relogram at the 20-day period. 17 of the 21 subjects showed a positive value at this point, statistically significant at the $p < 0.01$ level (sign test, two-tailed). Auto-correlation at the 21-day period is similarly statistically significant at the $p < 0.05$ level, with 15 out of 21 showing a positive value. There are no other significantly positive auto-correlations in the spectrum. Cross-correlation fails to show any clear peak. This indicates that the fluctuations producing positive auto-correlations at 20 and 21 days are by no means sinusoidal.

Figure 2 shows the results of frequency analysis on the time at which subjects made their recordings. The cross-correlogram shows a clear peak at 7 days, but no peak at 20 or 21 days. The auto-correlogram shows statistically significant peaks at the 7-day interval and subsequent harmonics.

The 'Alertness-Dullness' auto-correlogram showed small peaks at 21 and 23 days, and a significantly positive value at the 22-day interval ($p < 0.05$; sign test, two-tailed). Analyses of the other self-reported mood and sleep quality scales showed no clear evidence of periodicity.

Discussion. The correlograms shown in figure 2, of time of day, require some interpretation. In general, if the auto-correlation at 1 period is highly positive, then, inevitably, auto-correlations at every subsequent harmonic of that period will be similarly highly positive, unless there is a phase shift. The apparent peak in the 'Time' auto-correlogram at 21 days, like the slightly larger one at 14 days, is probably artefactual and produced by the high auto-correlation at the 7-day interval. (There would be no question of phase shift, since all subjects were almost certainly strongly entrained by our calender week.) Cross-correlation shows no hint of a peak at 21 days. It is therefore unlikely that the observed 20-day periodicity in temperature was mediated through variations in the time at which the recordings were made.

It is tempting to speculate that the reported periodicities in steroid excretion[1], and pitch perception[2], like the variations in temperature, and perhaps even mood, reported here, are manifestations of some stable intrinsic rhythm in the human male. It will be necessary to carry out prolonged series of measurements before anything can confidently be said about either the shape of the temperature cycle, or its phase relation to any other cycles. The evidence presented here, however, supports the notion of a temperature cycle of about 20 days period length.

Inhibition of growth in developing oocytes of the desert locust

S. S. Tobe[1]

Department of Zoology, University of Toronto, 25 Harbord Street, Toronto (Ontario, Canada M5S 1A1),
3 August 1976

Summary. The location of the terminal oocytes (T) in the ovary of the desert locust dictates whether the penultimate oocytes (T-1) will enter vitellogenesis – as long as the T oocytes are retained within the ovarioles, vitellogenesis in T-1 oocytes is prevented. When the T oocytes are ovulated into the oviduct, growth of the T-1 oocytes (new T) resumes. Inhibition of vitellogenesis in T-1 oocytes is not due to low rates of JH biosynthesis since high rates of JH biosynthesis were observed in animals in which T oocytes were retained in the ovarioles.

Oocyte growth in many insects is a highly coordinated synchronous process, with batches of oocytes maturing at the same rate for oviposition at the same time. In these insects, only the terminal oocytes in individual ovarioles reach maturity during any given reproductive cycle. This implies that the penultimate and younger oocytes are prevented from completing maturation. This inhibition usually involves the arrest of growth in the penultimate oocytes (T-1), as long as the terminal oocytes (T) are retained within the female reproductive system. Thus, in females bearing oothecae, in females retaining mature oocytes and in females reared in the absence of males, the ultimate effect of this inhibition is to prevent the growth and maturation of T-1 and younger oocytes[2]. There are at present two types of inhibition of oocyte maturation which have been postulated to operate in insects. The first of these, which has been hypothesized to function in houseflies, ovoviviparous cockroaches and the Hemipteran, Iphita limbata, suggests that a humoral factor is released from the reproductive system which then acts upon the corpus allatum (CA), preventing the release of the juvenile hormone (JH)[3–6]. Accordingly, egg production is reduced or stopped, since the maturation of oocytes in most insects requires the presence of 'active' CA[2]. The second hypothesis does not involve the release of JH from the CA but rather postulates that a factor is released from the ovaries which acts directly upon the follicular epithelium to prevent the uptake of vitellogenin by the maturing oocytes; such a mechanism has been postulated in Rhodnius prolixus[7,8].

