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The Effects of Estrogen, Progesterone, and Ionized Calcium on Seizures During the Menstrual Cycle of Epileptic Women

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Summary: Previous research suggested a positive relationship between levels of serum estrogen and seizures, a negative relationship between levels of serum Ca^{++} and seizures, and a negative relationship between serum levels of estrogen and Ca^{++}. This study sought a relationship between levels of serum estrogen, ionized calcium, progesterone, and its possible effect on seizures during the menstrual cycle of epileptic women. The negative relationship between serum estrogen and Ca^{++} was confirmed. However, a study of 15 institutionalized epileptic women, all with a diagnosis of primary generalized epilepsy, demonstrated that fewer seizures occurred in midcycle (when estrogen levels were elevated and Ca^{++} levels were decreased) than at other stages of the menstrual cycle. This is suggestive of a protective (anticonvulsant) aspect of the hypothalamo–hypophyseal negative feedback system, previously unreported. **Key Words:** Epilepsy—Seizures—Menstrual cycle—Hormones—Estrogen—Progesterone—Calcium—Hypothalamo–hypophyseal system.

The relationship between stages of the menstrual cycle and concomitant steroid/mineral fluctuations with seizure disorder has been the subject of intense study (Sieveking, 1857; Laidlaw 1956; Logothetis et al., 1959; Wooley and Timiras, 1962a,b; Marcus et al., 1966; Sanchez-Longo and Gonzales Saldana, 1966; Stitt and Kinnard, 1968; Timaras, 1969; Terasawa and Timaras, 1969; Zimmerman et al., 1973; Buntner and Rosciszewska, 1975; Backstrom, 1976; Striano et al., 1979; Newmark and Penry, 1980; Rosciszewska, 1980. Unfortunately, the use of subjects with a variety of seizure types, their observation for variable lengths of time, and the use of different data gathering/analytic techniques has resulted in dissimilar, often contradictory, results.

Early in this century, Bigwood (1924) posited an association between levels of serum ionized calcium (Ca^{++}) and seizures. More recently, Pitkin et al. (1978) observed a negative relationship between levels of serum estradiol (17B) and ionized calcium during the ovulatory stage of the menstrual cycle of healthy drug-free women. Although the relationship of ionized calcium levels to seizure disorder has been extensively studied (Brink et al., 1946; Chance, 1965; Corriol et al., 1969; Katz and Miledi, 1969, 1970; Zuckermann and Glaser, 1975; Lux and Heinemann, 1978; Dunwiddie and Lynch, 1979; Frankenhaeuser and Hodgkin, 1979; Benninger et al., 1980; Schwartzkroin and Prince, 1980; Prince and Connors, 1984; Woodbury et al., 1984), the role of this mineral in central nervous system function remains enigmatic at best. The possible synergistic role of estrogen and ionized calcium in seizure disorder during the menstrual cycle has never been examined. This study, then, attempts to unify two models of possible seizure generation and to examine the effects.

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THE SAMPLE

Fifteen institutionalized epileptic women, all with a diagnosis of primary generalized epilepsy

(Bancaud et al., 1981) and between 18 and 40 years of age, agreed to participate. Informed consent was obtained from the subjects' legal guardians, their institution of residence, and the subjects themselves where appropriate. The study was sanctioned by a committee examining ethical issues of human research. Each subject had been on antiepileptic drug polypharmacy, but was still experiencing seizures. None of the subjects participating in this study suffered from any syndrome associated with osteoclastic/osteoblastic disease, ovarian dysgenesis, or had been exposed (systemically or topically) to any adjunct hormonal/steroid preparations for a minimum of 6 months prior to the data collection stage of the study.

METHODS

Data collection spanned the length of one menstrual cycle. The morning following exhibition of menstrual flow was designated as data collection day 1. With the subject in a fasting state, blood was withdrawn from the antecubital fossa. Further withdrawals were carried out on alternate days from alternate arms, until the first day of the next menstrual flow. At that time, blood withdrawal ceased. Markowitz et al. (1981) demonstrated a circadian calcium rhythm, with highest determinations registered daily around 10:00 a.m. Thus, blood was withdrawn between 8:00 and 10:00 a.m. This effectively removed potential confounding time-of-withdrawal effects and minimized potential food/calcium level interactions. No attempt was made to restrict antiepileptic medication use at prescribed times.

