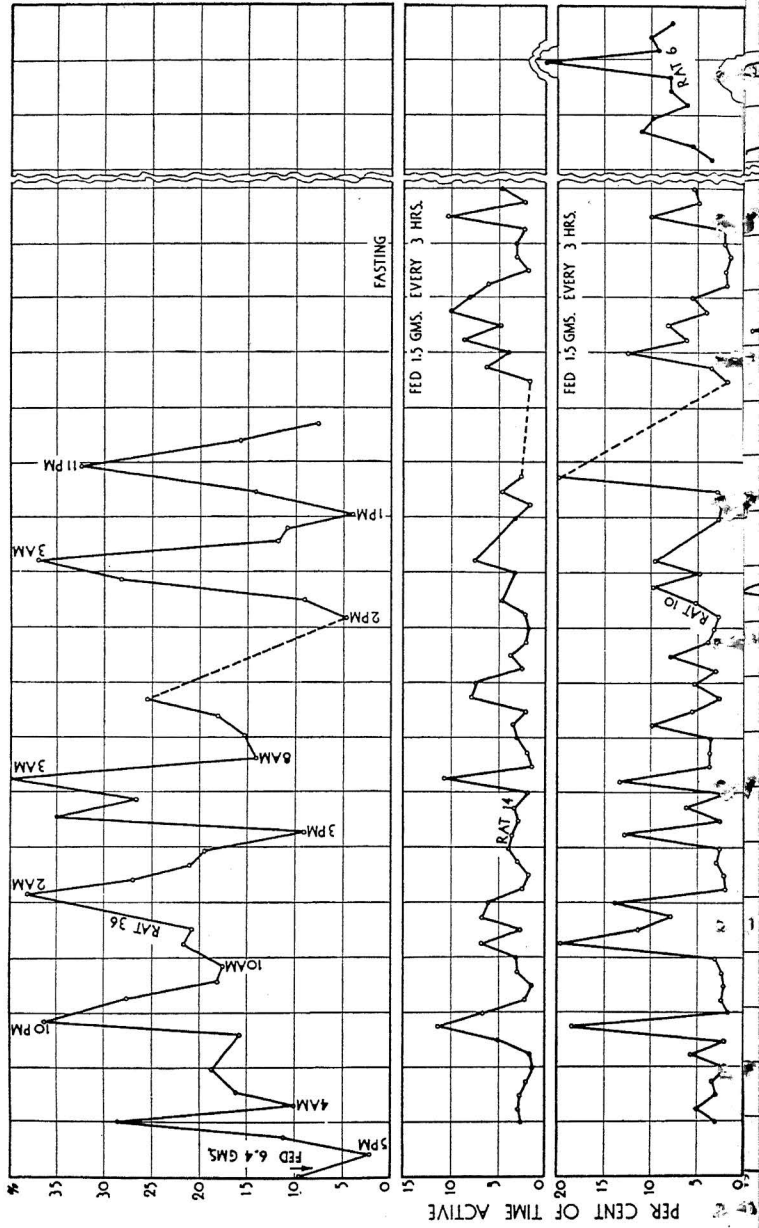
Fig. 5 shows that this method of 3-hourly feeding under continuous light does not extinguish all rhythms. It extinguishes only the diurnal rhythms, leaving other unexplained residual, inherent, and spontaneous rhythms discussed, among others, by Richter.

**3. Diurnal Activity Rhythm and its Extinguishment.**—After eliminating the diurnal heat increment of feeding effect by feeding the animals at 3-hour intervals, the remaining diurnal rhythm must be attributed to muscle tonicity as suggested by Kayser for his pigeons, and to muscular activity. While our rats were confined in a small chamber where muscular movements were greatly restricted, there was a certain amount of movement comparable to the movements of a person in bed. An attempt was made to record these movements by the apparatus indicated in Fig. 3. This apparatus does not of course record changes in muscle tone. Nor does it record all sensible movements. Nonetheless it is of some interest to observe as many of the movements as possible, with the results shown in Fig. 6, on the same rats for which the metabolism is indicated in Fig. 5.

