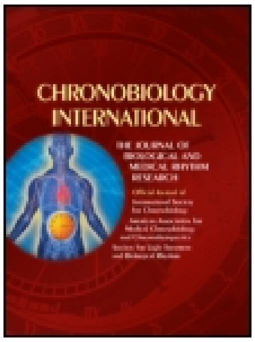


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# TEN-YEAR-REPLICATED CIRCADIAN PROFILES FOR 36 PHYSIOLOGICAL, SEROLOGICAL AND URINARY VARIABLES IN HEALTHY MEN

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**Abstract—At 3-hr intervals over a 24-hr span, 36 systemic, serologic and urinary variables were examined in 7 men in their mid 20's in the Spring of 1969, and again in the same 7 men in the Spring of 1979 under a similar chronobiologic protocol, using the same chemical and numerical analytical procedures. The variables examined for rhythms by cosinor were: vital signs—blood pressure (systolic, diastolic, pulse pressure and mean arterial pressure), heart rate, intraocular pressure (left and right), oral temperature; serum components—albumin, albumin/globulin ratio, total bilirubin, calcium, carbon dioxide, chlorides, bilirubin, cholesterol, globulin, glucose, potassium, sodium, sodium/potassium ratio, transaminase, triglycerides, total protein, urea nitrogen; and urine components—calcium, calcium/magnesium ratio, creatinine, magnesium, pH, potassium, sodium, sodium/potassium ratio, urea clearance, urea nitrogen, volume and zinc. Although all subjects appeared clinically healthy in 1969 and in 1979, certain inter-study differences were observed in a number of rhythm parameters of different variables. Statistically significant increases in mesor for the group as a whole were observed for serum Ca, cholesterol, Cl, $CO_2$, K, Na, and while statistically significant mesor decreases for a group as a whole were noted in serum glucose and transaminase. Statistically significant increases in amplitude for the group as a whole were observed in serum chloride and urinary Na/K ratio, while statistically signficiant decreases were observed in amplitude for blood pressure, heart rate, serum albumin, A/G ratio, globulin, glucose, protein, sodium and transaminase. For the group as a whole, a statistically significant advance in acrophase was observed in serum transaminase, while a statistically significant delay in acrophase was observed for serum A/G ratio, globulin, glucose, potassium, protein, sodium and for urinary magnesium. Statistically significant by sign test, but not by cosinor, was a numerical mesor increase for urinary urea clearance, a numerical decrease in mesor for urinary zinc; a numerical amplitude decrease for serum cholesterol; and a numerical delay in acrophase for oral temperature and serum cholesterol, $CO_2$, and globulin in all men examined. Only mesor changes in serum cholesterol and urinary Ca/Mg were positively correlated with the change in body size over the 10-year span between studies.**

## Introduction

Circadian variation in physiological variables, including vital signs and components of serum and urine had been examined in 13 young soldiers in their mid-20's in the Spring of 1969, in a study conducted at San Antonio, Texas. Most of the data obtained from that study are published (1–9). In the spring of 1979, a similar study was conducted at Hines, Illinois on 7 of the men studied 10 years earlier. Several reports on that study are also in the literature (10–14).

The 1969 and 1979 studies were conducted using the same experimental and analytical procedures. The circadian characteristics of 36 variables determined in the same subjects in each of two studies are here compared to offer a data base of reference values for circadian parameters in subjects presumably in the prime of their lives and for assessing possible clinical changes later in life. The changes noted in circadian characteristics within a 10-year span and validated by inferential statistical means will have to be checked by further longitudinal follow-up before they can be considered to reveal aging and/or an enhanced health risk, if not a predisposition for a variety of metabolic disorders or other pathology.

## Subjects, materials and methods

Seven men, 33–38 years of age, who served as subjects in our 14–15 May 1969 study conducted at 4th Army Medical Laboratory, Fort Sam Houston, San Antonio, Texas (latitude: 29.25N, 98.30W), served again as subjects in our 18–19 May 1979 study, conducted at the Special Diagnostic and Therapeutic Unit of the Veterans Administration Hospital, Hines, IL (latitude: 41.49N, 87.37W). The protocols followed in both studies were similar if not identical, as were the analytical procedures employed (1, 14). The Chicago area temperature for 18 May 1979 ranged from a high of 85°F (29°C) and a low of 60°F (15.5°C); for 19 May these were 72°F (22°C) and 48°F (9°C), respectively. The relative humidities were 34% and 57%, and the barometric pressures at 1500 were 29.23 and 29.15 inches (74.2 and 74.0 mm) of Hg, respectively.

All subjects were administratively admitted to two hospital wards of the Special Diagnostic Unit, immediately after leaving their daily routines of civilian life. No special standardization to the hospital environment was made. Each subject was given a physical examination by the physician member of our staff. Height and body weight were recorded. Participants were regularly diurnally active, taking sleep at night. The protocol of the study, familiar from earlier studies, was reviewed briefly with the subjects. Lights were turned off at 2215 and on at 0615. Meals consisted of a general hospital diet totaling 2500 calories and were served at 0730, 1330 and 1630. The food was prepared and served by the dietetic service of the hospital. The food actually consumed was monitored for each subject and any item not eaten was subtracted from the intended dietary intake. The average calories consumed by all subjects were 2250 and ranged between 1834 and 2516. The subjects were not restricted in their water intake, except for the half hour prior to sampling, but were required to abstain from other liquids and food between meals. Nutritional assessments, including anthropometric measurements, were made for each subject; all were found to have a good nutritional status.

Vital signs and specimens were collected at 3-hr intervals beginning at 1900 on 18 May. The sequence of sampling involved the following, in this order: heart rate, oral temperature, blood pressure, intraocular pressure, blood and urine. Blood for hematology was temporarily stored in a refrigerator while clotted blood was centrifuged, the serum separated and aliquoted for different tests before storage at -25°C.

Urine volume was recorded, pH measured and occult blood, protein, glucose, bilirubin, ketone were estimated by Multistix (Ames Division, Miles Laboratories, Elkhart, IN 46515). Each urine was then aliquoted into three equal portions and frozen at -25°C. All separations and aliquoting were completed before the next sequence of data collection.

Serum analyses in 1979 were by Technicon's SMAC-24, a newer generation system of the SMA-660 and SMA-1260 used in the 1969 study. The systems were thus similar in both studies.

Values computed from raw data include pulse pressure (difference between systolic and diastolic blood pressure), mean arterial pressure (diastolic blood pressure plus 1/3 pulse pressure) and ratios between serum albumin/globulin, sodium/potassium and urinary calcium/magnesium and sodium/potassium. Before statistical analyses, physiologic and serologic data were paired with time of collection, while urinary values were assigned to the midpoint of the 3-hr collection interval. All data were analyzed by computerized inferential statistical methods, including the fitting of a 24-hr cosine curve to individual data series by the method of least squares. A $P$-value for the rejection of the zero circadian amplitude assumption, the acrophase (timing), amplitude and the mesor were determined on each data series (15). Group results were summarized by population mean cosinor. Rhythm parameters found in the two studies were tested for differences both on an individual basis, as well as for the group as a whole (16). Changes in body size area were correlated with changes in circadian mesor and amplitude using linear regression.

## Results

The physical characteristics of the subjects are presented in Table 1. Over the 10-year time span between studies, 5 of 7 subjects gained an average of 13.2 kg. The other two lost 8.2 and 3.2 kg, respectively. Such changes in weight might have detectable consequences on metabolic variables.

Population mean cosinor summaries and an indication of statistical significance from parameter testing for eight vital signs in each study span are presented in Table 2. Chronograms for each variable studied in 1969 and 1979 are presented for each subject and for the group of 7 subjects in Figures 1–8. No statistically significant changes in mesor of any vital sign were found for the group as a whole. A statistically significant *decrease* in amplitude was found in heart rate and blood pressure for the group as a whole, while a *delay* in acrophase for oral temperature was statistically significant for the group as a whole. On a group basis there are thus changes in rhythm characteristics when there is no dynamic change in group mesor for the small number of subjects studied. On an individual basis, statistically significant differences between the two data sets on the same subject obtained 10 years apart consisted of a mesor *increase* of the intraocular pressure of 4 subjects, of the heart rate of 3 subjects and of the diastolic blood pressure of one subject.

Population mean cosinor summaries for the 16 variables of serum from subjects studied in 1969 and again in 1979 are presented in Table 3. Chronograms for each serologic variable in both studies are presented for each subject and for the group in Figures 9–24. As a group, a statistically significant *increase* in mesor was noted for Ca, cholesterol, Cl, $CO_2$, K and Na, while a statistically significant *decrease* in mesor was noted for glucose and transaminase. Statistically significant changes in amplitude for the group as a whole consisted of an *increase* for serum Cl, and a *decrease* for serum albumin, A/G ratio, Cl, globulin, glucose, total protein, sodium and transaminase. A numerical *decrease* in amplitude was observed in serum cholesterol for each subject. For group acrophase, a statistically significant *advance* was found in serum transaminase and a *delay* for serum A/G ratio, globulin, glucose, potassium, total protein and sodium. A numerical *delay* in acrophase was found in serum albumin, cholesterol and $CO_2$ for all subjects.

Population mean cosinor summaries for 12 variables in the urines of the 1969 and 1979 studies are presented in Table 4. Chronograms for each variable, for each subject and for the group, are presented in Figures 25–36. All subjects showed a numerical *decrease* in the mesor of zinc, with statistical significance reached for four of the men. For group acrophase, a statistically significant *delay* was found in urinary magnesium. A numerical *advance* in acrophase was present for the urinary Na/K ratio in all the men.

Results from a correlation of the change in body size with the change in mesor and amplitude between the two studies are presented in Table 5. A statistically significant *positive*

**Table 1. Physical measurements of men studied 10 years apart**

| Subject | Initials | Age at Start (yrs) | Height (cm) 1969 | Height (cm) 1979 | Weight (kg) 1969 | Weight (kg) 1979 | Weight (kg) Δ | Body surface ($m^2$) 1969 | Body surface ($m^2$) 1979 | Body surface ($m^2$) Δ |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | GRM | 23 | 170 | 169 | 77.1 | 98.0 | +20.9 | 1.88 | 2.09 | +0.21 |
| 2 | OJF | 25 | 183 | 183 | 69.4 | 76.2 | +6.8 | 1.92 | 1.96 | +0.04 |
| 3 | LMJ | 26 | 192 | 191 | 98.4 | 120.2 | +21.8 | 2.28 | 2.46 | +0.18 |
| 4 | LDM | 26 | 171 | 171 | 71.7 | 63.5 | -8.2 | 1.84 | 1.77 | -0.07 |
| 5 | MRL | 26 | 171 | 171 | 79.4 | 76.2 | -3.2 | 1.92 | 1.90 | -0.02 |
| 6 | BSA | 28 | 175 | 175 | 79.4 | 87.5 | +8.1 | 1.96 | 2.02 | +0.06 |
| 7 | MTJ | 28 | 173 | 171 | 71.7 | 81.2 | +9.5 | 1.85 | 1.94 | +0.09 |

**Table 2. Population mean-cosinor summary by year for vital signs of seven men**
(Group summary of circadian parameters derived from least-squares fit of 24-hr cosine curve to each data series)

| Year | Variable | Units | *P* | Mesor | Se | Amplitude | (95% Confidence limits) | Acrophase† | (95% Confidence limits) |
|---|---|---|---|---|---|---|---|---|---|
| 1969 | Oral temperature | °F | 0.002 | 97.80 | 0.24 | 1.01 | (0.54, 1.51) | 1430 | (1314, 1622) |
| 1979 | Oral temperature | °F | <.001 | 97.73 | 0.21 | 0.65* | (0.38, 0.95) | 1546 | (1438, 1738) |
| 1969 | Heart rate | bts/min | 0.023 | 62.88 | 3.77 | 7.29 | (1.38, 13.20) | 1414 | (1118, 1726) |
| 1979 | Heart rate | bts/min | 0.082 | 65.79 | 2.84 | 2.11* | | 1450 | |
| 1969 | Systolic blood | mmHg | 0.003 | 118.43 | 3.47 | 7.56 | (3.74, 11.77) | 1434 | (1130, 1658) |
| 1979 | Pressure (BP) | mmHg | 0.144 | 117.01 | 3.26 | 2.96* | | 1754 | |
| 1969 | Diastolic BP | mmHg | 0.014 | 74.86 | 3.03 | 6.94 | (1.74, 12.81) | 2150 | (1738, 2346) |
| 1979 | Diastolic BP | mmHg | 0.825 | 75.42 | 3.16 | 0.44** | | 2030 | |
| 1969 | Pulse P | mmHg | <.001 | 43.57 | 1.61 | 11.80 | (6.88, 17.61) | 1352 | (1244, 1650) |
| 1979 | Pulse P | mmHg | 0.143 | 41.58 | 2.48 | 2.61** | | 1900 | |
| 1969 | Mean arterial P | mmHg | 0.028 | 89.34 | 3.09 | 4.50 | (0.65, 8.53) | 2112 | (1628, 0100) |
| 1979 | Mean arterial P | mmHg | 0.212 | 89.24 | 2.96 | 1.22** | | 1956 | |
| 1969 | Left intraocular P | mmHg | 0.805 | 16.15 | 1.02 | 0.26 | | 0302 | |
| 1979 | Left intraocular P | mmHg | 0.677 | 18.33 | 0.47 | 0.32 | | 0430 | |
| 1969 | Right intraocular P | mmHg | 0.573 | 16.71 | 1.11 | 0.55 | | 0626 | |
| 1979 | Right intraocular P | mmHg | 0.514 | 18.42 | 0.49 | 0.32 | | 0346 | |

*Inter-group difference from parameter test: $*P \leq 0.05$, $**P \leq 0.01$.
†Hour and minute referred to local midnight.

**Table 3. Population mean-cosinor summary by year for serum variables of seven men**
(Group summary of circadian parameters derived from least-squares fit of 24-hr cosine curve to each data series)

| Year | Variable | Units | P | Mesor | Se | Amplitude | (95% Confidence limits) | Acrophase† | (95% Confidence limits) |
|---|---|---|---|---|---|---|---|---|---|
| 1969 | Albumin | g% | 0.010 | 4.55 | 0.04 | 0.15 | ( 0.04, 0.30) | 1430 | (1206, 1954) |
| 1979 | Albumin | g% | 0.028 | 4.61 | 0.06 | 0.05* | ( 0.01, 0.10) | 1854 | (1450, 0126) |
| 1969 | Albumin/globulin | ratio | 0.046 | 1.78 | 0.07 | 0.08 | ( 0.01, 0.17) | 0410 | (2014, 0626) |
| 1979 | Albumin/globulin | ratio | 0.006 | 1.81 | 0.06 | 0.03* | ( 0.01, 0.06) | 1218** | (0722, 1618) |
| 1969 | Total bilirubin | mg% | 0.022 | 0.58 | 0.03 | 0.05 | ( 0.01, 0.29) | 1138 | (0558, 1710) |
| 1979 | Total bilirubin | mg% | 0.019 | 0.76 | 0.11 | 0.19 | ( 0.04, 0.35) | 0638 | (0418, 1022) |
| 1969 | Calcium | mg% | 0.230 | 90.69 | 0.08 | 0.09 | | 1726 | |
| 1979 | Calcium | mg% | 0.679 | 10.03 | 0.08* | 0.05 | | 1450 | |
| 1969 | Chlorides | mEq/l | 0.074 | 104.43 | 0.16 | 0.69 | | 2158 | |
| 1979 | Chlorides | mEq/l | 0.006 | 105.30 | 0.32* | 1.51* | ( 0.62, 2.45) | 0006 | (2214, 0242) |
| 1969 | Cholesterol | mg% | 0.164 | 196.11 | 11.81 | 9.31 | | 1714 | |
| 1979 | Cholesterol | mg% | 0.085 | 252.28 | 17.63* | 4.21 | | 2230 | |
| 1969 | $CO_2$ | % content | 0.572 | 26.49 | 0.21 | 0.22 | | 0426 | |
| 1979 | $CO_2$ | % content | 0.845 | 29.56 | 0.41** | 0.31 | | 1230 | |
| 1969 | Globulin | g% | 0.014 | 2.61 | 0.10 | 0.19 | ( 0.05, 0.33) | 1530 | (1402, 1746) |
| 1979 | Globulin | g% | 0.025 | 2.56 | 0.08 | 0.05** | ( 0.01, 0.11) | 2334** | (1818, 0330) |
| 1969 | Glucose | mg% | 0.020 | 104.20 | 1.69 | 10.46 | ( 1.83, 19.29) | 1526 | (1426, 1806) |
| 1979 | Glucose | mg% | 0.050 | 94.22 | 2.42** | 2.89** | | 0018** | |
| 1969 | Potassium | mEq/l | 0.284 | 4.10 | 0.06 | 0.09 | | 2346 | |
| 1979 | Potassium | mEq/l | 0.042 | 4.32 | 0.05* | 0.09 | ( 0.01, 0 .20) | 0642** | (0110, 1150) |
| 1969 | Total protein | g% | <.001 | 7.17 | 0.14 | 0.34 | ( 0.14, 0.59) | 1542 | (1502, 1834) |
| 1979 | Total protein | g% | 0.009 | 7.16 | 0.09 | 0.09** | ( 0.03, 0.17) | 2106** | (1818, 0158) |
| 1969 | Sodium | mEq/l | 0.002 | 139.72 | 0.21 | 1.29 | ( 0.68, 2.14) | 1910 | (1642, 2250) |
| 1979 | Sodium | mEq/l | <.001 | 143.75 | 0.42** | 0.95* | ( 0.61, 1.38) | 2310** | (2026, 0114) |
| 1969 | Sodium/potassium | ratio | 0.344 | 34.24 | 1.09 | 0.75 | | 1346 | |
| 1979 | Sodium/potassium | ratio | 0.034 | 33.42 | 1.11 | 0.84 | ( 0.33, 1.35) | 1926 | (1546, 2230) |
| 1969 | Transaminase | KU | 0.007 | 23.14 | 1.09 | 3.18 | ( 1.21, 5.15) | 0734 | (0430, 1026) |
| 1979 | Transaminase | KU | 0,021 | 19.68 | 1.09* | 1.17** | ( 0.24, 2.09) | 2301** | (1914, 0250) |
| 1969 | Triglycerides | mg% | <.001‡ | 150.13 | 37.84 | 66.09 | (47.67, 98.63) | 1602 | (1310, 1810) |
| 1979 | Triglycerides | mg% | <.001 | 201.34 | 43.92 | 60.01 | (39.46, 80.56) | 1702 | (1514, 1850) |
| 1969 | Urea nitrogen | mg% | 0.043 | 17.98 | 1.34 | 1.58 | ( 0.06, 3.14) | 2038 | (1750, 0158) |
| 1979 | Urea nitrogen | mg% | <.001 | 17.80 | 0.89 | 1.12 | ( 0.76, 2.21) | 1926 | (1434, 2138) |

*Inter-group difference from parameter test: *$P \leq 0.05$, **$P \leq 0.01$.
†Hour and minute referred to local midnight.
‡Results determined using amplitude as % of mesor.