Blood destined for calcium levels analysis was withdrawn first, anaerobically, into royal-blue-top siliconized "Vacutainers." Blood for all other assays was withdrawn into red-top Vacutainers with no additives. Aliquots were left exposed to ambient temperature for 30 min prior to centrifugation and anaerobic extraction of serum. Serum was then fast-frozen and stored at −80°C prior to assay.

Aliquots were assayed randomly in duplicate. Estradiol (17B) levels were measured by radioimmunoassay (RIA) techniques described by Challis et al. (1980). Interassay coefficient of variation was reported as 6%. Serum progesterone levels were obtained using RIA techniques described by Garfield et al. (1979). An 8% interassay coefficient of variation was observed. Ca^{++} levels were obtained by assay using a flow-through ion-specific electrode (ORION SS-20), with a resultant interassay coefficient of variation of 1.4%.

A daily record of seizure activity was kept by the subject's counsellor and the subject herself where appropriate. Seizures were recorded on a daily basis. For the purpose of the study, determinations for each subject (both serum levels as well as seizures experienced) were forced into an artificially constructed 30-day cycle, with the estrogenic peak assigned to day 0. The cycle was then subdivided into three stages. Day 0 plus two determinations was labeled the ovulatory stage, determinations −3 to −7 were labeled the follicular stage, and determinations +3 to +7 the luteal stage of this cycle. As seizures were recorded on a daily basis, some seizures occurred when no serum level determinations were recorded. These events were lagged forward 1 day to conform to the model.

The raw data for estradiol (17B) and progesterone were transformed into their square root for computational ease. In addition, the repeated measurement nature of this data necessitated the use of a correction factor in analyses, as described by Donald (1984).

RESULTS

Subjects' institutional records were searched to determine the length of menstrual cycles previous to the study cycle. The length of 4 cycles did not differ significantly ($p > 0.05$) from the length of the study cycle. The study cycle did not represent an unusual event in terms of its length. Mean serum estradiol (17B) levels for all subjects in each of the previously described cycle stages presented the following values: follicular stage 67.976 pg/ml (SEM ± 4.987 pg/ml), ovulatory stage 202.0 pg/ml (SEM ± 12.54 pg/ml), luteal stage 142.536 pg/ml (SEM ± 11.279 pg/ml). These levels fell within standard normal ranges expected (Krupp et al., 1985). Mean serum progesterone levels for all subjects in each of the cycle stages were recorded at follicular stage, 67.58 ng/dl (SEM ± 11.32 ng/dl), ovulatory stage, 112.86 ng/dl (SEM ± 16.9 ng/dl), and luteal stage, 343.2 ng/dl (SEM ± 37.79 ng/dl) (Fig. 1). Although the mean serum progesterone level for the luteal stage of the cycle approached the lower limits of the standard normal range expected for that cycle stage, the other two mean levels fell within these standard ranges (Krupp et al., 1985). It appears that mean serum levels for estradiol (17B) during the ovulatory stage of the cycle were sufficient to promote folliculogenesis. On the other hand, the somewhat low mean level of serum progesterone during the luteal stage, in addition to a high SEM, strongly suggests that not all of the subjects experienced a fertile cycle.

Previously cited research (Pitkin et al., 1978)