As in Fig. 5, the top curve represents activity of the fasting rat under continuous light, the bottom curve represents *average* activity under continuous light fed at 3-hour intervals; the others are individual curves. It appears from Fig. 5 that the activity is greater in fasting than feeding rats and that the recordable activity rhythm seems to be extinguished more rapidly than the metabolic rhythm, *indicating that non-recordable activity, especially muscle tonus, is a major item in the diurnal metabolic rhythm.*

**4. Diurnal R. Q. Rhythm.**—Fig. 7 shows the time course of the respiratory quotient of the fasting rat under continuous light (top curve), the average of 6 rats under continuous light and continuous (3-hourly) feeding (bottom curve), and the individual curves.

Fig. 8 presents a comparison of the average time curves of the respiratory quotient (bottom curve), metabolism per $W^{.73}$ (middle curve), and activity rhythms (top curve). The activity and metabolism curves obviously parallel each other. The R. Q. peak tends to precede the metabolic peak. We found similar precedence of R. Q. to metabolism peak in cattle. Following feeding in cattle, the R. Q. rapidly rises, and the change from a rising to a declining course occurs while the metabolism increase due to feed intake continues. In most cases there was increase in R. Q. during the mus-
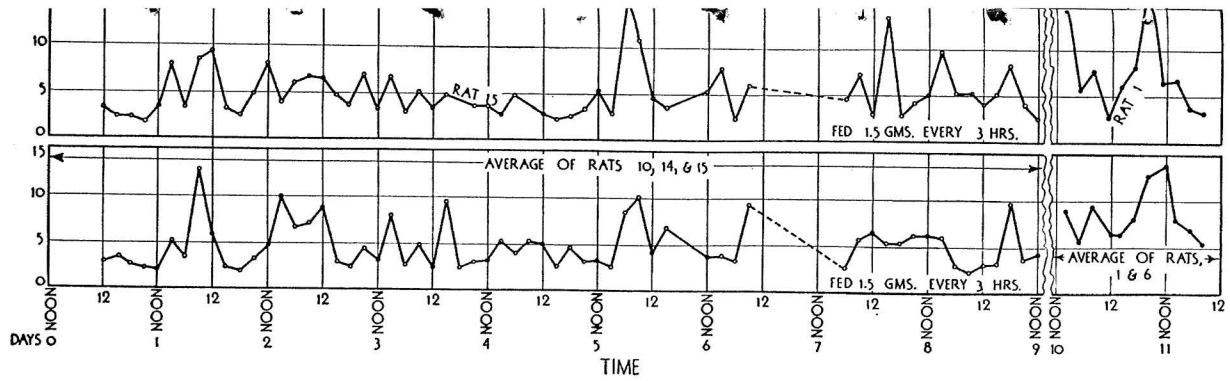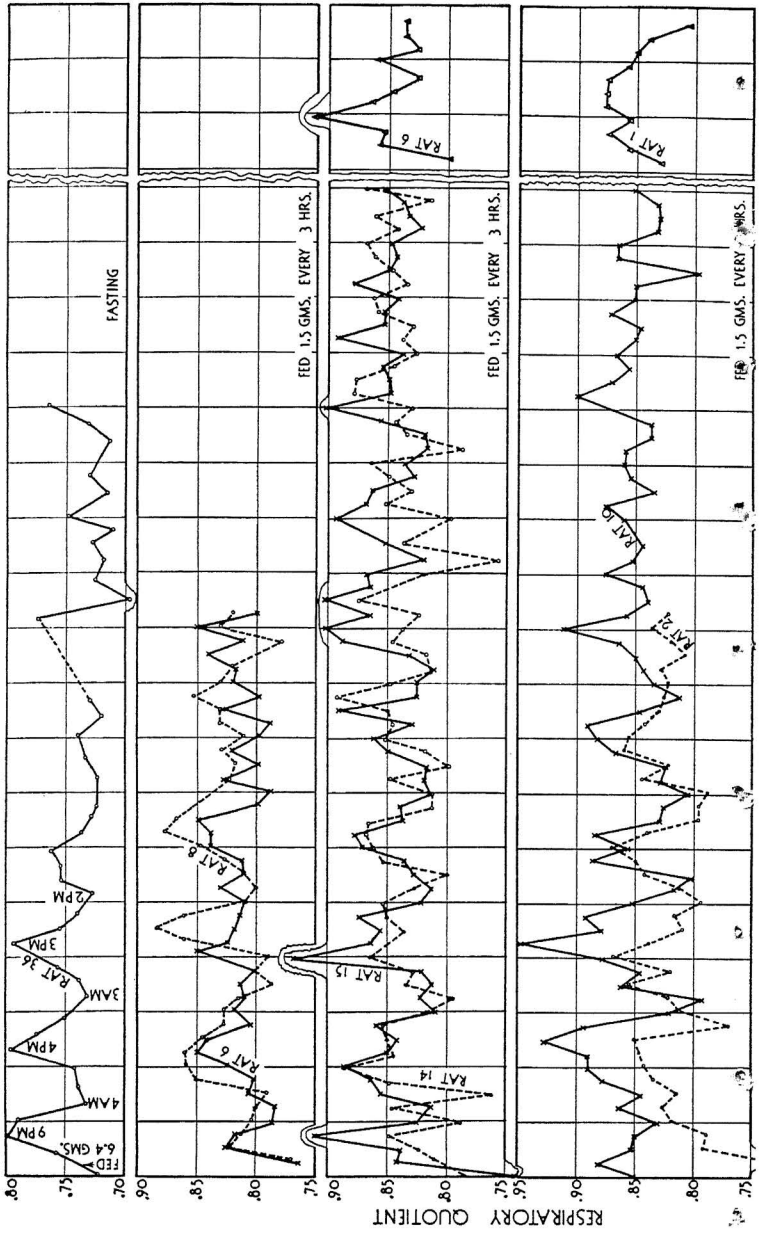
Fig. 6.—Diurnal muscle-activity rhythms of the rats represented in Fig. 5. These curves of course represent only such muscular activities as are recorded by the apparatus shown in Figs. 2 and 3. The metabolically important muscle-tonus changes are here represented not at all or only to a small extent. Note the extinguishment of the rhythms under constant light and 3-hourly feeding conditions.