**Table 4. Population mean-cosinor summary by year for urinary variables for seven men**
(Group summary of circadian parameters derived from least-squares fit of 24-hr cosine curve to each data series)

| Year | Variable | Units | P | Mesor | Se | Amplitude | (95% Confidence limits) | Acrophase† | (95% Confidence limits) |
|---|---|---|---|---|---|---|---|---|---|
| 1969 | Calcium | mg | 0.060 | 19.40 | 2.57 | 9.17 | | 1210 | |
| 1979 | Calcium | mg | 0.009 | 21.82 | 2.94 | 11.64 | (4.15, 19.38) | 1330 | (1118, 1626) |
| 1969 | Calcium/magnesium | ratio | 0.229 | 1.62 | 0.55 | 0.25 | | 1542 | |
| 1979 | Calcium/magnesium | ratio | 0.110 | 1.23 | 0.65 | 0.28 | | 1408 | |
| 1969 | Creatinine | mg | 0.694 | 247.63 | 16.91 | 15.16 | | 1410 | |
| 1979 | Creatinine | mg | 0.130 | 257.45 | 17.57 | 18.30 | | 1954 | |
| 1969 | Magnesium | mg | 0.016 | 12.51 | 1.57 | 3.98 | (0.98, 7.38) | 1142 | (0710, 1446) |
| 1979 | Magnesium | mg | 0.014 | 15.58 | 1.55 | 6.10 | (1.87, 10.56) | 1450* | (1126, 1658) |
| 1969 | pH | — | 0.088 | 6.01 | 0.14 | 0.29 | | 1338 | |
| 1979 | pH | — | 0.390 | 5.78 | 0.11 | 0.20 | | 1222 | |
| 1969 | Potassium | mg | 0.003 | 370.5 | 43.8 | 149.8 | (72.8, 229.2) | 1510 | (1158, 1810) |
| 1979 | Potassium | mg | <.001 | 370.9 | 27.1 | 138.2 | (78.0, 246.4) | 1334 | (1114, 1746) |
| 1969 | Sodium | mg | 0.018 | 571.9 | 80.3 | 223.8 | (51.4, 409.4) | 1422 | (1150, 1930) |
| 1979 | Sodium | mg | 0.014‡ | 640.0 | 83.9 | 256.0 | (67.5, 479.0) | 1658 | (1314, 2142) |
| 1969 | Sodium/potassium | ratio | 0.733 | 1.63 | 0.27 | 0.17 | | 1438 | |
| 1979 | Sodium/potassium | ratio | 0.048 | 1.88 | 0.55 | 0.53* | (0.10, 0.97) | 2038 | (1522, 2350) |
| 1969 | Urea | gm | 0.198 | 1654.3 | 150.2 | 204.1 | | 1806 | |
| 1979 | Urea | gm | 0.008 | 1867.6 | 131.0 | 262.7 | (88.5, 591.5) | 1638 | (1338, 2214) |
| 1969 | Urea clearance | % | 0.590 | 70.32 | 9.41 | 6.40 | | 1346 | |
| 1979 | Urea clearance | % | 0.134 | 80.39 | 8.46 | 7.58 | | 1446 | |
| 1969 | Volume | ml | 0.389 | 154.25 | 29.94 | 37.26 | | 1322 | |
| 1979 | Volume | ml | 0.185 | 204.44 | 37.91 | 52.24 | | 1350 | |
| 1969 | Zinc | μg | 0.542 | 150.1 | 29.2 | 20.8 | | 1402 | |
| 1979 | Zinc | μg | 0.112 | 107.4 | 14.5 | 11.2 | | 0954 | |

*Inter-group difference from parameter test: $*P < 0.05$, $**P < 0.01$.
†Hour and minute referred to local midnight.
‡Results determined using amplitude as % of mesor.

**Table 5. Pearson product-moment correlation of differences between study 1 and 2 in body surface area and circadian mesor and amplitude of each variable investigated**

| | | for: | mesor | | Amplitude | |
|---|---|---|---|---|---|---|
| Variable | n | | *r* | *P* | *r* | *P* |
| Vital signs | | | | | | |
| Oral temperature | 7 | | −0.22 | 0.642 | +0.63 | 0.128 |
| Heart rate | 7 | | +0.12 | 0.805 | +0.79 | 0.035 |
| Systolic BP | 7 | | +0.70 | 0.081 | +0.40 | 0.370 |
| Diastolic BP | 7 | | +0.60 | 0.150 | +0.02 | 0.959 |
| Mean arterial pressure | 7 | | +0.24 | 0.599 | +0.26 | 0.576 |
| Pulse pressure | 7 | | +0.04 | 0.940 | −0.18 | 0.704 |
| Left introaocular pressure | 7 | | +0.14 | 0.772 | +0.52 | 0.212 |
| Right intraocular pressure | 7 | | +0.30 | 0.511 | +0.06 | 0.891 |
| Serum | | | | | | |
| Albumin | 7 | | +0.14 | 0.762 | −0.27 | 0.554 |
| Albumin/globulin ratio | 7 | | −0.16 | 0.724 | +0.33 | 0.467 |
| Bilirubin | 7 | | +0.31 | 0.500 | −0.12 | 0.803 |
| Calcium | 7 | | +0.51 | 0.215 | +0.02 | 0.962 |
| Chlorides | 7 | | +0.73 | 0.063 | +0.86 | 0.013 |
| Cholesterol | 7 | | +0.78 | 0.039 | −0.29 | 0.527 |
| $CO_2$ | 7 | | −0.65 | 0.112 | +0.65 | 0.114 |
| Globulin | 7 | | −0.15 | 0.740 | +0.40 | 0.378 |
| Glucose | 7 | | −0.02 | 0.964 | +0.38 | 0.398 |
| Potassium | 7 | | −0.37 | 0.407 | −0.22 | 0.640 |
| Protein | 7 | | −0.22 | 0.635 | +0.14 | 0.771 |
| Sodium | 7 | | +0.48 | 0.235 | +0.53 | 0.206 |
| Sodium/potassium ratio | 7 | | +0.43 | 0.256 | −0.51 | 0.218 |
| Transaminase | 7 | | +0.51 | 0.214 | +0.04 | 0.938 |
| Triglycerides | 7 | | +0.50 | 0.224 | −0.48 | 0.235 |
| Urea nitrogen | 7 | | −0.69 | 0.087 | −0.40 | 0.380 |
| Urine | | | | | | |
| Volume | 7 | | +0.24 | 0.603 | +0.12 | 0.792 |
| pH | 7 | | −0.11 | 0.814 | −0.08 | 0.860 |
| Urea | 7 | | −0.01 | 0.986 | −0.22 | 0.632 |
| Urea clearance | 7 | | −0.22 | 0.636 | +0.05 | 0.913 |
| Creatinine | 7 | | +0.37 | 0.415 | +0.45 | 0.253 |
| Sodium | 7 | | −0.06 | 0.901 | +0.34 | 0.455 |
| Potassium | 7 | | −0.54 | 0.195 | −0.52 | 0.211 |
| Sodium/potassium ratio | 7 | | −0.01 | 0.994 | −0.64 | 0.116 |
| Calcium | 7 | | −0.20 | 0.674 | +0.53 | 0.207 |
| Magnesium | 6 | | −0.61 | 0.192 | +0.59 | 0.208 |
| Calcium/magnesium ratio | 6 | | +0.93 | 0.007 | −0.50 | 0.272 |
| Zinc | 7 | | −0.64 | 0.118 | −0.02 | 0.963 |

correlation was observed with mesor change in serum cholesterol (Figure 37, top) and in the urinary Ca/Mg ratio (Figure 37, bottom), while significant *positive* correlations between body size change and change in amplitude were observed for heart rate and serum Cl. A *positive* correlation with borderline statistical significance ($P \leqslant 10$) was found for change in systolic blood pressure, serum Cl and urea nitrogen mesors.

## Discussion

It is important to note that the data of 1969 and 1979 are well within the so-called "normal limits" given for physiologic variables in various texts. Hence, any difference in the mesor of electrolytes between the 1969 and 1979 data may reflect changes with age, e.g. in the buffer system coordinating the pH of body fluids, that would otherwise remain undetected. While our findings relate to a small number of individuals, their statistical significance, when present, describes that particular individual. By the chronobiologic approach used herein, a $P$-value becomes a useful tool for the individual's health assessment, rather than merely a result for the description of some idealized group. Statistical significance for the individual or the group in chronobiology is a necessary but not a sufficient condition for the assessment of a given problem such as aging. Even statistical significance presented herein must be qualified, since very many tests were carried out on the data set of each individual, as well as of the group as a whole. Only if the findings are confirmed in future studies can the statistically significant variation in the same group of subjects, studied at a 10-year interval, be accepted to reveal physiologic aging in a longitudinal fashion. Moreover, if this is possible only by virtue of the chronobiologic design of these studies, there remains the task of ascertaining with statistical significance the biologic importance of a given change.

The "across the board" higher cholesterol mesor in 1979 versus 1969, in all subjects over a span of 10 years, exceeds the so-called "usual limits" and should constitute a serious concern, especially if such probable increases are allowed to continue with age. The group mesor for cholesterol, 196 mg/dl in 1969, is 252 mg/dl in the 1979 study. The group mesor for triglycerides also differs, from 150 in 1969 and 201 mg/dl in 1979. The National Heart, Lung and Blood Institute in the United States currently established 260 mg/dl as the cholesterol concentration at which drug therapy should be initiated in subjects over 40 years of age. Four of our subjects in the 1979 study substantially exceeded the 260 mg/dl criterion at 33–38 years of age, apart from any chronobiologic considerations awaiting study on additional subjects.

It is an intriguing speculation that the statistically significant positive correlation noted for the mesor change in body size ($M^2$) with that of the urinary calcium/magnesium ratio may be more important clinically than the similar correlation with changes in serum cholesterol. This may be so because magnesium deficiency has been associated with lower magnesium levels in serum, with coronary vasospasm, acute infarction and sudden death (17). This relative magnesium deficiency may contribute to the loss of myocardial potassium stores, resulting in greater myocardial irritability as reflected by a higher urinary Ca/Mg ratio (18). Our data appear to indicate that the greater the change in body surface area (here due to increase in weight), the greater the increase in urinary Ca/Mg ratio ($P = 0.007$, Figure 37), indicating lower Mg exertion and suggesting depletion of magnesium stores. However, the relationship of the Ca/Mg ratio in serum to that in urine has not been clearly defined and therefore needs to be more extensively studied metabolically and clinically before such speculation could be ascertained.

The number of mesor differences observed for each subject is summarized in Table 6, together with each subject's medical assessment in 1979. It is important to point out that the physical examinations given each subject in the mid-to-late 1960s, prior to induction into the army, were routine and did not include any extensive physiological studies and it was on such a limited basis that all subjects were considered medically fit by Army standards. Out of a total of 251 time series collected and analyzed, there was a numerical *increase* for 148 mesors, a *decrease* for 102 mesors, with 1 mesor unchanged. There was also a numerical *decrease* for 162 amplitudes and 89 *increases* in amplitude. While these overall numerical changes may be equated to "no change" or at least to "no detectable change," they should be noteworthy for the group (not only for the individual) when 7 out of 7 are in the same direction and should be a particular focus of further study. Future work should involve subject groups several orders of magnitude larger than those here studied. Invariably included should be those variables that showed cosinor-validated changes within 10 years. On an individual basis, statistically-significant ($P \leq 0.05$) mesor *increases* were found in 69 series, statistically-significant mesor *decreases* were found in 25 series. These changes may relate to some metabolic deviations which may surface clinically in some subjects. A closer examination

**Table 6. Individual mesor differences between 1969 and 1979 and clinical assessment of each subject in 1979**

| Subject # | Age (years) | N of statistically; significant mesor* increases | N of statistically; significant mesor* decreases | Clinical impression in 1979 |
|---|---|---|---|---|
| 1 | 33 | 12 | 7 | Moderately obese, otherwise healthy |
| 2 | 35 | 10 | 1 | Symptomatic hiatus hernia |
| 3 | 36 | 8 | 2 | History of hyperlipemia, asymptomatic |
| 4 | 36 | 13 | 3 | Chronic asthma; arthritis of shoulder and elbow; somewhat depressed from marital difficulties |
| 5 | 36 | 8 | 4 | Clinically healthy; left ventricular conduction delay in ECG |
| 6 | 38 | 11 | 3 | Clinically healthy |
| 7 | 38 | 7 | 5 | Clinically healthy; slightly overweight |

*Out of 36 variables investigated for subjects 1–6 and 34 variables for subject 7. Conventional electro-cardiographic spotcheck (ECG) carried out on each subject, unremarkable unless otherwise noted.

of the circadian dynamics of physiological variables in future studies may prove essential for an efficient health assessment. The continued accumulation of data similar to those presented here and elsewhere (19–31) should provide reference standards which may prompt dietary or other preventive intervention. Deviations from the chronobiologically determined reference standards would then serve as harbingers of an increased risk of developing diseases of our civilization—those of circulation, cancer and metabolism, in particular.

It has been reported that with advancing age there is a tendency toward a decrease in the circadian amplitude, as well as some modifications of the mesor for many of the variables presented in our studies (32). It is in view of the need for instituting timely preventive measures that circadian time-specified reference standards may well be constructed for peer-groups at least at 10-year intervals, whenever, as shown herein (and to be confirmed), one can anticipate statistically significant changes within such a span. Moreover, such reference standards should be not only circadian, but also circannual rhythm stage specified, as suggested for the case of cholesterol earlier (33). This suggestion applies not only to serum cholesterol, but it will be necessary to add standards for high and low density lipoprotein fractions (recently reported to be circadian rhythmic, but with reduced amplitude, in the elderly [34]), as well as for other variables of particular interest to physiologic aging.

## Summary

From a circadian chronobiologic perspective, the immense amount of data uniquely reviewed in this report across a 10-year span in seven healthy individuals serves a useful beginning to the study of the effects of normal aging upon commonly measured physiologic and biochemical variables and, more importantly, upon the circadian rhythm characteristics of these variables. A great deal of supposition about what happens to the mesor, amplitude and acrophase of an individual's circadian rhythms in a variety of endpoints has been based upon transverse studies of short duration and relatively few long-term studies. The further accumulation of data such as presented here and similar long-term longitudinal time series can have no adequate substitute for truly understanding whether reproducible age-related changes in circadian rhythms occur as individuals age.

With these qualifications and with the further qualification that the timing of our observations

Figure 1

Chronograms of Temperature

(□ = 1969, ■ = 1979)

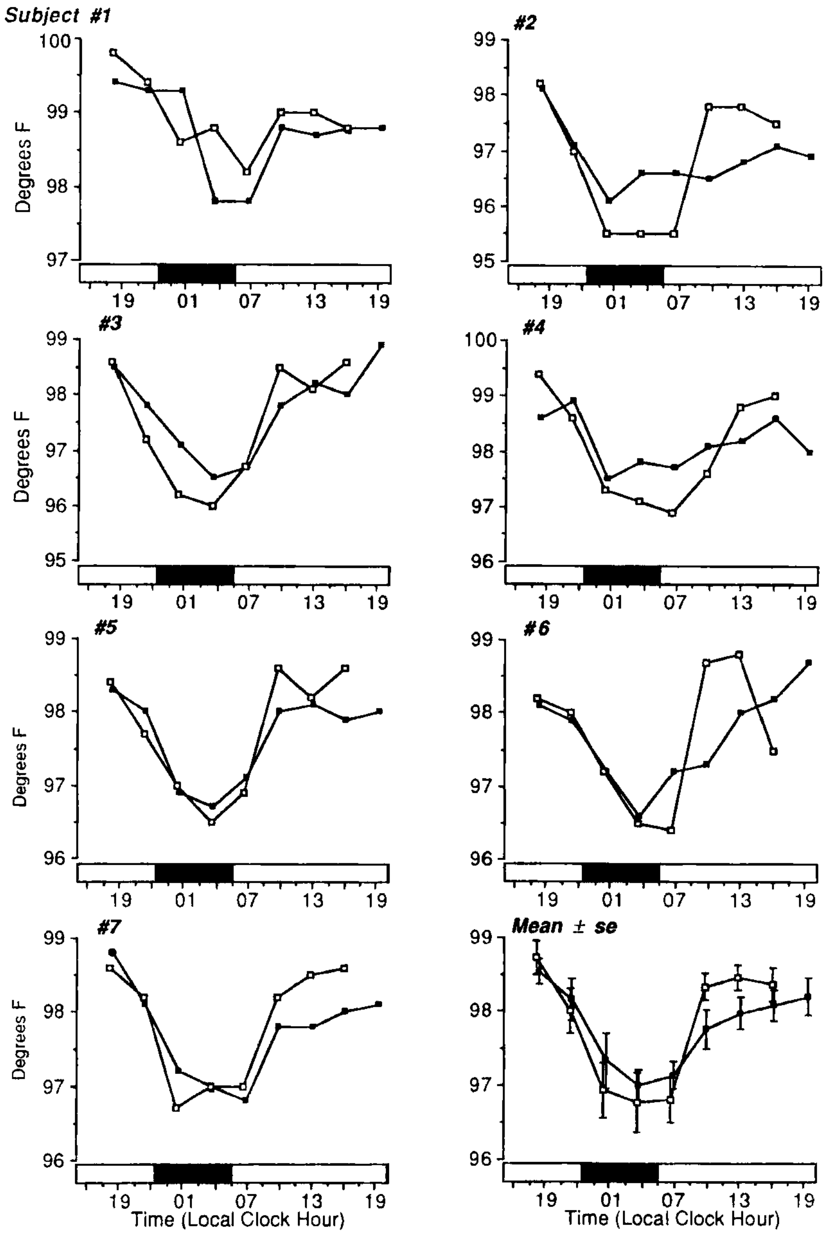


Figure 2

Chronograms of Heart Rate

(□ = 1969, ■ = 1979)

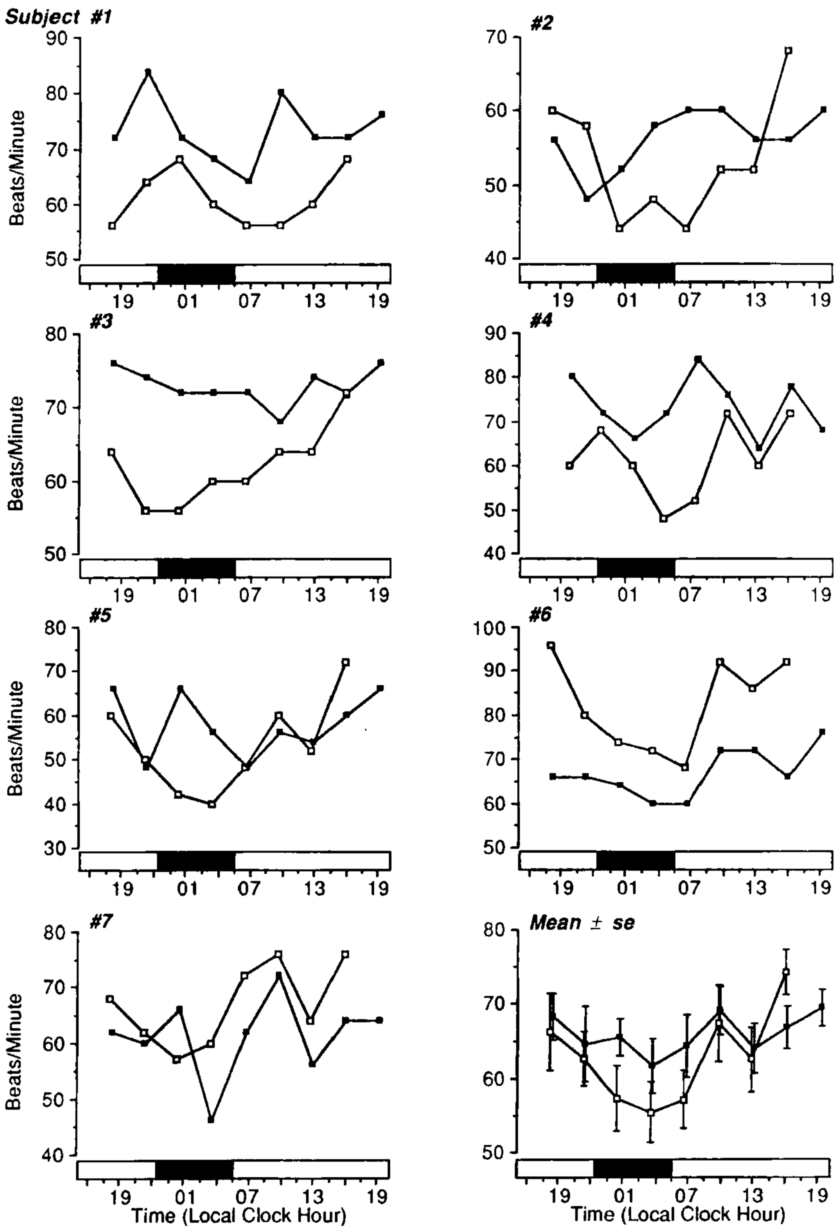


Figure 3

## Chronograms of Systolic Blood Pressure

( □ = 1969, ■ = 1979)