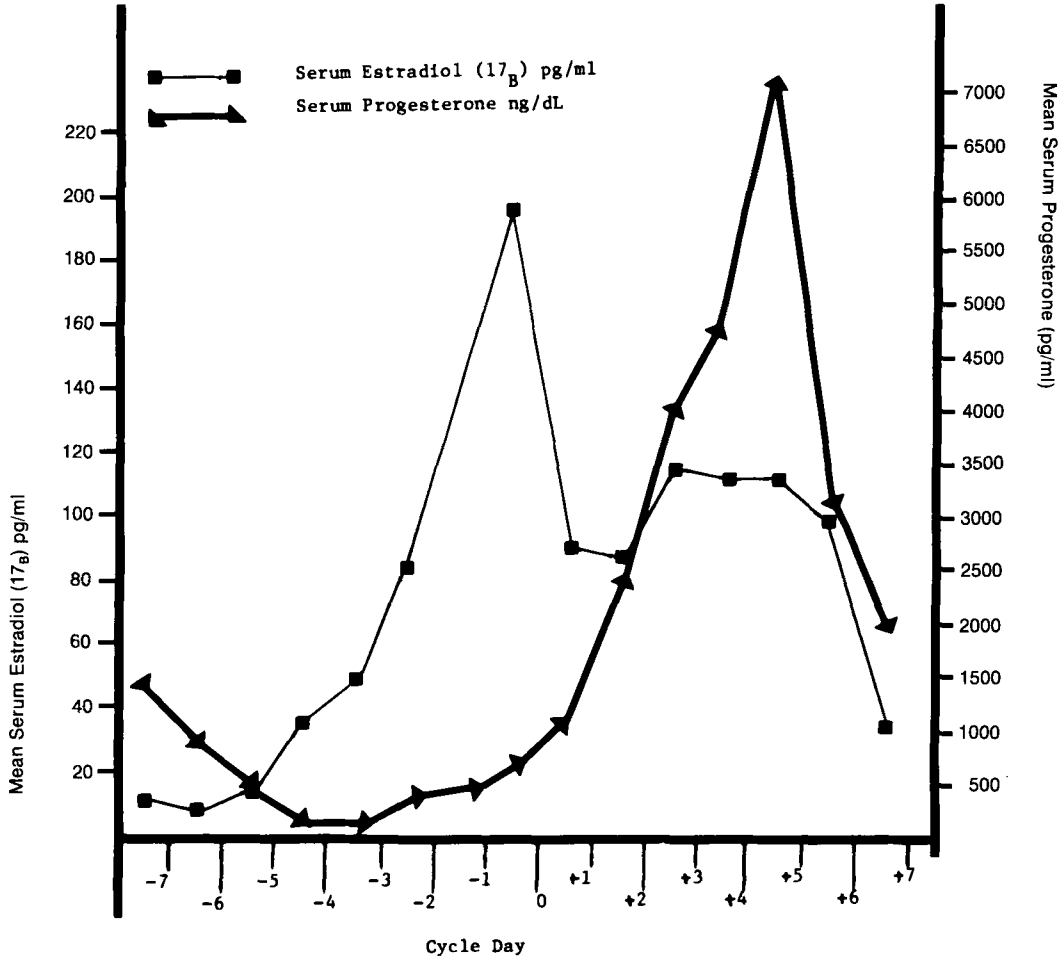

FIG. 1. Alternate-day determinations of serum estradiol (17B) and progesterone during one menstrual cycle (n = 15). Alternate-day mean serum values of progesterone and estradiol were obtained from 15 epileptic women during one menstrual cycle. Values were forced into a standardized 30-day cycle.

demonstrated an inverse relationship between levels of serum estradiol (17B) and Ca^{++} during the ovulatory stage of the menstrual cycle. This relationship was confirmed in these subjects ($t_{227\ df} = 3.881$, $p < 0.001$) (Fig. 2). Ca^{++} serum levels ranged between a low of 0.858 mmol/L and 1.422 mmol/L. Although both the lower determination and the higher determination lay outside the expected standard normal ranges (Krupp et al., 1985), the differences observed were not statistically significant ($p > 0.05$). This is particularly important, as epileptic patients receiving long-term antiepileptic therapy had been observed to suffer from a variety of rachitic disorders. All of the subjects had been receiving an antiepileptic regimen with phenytoin, primidone, carbamazepine, and phenobarbital predominantly used in varying combinations. No serum level determination for these chemicals was undertaken. Clearly, however, these 15 subjects who had been receiving antiepileptic drug polypharmacy for a considerable length of time did not demonstrate chronic low calcium levels. This was further confirmed by a mean alkaline phosphatase level (for all subjects) of 92.672 U/L, which lies within normal limits (Krupp et al., 1985). As anticipated, the relationship of Ca^{++} to alkaline phosphatase exhibited a slope b_1 of -52.360 ($t_{227\ df} = 1.2000$, $p > 0.05$). There was no significant difference between the means for alkaline phosphatase in each of the three cycle stages. More importantly, the variance for each of these means decreases by cycle stage. This is indicative of the response to ionized calcium level decrease experienced by these subjects during the ovulatory stage of the cycle (when estrogen levels were elevated and ionized calcium levels reached a nadir).