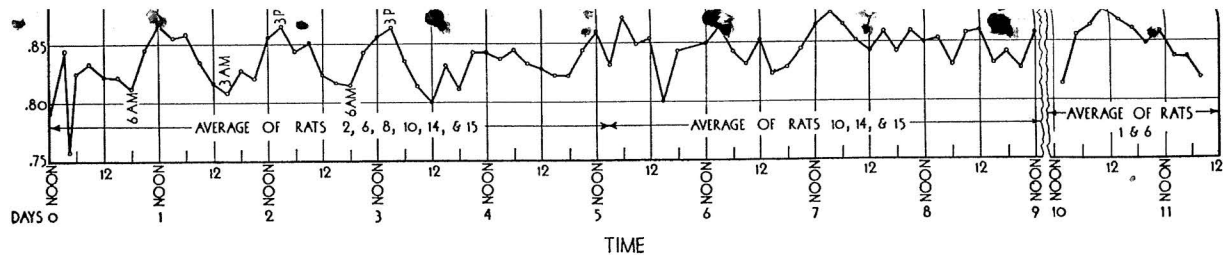
Fig. 7.—Diurnal R. Q. rhythms of the rats represented in Figs. 5 and 6. Note that even the fasting rat shows a diurnal rhythm during the first 3 days of fast, and recall that during the activity peak 17 to 20% of the heat is dissipated by water evaporation, and during the metabolic trough, 13% to 16% of the heat is dissipated by water evaporation. As with metabolic and muscular-activity rhythms, the R. Q. rhythms tend to be extinguished.
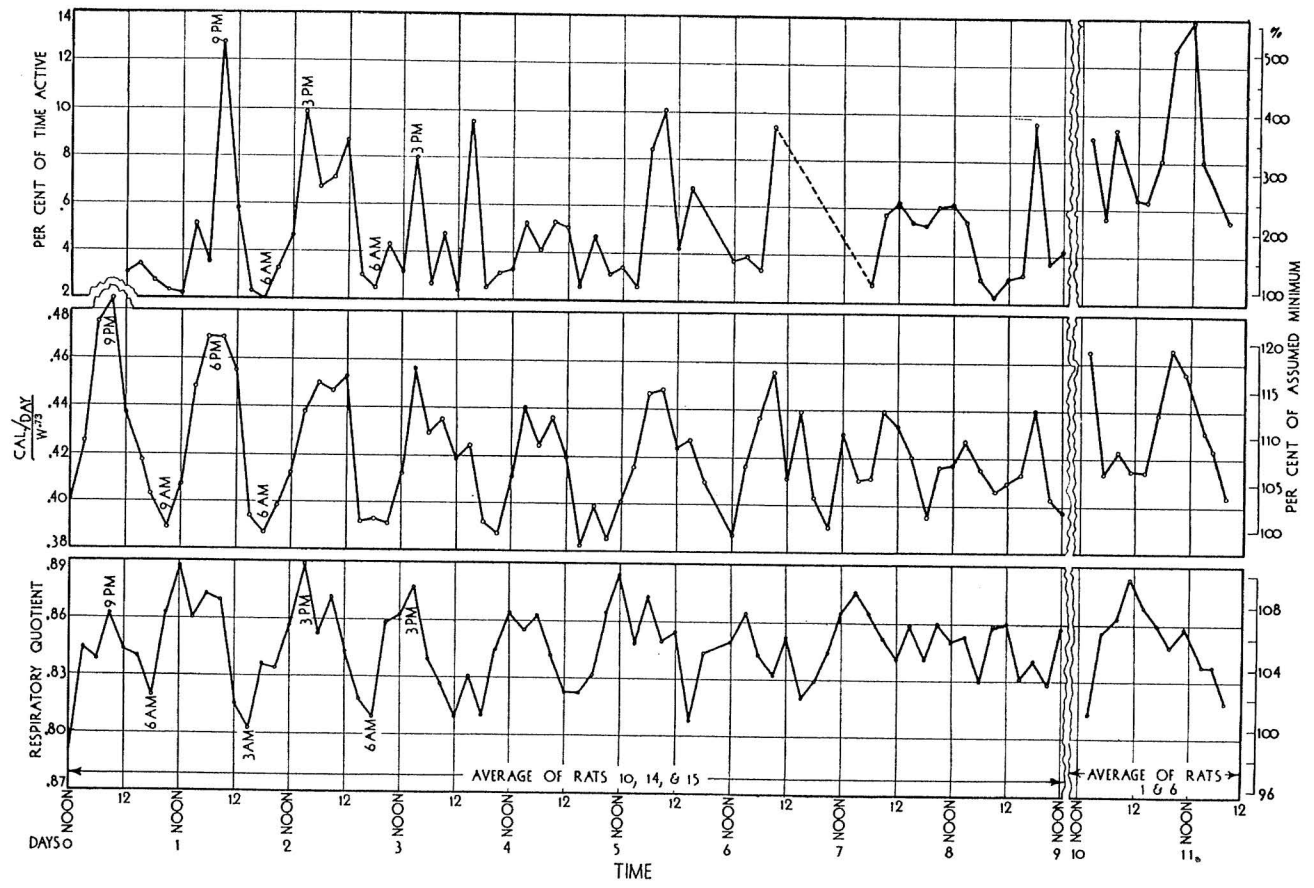
Fig. 8.—A comparison of the average time curves of the diurnal rhythms of R. Q., metabolic level, and muscular activity shown in Figs. 5 to 7. The rats were under continuously uniform light day and night, and fed every 3 hours ⅛ of the day's maintenance ration.