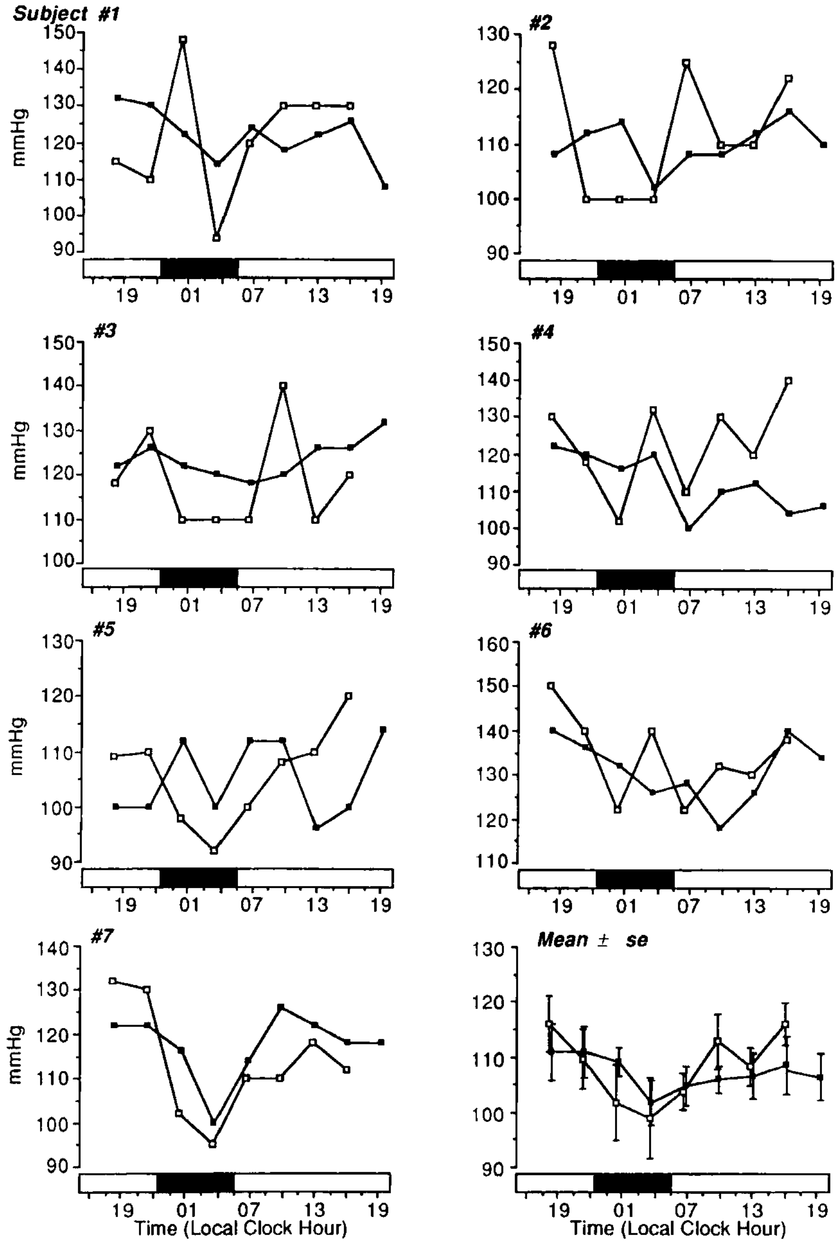


Figure 4

## Chronograms of Diastolic Blood Pressure

( □= 1969,■ = 1979)

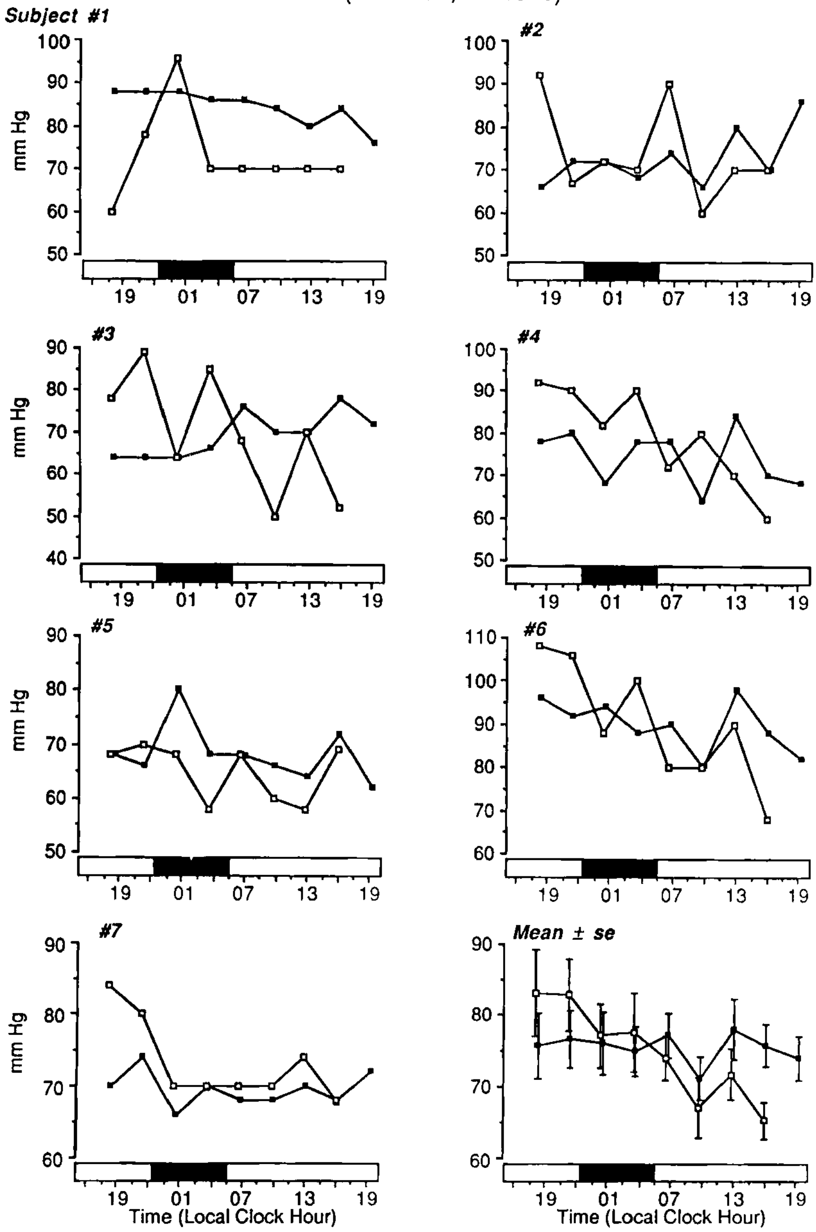


Figure 5

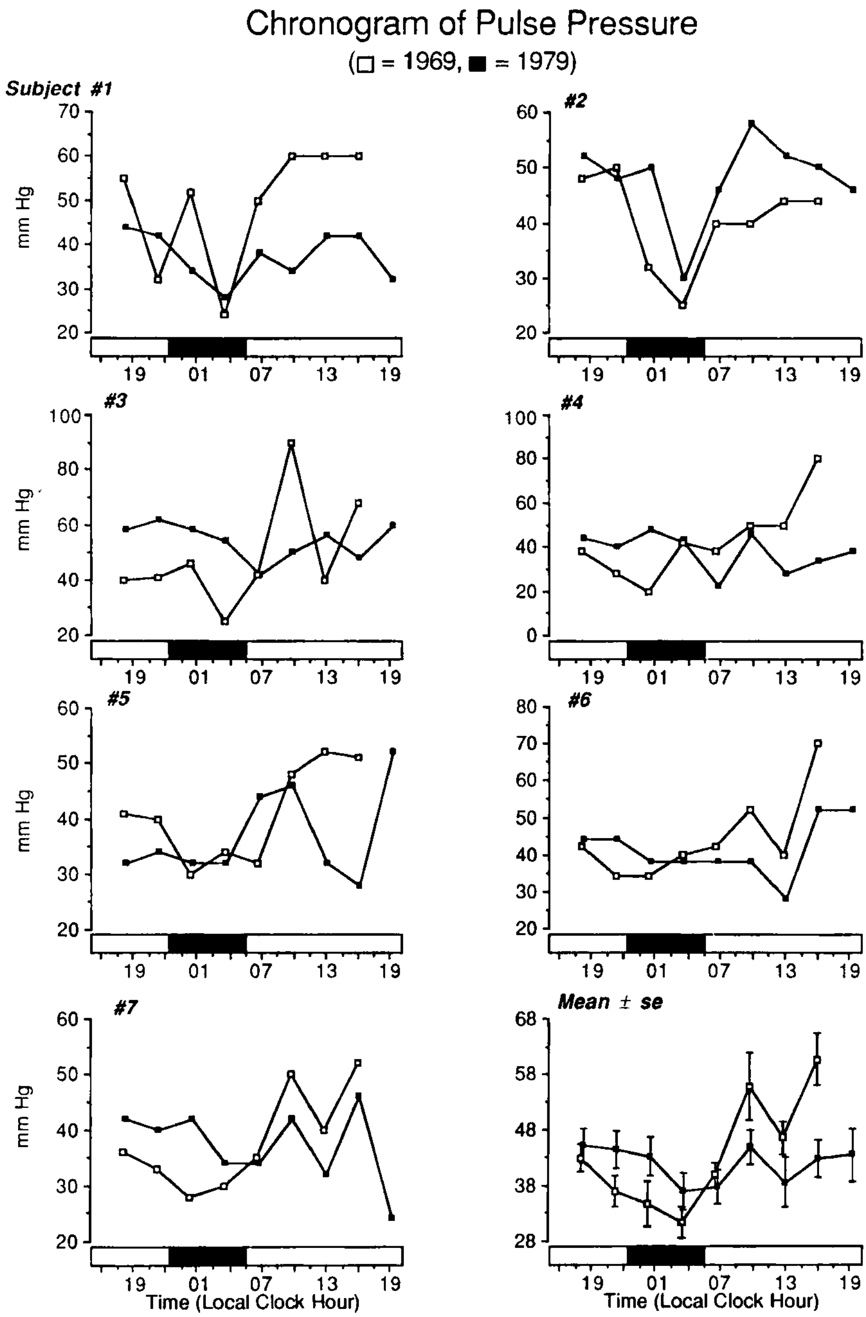

Chronogram of Pulse Pressure
(□ = 1969, ■ = 1979)
Subject #1
#2
#3
#4
#5
#6
#7
Mean ± se
mm Hg
19 01 07 13 19
Time (Local Clock Hour)

Figure 6

Chronograms of Mean Arterial Pressure

(□ = 1969, ■ = 1979)

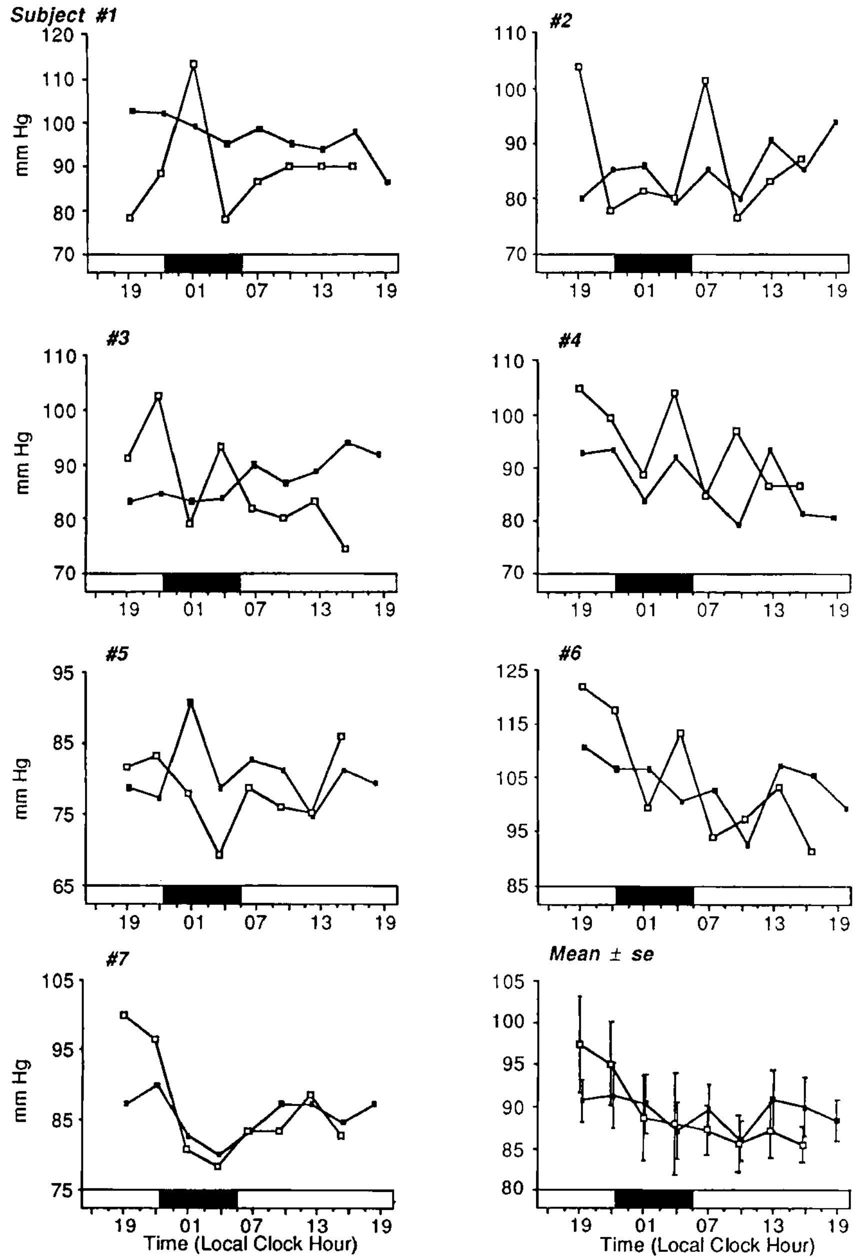


Figure 7

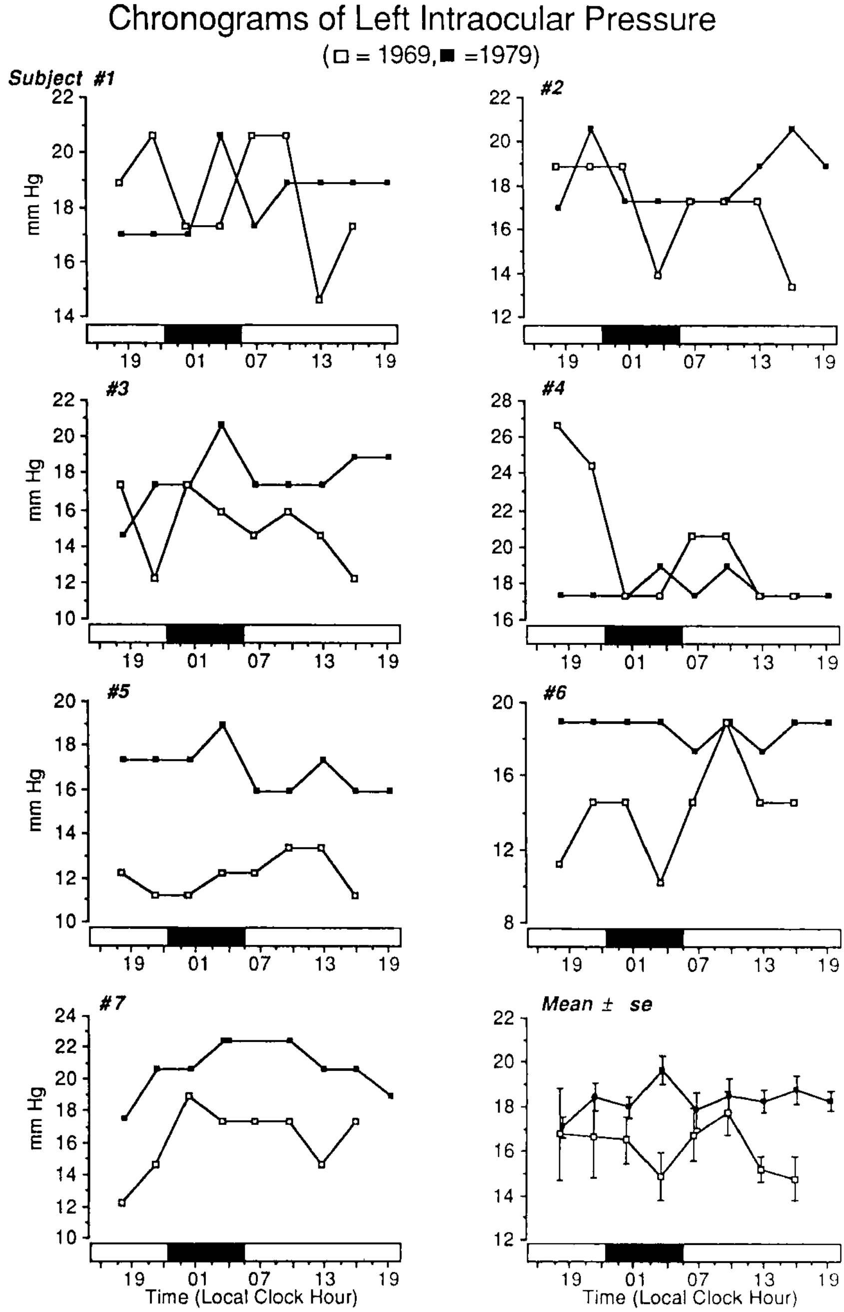

Chronograms of Left Intraocular Pressure
(□ = 1969,■ =1979)
Subject #1
#2
#3
#4
#5
#6
#7
Mean ± se
mm Hg
19 01 07 13 19
Time (Local Clock Hour)

Figure 8

Chronograms of Right Intraocular Pressure

(□ = 1969, ■ = 1979)

