fore constitute a relatively larger part of the hemispheres in the hedgehog than in the dog. Particularly in rhinencephalic structures 5HT is contained preferentially[9].

The immediate cause of the brain 5HT increase is probably due to such changes in enzyme activities that relatively more 5HT is synthetized than destroyed. The oxygen consumption of the tissues is greatly decreased during hibernation[10]. The monoamine oxidase activity is largely dependent upon oxygen tension[11]. The amino acid decarboxylases, on the other hand, are more efficient in anaerobic conditions[12]. Variations in the oxygen and carbon dioxide tensions, however, may not be the cause of 5HT increase, since ANDERSON and BONNYCASTLE[13] found no effect of deprivation of oxygen or accumulation of carbon dioxide, or of both, on the brain 5HT in rats. Direct evidence is so far not available concerning the possible changes of the activities of enzymes forming or destroying 5HT in different states of activity in hedgehogs.

The physiological significance of the changes of the brain 5HT is difficult to interpret. The predominant appearance of bound 5HT in synaptic vesicles of nerve terminals in the guinea-pig brain seems strongly to suggest its participation in synaptic transmission[14]. Several observations suggest that 5HT more suitably functions as a trophotropic than as an ergotropic transmitter[6,15,16]. Our own observations with hibernating hedgehogs point in the same direction. The 5HT precursor, 5-hydroxytryptophan (40 mg/kg of *dl*-5HTP s.c.) did not arouse the hibernating hedgehog, although it was decarboxylated in the brain and increased the brain 5HT content. On the other hand, the equivalent dose of *l*-DOPA started the arousal mechanism, and the hedgehog was aroused within a few hours[17].

According to AZZALI[18], SUOMALAINEN, and NYHOLM[19] the neurosecretion from the supraoptic nuclei is enhanced during the cold season, especially during the last period of hibernation. This is an important observation since it demonstrates that not all brain functions are depressed during hibernation. From this it may be concluded that also the production of an agent functioning as a transmitter at the supraoptic nuclei is probably enhanced during the cold season. PICKFORD[20] and DUKE et al.[21] have suggested that acetylcholine (ACh) is this transmitter in the dog. It would be attractive to suggest a possibility of the activation of a central cholinergic transmitter system in the hedgehog during the cold season and hibernation. Parallel changes in the 5HT and ACh contents in the brain have been reported in different activity states. Thus the ACh content of the brain is higher during sleep than in wakefulness, being at its highest during anesthesia[22,23]. The brain 5HT also increases in rats during anesthesia[13] and during the sleep period in mice[24].

The electric activity in the brain is greatly decreased during hibernation, 90% in the ground squirrel according to STRUMWASSER[25]. The biochemical processes taking place in synaptic transmission, however, need not be decreased to the same extent. Our experiments with precursor amino acids and monoamine oxidase inhibitors show a considerable amine formation in the brain and some other organs, especially in the kidney and liver of the hibernating hedgehog. The animals were aroused within a few hours after the administration of monoamine oxidase inhibitors[17].

Our studies on the catecholamine content in the brains of hedgehogs in different activity states indicate that the noradrenaline content is decreased during profound hibernation[17]. For the present we pay attention only to the opposing changes of 5HT and noradrenaline in the hibernating brain. In conclusion, we suggest that these supposed transmitter substances belong to functionally different systems in the brain, one of which may be functioning more effectively while the other is less active[26].

Zusammenfassung. Der 5-Hydroxytryptamingehalt des Igelhirns während der kalten Jahreszeit und besonders während des Winterschlafs war stets höher als mitten im Sommer. Die grösste Zunahme wurde in den Grosshirnhemisphären gefunden. Mechanismus und physiologische Bedeutung der Änderung wurde diskutiert und dabei angenommen, dass diese im Zusammenhang mit einer autonomen Regelung des Hibernationszustandes stehe.

