19. R. E. Kouri, H. Ratrie, C. E. Whitmire, *J. Natl. Cancer Inst.* **51**, 197 (1973); P. E. Thomas, J. J. Hutton, B. A. Taylor, *Genetics* **74**, 655 (1973).
20. B. Dowty, D. Carlisle, J. L. Laseter, J. Storer, *Science* **187**, 75 (1975).
21. *New York Times*, 19 April 1975, p. 1; *ibid.*, 18 May 1975, p. 63; *Science News*, 26 April 1975, p. 269.
22. E. S. Vesell and J. G. Page, *Science* **159**, 1479 (1968); *ibid.* **161**, 72 (1968); *J. Clin. Invest.* **47**, 2657 (1968); B. Alexanderson, D. A. Price Evans, F. Sjöqvist, *Br. Med. J.* **4**, 764 (1969); P. B. Andreasen, A. Frøland, L. Skovsted, S. A. Andersen, M. Hauge, *Acta Med. Scand.* **193**, 561 (1973).

10 April 1975; revised 21 May 1975

Monthly Gonadotropin Cycles in Premenarcheal Girls

Abstract. *Patterns of nocturnal excretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were investigated in 11 girls. Autoregressive digital filtering of low- and high-frequency variations was used to make patterns more apparent. Coincident FSH and LH surges, separated by an interval of 20 to 40 days, were seen in specimens from three of six postmenarcheal girls and three to five premenarcheal girls. This suggests that cyclic hypothalamic-pituitary-ovarian interactions occur before menarche.*

After menarche, follicular maturation which results in both ovulation and atresia are associated with distinctive patterns of change in gonadotropin secretion including a gonadotropin surge during each cycle (*1*). In premenarcheal girls surges in gonadotropin secretion have been reported (*2, 3*), but cyclic variations in pituitary gonadotropin secretion similar to those seen in sexually mature girls have not been demonstrated. Having found that gonadotropin concentrations in first morning urine voidings from normally cycling women show patterns consistent with those seen in 24-hour urine specimens and daily blood samples (*4*), we investigated gonadotropin secretion patterns utilizing first morning urine voidings in prepubertal, pubescent, and postpubertal girls. These studies revealed monthly gonadotropin cycles in premenarcheal as well as in postmenarcheal girls.

Eleven healthy girls (five premenarcheal and six postmenarcheal), ages 8 years 11 months to 16 years 7 months, volunteered to collect timed nocturnal and first morning urine specimens for 60 consecutive days. No abnormalities were found in their histories, physical examinations, routine laboratory studies, or endocrine evaluations. None were taking medications or oral contraceptives during the study period. On each of the 60 days, the girls just before retiring would void and discard the urine, noting the time precisely. The next morning, the first urine voiding was added to any urine which may have been collected during the night and the time again was noted precisely. The samples were refrigerated and delivered to the laboratory, where the gonadotropins were precipitated with acetone after *p*H adjustment with acetic acid (*4*). Precipitates, containing both follicle-stimulating hormone (FSH) and luteinizing hormone (LH), were dissolved in buffer and measured by specific radioimmunoassay (*5, 6*). To minimize the effects of interassay variation, all samples from a given subject were examined in the same radioimmunoassay, with IRP2HMG (*7*) as reference preparation.

The excretion rates in these peripubertal girls represent pituitary gonadotropin secretion during sleep. This may be the optimum time to examine gonadotropin activity since serum levels in some pubescent girls have been shown to be higher when the girls were asleep than when they were awake (*8*). Since each specimen was obtained at the same time during the day, the effect of diurnal variation on the data was minimized.

The total gonadotropin excretion during each night was divided by the collection interval and expressed as international units (I.U.) per hour to reduce variation resulting from different collection times. Nevertheless, significant variability remained which may be attributed to (i) variance between assays that is amplified by the small quantities of gonadotropin excreted by premenarcheal girls, (ii) nonsystematic biological variation, in addition to (iii) underlying physiological cycles.