cularly quiet or low-metabolism phase and a decrease in R. Q. during the muscularly active or high-metabolism phase.

Krogh and Lindhard[46] discovered that a high R. Q. tends to be reduced by muscular exercise. The peculiar relation between the time course of the R. Q. and metabolism shown in Fig. 8 substantiates their conclusions. The curious aspect about Figs. 6 and 7 is that this relation continues during fast and during continuous (3-hourly) feeding.

It may be noted here that the insensible water loss from cutaneous and respiratory surfaces parallel's the metabolism curve. At the metabolic (and activity) peak there was also a water-loss peak when 17% to 20% of the heat loss was calculated to be due to evaporation of water; at the metabolic trough there was also a water evaporation trough when 13% to 16% of the calculated heat loss was dissipated by evaporation.

## III.  SUMMARY AND CONCLUSIONS

Some species, such as rats, can see better in normal night light than in normal daylight; others, such as man, can see better in daylight than night light. Of course even light-loving man is blinded by dazzling light: light is a relative concept which lights one's path and blinds another. This diurnal difference in vision gradually leads to a diurnal rhythm in rest (especially sleep) and activity, and to diurnal rhythms of many other processes.

The nocturnal rat for example gradually develops the habits—conditioned reflexes—of sleeping during the day, and feeding, etc. during the night. These conditioned reflexes gradually come to include also muscle tone, urination, defection, intermediate metabolism including blood composition, and even perhaps body composition. All these diurnal differences are reflected in a diurnal metabolic rhythm which is so persistent that in the rat it can not be extinguished by a month of continuous light alone, by continuous feeding (at 3-hour intervals 1/8 of day's maintenance ration) alone, or by a combination of continuous lighting and fasting. But a combination of continuous lighting and continuous feeding (1/8 of daily ration every 3 hours) extinguished the metabolic rhythm in about a week.

The fact of the presence of such rhythms, their natural history and mechanisms, and their ultimate dependence on the sun rhythm, is scientifically and philosophically (from the organismic viewpoint)

[46]Krogh, A., and Lindhard, J., The relative value of fat and carbohydrate as sources of muscular energy. Biochem. J., *14*, 290, 1920. See also Higgins, Berkson, and Flock, l. c., reporting bimodal curves of glycogen storage.

interesting, and it is practically important in studies on the influence of given factors on metabolism. Unless the diurnal metabolic rhythm is taken into consideration, or unless the rhythm is extinguished as described in the text, the metabolic difference due to the conditioned diurnal and related rhythms may exceed the metabolic differences caused by the factors under investigation, and confuse the problem.

While the laboratory part of this research was concerned largely with the diurnal metabolic rhythm, the review of literature indicates the presence of many other rhythms. There are shorter rhythms in the rat, such as Richter's two to four-hour "spontaneous" rhythms, 3-hour feeding and "washing-up" rhythms, and numerous digestive, circulatory, respiratory, excretory and secretory rhythms, blood and tissue (especially liver and adrenal) composition and brain potential rhythms which influence the the metabolic level. There are also longer rhythms. Activities of rats in revolving cages[47] indicate the presence of 4-day rhythms, and longer sex and seasonal-cycle rhythms.

[47]Cf., Wang, G. H., The relation between spontaneous activity and the oestrus cycle in the white rat. Comp. Psychol. Monographs, 2, 1, 1923 ; Slonaker, J. R., The effect of pubescence, oestration, and the menopause on the voluntary activity of the albino rat. Am. J. Physiol., 68, 294, 1924 ; Richter, l. c., 1927; Sherwood, T. C., The relation of season, sex, and weight to the basal metabolism of the albino rat. J. Nutrition, 12, 223, 1936 ; Smith, A. H., and Anderson, J. E., The effect of quantitative and qualitative stunting on maze learning. J. Comp. Psych., 6, 337, 1926 ; relation of performance to age and nutritive condition in the white rat. Id., 13, 409, 1932.

## ADDENDA

After the foregoing material was in final proof, H. G. Barrott, J. C. Fritz, E. M. Pringle, and H. W. Titus reported beautiful and surprising data on a diurnal rhythm in growing fowls. (The article entitled "Heat production and gaseous metabolism of young male chickens" appeared in the February 10 issue of the Journal of Nutrition, vol. 15, p. 145, 1938). The surprising aspect about the data is that "the amplitude of the diurnal rhythm of the energy metabolism is greatest in the very young chick and decreases rapidly with age." "At the average age of 1 week . . . . . an average maximum difference of about 24% was observed between the oxygen consumption at 8 a. m. and 8 p. m. After the age of 14 weeks was reached the amplitude of the diurnal rhythm tended to remain constant at a value . . . . . . of about 11.4% between the 8 a. m. and 8 p. m. values." This apparently contradicts our adopted theory that the diurnal rhythm develops with increasing age as a conditioned reflex. The trough of the rhythm occurred at 8 p. m., and the peak at 8 a. m. These times of high and low metabolic levels presumably vary with season.