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In vitro Exploration of a Circadian Rhythm in Adrenocorticotropic Activity of C Mouse Hypophysis

In vitro[1], as well as *in vivo*[2,3] a circadian (circa, dies[4–6]) rhythm characterizes the reactivity of certain C mouse adrenals to adrenocorticotropic hormone (ACTH). Thus, the amount of corticosterone produced as a result of

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ACTH added *in vitro* depends predictably upon the timing of adrenal removal. This adrenal reactivity rhythm to ACTH is grossly out-of-phase with the spontaneous circadian periodic changes in corticosterone content of serum from the same mice. When serum corticosterone levels are low, the adrenal responsiveness to large doses of ACTH is high.

Against this background, a rhythm in adrenocorticotropic activity of the inbred C (Bagg albino) mouse pituitary and the time relations of this rhythm to other aspects of the adrenal cycle will be described herein. Corticosterone production was estimated, following incubations of adrenal tissue removed from animals at one time-point with pituitary tissue[7] which had been removed from separate yet comparable groups of standardized mice at 4 h intervals during a 24 h period.

Female C mice, 2 to 3 months of age, served as donors of pituitaries. These mice had been standardized for 1 week on a regime involving, among several other precautions[8], a schedule of light from 0600 to 1800, alternating with darkness. Groups of these mice were killed at 4 h intervals, starting at 0800 of one day and ending at 0800 of the next. At each time point pools of 4 pituitary glands were prepared and immediately frozen and stored in buffer medium used for incubation.

Within 2–4 weeks after the collection of pituitaries, male C mice, previously standardized for one week in light from 0600 to 1800, were killed on the day of the incubation at 0400 ($\pm$ $^1/_2$ h). The adrenals of this latter group of mice were immediately removed, defatted, quartered[9] and added in pools, each of 10 glands, into 25 ml. Erlenmeyer flasks containing the pools of 4 frozen pituitary glands which had been removed several weeks earlier. The medium consisted of 2.0 ml of a Krebs-Ringer bicarbonate buffer adjusted to pH 7.4 and 200 mg% glucose. Additional duplicate sets of flasks contained (a) adrenals alone, (b) adrenals with ovine ACTH (Parke, Davis and Co.) in doses of 0.04, 0.4 or 4.0 I.U., (c) fresh brain surface or (d) a pool of 5 fresh pituitaries obtained at 0400 from the adrenal donor mice. The flasks were placed for 2 h in a Dubnoff incubator kept at 37°C with a gas phase of 95% oxygen and 5% CO_2. Corticosterone content per flask after the 2 h incubation period was estimated as described previously[1] according to the method of SILBER, BUSCH, and OSLAPAS[10].

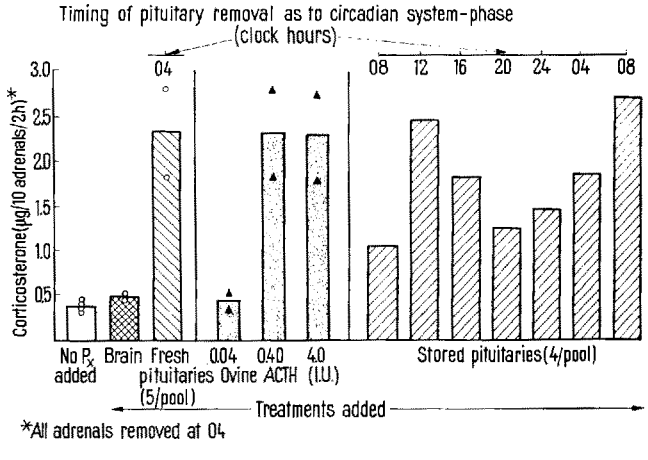

Fig. 1. Corticosterone production during 2 h incubations of mouse adrenals is raised by the addition of (1) fresh mouse pituitaries, of (2) ovine ACTH, in doses of 0.4 or 4.0 I.U., or of (3) pituitaries stored frozen for several weeks. All incubation flasks contained 10 quartered C mouse adrenals in 2.0 ml Krebs-Ringer-bicarbonate buffer, pH 7.4, with 200 mg% glucose. Incubations in 95% oxygen and 5% carbon dioxide.