To examine the data for monthly physiological cycles and reduce other sources of variability, a two-stage autoregressive filter in discrete time was used to remove general trends of long duration (periods greater than 45 days) and high-frequency variations (periods less than 10 days). This technique involved transforming the data, $X_1, X_2, \ldots, X_{60}$, according to the following scheme (*9*):

$$Y_i = (1-\alpha)Y_{i-1} + \alpha X_i, \quad i = 2 \text{ to } 60,\ Y_1 = X_1 \quad (1)$$

$$Z_{i-1} = (1-\alpha)Z_i + \alpha Y_{i-1}, \quad i = 60 \text{ to } 2,\ Z_{60} = Y_{60} \quad (2)$$

$$U_i = Z_i - \frac{\alpha^2}{1+(1-\alpha)^2} X_i,\ i = 1 \text{ to } 60 \quad (3)$$

$$V_i = X_i - U_i,\ i = 1 \text{ to } 60 \quad (4)$$

First, α was chosen to be 0.2, and the autoregression equations, Eqs. 1 through 3, applied twice (the X_i in the second application being the U_i from the first). The residuals V_i were then calculated as the difference between U_i, the final trend, and X_i, the initial data (*10*). This procedure results in removing long-term trends with periods of greater than 45 days. Next, the residuals, V_i, were similarly treated once (V_i was substituted for X_i in Eq. 1) with α set at 0.4 so as to remove variations with periods of less than 10 days.

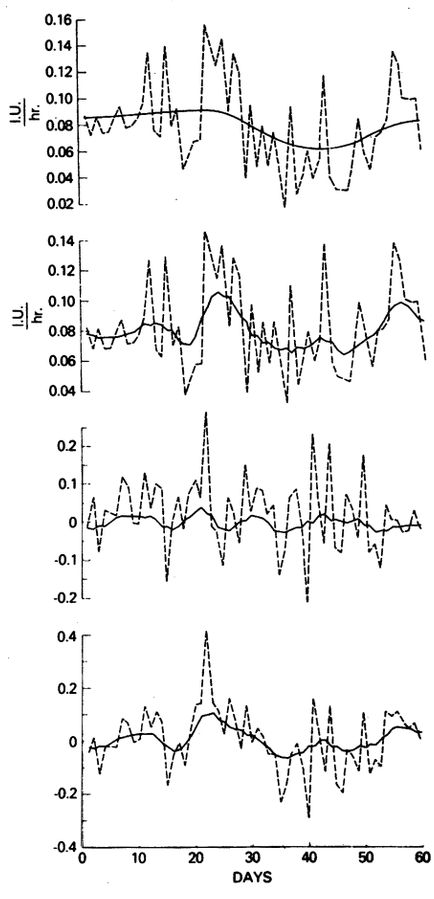

Fig. 1. Autoregression analysis of data and random numbers. The top panel shows FSH data from subject 1 as a broken line and the general trend after double autoregression with $\alpha = 0.2$ as the solid line; periods less than 45 days do not contribute substantially to this trend. The second panel shows the residual between this general trend and the data as a broken line; the solid line represents autoregression of these residuals using $\alpha = 0.4$ and hence emphasizes the remaining components of the data with periods of greater than 10 days. The third panel shows a set of 60 random numbers as the broken line and the ultimate result of analyzing these numbers with the same program used to analyze the data; thus the solid line is comparable to the solid line in panel 2 but shows no evidence of periodicity with wave lengths between 20 and 40. The broken line in the bottom panel shows the same set of random numbers but with the derived signal (solid line) from panel 2 added to it after scaling appropriately; the autoregression analysis results in the solid line in which the signal is seen to reemerge at a decreased amplitude.

Table 1. Median nocturnal gonadotropin excretion.

Subject	Pubertal stage	Age (yr-mo)	FSH (I.U./hr)	LH (I.U./hr)	FSH/LH
1	I	8–11	0.08	0.02	4.4
2	I	9– 9	0.30	0.03	10.8
3	II–	12– 2	0.28	0.14	1.9
4	II	11– 0	0.18	0.06	3.4
5	II+	11– 4	0.35	0.24	1.4
6	III	13– 8	0.29	0.92	0.34
7	IV	13– 9	0.27	0.26	0.99
8	IV	15– 2	0.34	1.28	0.26
9	V	14–11	0.20	0.30	0.68
10	V	16– 5	0.40	0.55	0.60
11	V	16– 7	0.55	1.28	0.41

Figure 1 shows the application of this data reduction technique with the use of the FSH data from subject 1, an 8-year 11-month old prepubertal (Tanner stage I) girl (*11*). The broken line in the top panel shows the initial data and the superimposed solid line trend which was found by the two-stage autoregression procedure, applied twice, with $\alpha = 0.2$. The difference between these two curves, the residuals after removal of the trend, are plotted as the broken line in the second panel. The final filtered output, the "signal," which results after removal of the high-frequency components by using the two-stage autoregression once ($\alpha = 0.4$) is shown as the solid line in the second panel. This technique permits one to examine the monthly cyclic patterns without being distracted by the very high- and low-frequency components in the initial data.