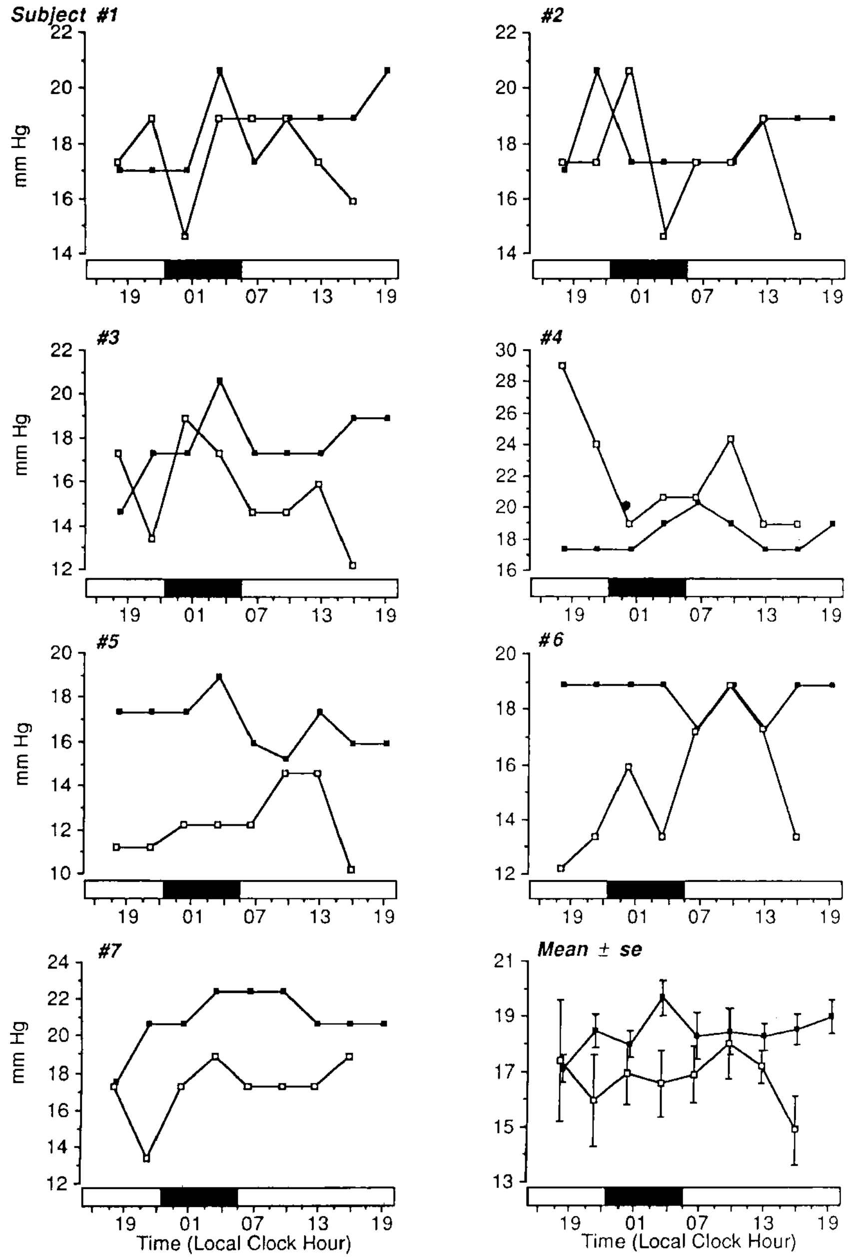


Figure 9

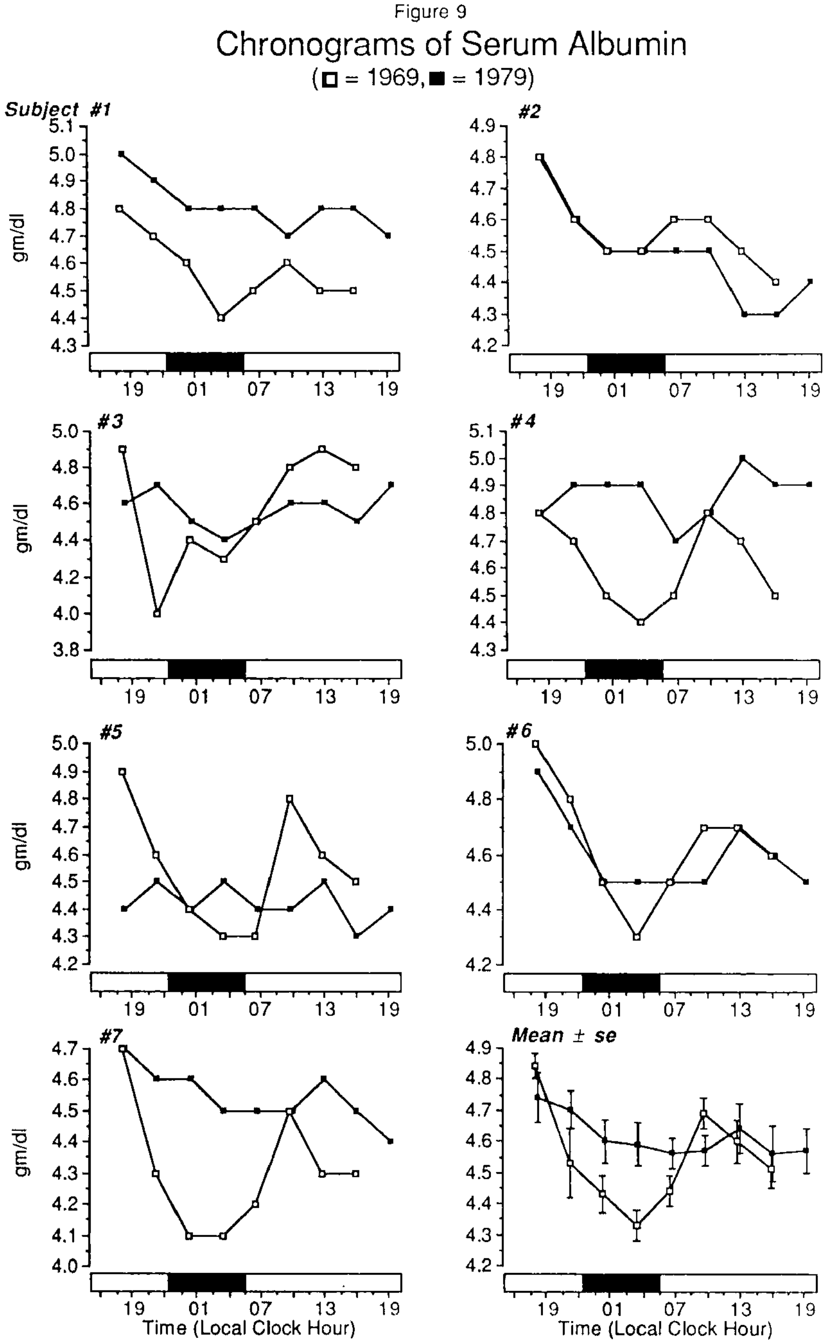
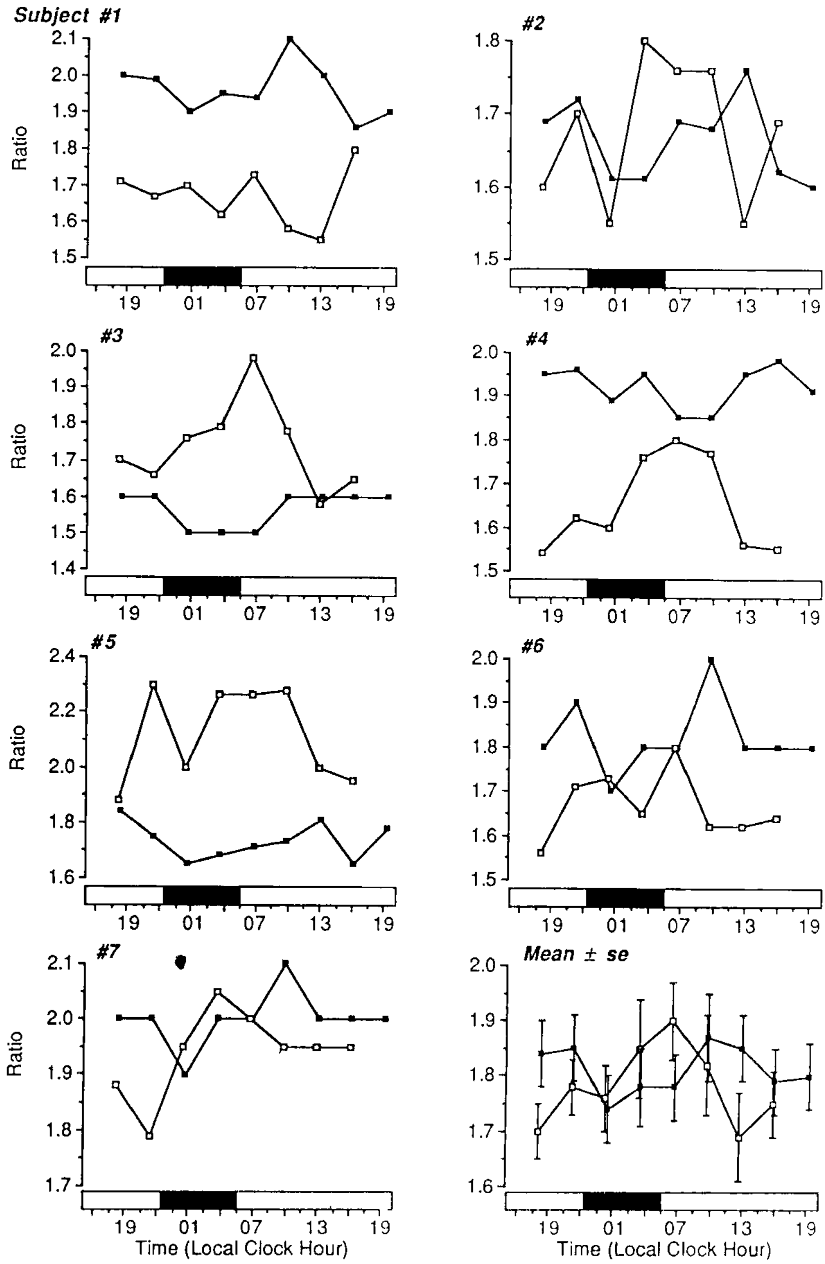

Chronograms of Serum Albumin
(□ = 1969, ■ = 1979)
Subject #1
#2
#3
#4
#5
#6
#7
Mean ± se
gm/dl
19 01 07 13 19
Time (Local Clock Hour)

Figure 10
## Chronograms of Serum Albumin/Globulin Ratio
(□ = 1969, ■ = 1979)



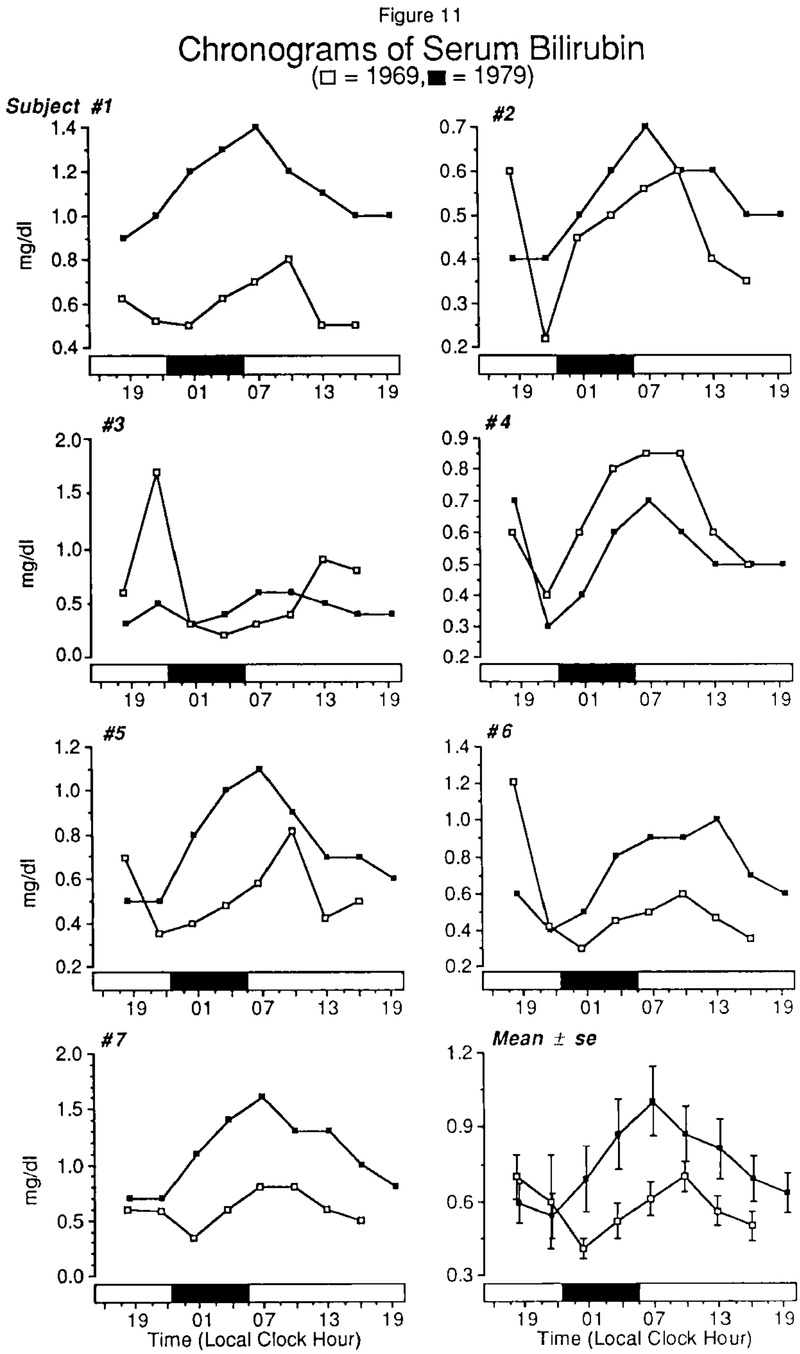


Figure 11
Chronograms of Serum Bilirubin
(□ = 1969,■ = 1979)
Subject #1
#2
#3
#4
#5
#6
#7
Mean ± se
mg/dl
Time (Local Clock Hour)
19 01 07 13 19

Figure 12

Chronograms of Serum Calcium

(□ = 1969, ■ = 1979)

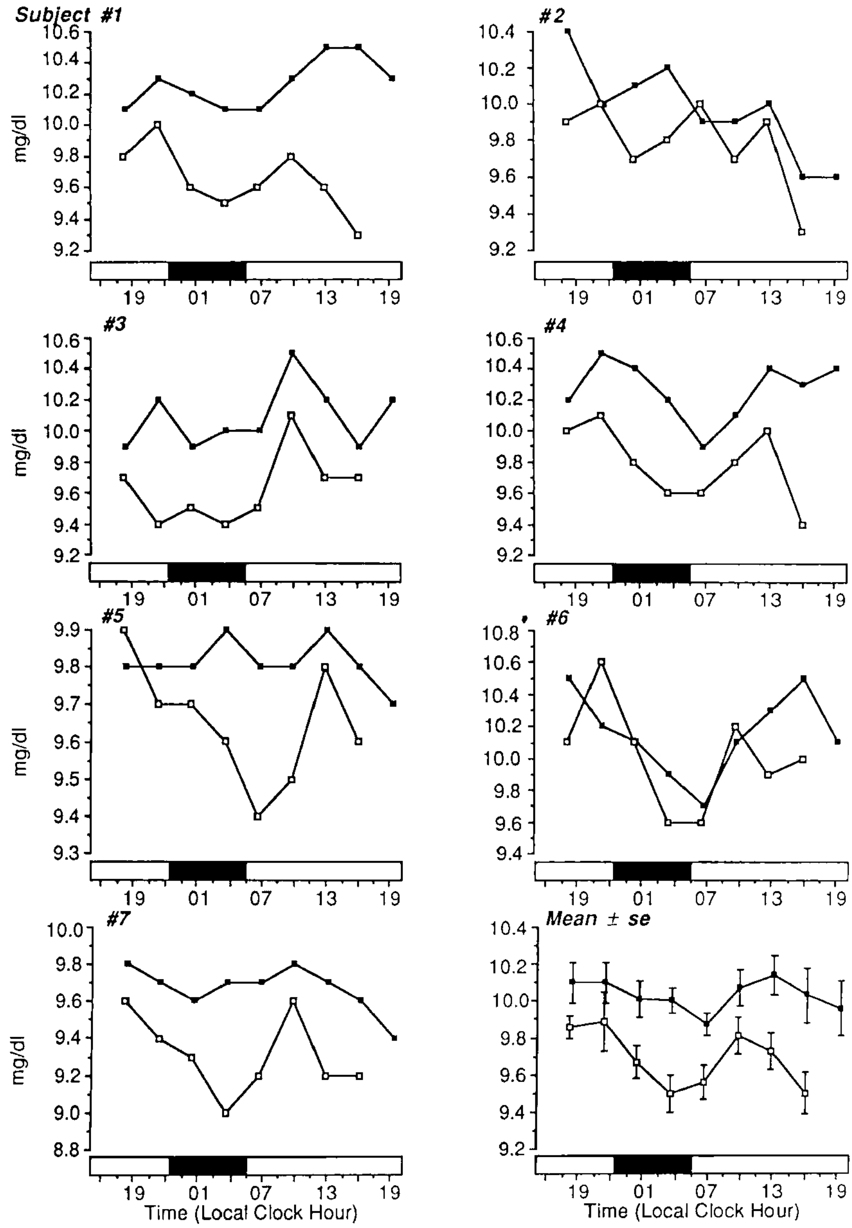


Figure 13

# Chronograms of Serum Chlorides

(□ = 1969, ■ = 1979)

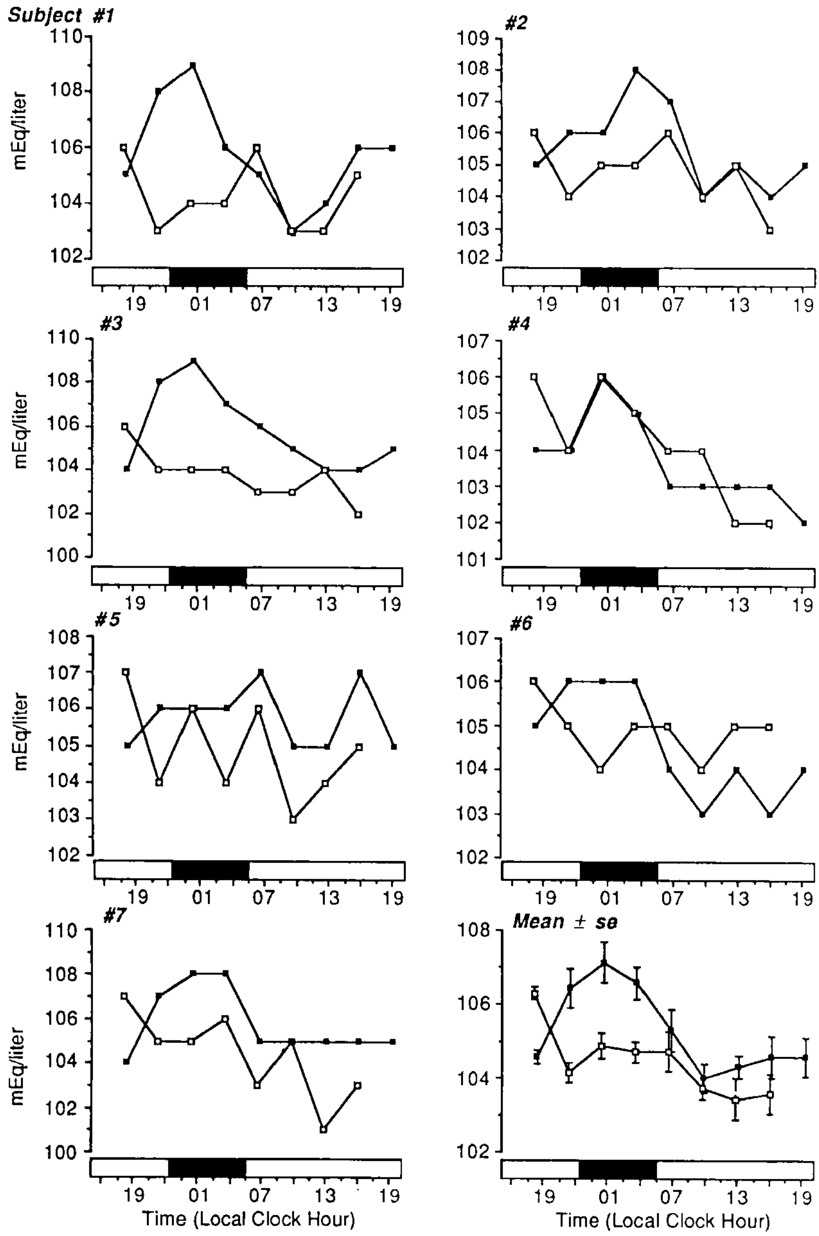


Figure 14

Chronograms of Serum Cholesterol

(□ = 1969, ■ = 1979)

