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A Solar Daily Variation in Oxygen Consumption of the Embryonated Egg.* (26094)

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For a number of years this laboratory has pursued an extensive comparative study of the persistent metabolic rhythms in algae, angiosperms, invertebrates, and vertebrates. These metabolic rhythms possess basic, component, solar and lunar daily variations which predominate or underlie the phase-labile, adaptive rhythms of special processes when the latter are present. The occurrence of such variations is called extrinsic rhythmicality because the periods, and at least to some extent, the amplitudes and forms, are derived in direct response to some subtle geophysical rhythm(1). The disclosure of these variations in all organisms studied suggests that this is a universal phenomenon. If there were present such fluctuations in the metabolic state of the embryonated egg, they might constitute a variable whose importance has not been recognized to date. Therefore, the following study was undertaken to learn (i) if systematic fluctuations did indeed occur and (ii) something of their character were this the case.

Materials and methods. O_2-consumption of individual eggs was measured with a Brown automatic recording respirometer(2, 3) in constant illumination, temperature, humidity, pressure, CO_2- and O_2-tension, etc. It was necessary to modify the respirometer chamber somewhat. A glass tumbler having a volume of 110 cc was fitted with a No. 11 rubber stopper. To the upper end of this stopper was sealed a plastic (Saran) sack of about 60 cc capacity which was the oxygen reservoir. The rubber stopper was penetrated by a 26-gauge hypodermic needle which served as a capillary connection between the collapsible oxygen reservoir and the chamber beneath. In operation 10 cc of water, a vial of KOH solution, and a small glass frame were placed in the bottom of the tumbler. The egg was rested air sack uppermost on the 4 tips of the glass support. The flask was stoppered, leaded until it just submerged, and suspended beneath the recorder in the water bath of a barostat at 38 $\pm$ .05° C. The unit was hermetically sealed and aspirated to a constant pressure of 28.7 in. Hg.

Eggs were obtained periodically from a local commercial source between Nov. 1959, and April, 1960. Before its oxygen consumption was recorded, each egg was allowed to warm to room temperature and was then placed in a forced-draft incubator at 38°C at about 4 P.M. C.S.T. The day on which incubation was initiated was designated Day 1. During the morning or afternoon of Day 2 or 3 the egg was transferred to the respirometer in which incubation was continued until termination of the experiment on Day 7. The egg was then opened and inspected. If an embryo did not appear to have developed normally its record was discarded. Ordinarily the oxygen supply was depleted on Day 7. Some data for Day 8 were obtained by opening the barostat for a 10-minute interval on Day 7 and replenishing the supply of oxygen and of CO_2-absorbent. This study is restricted to a 4-day period in development. The onset of the period is determined by the sensitivity of the recorder and the end, by the capacity of the O_2-reservoir.

Results. About 1800 hours of respiration

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TABLE I. Rates of Oxygen Consumption for Normal Embryonated Eggs as Determined by Various Investigators.

Hr of incubation	This study	Romanoff(4)	McLimans *et al.*(5) Series A‡	McLimans *et al.*(5) Series B‡	Murray (6)*	Hasselbalch (7)†	Greiff *et al.* (8) Spring setting
72	.226 ±.066§(6)‖	.253 ±.040 (4)					
96	.427 ±.110 (25)	.381 ±.100 (6)	.34 ±.11 (12)				
120	.680 ±.131 (26)	.524 ±.052 (6)	.44 ±.05 (25)			.63	.61
144	.826 ±.178 (9)	.831 ±.134 (9)	.89 ±.07 (11)	.91 ±.06 (3)	.88	.92	1.54
168	1.423 ±.191 (14)	1.421 ±.223 (6)		1.19 ±.14 (6)	1.32	1.77	2.08
192	1.922 ±.173 (4)	1.671 ±.173 (4)	1.23 ±.26 (33)	1.51 ±.03 (5)	1.99	2.43	2.26

Rate as cc of O_2/egg/hr. * Calculation of Needham(9) from Murray's data. † As cited by McLimans *et al.* ‡ Series of different genetic patterns. § Stand. dev. ‖ No. of observed eggs.

data were collected and distributed as follows, 24 complete Day 5's, 23 complete Day 6's, 8 complete Day 8's, and several incomplete days from slightly earlier and later stages. The validity of results obtained under the conditions of this experiment was tested by comparing them with results of other investigators who have employed a variety of technics to measure O_2-consumption in the intact egg (Table I). These data are in such consistently good agreement over the 6-day period that one may safely conclude the Brown recording respirometer to be a reliable instrument for purposes of this study.