This method of data analysis also was applied to nine independent sets of 60 random normal deviates (white noise) (*12*). A typical one of these random noise series is shown as the broken line in the third panel of Fig. 1. The final filtered signal which results from the use of the autoregression technique described above for the experimental data is shown as the solid line in the third panel. This example illustrates that the filtering technique itself does not tend to introduce periodicity. On this same set of random numbers we superimposed the smoothed FSH curve (see second panel of Fig. 1) from the first subject, and plotted the combined data in the broken line in the bottom panel of Fig. 1. This set of data containing the known added signal plus random noise was then analyzed and the results are shown as the solid line in the bottom panel of Fig. 1. As can be seen, the signal of the first subject reemerged, but at a reduced amplitude (*13*).

Figure 2 summarizes our data after autoregressive filtering. Twenty- to 40-day cycles in FSH excretion can be seen in three premenarcheal subjects (numbers 1, 2, and 4) and may be present in the other two. That periodicity in gonadotropin excretion exists in premenarcheal girls is further suggested by the occurrence of LH elevations associated with the FSH elevations in at least three of these five subjects. Hayes and Johanson (*3*) have also shown simultaneous surges of urinary FSH and LH excretion in a premenarcheal girl although monthly periodicity was not observed in their raw data. Winter and Faiman (*14*) have presented serum gonadotropin data from four premenarcheal girls; the data for two of the girls is consistent with a biphasic FSH pattern during the 1-month observation period. In addition, the process of follicular maturation and atresia that occurs from fetal life until the menopause suggests that the process may reflect periodic waxing and waning of hormone stimulation even in premenarcheal girls.

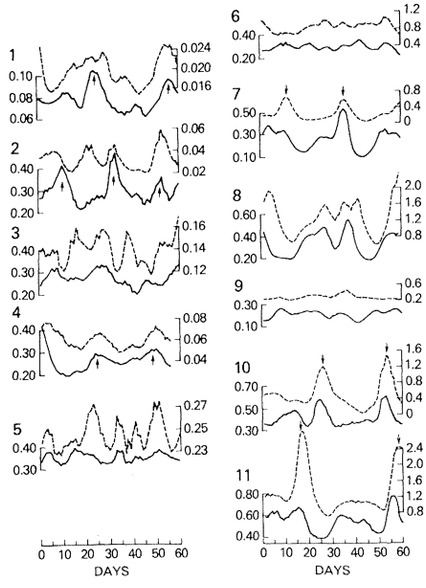

Fig. 2. Gonadotropin data in peripubertal girls after autoregression analysis. The broken lines are LH and the solid lines FSH excretion rate patterns determined over a 60-day period in each of eleven peripubertal girls numbered in order of increasing pubertal stage and age within a given stage. The five panels on the left are from premenarcheal girls and the six panels on the right from postmenarcheal girls showing evidence of monthly gonadotropin cycles (surges indicated by arrows) both before and after menarche.

Monthly LH surges typical of sexually mature females are seen in three of the six postmenarcheal girls (subjects 7, 10, and 11). The cyclic pattern is more evident in the mature girls because of the amplification of the LH peaks achieved by the eightfold increase in this hormone. These girls also show FSH elevations corresponding to the LH surges similar to those of the premenarcheal girls. In one of the postmenarcheal girls (subject 8) there is a suggestion of cycling, but no evidence of monthly periodicity was found in the other two postmenarcheal girls. The absence of hormone surges in up to half of these postmenarcheal girls may be attributable to the irregularities in the menstrual cycle seen just after menarche (*15*). It is clear, however, that the technique used in this work does demonstrate the normal adult type pattern of gonadotropin excretion and secretion and provides evidence of similar patterns in premenarcheal girls. In this small series, such patterns are demonstrable as frequently in premenarcheal as in postmenarcheal girls.

Since FSH production, excretion, and serum levels are greater than those for LH in premenarcheal girls, it is not surprising that their FSH patterns are stronger than those of LH. In our study, the median of 60 daily gonadotropin excretion rates was used as a single measure of gonadotropin activity for each subject, and the median of 60 daily FSH : LH ratios was used as a measure of relative activity. The mean of the median FSH activities for the five premenarcheal girls was 0.24 I.U. per hour while the corresponding mean LH activity was 0.10 I.U. per hour with a relative activity of 4.4. This dominance of FSH in prepubertal children is well known (*5*) and is consistent with the occurrence of incomplete follicular maturation, limited estrogen production, and atresia similar to that seen during anovulatory cycles in sexually mature women (*16*).