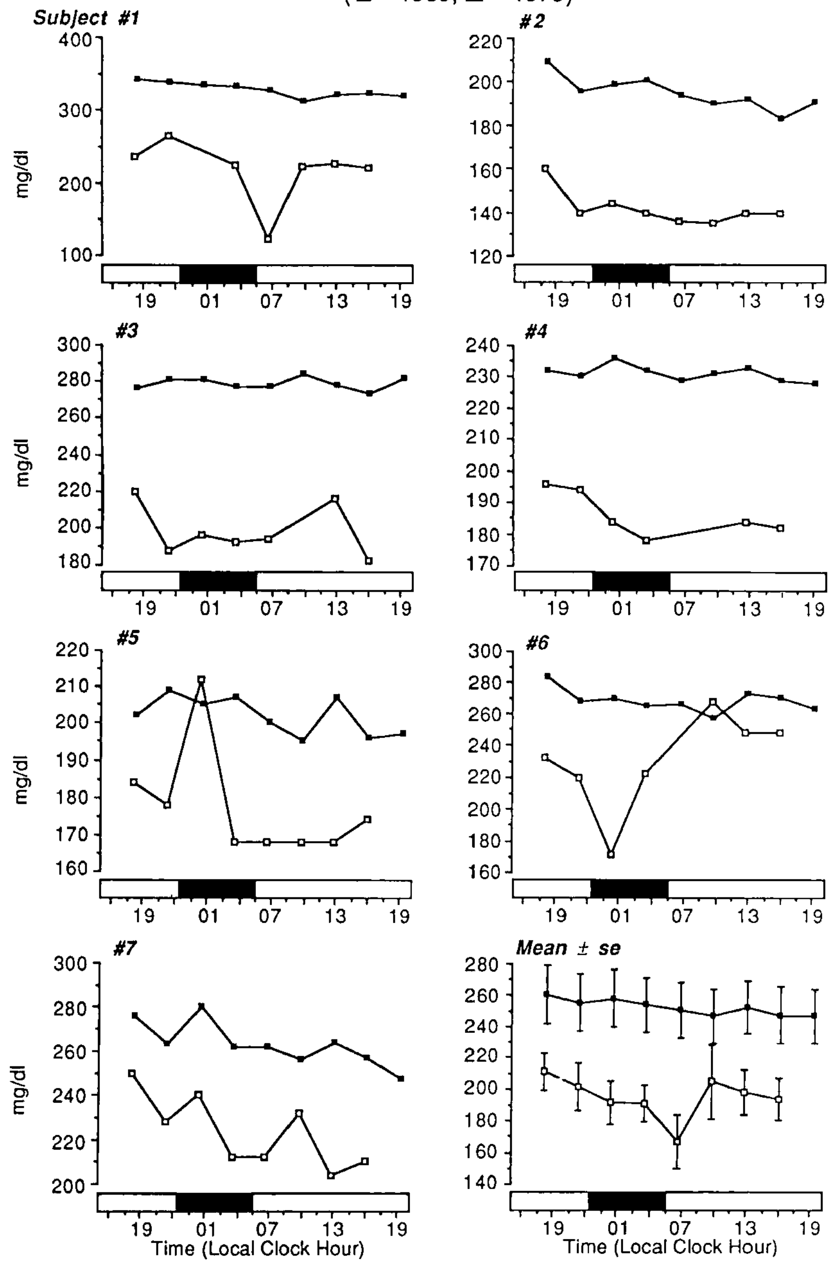


Figure 15

# Chronograms of Serum CO2

(□ = 1969,■ = 1979)

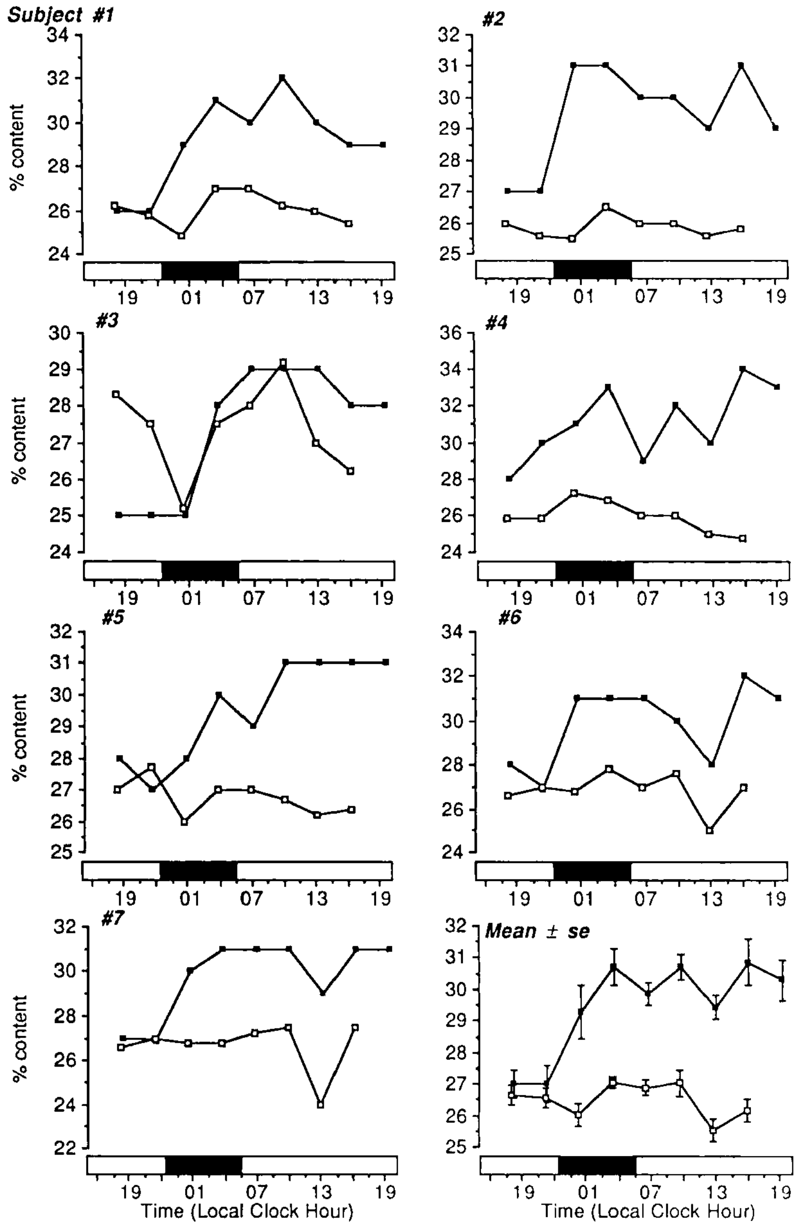


Figure 16
Chronograms of Serum Globulin
(□ = 1969, ■ = 1979)

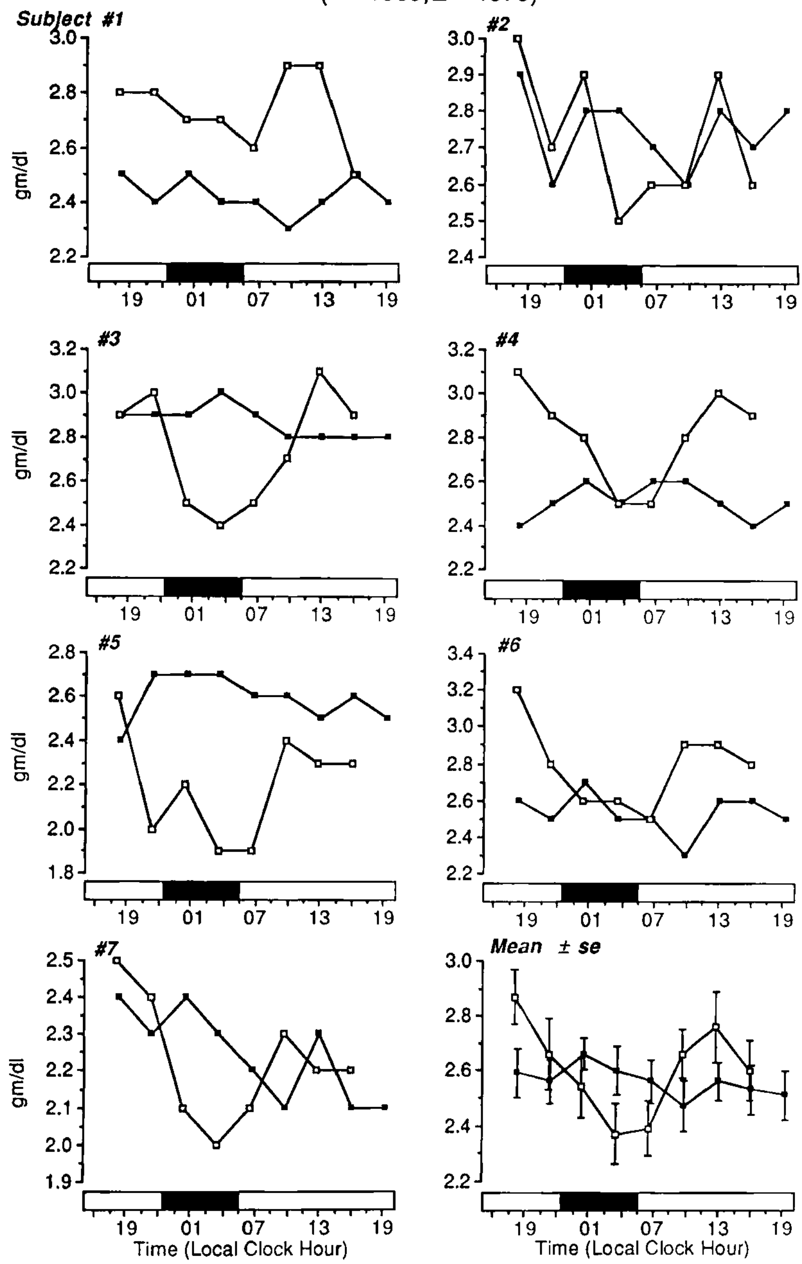


Figure 17

## Chronograms of Serum Glucose

(□ = 1969, ■ = 1979)

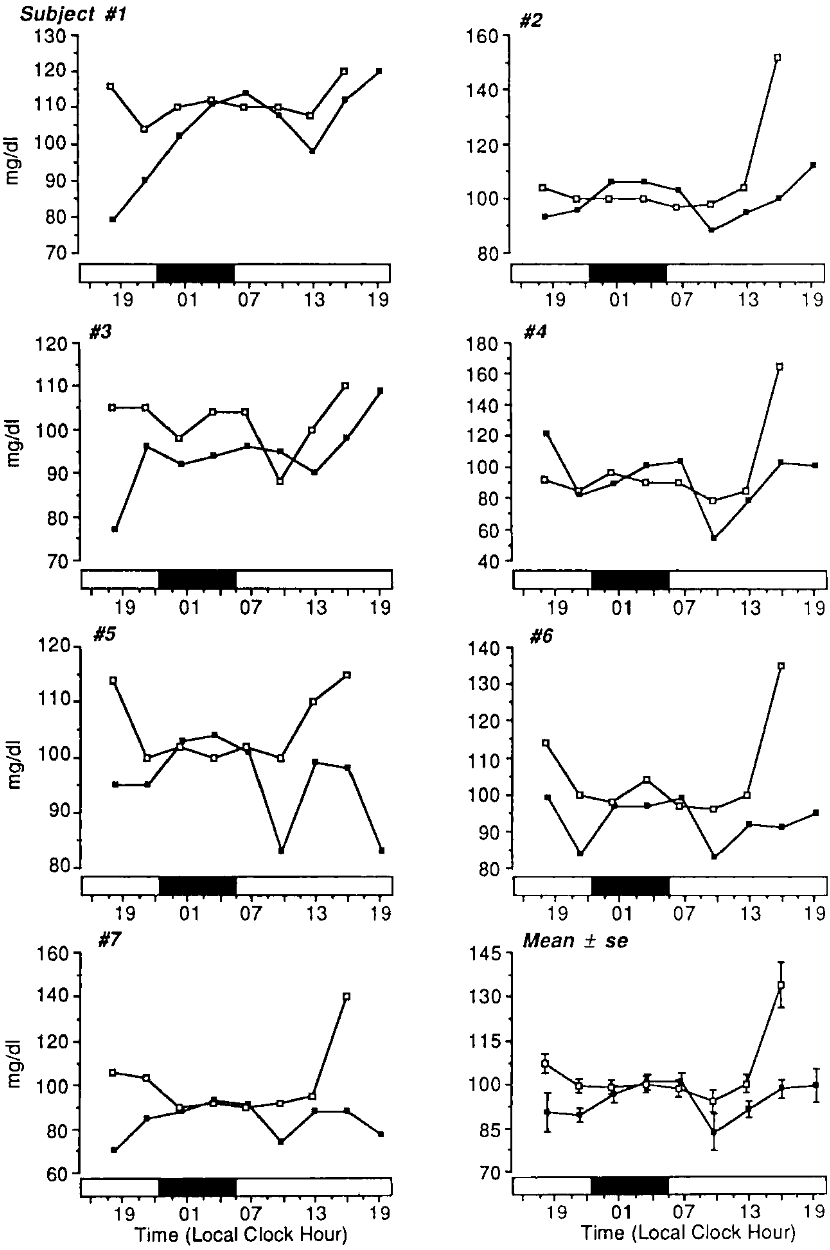


Figure 18

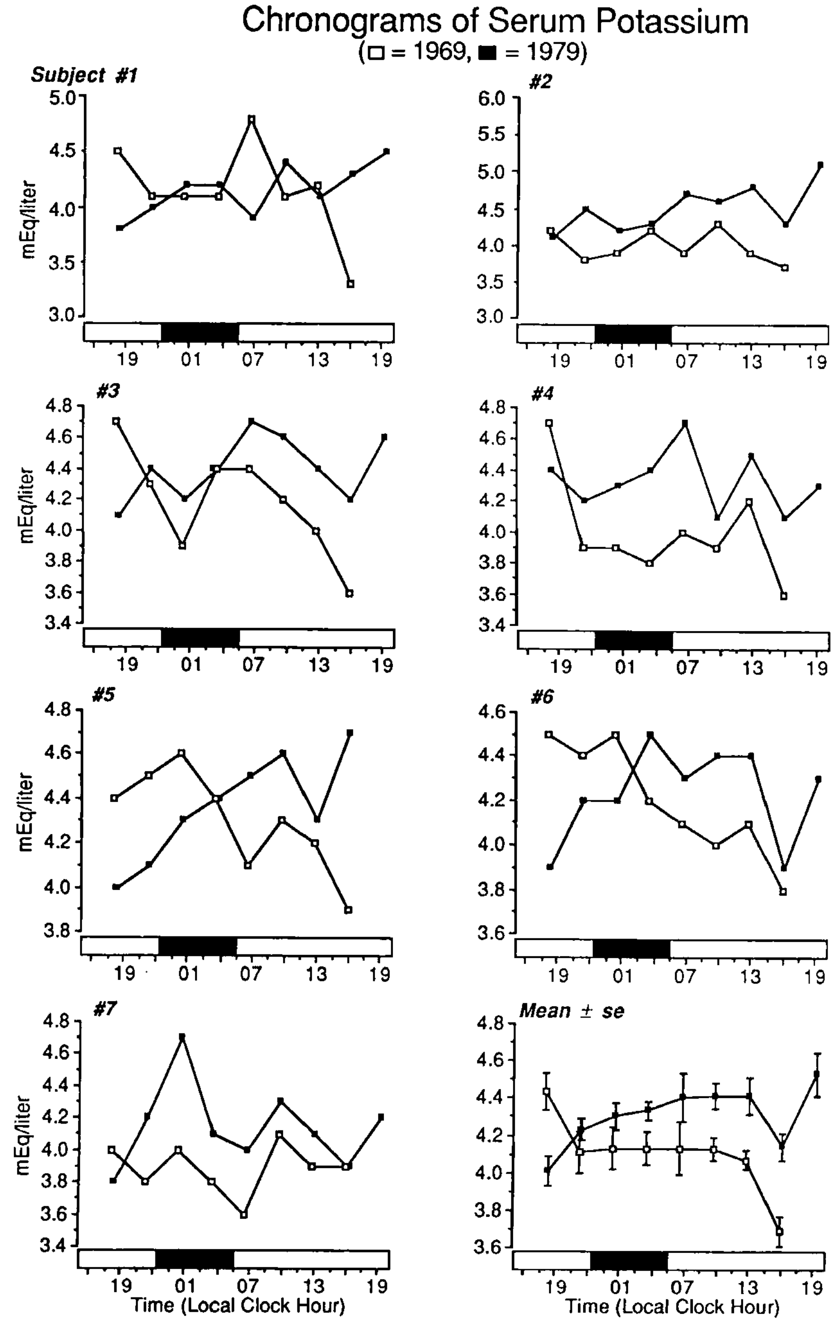

Chronograms of Serum Potassium
(□ = 1969, ■ = 1979)
Subject #1
#2
#3
#4
#5
#6
#7
Mean ± se
mEq/liter
19
01
07
13
Time (Local Clock Hour)

Figure 19

Chronograms of Serum Total Proteins

(□ = 1969, ■ = 1979)

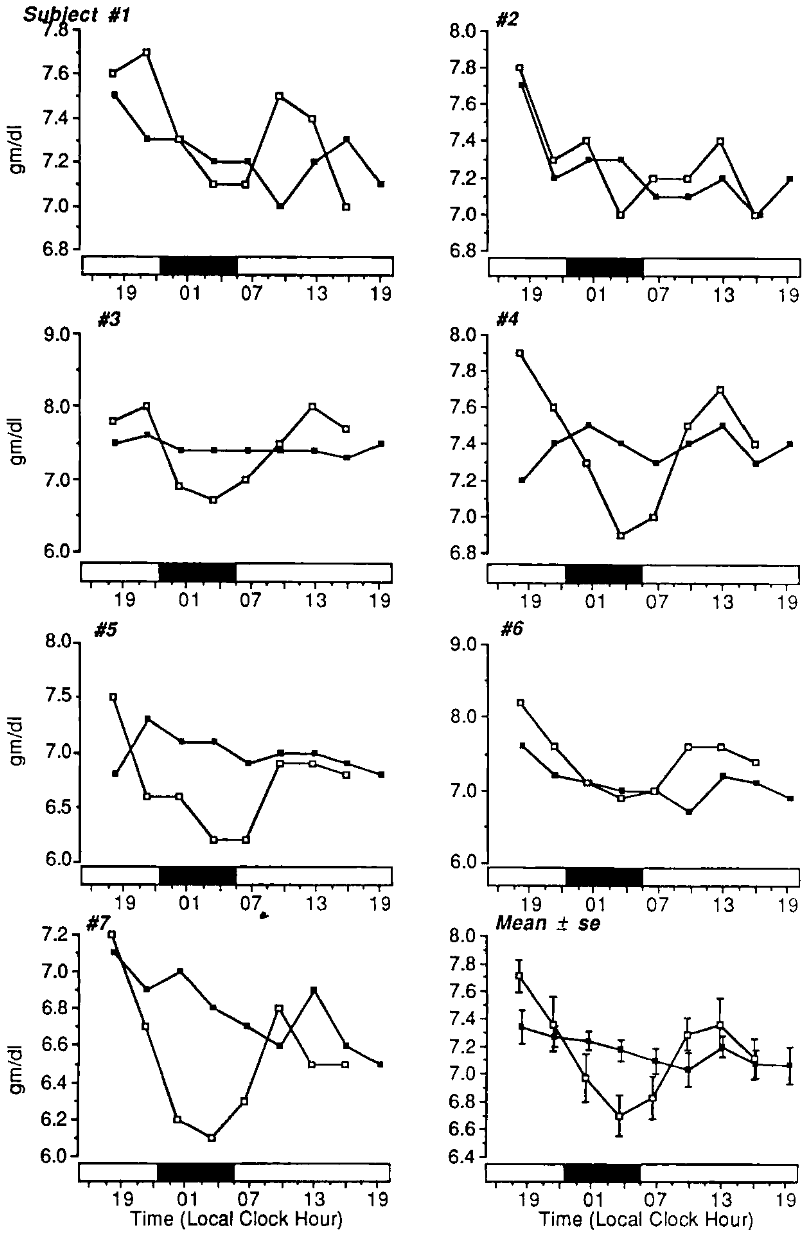


Figure 20

## Chronograms of Serum Sodium

(□ = 1969, ■ = 1979)