Previous research suggested that fewer seizures occurred when serum levels of estradiol (17B) were low and serum Ca^{++} levels elevated. In general, this tended to occur in the previously defined follicular and luteal stages of the cycle. These results were not confirmed in this group of subjects (Fig.

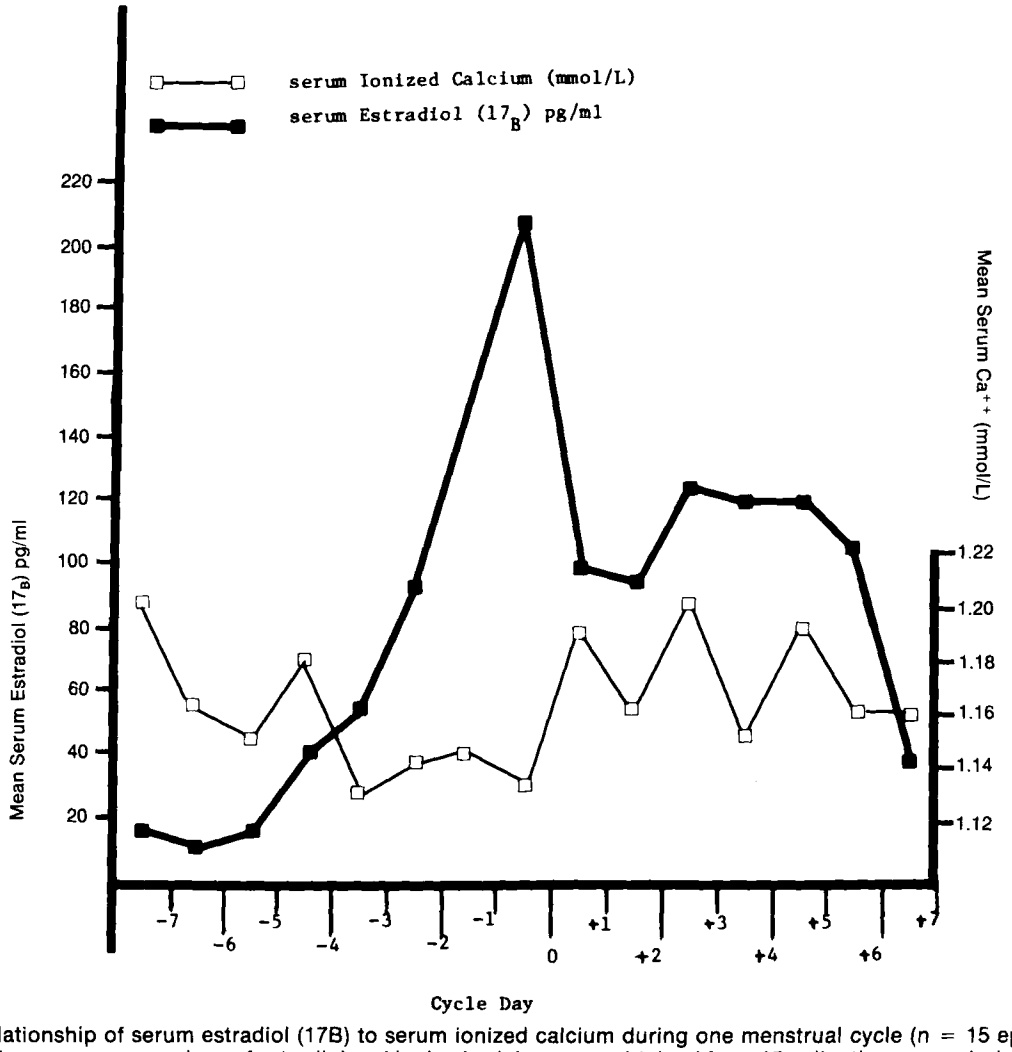

FIG. 2. Relationship of serum estradiol (17B) to serum ionized calcium during one menstrual cycle (n = 15 epileptic women). Alternate-day mean serum values of estradiol and ionized calcium were obtained from 15 epileptic women during one menstrual cycle. Values were forced into a standardized 30-day cycle.