In postmenarcheal girls the secretion, excretion, and blood levels of FSH are less than those of LH which is not the case in premenarcheal girls. The mean of the median FSH activities for the six postmenarcheal girls was 0.34 I.U. per hour and the corresponding mean LH activity was 0.77 I.U. per hour with a mean urinary FSH : LH relative activity of 0.55. The inversion of the FSH : LH ratio occurred at menarche and was due principally to the eightfold rise in LH at that time with but a small increase in FSH. The abruptness of the inversion of this ratio is emphasized by the fact that in all of the premenarcheal

girls the ratio was greater than unity while in all of the postmenarcheal girls it was less than unity, even though all stages of puberty were represented (Table 1).

Previous studies have not disclosed the presence of monthly gonadotropin cycles in premenarcheal girls. However, we found that by using the described autoregressive smoothing techniques on data collected for 60 consecutive days we were able to suppress extraneous variation and allow monthly gonadotropin surges to become more apparent. The validity of the technique is supported by the observation of normal cycles in postmenarcheal girls. By studying five premenarcheal girls, in whom the FSH : LH ratio was appropriate to the pubertal stage, we have observed monthly gonadotropin cycles in at least three of them. This suggests that gonadotropin cycles may have a role even prior to menarche.

JAMES W. HANSEN
HOWARD J. HOFFMAN
GRIFF T. ROSS
Reproduction Research Branch and *Biometry Branch, National Institute of Child Health and Human Development, Bethesda, Maryland 20014*

References and Notes

1. G. T. Ross, *Fertil. Steril.* **25**, 522 (1974).
2. A. B. Rifkind, H. E. Kulin, P. L. Rayford, C. M. Cargille, G. T. Ross, *J. Clin. Endocrinol. Metab.* **31**, 517 (1970).
3. A. Hayes and A. Johanson, *Pediatr. Res.* **6**, 18 (1972).
4. J. W. Hansen and G. T. Ross, in preparation.
5. E. O. Reiter, H. E. Kulin, S. M. Hamwood, *J. Clin. Endocrinol. Metab.* **36**, 661 (1973).
6. Specific FSH antiserums were supplied by the National Institute of Arthritis, Metabolic, and Digestive Diseases.
7. Second international reference preparation of human menopausal gonadotropin was supplied by Derek Bangham for the World Health Organization.
8. R. Boyar, J. Finkelstein, H. Roffwarg, S. Kapen, E. Weitzman, L. Hellman, *N. Engl. J. Med.* **287**, 582 (1972).
9. Y_i is the time series resulting from autoregressing the original data, X_i, in the time forward direction. Z_i, is the time series resulting from autoregressing the derived series, Y_i, in the opposite direction. By performing the autoregression in both directions, the phase shift of the first autoregression is canceled by the exactly opposite phase shift of the second autoregression. The trend is specified by U_i, which is preferred to Z_i, in part because of the resulting simplification in the overall transfer function. W. C. Orr and H. J. Hoffman, *IEEE (Inst. Electr. Electron. Eng.) Trans. Bio-Med. Eng.* **BME-21**, 130 (1974).
10. H. J. Hoffman, D. Guerry IV, D. C. Dale, *J. Interdiscipl. Cycle Res.* **5**, 1 (1974).
11. W. A. Marshall and J. M. Tanner, *Arch. Dis. Child.* **44**, 291 (1969).
12. The Rand Corporation, *A Million Random Digits with 100,000 Normal Deviates* (Free Press, Glencoe, Ill., 1955).
13. The method of data analysis used to define the signal has been accomplished by a combination of autoregressive filters. Each of these autoregressions is a linear filter, and their combined characteristics can be calculated as a transfer function in the frequency domain. The peak frequency admitted by this transfer function is located at $\pi/15$ radians which is equivalent to a period of 30 days. The filter characteristic is broad; the bandwidth, or half-power width, extends from $2\pi/45$ radians (45-day period) to $\pi/5$ radians (10-day period). Also, it should be pointed out that the transmission at the peak frequency is approximately one-third, rather than unity. This attenuation will result in any periodic monthly component in the time series data being reduced to one-third of its true amplitude. For a periodic component near 45 days in length the amplitude is reduced to one-sixth the true amplitude. Since no adjustment has been made for this change in amplitude of the signal due to the filtering procedure, the relative scales provided in the figures do not accurately reflect the amplitude of the LH and FSH surges in the original data.
14. J. S. D. Winter and C. Faiman, *J. Clin. Endocrinol. Metab.* **37**, 714 (1973).
15. A. E. Treloar, R. E. Boynton, B. G. Behn, B. W. Brown, *Int. J. Fertil.* **12**, 77 (1967).
16. B. M. Sherman and S. G. Korenman, *J. Clin. Endocrinol. Metab.* **39**, 145 (1974).
17. We acknowledge the technical assistance of R. V. Reid in analyzing the specimens for gonadotropins and the constructive comments of Dr. D. Alling during the preparation of the manuscript.