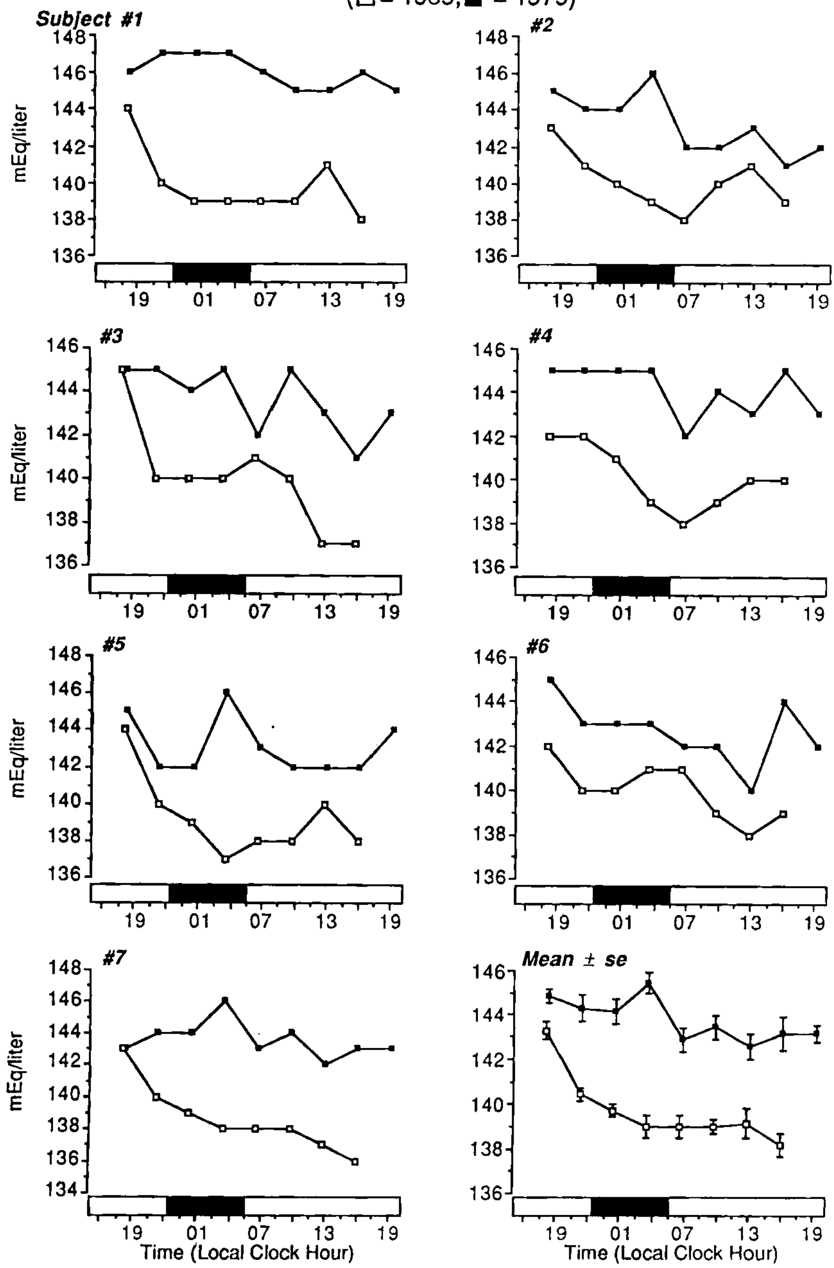


Figure 21

Chronograms of Serum Sodium/Potassium Ratio

(□ = 1969, ■ = 1979)

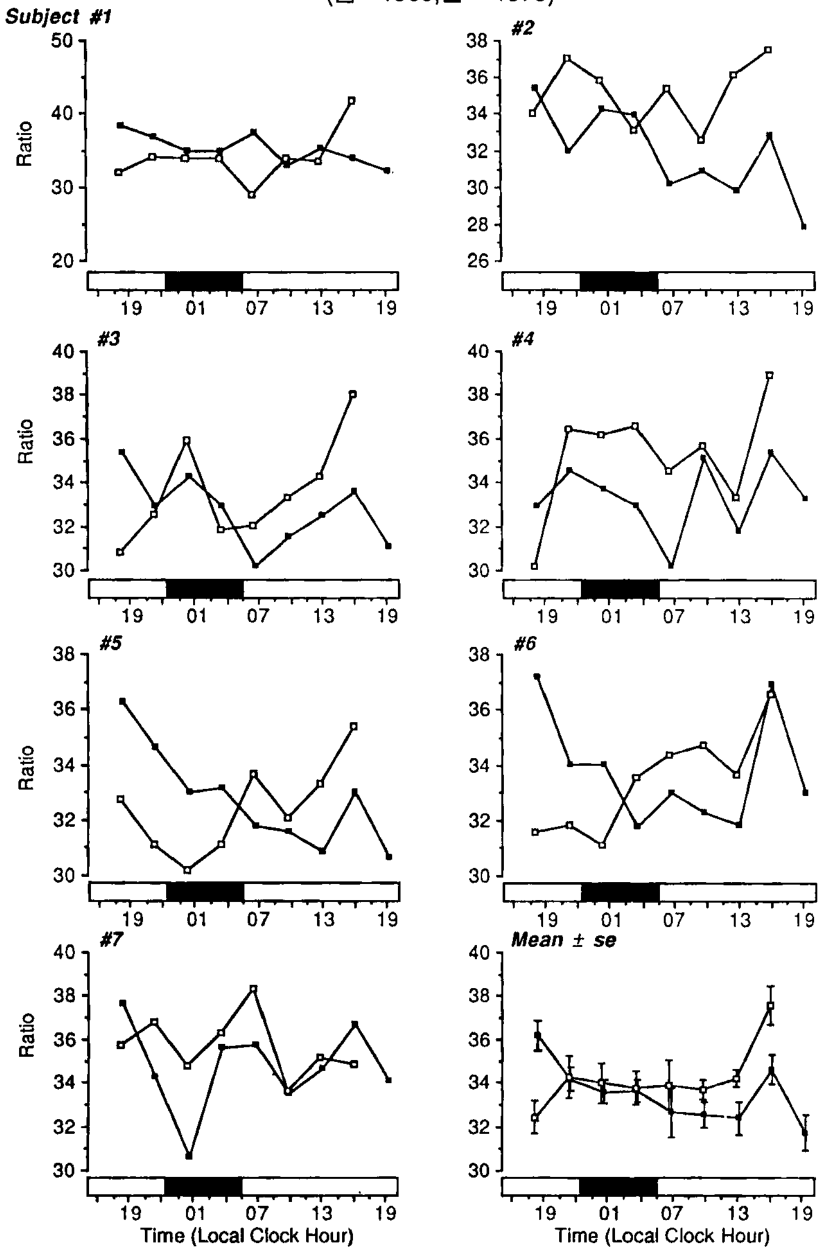


Figure 22

Chronograms of Serum Transaminase

(□ = 1969, ■ = 1979)

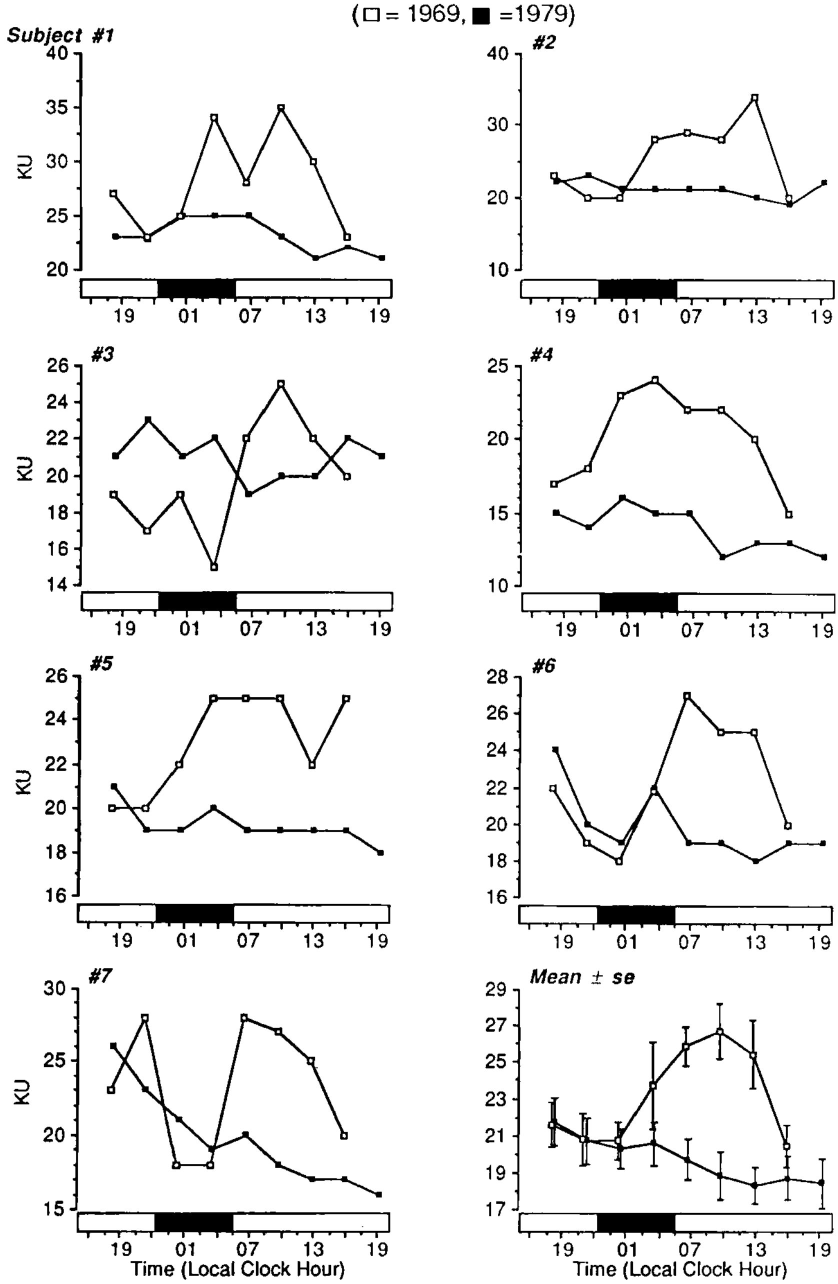


Figure 23

Chronograms of Serum Triglycerides

(□ = 1969, ■ = 1979)

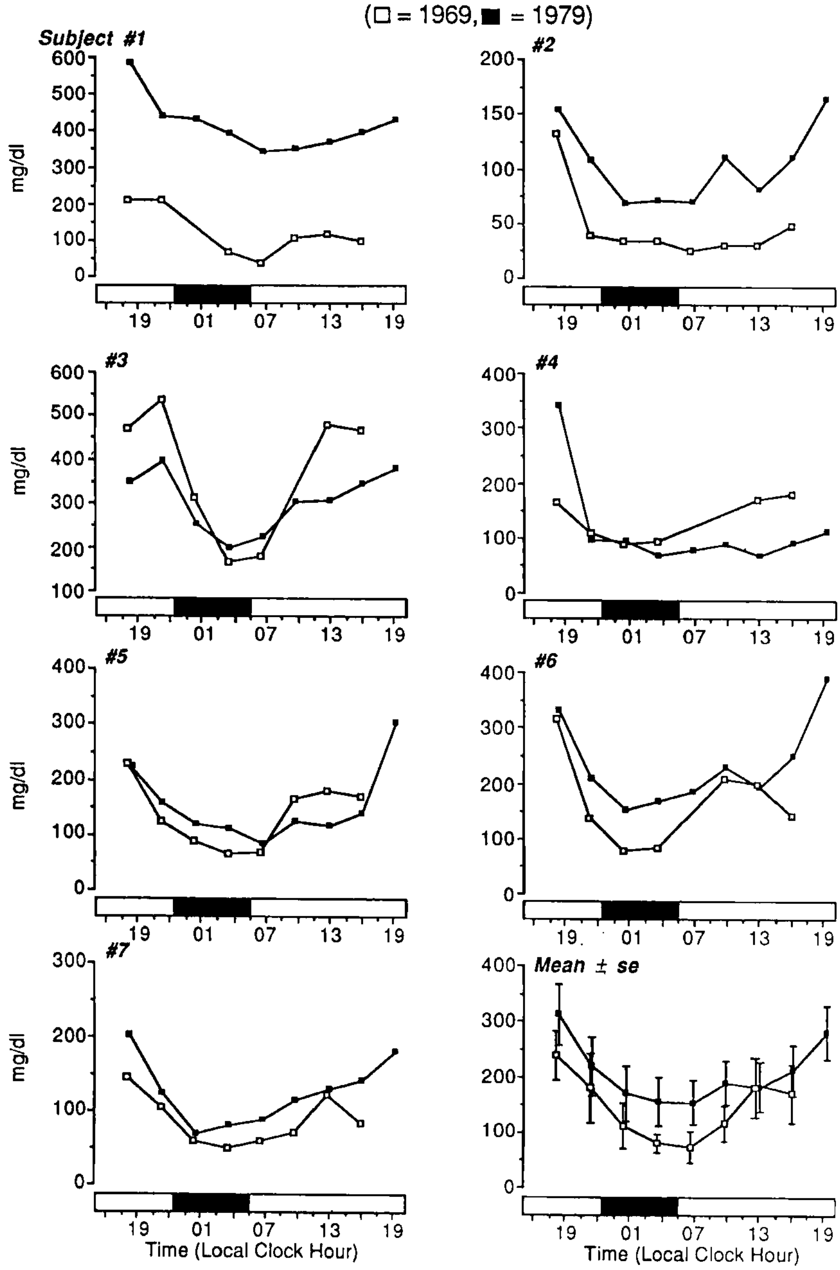


Figure 24

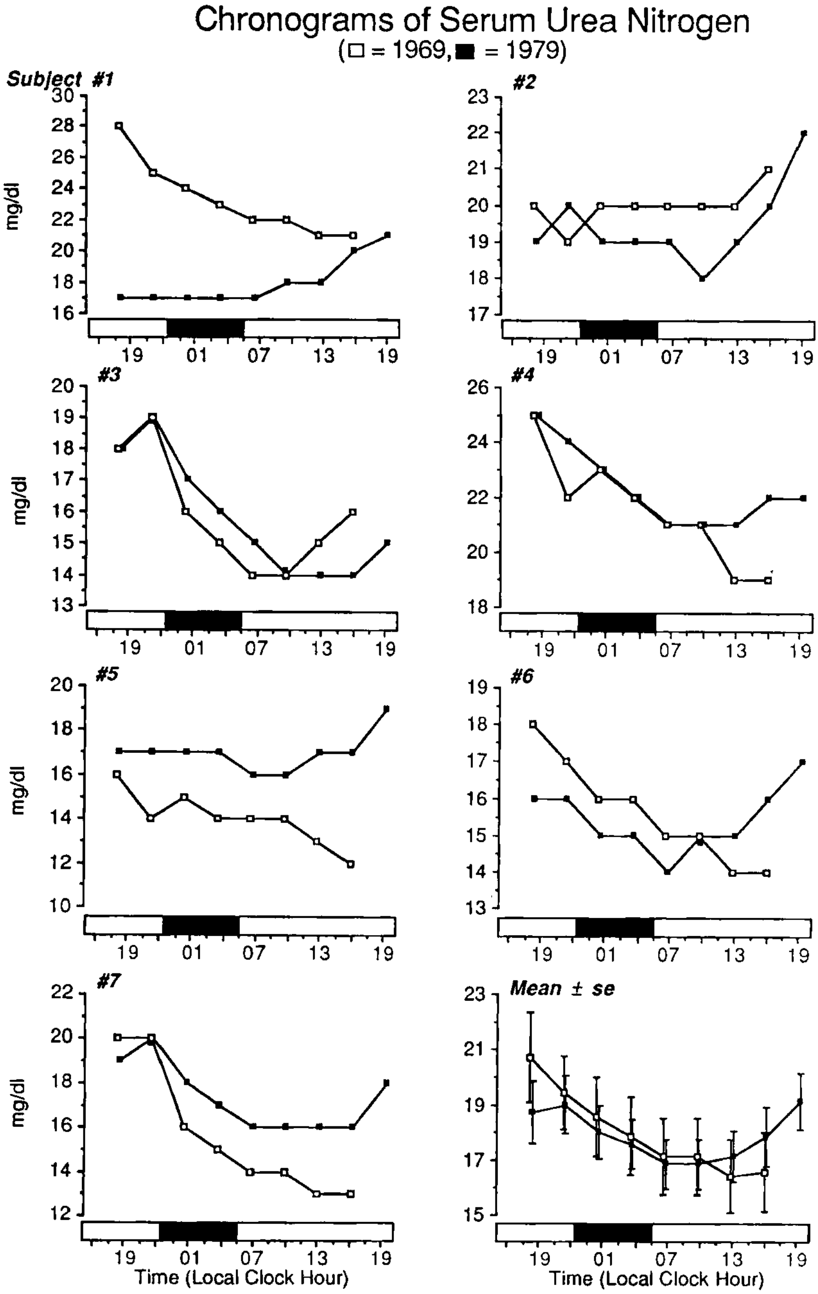

Chronograms of Serum Urea Nitrogen
(□ = 1969, ■ = 1979)
Subject #1
#2
#3
#4
#5
#6
#7
Mean ± se
mg/dl
19 01 07 13 19
Time (Local Clock Hour)

Figure 25

Chronograms of Urinary Calcium

(□ =1969, ■ =1979)

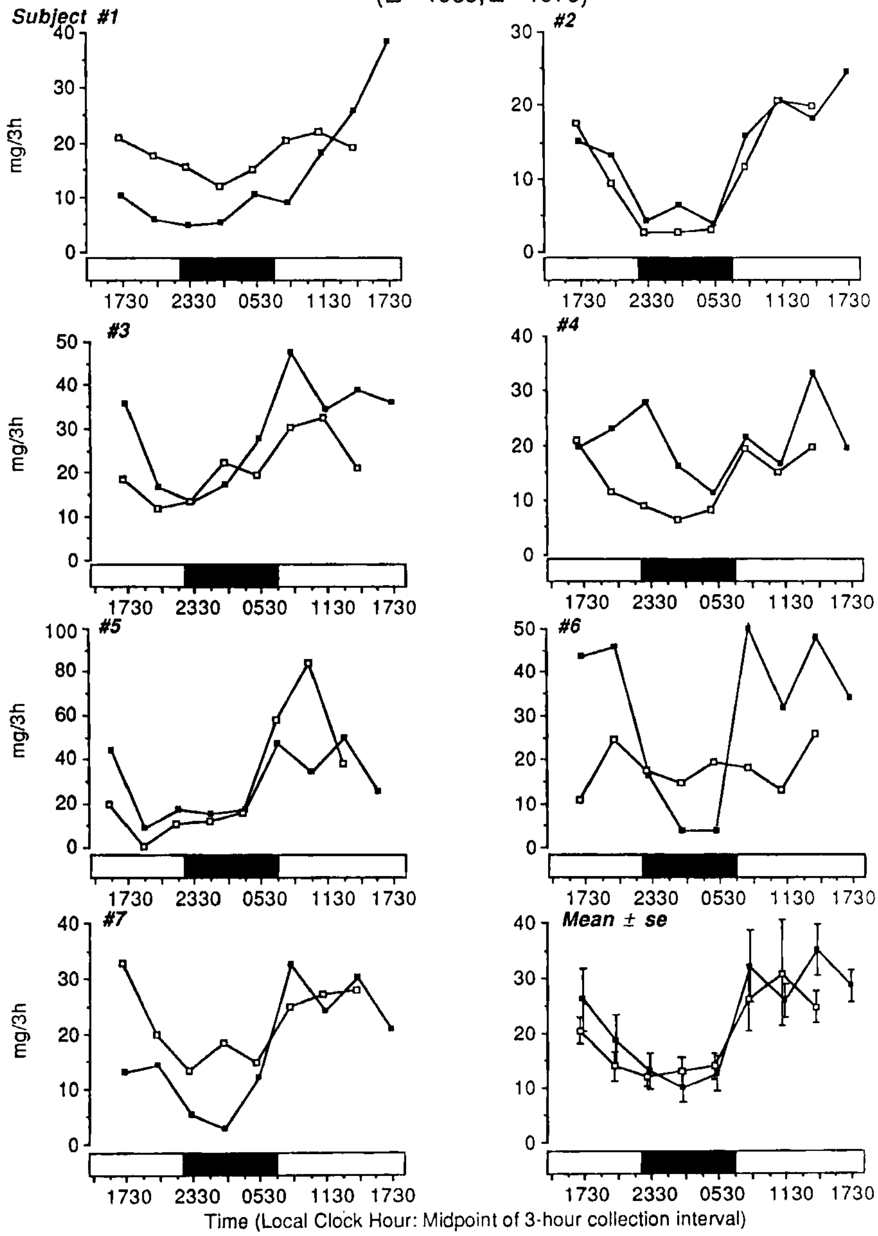


Figure 26

Chronograms of the Calcium/Magnesium Ratio in Urine

(□ = 1969, ■ = 1979)