In the desert locust Schistocerca gregaria, T-1 oocytes do not enter vitellogenesis in normal females as long as either maturing or mature T oocytes are present[9,10]. Thus it seemed likely that some type of inhibition operated to prevent T-1 oocytes becoming vitellogenic. Highnam[11] suggested that the presence of mature oocytes at least partially restricted the activity of the CA. The hypothesized mode of action of this inhibitory factor in S. gregaria

1 I thank S. Honsl for technical assistance and Prof. K. G. Davey and Dr G. E. Pratt for critically reading the manuscript. This work was supported by National Research Council of Canada Operating, grant A9408.
2 F. Engelmann, in: The Physiology of Insect Reproduction. Pergamon Press, Oxford 1970.
3 T. S. Adams, A. M. Hintz and J. G. Pomonis, J. Insect. Physiol. *14*, 983 (1968).
4 T. S. Adams, J. Insect. Physiol. *16*, 349 (1970).
5 F. Engelmann, Arch. Anat. microsc. *54*, 387 (1965).
6 K. K. Nayar, Proc. Ind. Acad. Sci. B *47*, 233 (1958).
7 G. E. Pratt and K. G. Davey, J. exp. Biol. *56*, 223 (1972).
8 E. Huebner and K. G. Davey, Can. J. Zool. *51*, 113 (1973).
9 K. C. Highnam, O. Lusis and L. Hill, J. Insect. Physiol. *9*, 827 (1963).
10 S. S. Tobe and G. E. Pratt, J. exp. Biol. *62*, 611 (1975).
11 K. C. Highnam, Roy. Ent. Soc. Lond., Symp. No. 2, 26 (1964).

is similar therefore to that subsequently suggested for the housefly, Musca domestica[3,4]. However, recently Lea[12] has cast doubt on the nature of the inhibition in M. domestica and has suggested, on the basis of allatectomy experiments, that the factor(s) involved in preventing oocyte growth in penultimate oocytes does not operate through the inhibition of JH release from the CA. Similarly, in R. prolixus, the antigonadotropin has been postulated *not* to act at the level of the CA to prevent JH release[7,8]. I report here on the inhibition of growth in T-1 oocytes of S. gregaria and show conclusively that this factor does *not* operate by inhibiting the synthesis and release of JH by CA.

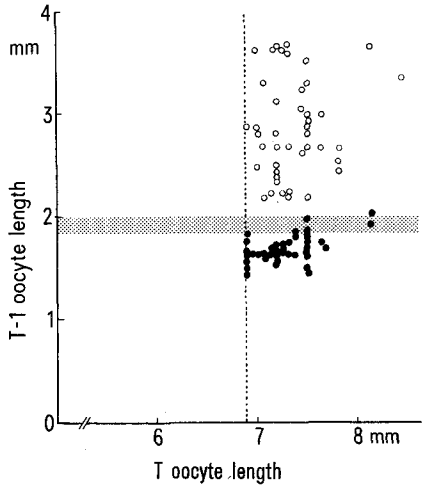

Fig. 1. Relationship between lengths of *mature* terminal (T) oocytes and penultimate oocytes (T-1) in Schistocerca gregaria. Dotted line represents the length at which chorionation occurs in T oocytes and hatched area indicates size range over which oocytes begin active vitellogenesis. Solid circles represent animals in which T oocytes have been retained *within* the ovarioles and open circles represent animals in which T oocytes have been ovulated into the oviducts.

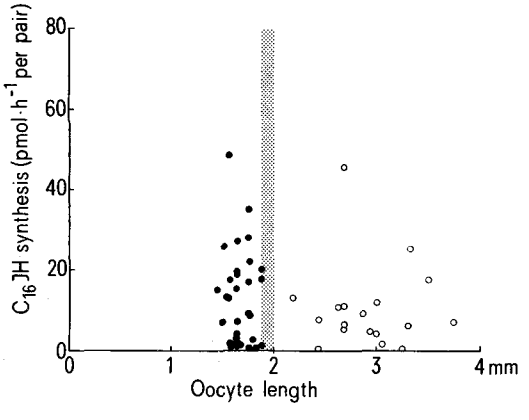

Fig. 2. Relationship between length of T-1 oocytes (solid circles) or new T oocytes (open circles) and rate of synthesis of C_{16} juvenile hormone by individual pairs of corpora allata from S. gregaria, as revealed by the incorporation of [methyl-^{14}C]-methionine in vitro. Hatched area indicates size range over which oocytes begin vitellogenesis. In animals in which vitellogenesis in new T oocytes has begun (open circles), one wave of oocytes has been matured and ovulated into the oviducts while in animals in which the T-1 oocytes have not become vitellogenic (solid circles), the mature T oocytes have been retained *within* the ovarioles.