To disclose any daily pattern which might be superimposed upon the sigmoid curve characteristic of O_2-consumption during development, the data were reduced as follows. A straight line was constructed arbitrarily between the average 1-2 A.M. value and the average 11-12 P.M. value for each complete day of data. The deviation of each hourly reading from this linear daily trend-line was measured. In Fig. 1 are depicted the mean daily patterns of O_2-consumption for 3 days of incubation obtained as mean hourly deviations from daily trend. The mean linear trend expressed in cc/egg/hr is from 0.226 to 0.497 on Day 5, 0.518 to 0.747 on Day 6, and 1.186 to 1.508 on Day 8. It is readily apparent that the 3 days possess a common diurnal pattern in average hourly rate of O_2-consumption. Through the morning hours rates are characteristically low but rise in the afternoon to a sharply defined maximum at 7 P.M. and then return rapidly to the trend-line. This pattern is indicated by the following statistically significant differences between means of maximal and minimal hourly rates: 5 A.M. and 7 P.M. on Day 5, $P<.001$; 5-6-7 A.M. and 6-7-8 P.M. on Day

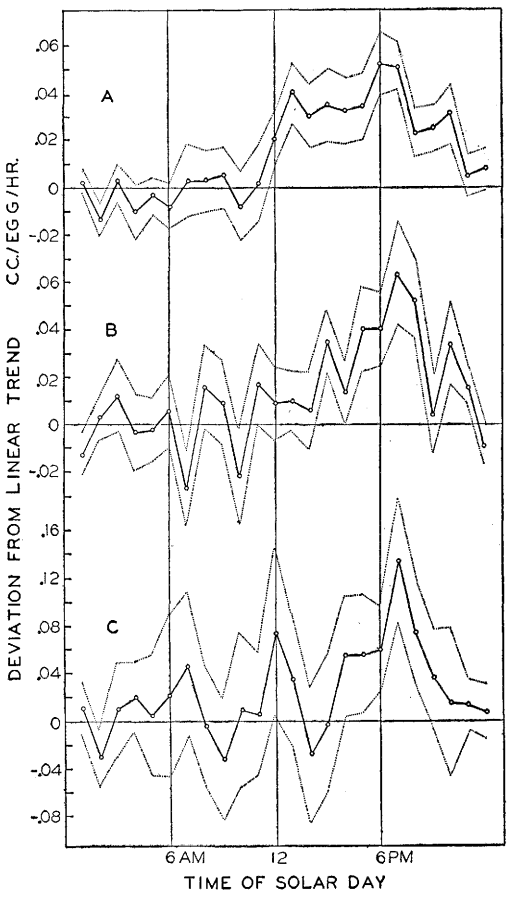

FIG. 1. Daily variations in O_2-consumption for 3 days of incubation, as mean hourly deviations from daily trend. A. Day 5, avg of 24 eggs. B. Day 6, avg of 23 eggs. C. Day 8, avg of 8 eggs. Stand. errors for all hours are indicated. Time is CST.

6, $P<.001$; and 8-9 A.M. and 7-8 P.M. on Day 8, $P<.02$. The mean range of the fluctuation is 17.1% of the mean daily rate on Day 5, 13.0% on Day 6, and 12.3% on Day 8.

It will be recalled that all data were obtained while the recorder and organism were hermetically sealed in a large rigid container maintained at constant ambient pressure. During this study the barometric pressure was recorded with a microbarograph at the laboratory in Evanston. Analysis of the O_2-consumption data revealed that the 7 P.M. deviation from linear trend on Days 5 and 6 was correlated with the 2-6 P.M. rate of barometric pressure change on the same day within the limits −0.12 to +0.08 in. Hg ($r = +0.446$, $N = 41$, $P<.005$). Range in metabolic deviation was substantial, −0.12 cc/egg/hr to + 0.28 cc/egg/hr.