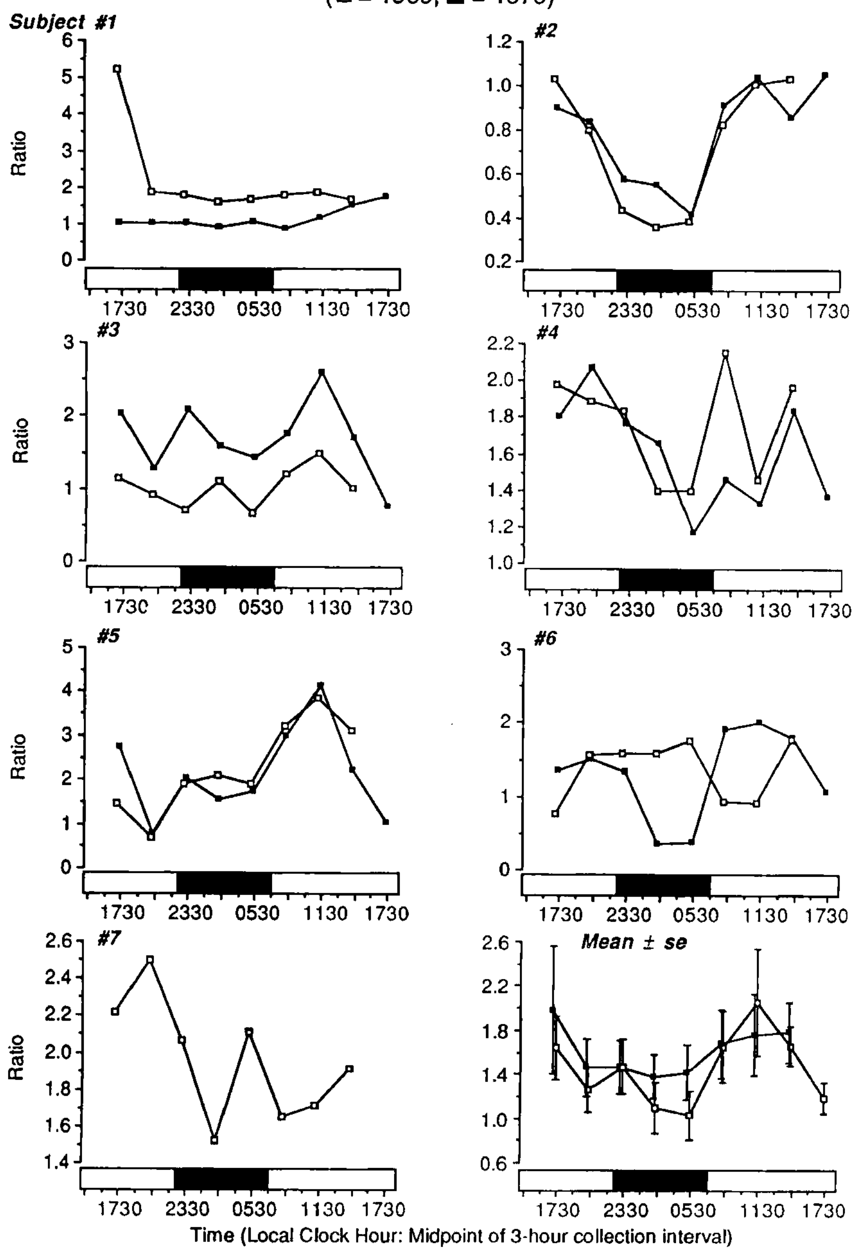


Figure 27

Chronograms of Urinary Creatinine

(□ = 1969, ■ = 1979)

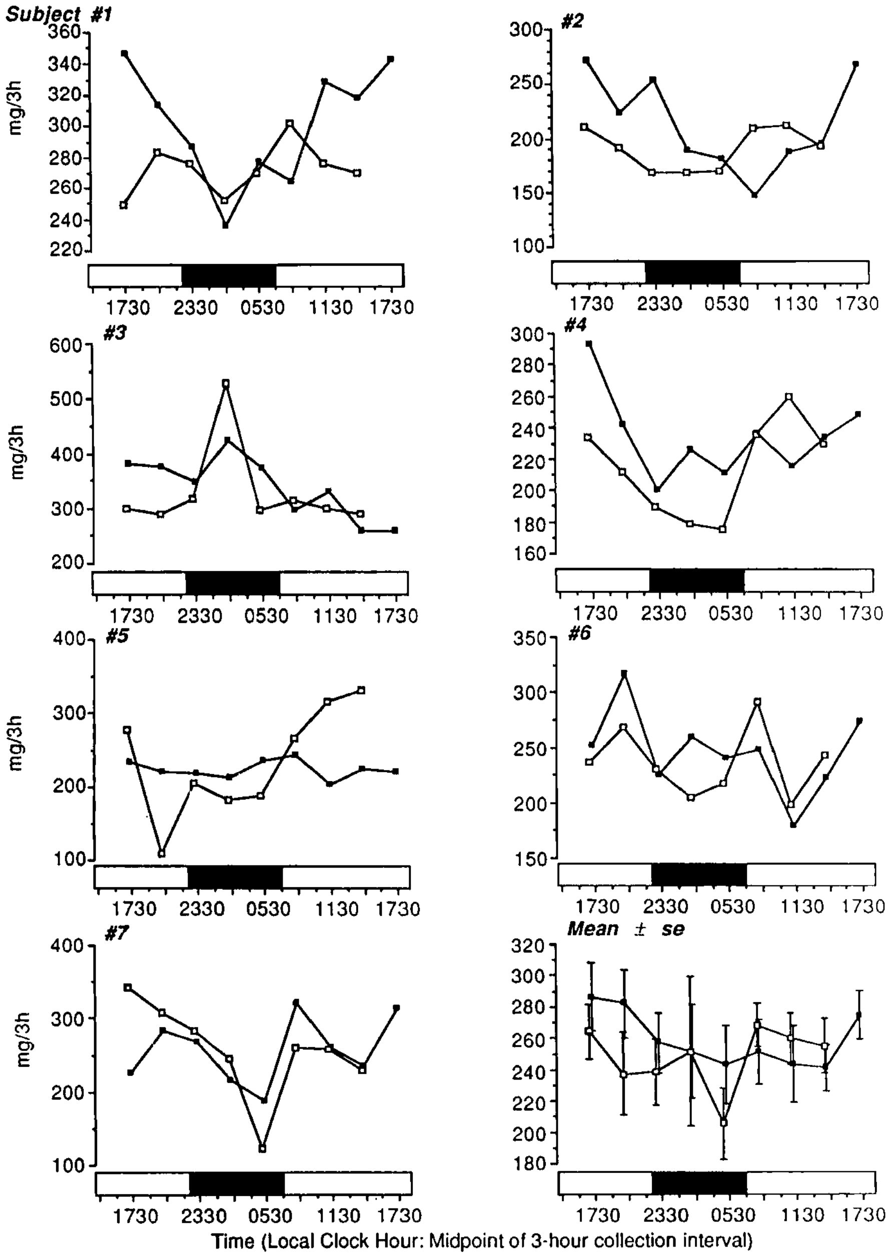


Figure 28

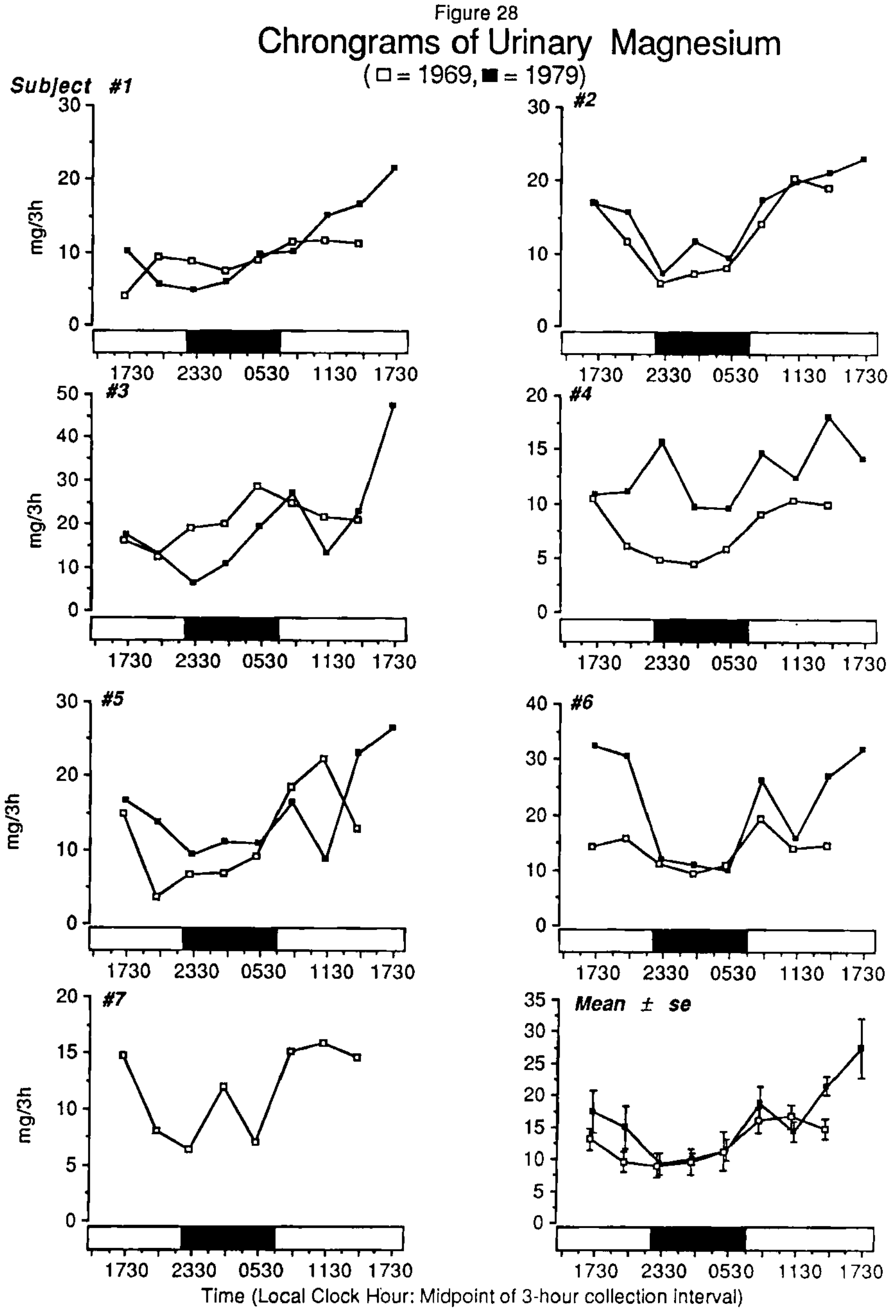
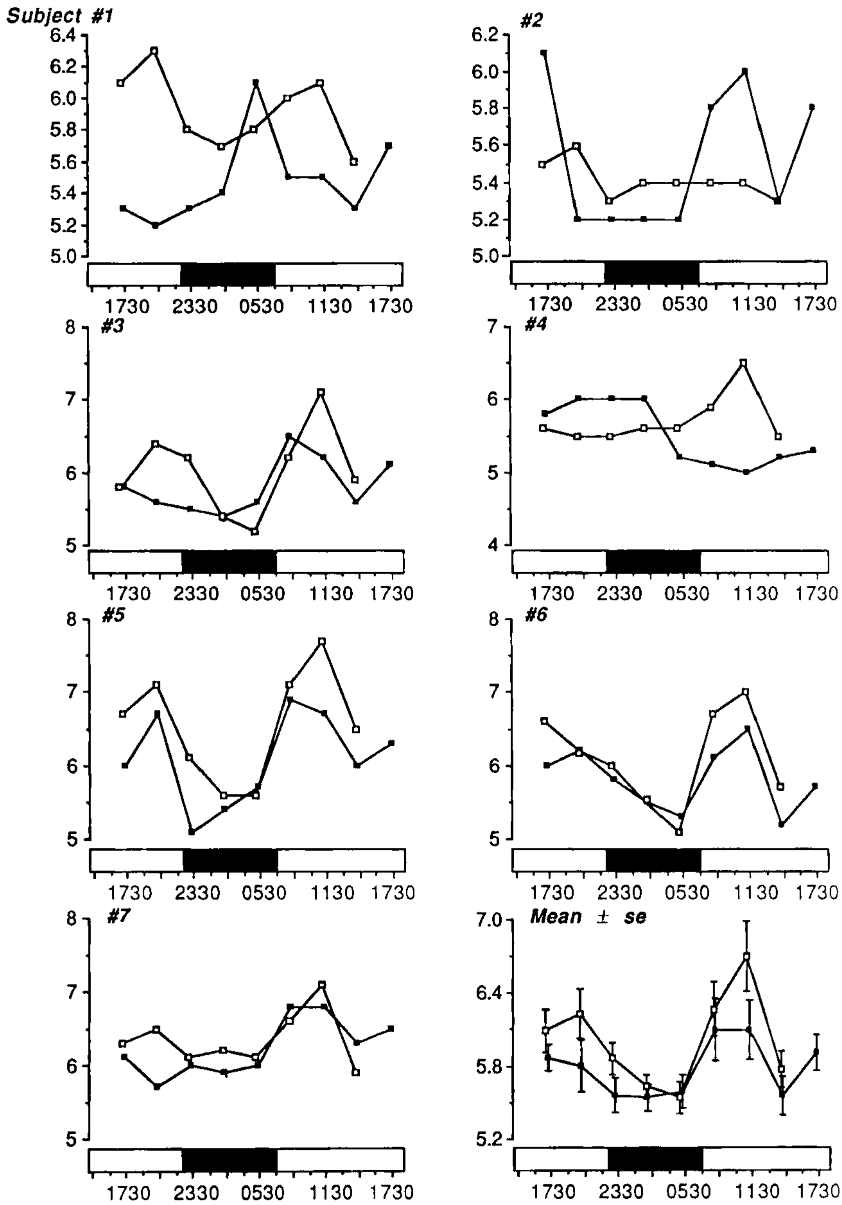

Chrongrams of Urinary Magnesium
(□ = 1969, ■ = 1979)
Subject #1
#2
#3
#4
#5
#6
#7
Mean ± se
mg/3h
1730 2330 0530 1130 1730
Time (Local Clock Hour: Midpoint of 3-hour collection interval)

Figure 29
Chronograms of Urinary pH
(□ = 1969, ■ =1979)

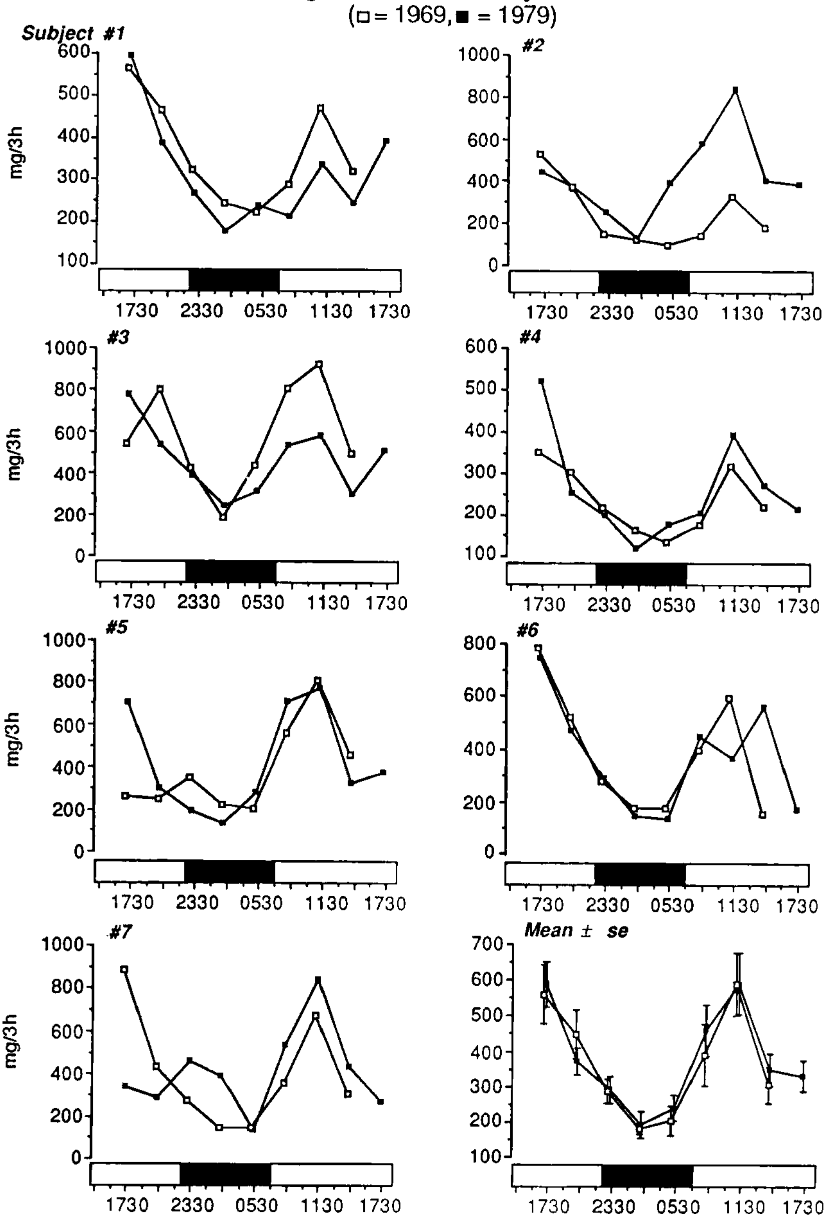



Time (Local Clock Hour: Midpoint of 3-hour collection interval)

Figure 30

Chronograms of Urinary Potassium

(□ = 1969, ■ = 1979)