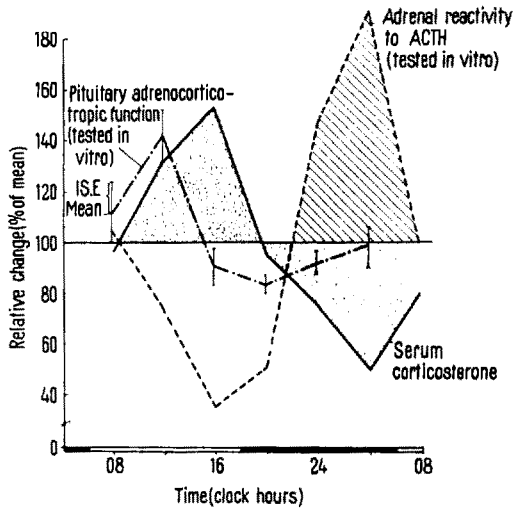

Fig. 2. Time relations of circadian rhythms in pituitary adrenocorticotropic activity (---); in adrenal reactivity to ACTH tested *in vitro* ////////; and in serum corticosterone ::::::::. All values expressed as relative change in % of the mean plotted against time in clock hours. Pituitary value based on total of 340 inbred male C mice.

The results of one series of incubations are shown in Figure 1. The addition to the adrenal incubation of pituitary glands, that had been stored for several weeks in the frozen state, significantly raised the corticosterone values *in vitro*, as did also the addition of fresh pituitaries or of ACTH, in doses of 0.4 or 4 U. The addition of brain surface tissue, or of 0.04 U of ACTH, gave results comparable to the control incubations containing adrenals alone.

Moreover, pituitary adrenocorticotropic activity *in vitro* appeared to depend upon the time of pituitary removal. While all values obtained in incubations of pituitaries with adrenals were higher than those from the control incubations, those values obtained with pituitaries removed at the 1200 time-point and the second 0800 time-point were higher than those obtained with pituitaries removed at other time-points (Figure 1). In two additional experiments[11], the highest values again were obtained with pituitaries removed at 0800 or 1200. These data suggested a circadian rhythm in the ACTH content of the hypophysis and, furthermore, a possible lead-in-phase of this pituitary rhythm over the corticosterone rhythm in serum and adrenal, which usually peaks around 1600, on a regime of light from 0600 to 1800 alternating with darkness[1,12].

To explore these possibilities, those values obtained in a given experiment with pituitaries removed at 0800 and 1200 were averaged and compared with the mean of values obtained with pituitaries removed at all the other time

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points. When the latter mean was equated to 100% for three such experiments, the average of the 0800 and 1200 values was higher, by 21, 43, and 43% respectively, than the mean of the remainder of values. In the first experiment depicted in Figure 1, values were based on adrenal incubation with only a single pool of pituitaries at each time point. A second and third experiment which gave comparable results, involved several pituitary pools at each time point and the corresponding P values for the latter experiments were 0.013 and 0.036.

The present studies and different earlier work[1] both explore complementary facets of the *in vivo* timing of spontaneous pituitary-adrenal interactions by an *in vitro* approach. Continuance of such *in vitro* investigation will serve to examine in further detail the periodic interactions of pituitary and adrenal factors, in the absence of complicating effects, e.g., from other hormonal, neural or circulatory controls. Such work may then serve for a more rigorous analysis of related observations and postulates on the adrenal cycle based upon *in vivo* work exclusively[2,3,13,14].

In any event, the *in vitro* approaches show that the reactivity of the adrenal to ACTH, on the one hand, and the adrenocorticotropic activity of the pituitary, on the other, both function periodically in a predictable fashion under standardized conditions, and, also, that these two rhythms do not peak simultaneously[4]. Figure 2 visualizes this point. The parameters of pituitary-adrenal function studied reveal the same frequency. Important differences-in-phase among these circadian periodic functions are also apparent.