14 March 1975; revised 20 June 1975

Somatostatin Suppresses Secretin and Pancreatic Exocrine Secretion

Abstract. *Somatostatin, a hypothalamic peptide, suppresses hydrochloric acid–stimulated release of secretin, pancreatic flow rate, and bicarbonate and protein secretion in fasted, conscious dogs. It also reduces nonstimulated pancreatic exocrine secretion but does not affect basal secretin concentrations. Suppression of HCl-stimulated secretin release is complete, whereas pancreatic flow rate and bicarbonate and protein secretions are only partially inhibited. The action of somatostatin is rapid in onset and quickly reversible.*

Somatostatin (somatotropin-release inhibiting factor, SRIF), a tetradecapeptide isolated from ovine hypothalamic extracts, inhibits the release of growth hormone from the pituitary gland (*1*). SRIF was also found to suppress thyrotropin (TSH) release stimulated by thyrotropin-releasing factor (TRF) (*2*), both basal and arginine-stimulated insulin and glucagon release from the pancreas (*3*), and basal as well as food-stimulated gastrin release from the stomach (*4*).

We have investigated the effect of SRIF on the release of immunoreactive secretin (IRS) from the proximal small intestine and have found that SRIF suppresses both the HCl-mediated IRS release and the pancreatic secretion of water, bicarbonate, and protein. All experiments were performed on conscious dogs that had been fasted overnight and that had long-term pancreatic fistulas. The fistulas had been prepared 3 weeks earlier according to the technique of Herrera (*5*) with two modifications: (i) the minor pancreatic duct was ligated and (ii) the continuity of stomach, duodenum, and jejunum was reestablished after the preparation of the duodenal pouch.

Figure 1 shows data obtained from six experiments in four dogs. Synthetic SRIF (200 μg, the cyclic form) infused continuously for 30 minutes into a hind leg vein had no significant effect on IRS concentrations (*6*). However, somatostatin suppressed further the low basal pancreatic flow and bicarbonate (*7*) and protein (*8*) secretion to barely detectable amounts ($P < .05$). This observation raised the possibility that SRIF may inhibit basal pancreatic secretion through secretin-independent mechanisms. In contrast, HCl-mediated IRS release was completely suppressed by SRIF. When SRIF (200 μg/30 minutes) was infused concurrently with HCl (9.6 meq/30 minutes, intraduodenally), there was no statistically significant rise in IRS concentrations. By comparison, when HCl was infused without SRIF, IRS rose from 610 ± 61 μunit/ml to 1110 ± 187 μunit/ml ($P < .001$) (*9*).

HCl-stimulated pancreatic flow rate and bicarbonate and protein secretion were all partially suppressed by somatostatin. When SRIF was infused concurrently with HCl, the incremental increases were 7.3 ± 2.6 ml/15 minutes for flow rate, 0.60 ± 0.26 meq/15 minutes for bicarbonate secretion, and 83 ± 22 mg/15 minutes for protein secretion. However, considerably greater increases were observed when HCl was infused alone. Flow rate increased by 21.8 ± 1.2 ml/15 minutes, bicarbonate secretion increased by 2.06 ± 0.31 meq/15 minutes, and protein secretion increased by 232 ± 25 mg/15 minutes. Thus, SRIF had reduced the HCl-stimulated increase in pancreatic flow rate by 67 percent, bicarbonate secretion by 72 percent, and protein secretion by 64 percent. These reductions were statistically significant ($P < .001$ for flow rate and bicarbonate secretion and $P < .05$ for protein secretion). Therefore, it appeared that SRIF affected HCl-stimulated IRS release more than pancreatic exocrine function. This was particularly evident in three dogs whose IRS responses were completely abolished but, nevertheless, showed distinct increases in pancreatic secretions during HCl plus SRIF infusions. The observation that the pancreatic secretions were only partially suppressed was not surprising. The stimulation of pancreatic exocrine function by HCl is a complex phenomenon. It involves the release of secretin, cholecystokinin-pancreozymin, and prob-