3). In fact, more seizures occurred during the follicular stage (54 seizures) and in the luteal stage (46 seizures) than during the ovulatory stage of the cycle. Differences in numbers of seizures during each of the cycle stages were statistically significant ($p < 0.01$), using an adjusted χ^2 (Donald, 1984). A negative relationship between mean levels of estradiol (17B) and seizures ($b_1 = -3.197 \times 10^{-3}$; $t_{227\ df} = 5.241$, $p < 0.001$) is further reinforced by the relationship of seizures to ionized calcium during the ovulatory stage of the cycle.

DISCUSSION

In this study, 15 epileptic women exhibited the expected cyclic fluctuations in estrogen levels. In addition, concomitant decrements in levels of ionized calcium (when estrogen levels are high) were confirmed. The ascription of anticonvulsant properties to progesterone was not sustained in this sample of subjects ($t_{227\ df} = 1.751$, $0.10 > p < 0.05$). The presence of a high variation in the level of serum progesterone during the luteal stage may have attenuated statistical significance of the relationship in this sample population.

Alkaline phosphatase, an index of calcium homeostasis, is expected to rise in the presence of lowered serum ionized calcium levels. A negative but statistically nonsignificant relationship between Ca^{++} and alkaline phosphatase levels confirms the adequacy of serum calcium levels in these subjects. A compensatory rise in alkaline phosphatase levels immediately following the estrogen peak and resultant ionized calcium decrease was observed during the ovulatory stage.

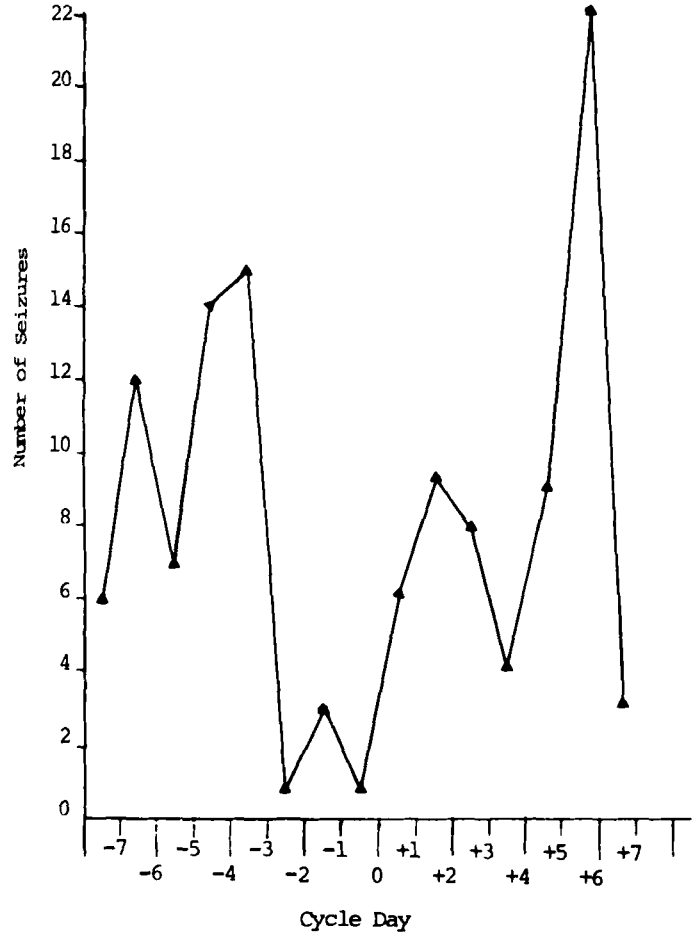

FIG. 3. Distribution of seizures by cycle day. Total number of seizures for all subjects by cycle day forced into a 30-day cycle.