Methods. Locusts were reared as previously described[10]. All locusts employed were between 12 and 14 days old, since we have previously demonstrated that under our rearing regime, maturation of the first wave of oocytes is completed by 12 days after fledging[10]. Locusts were not provided with damp sand for oviposition so that they would be forced to retain their mature oocytes. Ovaries and CA were dissected from unanaesthetized animals under citrate-fortified Ringer solution[13] in a sterile environment and the lengths of T and T-1 oocytes were measured using an ocular micrometer. Only locusts which contained chorionated oocytes in either the oviducts or within the ovarioles were employed (see below); the presence of the chorion on oocytes retained within the ovarioles can be easily determined by the presence of a distinctive white cap on the proximal end of each oocyte and by the distinctive surface sculpturing observed on oocytes dissected from ovarioles.

The biosynthetic activities of the CA from individual animals were determined from the incorporation of the methyl moiety of [methyl-^{14}C]-methionine (Amersham-Searle; final specific activity 36 mCi/mmole) into C_{16}JH (JH III; methyl, 10,11-epoxy-3,7,11-trimethyl-trans, trans-2,6-dodecadienoate) during a 3-h-incubation in vitro at 37 °C. In some experiments, the medium and CA were separated at the end of the incubation and extracted separately, while in others they were extracted together. The procedures for preparation of the incubation medium and for the extraction, separation and quantitation of the radiolabeled C_{16}JH have been described previously[10,14].

Results and discussion. Each of the paired ovaries of S. gregaria consists of approximately 50 individual ovarioles. Mature chorionated T oocytes are ovulated into the lateral oviducts (to await oviposition) after the completion of chorionation; however, these chorionated oocytes are retained within the ovarioles for a short time prior to the rupture of the follicular epithelium and the subsequent ovulation of the T oocytes into the oviducts. By dissecting large numbers of animals of appropriate age, animals with retained chorionated oocytes can be obtained. The location of the mature T oocytes is important to the development of the T-1 oocytes. Figure 1 shows that as long as mature T oocytes are retained *within* the ovarioles, the T-1 oocytes do not become vitellogenic (solid circles). We have previously established that oocytes become vitellogenic over the size range 1.84–2.00 mm[10] and figure 1 shows that in only one instance did the T-1 oocytes exceed the 2.00 mm range, as long as mature T oocytes were present in the ovarioles. However, as soon as ovulation had occurred, the former T-1 oocytes (new T oocytes) entered vitellogenesis and rapidly increased in length (figure 1, open circles). Thus figure 1 shows conclusively that the presence of mature oocytes within the ovarioles prevents the T-1 oocytes from entering vitellogenesis; the location of the mature T oocytes is therefore an indicator of the vitellogenic state of the T-1 oocytes. Previous reports have demonstrated that little growth occurs in the T-1 oocytes as long as the T oocytes are vitellogenic[9,10] and it has been suggested that the absence of growth in the T-1 oocytes is due in part to a competition between T and T-1 oocytes for available nutrients[15,16]. Figure 1, however, clearly shows a unique situation where

12 A. O. Lea, J. Insect. Physiol. *21*, 1747 (1975).
13 G. E. Pratt and S. S. Tobe, Life Sci. *14*, 575 (1974).
14 S. S. Tobe and G. E. Pratt, Biochem. J. *144*, 107 (1974).
15 K. C. Highnam, O. Lusis and L. Hill, J. Insect. Physiol. *9*, 587 (1963).
16 K. C. Highnam, Proc. int. Study Conf. Future Probl. Acridol., Lond., p. 123 (1970).

there is no longer competition for nutrients and yet growth of the T-1 oocytes is prevented. This suggests that the event of ovulation is physiologically important to the animal in releasing the inhibition of the T-1 oocytes. The nature of this inhibition is unknown, although in other insects it has been suggested to be humoral[3,8]. It would be of interest to determine if the location of the T oocytes in M. domestica reproductive tract influences the production or mode of action of the oostatic factor. However, it is possible that the nature of the inhibition differs in houseflies and locusts, particularly in view of the fact that in other flies (Glossina), the presence of mature oocytes within the ovarioles does not prevent the maturation of other oocytes in the same and other ovarioles[17].