All data shown in Figure 2 were converted first into a percentage of the mean for a given series. The means of these relative values plotted against time for three experiments involving the pituitary incubations with adrenals (------) are compared with those for serum corticosterone and adrenal responsiveness to ACTH tested *in vitro* as documented earlier[1] in the same strain of mice and under the same conditions. In the C mice studied, all three functions show clearly a circadian rhythm. Circadian periodic adrenocorticotropic activity of the C mouse pituitary leads-in-phase the rhythm in serum corticosterone which, in turn, is about 180° out-of-phase with the rhythm in adrenal reactivity to ACTH added *in vitro*.

Further work will have to be done before possible rhythms in hormone production and/or release can be segregated from the contribution of changes in hypophyseal ACTH content as such to the pituitary adrenocorticotropic rhythm here reported. Further questions deserving added study in themselves relate to the degree of generality of pituitary adrenocorticotropic rhythm and to its time relations in other strains or species. Nevertheless, these *in vitro* data as a whole reveal a set of interesting temporal parameters for students of pituitary-adrenal physiology and related bioassays.

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Zusammenfassung. Ein circadianer Rhythmus in der adrenocorticotropen Wirkung der Hypophyse bei Inzucht-C-Mäusen wird durch ein *in vitro*-Verfahren nachgewiesen. Es werden Angaben der Phasenunterschiede zwischen dem hypophysären Rhythmus und den gleichfalls circadian-periodischen Schwankungen gemacht: (1) im Serum-Corticosteron und (2) in der Reaktion der Nebenniere auf ACTH, *in vitro*.

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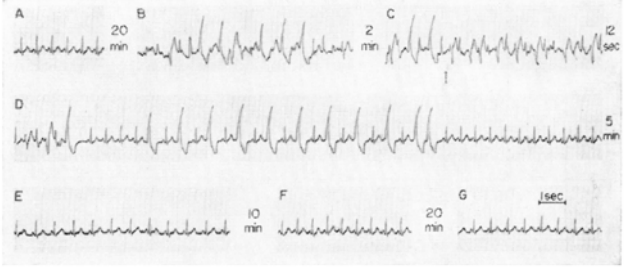
Antiarrhythmic Action of Synthetic Oxytocin in Anesthetized Man

The purpose of this communication is to present preliminary work on the use of synthetic oxytocin (Syntocinon®) in the treatment of electrocardiographically observed ventricular arrhythmias occurring under general anesthesia. Ten patients, eight of whom were anesthetized with halothane and two with methoxyfluorane were studied. The arrhythmias observed were frequent premature ventricular contractions (three patients), bigeminy (five patients) and multifocal ventricular contractions (two patients). The arrhythmias were associated with endotracheal intubation (two cases), carbon dioxide retention (one case), attempted coughing in response to the endotracheal tube (two cases), breath holding (one case), and adrenal manipulation (two cases). In two cases the etiology of the arrhythmia was not known.

The rapid intravenous infusion of 10 U of synthetic oxytocin restored normal sinus rhythm in seven cases while 20 U was required in one case. Conversion of the arrhythmias occurred within 30–60 sec. Figure 1 shows a representative case. In two cases a total dose of 20 U of synthetic oxytocin had no effect on the arrhythmia. A rise in pulse rate of 10–20 beats per min was observed in three patients and lasted 60–90 sec. In five patients a fall in mean arterial blood pressure of 15–20 mm Hg was observed (60 sec duration).



Lead 2 of electrocardiogram during halothane anesthesia in a 62 year old male. (A) Control record after 30 min of anesthesia. Normal sinus rhythm. (B) Ventricular arrhythmia associated with adrenal manipulation. (C) 2 min later, arrhythmia still present. At arrow 10 units of synthetic oxytocin given intravenously. (D) Normal sinus rhythm 33 sec after synthetic oxytocin infused. (E) (F) (G) Normal sinus rhythm during remainder of operation.