Gonadotropin production levels are controlled by a feedback system operating between the hypothalamo–hypophyseal axis. The hypothalamic region and its widely spread neuronal arborizations undergo dramatic changes in activity levels as well as changes to the microenvironment (Silverman et al., 1977). Release of growth hormone releasing hormone (GHRH) is strongly calcium dependent and is reputed to stimulate local, significant, and sustained action potentials, with a propensity to spread (Fritz and Speroff, 1982). In essence, the local neuronal estrogenic effect may have increased neuronal activity, and a change in ionic milieu could combine and facilitate spread of synchronized burst action potentials (Prince and Connors, 1984). This paradigm complements the model advanced by Schwartzkroin and Wyler (1980). These authors advance the hypothesis that "epileptogenic" cells are rather unstable due to the loss of IPSP protection. Under these circumstances, they are highly reactive to minimal ionic changes and conductance shifts. Although their work was mainly concerned with chemically created foci, a highly stimulated hypothalamic region with its extensive neuronal arborizations could, during the follicular and luteal stage of the cycle, replace this artificially created phenomenon. Levels of Ca^{++} and estrogen are both quantitatively sufficient during both the follicular and luteal stages of the cycle to exacerbate the general propensity to excitability. Local increased (if transient) excitation would provide the impetus to spread of burst discharges. During the ovulatory stage, the hypothalamo–hypophyseal feedback system is in a quiescent state, which may be actually inhibitive of spread of burst action potentials, or at least significantly less stimulating.

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RÉSUMÉ

Des travaux antérieurs ont suggéré l'existence d'une corrélation positive entre les taux d'œstrogènes sériques et les crises, d'une corrélation négative entre les taux sériques de Ca^{++} et les crises et d'une corrélation négative entre les taux respectifs de Ca^{++} et d'œtrogènes. Ce travail étudie les relations entre les taux sériques d'œstrogènes, de calcium ionisé et de progestérone, et l'influence éventuelle de ces facteurs sur la survenue de crises au cours du cycle menstruel chez les patientes épileptiques. Nous avons pu confirmer la corrélation négative entre œstrogènes et Ca^{++}. Cependant, l'étude de 15 patientes institutionnalisées, présentant des crises généralisées considérées comme l'expression d'une épilepsie généralisée idiopathique, a montré que les crises étaient plus rares en milieu de cycle (alors que les taux d'œstrogènes étaient élevés et les taux de Ca^{++} abaissés) que pendant les autres périodes du cycle menstruel. *Ceci suggère que le système de rétro-contrôle négatif hypothalamo-hypophysaire joue un rôle protecteur (anti-convulsivant) qui ne semble pas avoir été rapporté jusqu'ici.*

(P. Genton, *Marseille*)

RESUMEN

Informaciones previas sugieren que existe una relación positiva entre los niveles séricos de estrógenos y ataques epilépticos, una relación negativa entre los niveles séricos de Ca^{++} y

ataques y, finalmente, una relación negativa entre los niveles séricos de estrógenos y Ca^{++}. El estudio actual estudió la posible relación existente entre los niveles séricos de estrógenos, el calcio ionizado, la progesterona y su posible efecto sobre los ataques durante el ciclo menstrual de las mujeres epilépticas. Se ha confirmado la relación negativa entre los niveles séricos de estrógenos y el Ca^{++} sin embargo, un estudio de 15 mujeres epilépticas institucionalizadas, y todas con el diagnóstico de epilepsía generalizada primaria (epilepsía generalizada idiopática) demostraron que los ataques eran menos frecuentes en la porción media del ciclo (cuando los niveles de estrógenos estaban elevados y los niveles de Ca^{++} estaban reducidos), que en otros estadíos del ciclo menstrual. Este hallazgo sugiere una acción protectora (anticonvulsiva) del sistema de retroalimentación negativa hipotálamo-hipofisario que no ha sido publicado previamente.

(A. Portera-Sánchez, *Madrid*)

ZUSAMMENFASSUNG

Vorangegangene Arbeiten lassen eine positive Beziehung zwischen den Serum- Oestrogenspiegeln und Anfällen vermuten; eine negative Beziehung zwischen Serum-Ca^{++}-—und Anfällen und eine negative Beziehung zwischen Serumspiegel von Oestrogen und Calcium. In dieser Studie sollte der Beziehung zwischen diesen Größen und ihrer Auswirkung auf die Anfälle nachgegangen werden. Die negative Beziehung zwischen Serum-Oestrogen und Calcium wurde bestätigt. An 15 Frauen mit primär generalisierter Epilepsie konnte gezeigt werden, daß in der Mitte des Zyklus, wenn die Oestrogenspiegel erhöht und Calciumspiegel erniedrigt waren, weniger Anfälle auftraten als in den anderen Phasen des Zyklus. Dies läßt auf eine protektiv-antikonvulsive Wirkung des hypothalamo-hypophysären Feedback-Systems schließen, der bislang nicht berichtet wurde.

(Ch. Benninger, *Heidelberg*)