The results presented above demonstrate that in S. gregaria, the growth of the T-1 oocytes is inhibited by the presence of mature chorionated T oocytes *within* the ovarioles but *not* by T oocytes in the oviduct. Using this information, I have examined the activity of the CA in animals in which the growth of T-1 oocytes was inhibited and in animals in which ovulation had occurred, to determine if inhibition of oocyte growth was the result of 'inactivity' of the CA and conversely, if active vitellogenesis and growth of oocytes could be associated with high CA 'activity'. Figure 2 shows that the rate of synthesis of C_{16}JH in animals with inhibited T-1 oocytes is high in many instances (solid circles) while in animals in which ovulation had occurred, the rate of JH synthesis is lower (open circles). The mean rate of JH synthesis in animals with inhibited T-1 oocytes = 13.5 pmole h^{-1} per pair (n = 31) whereas in animals which had ovulated, mean JH synthetic rate = 8.0 pmole h^{-1} per pair (n = 18). Figure 2 demonstrates conclusively that in S. gregaria, the inhibition of growth in T-1 oocytes is not the result of CA inactivity, contrary to previous suggestions[11]. It might be argued that our assay procedure in vitro does not accurately reflect the synthetic capabilities of the CA in vivo. The validity of our assay has been discussed elsewhere[10] and we are confident that it accurately reflects the activity of the glands in vivo. It should also be noted that under all experimental conditions used to date, synthesis of JH in the CA of S. gregaria is followed immediately by release[18] – thus, there is no storage of JH within the CA. In the present experiments, by determining the C_{16}JH content of glands and incubation media separately, a similar relation has been observed.

Because inhibition of T-1 oocyte growth is not the result of low rates of JH biosynthesis, it is necessary to look elsewhere for the nature of the inhibition. At present, this is unknown but is has been suggested that an antigonadotropin emanating from the ovary is responsible for inhibition of T-1 oocytes in R. prolixus[8]. It is possible that a similar factor is operative in S. gregaria and in fact, the observation that T-1 oocytes become vitellogenic in partially ovariectomized animals[9] might be interpreted to indicate that an inhibitory factor is present in the ovary – when part of the ovary is removed, the inhibition is no longer effective. Work is in progress to define the nature of this inhibitory factor.

17 H. Mellanby, Parasitology *29*, 131 (1937).
18 S. S. Tobe and G. E. Pratt, Nature, Lond. *252*, 474 (1974).

Effects of cerebral lateral, ventricular infusions of phloridzin on feeding and body weight in Gallus domesticus (L.)

G. Apostol[1], Constanţa Matei-Vladescu and Valeria Popescu

Department of Animal Physiology and Biophysics, Institute of Biological Sciences, Bucharest (Romania), 18 June 1976

Summary. Contrary to earlier findings in rats, cerebral lateral ventricular infusions of 1×10^{-3} M or 2×10^{-3} M solutions of phloridzin at a rate of 2.5 µl/min for 90 min had no significant stimulating effects on food intake and weight gain in hens and cocks. These different responses to intraventricular phloridzin might reflect a difference of sensitivity to the inhibitory action of phloridzin on glucose transport in cerebral cells or certain peculiarities of mechanisms controlling food intake in chickens.

Glick and Mayer[2] have found that cerebral lateral, ventricular infusions of phloridzin caused marked hyperphagia and excessive weight gain in rats. They attributed the overeating to inhibition of glucose uptake in neural tissue by phloridzin and interpreted the phenomenon as definite proof for the existence of cerebral glucoreceptors which are involved in the regulation of food intake.

The purpose of the present experiments was to investigate the effects of intraventricular phloridzin on feeding and body weight in Gallus domesticus. This is a species of bird which, taking into account its anorectic response to insulin, seems to have certain peculiarities in the mechanisms controlling food intake[3–5].

Material and methods. The experiments were carried out on 6 laying hens (White Leghorn) and 6 4-month-old cocks (White Rock × Cornish) housed individually and fed commercial chicken mash and water ad libitum.

To perform the cerebral ventricular infusions on unanesthetized birds, a technique developed by Goodrich et al.[6], and modified by us, was used. According to this technique, the lateral ventricle is punctured at the time of each infusion through an extradural guide-tube mounted chronically in the parietal bone. Details of construction for our modified guide-tube and probe assembly are shown in figure 1. Implantation of guide-tube was conducted under pentobarbital anaesthesia, using stereotaxic coordinates from the stereotaxic atlas of van Tienhoven and Juhasz[7].

1 Present address: Institute of Endocrinology, Department of Neurobiology, Bul. Aviatorilor, 34, Bucharest 1, Romania.
2 Glick and J. Mayer, Nature *219*, 1374 (1968).
3 S. Lepkovski, R. Len, T. Koike and R. Bouthillet, Am. J. Physiol. *208*, 589 (1965).
4 S. Lepkovski, M. K. Dimick, F. Furuta, N. Snapir, R. Park, N. Narita and K. Komatsu, Endocrinology *81*, 1001 (1967).
5 C. Matei-Vladescu, Revue roum. biol. Ser. zool. *6*, 383 (1971).
6 C. A. Goodrich, B. Greeney, T. B. Miller and J. R. Pappenheimer, J. appl. Physiol. *26*, 137 (1969).
7 A. van Tienhoven and L. P. Juhasz, J. comp. Neurol. *118*, 185 (1962).