Discussion. It is well-known that the period of laboratory incubation cannot be used to determine embryonic stage of development. Among eggs incubated for the same period variation in stage may be considerable, particularly in the first 4 or 5 days of incubation(10). Individual or chance factors which contribute to this variability are genetic differences(11), differences in length of the latent period(10), uneven retention of the egg in the oviduct, and temperature of environment in storage and transport prior to incubation(12). In this study the investigator made no attempt to control these factors. Thus, recurrence of a sharply defined maximum in O_2-consumption at the same time on successive days is prima-facie evidence that the observed diurnal fluctuations are *not* correlated with any particular developmental process (morphogenesis, differentiation). The direct relationship of metabolic activity to time of solar day indicates that one is dealing with a protoplasmic response identical to manifestation of a persistent rhythm. The daily pattern in O_2-consumption appears to be independent of morphological development within limits of variation for embryos of the same incubational age. However, in that new levels of development are rapidly being achieved, it is conceivable that the form of daily fluctuations, other than the ones studied here, may be a function of the incubation period. Such a possibility is clearly suggested by diversities in minor components of the 3 mean fluctuations in Fig. 1.

The correlation of 7 P.M. metabolic activity with coincident 2-6 P.M. rate of barometric pressure change can be adduced as further support for identification of these diurnal fluctuations with extrinsic rhythmicality. Such correlations with this specific pressure parameter have been described for metabolic rhythms in organisms as diverse as the potato(3), fiddler crab(13), and mud snail (14). The existence of this relationship to atmospheric pressure change in an organism which so nearly approaches a closed system is obviously further evidence for the universal nature of this phenomenon. Not only does the metabolic state of the organism vary with time of day, but also from one day to the next, as it responds to its varying geophysical environment.

Summary. The O_2-consumption of individual embryonated eggs was measured continuously for 3 days in constant conditions, including pressure. Superimposed on linear daily trend for Days 5, 6, and 8 was a mean pattern in hourly rates possessing a characteristic morning minimum and 7 P.M. maximum. This 7 P.M. deviation from daily trend was related to the concurrent 2-6 P.M. rate of atmospheric pressure change. This indicates that the solar daily variations of oxidative metabolism in the intact egg are a manifestation of extrinsic rhythmicality.

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Observations on the Role of Lipids in Serum-Induced Intravascular Thrombosis.* (26095)

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Thrombus formation in diseased coronary arteries is the most common clinically important complication of atherosclerosis. Moreover, it has been suggested that the atherosclerotic process itself may result from endothelialization of intravascular thrombi(1). Dietary and serum lipids have been shown to be associated with the incidence and prevalence of coronary heart disease in human populations. Whether lipid alterations can affect coagulability of blood leading to thrombosis, as well as possibly contributing to the development of the underlying atherosclerosis, is not known. Alterations of serum lipids can produce marked atherosclerosis in experimental animals, yet there are few reports of induced thrombosis in such experiments(2). To investigate the possible role of lipids in coagulability of blood, there have been many studies utilizing various *in vitro* technics. The controversial results have been well summarized(3,4).

Wessler(5) reported on a method of inducing intravascular thrombi in dogs in a venous segment isolated after systemic injection of homologous serum. A later communication(6) presented an extension of this *in vivo* technic to production of thrombi in rabbits following infusion of heterologous (human) serum. This communication also presented evidence to show that the active serum factors necessary in this system are Factor IX (Plasma Thromboplastic Component), Hageman Factor, and possibly Plasma Thromboplastin Antecedent(7).

In an effort to quantitate the dose-response-time interrelationships we have adapted Wessler's method to the use of chickens as the assay animals. Chickens are cheap, grow rapidly, and are easily maintained. Some preliminary findings of the effects of lipids on this system are presented.

Materials and methods. White Leghorn cockerels approximately 3 to 4 weeks of age (200 to 300 g) were used. Some of the birds were fed a commercial Chick Starter mash, others received purified diets containing 0.8% cholesterol in which the source of fat was varied. The composition of these has been described(8).

Pooled human serum lots were stored at 4°C and used within one week of collection. Pooled chicken serum from birds on the mash diets was similarly stored for use.

Cholesterol determinations on the assay birds' sera and on the infused test sera were performed by the method of Carpenter *et al.* (9).

Chickens were anesthetized with intraperitoneal sodium pentobarbital (Nembutal) and a 5 cm segment of non-branching jugular vein was exposed. The test serum was infused into the contra-lateral jugular vein and al-

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