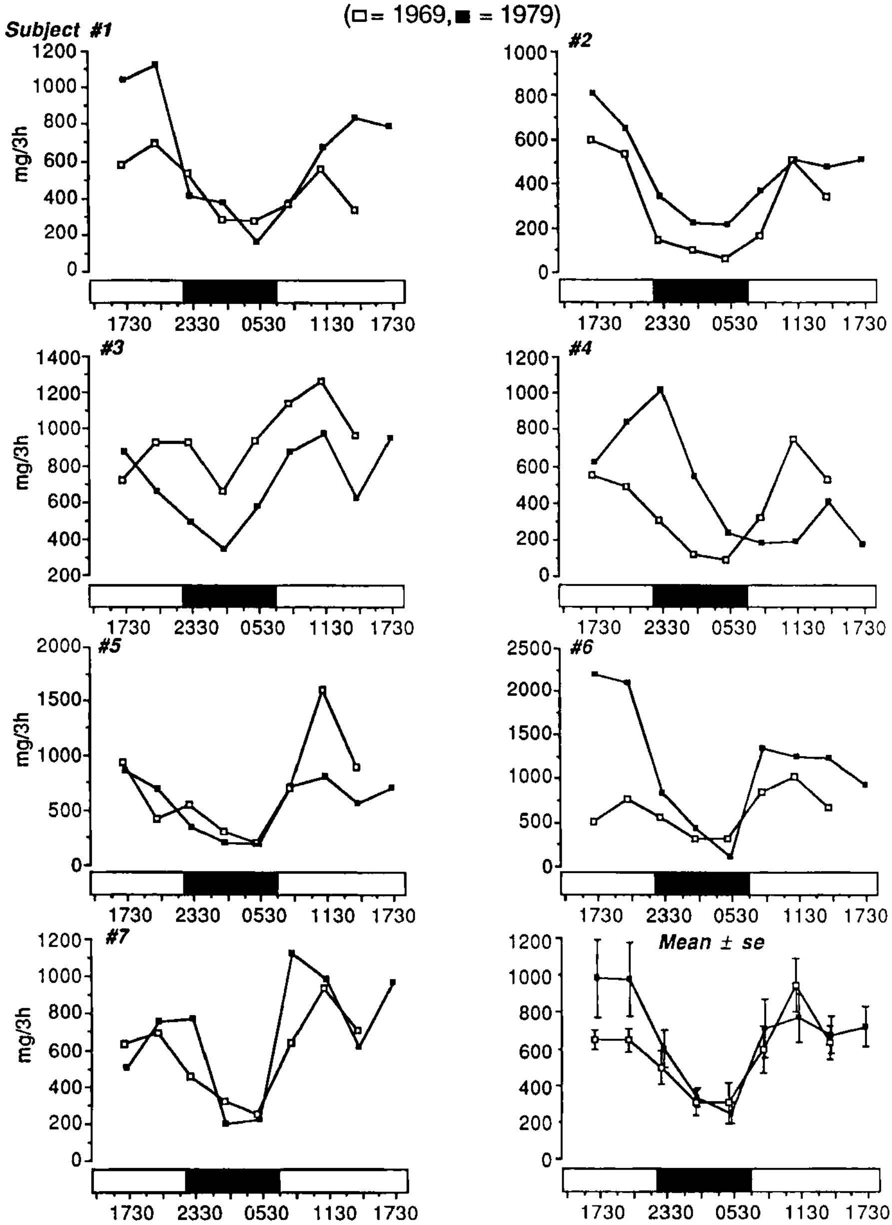



Time (Local Clock Hour: Midpoint of 3-hour collection interval)

Figure 31

Chronograms of Urinary Sodium

(□ = 1969, ■ = 1979)




Time (Local Clock Hour: Midpoint of 3-hour collection interval)

Figure 32

## Chronograms of Urinary Sodium/Potassium Ratio

(□ = 1969, ■ = 1979)

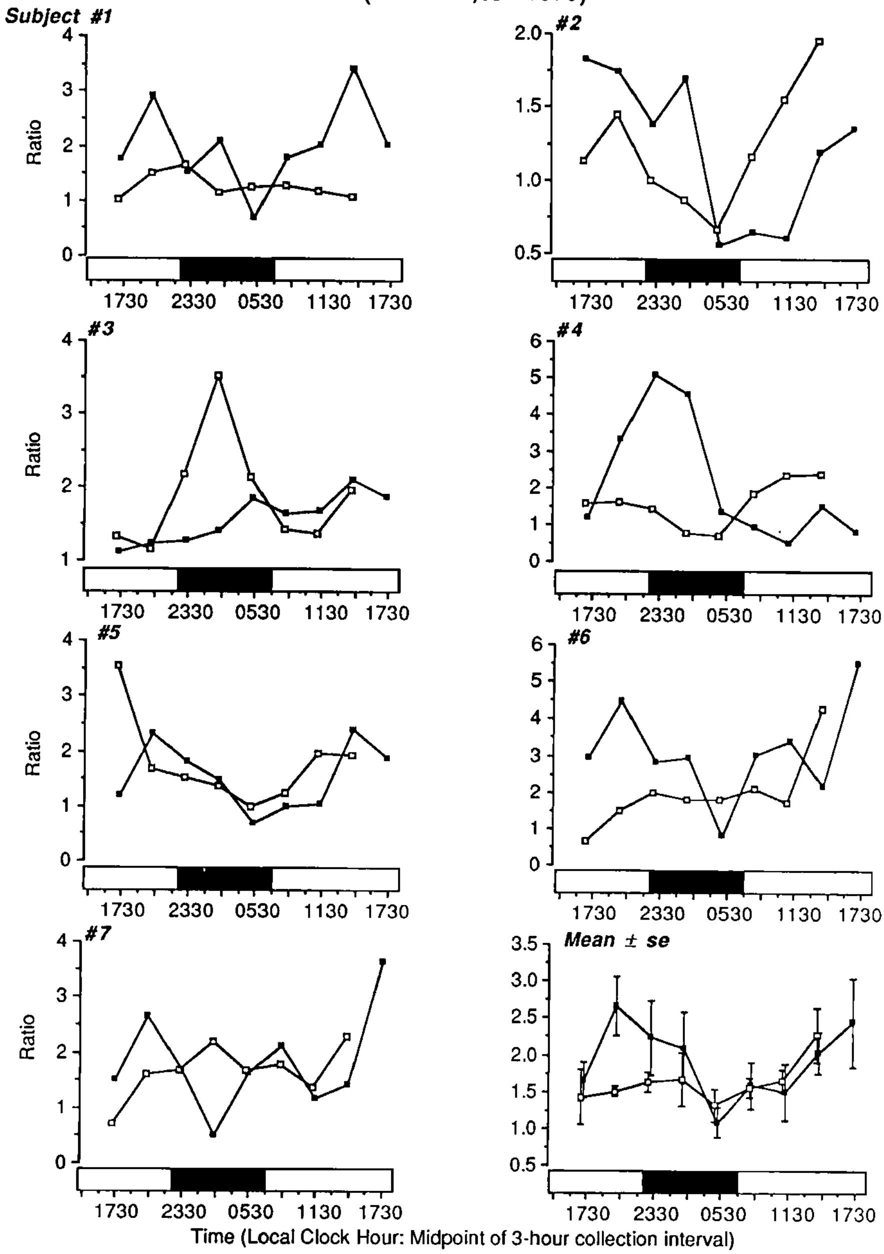


Figure 33

# Chronograms of Urinary Urea

(□ = 1969,■ = 1979)

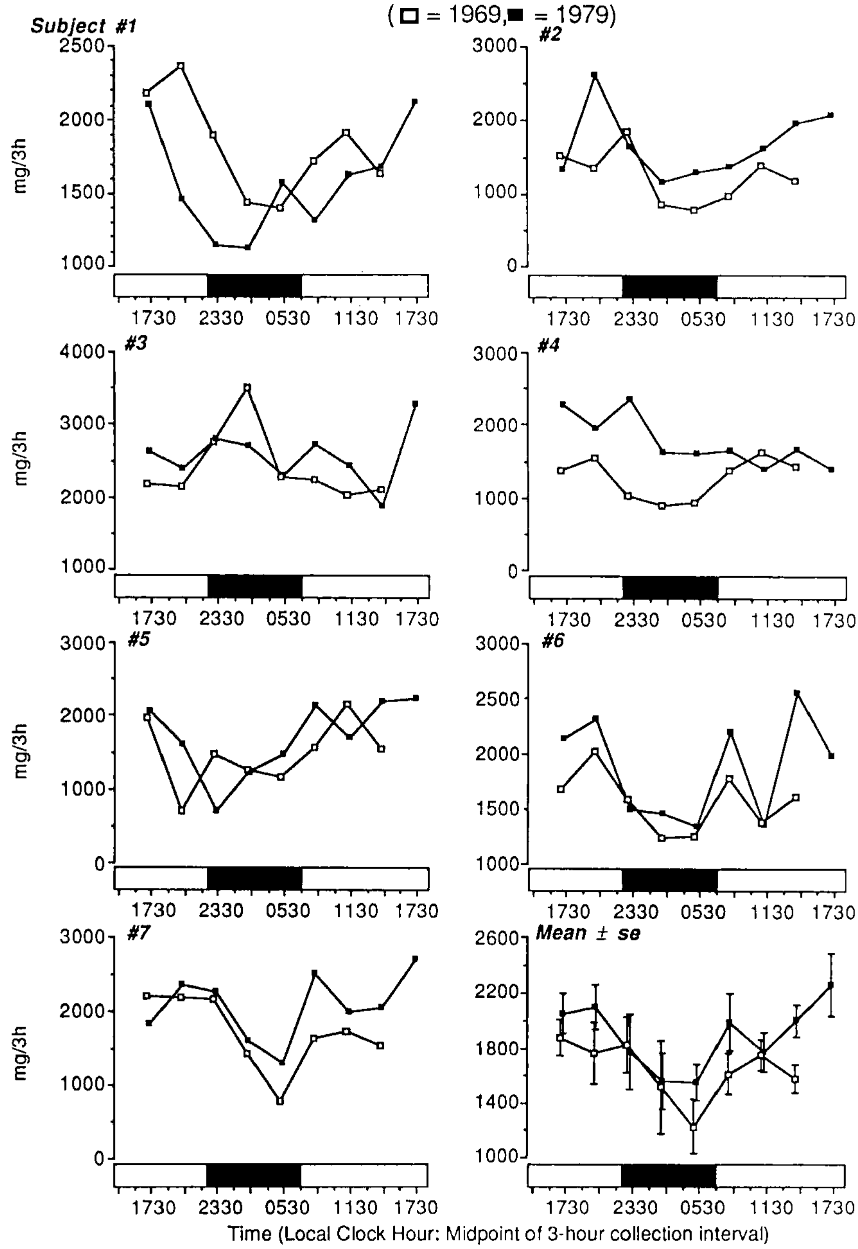
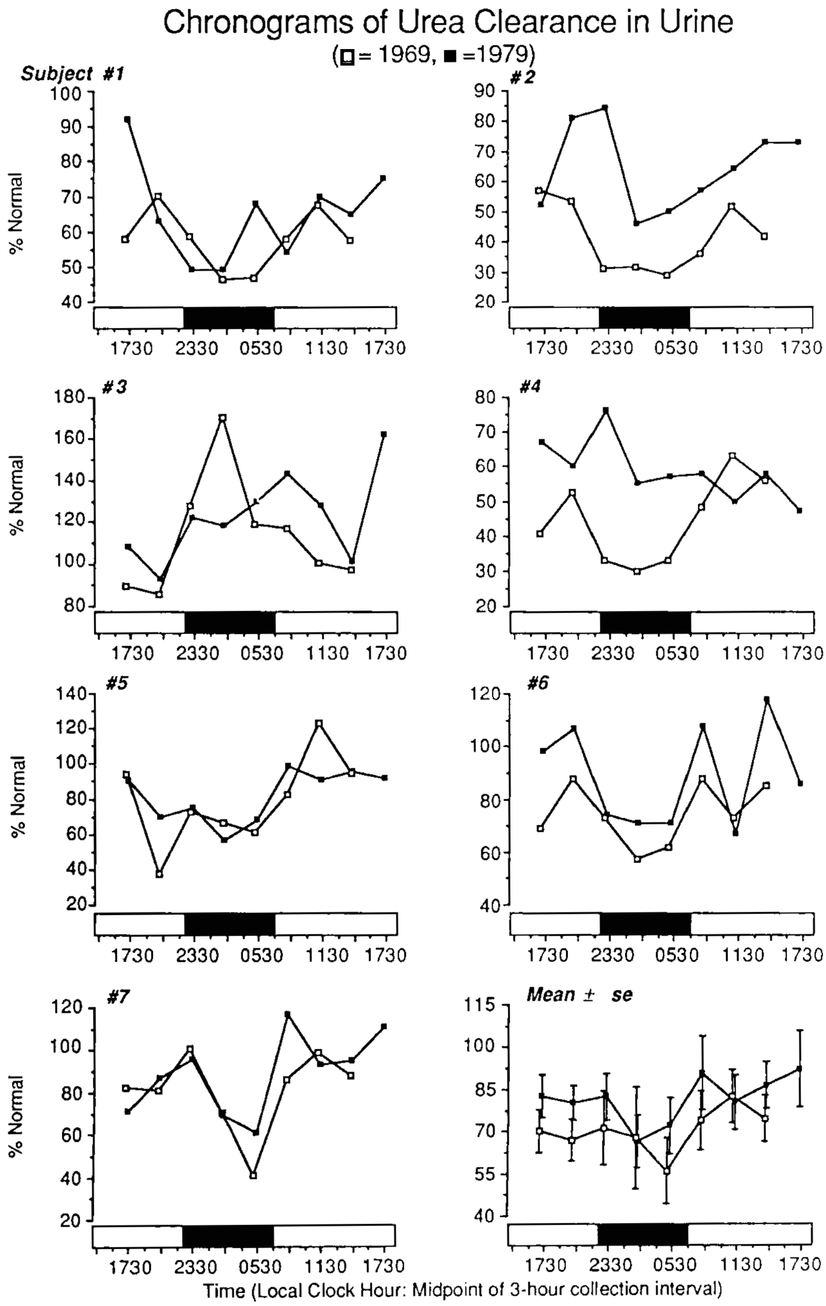


Figure 34

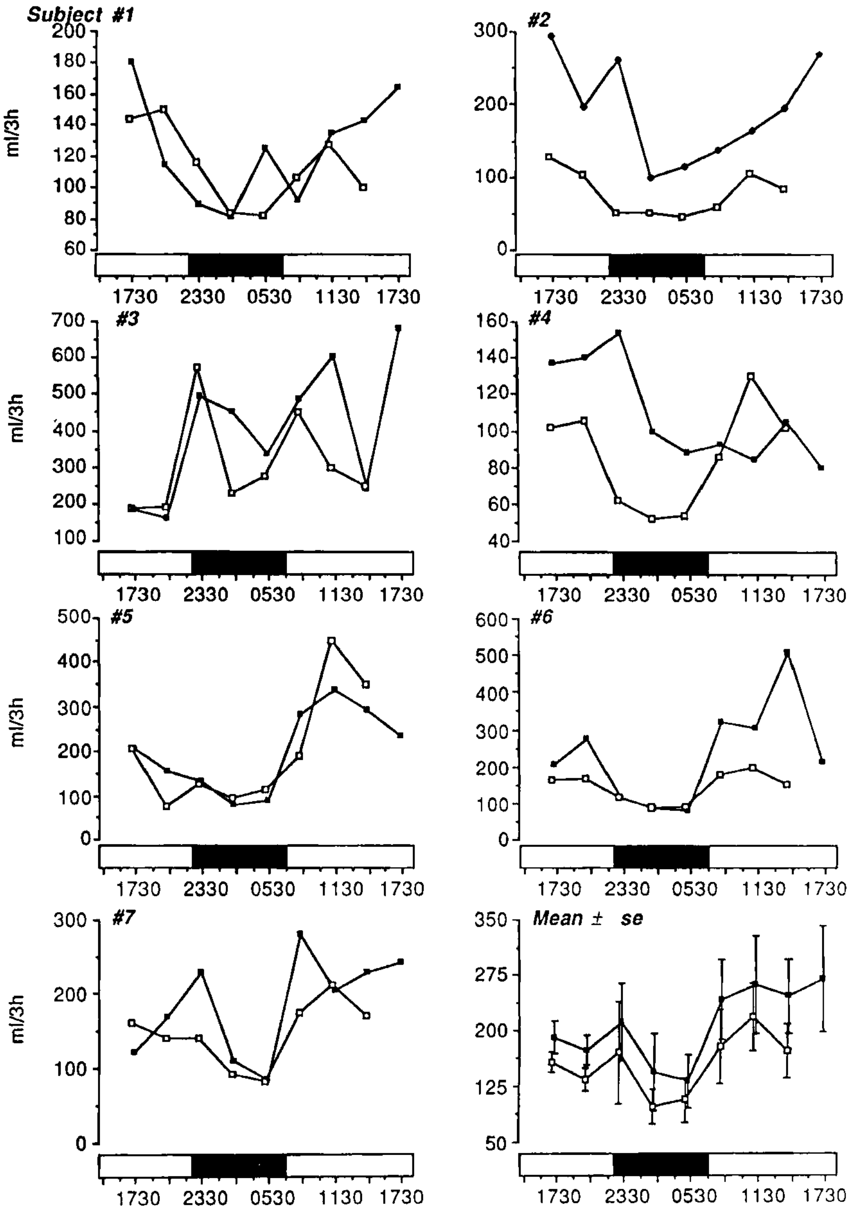


Chronograms of Urea Clearance in Urine
(□ = 1969, ■ = 1979)
Subject #1
#2
#3
#4
#5
#6
#7
Mean ± se
% Normal
1730 2330 0530 1130 1730
Time (Local Clock Hour: Midpoint of 3-hour collection interval)

Figure 35

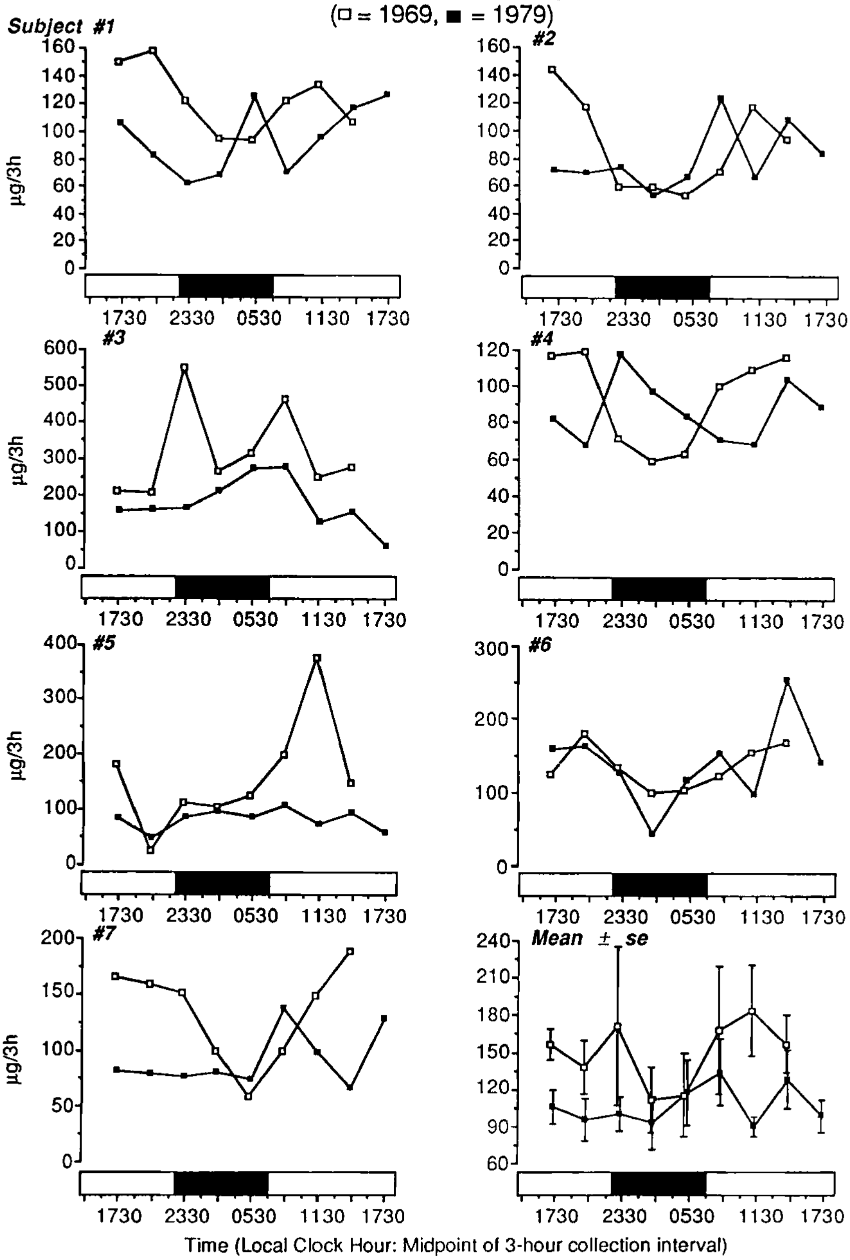
## Chronograms of Urine Volume

(□ = 1969, ■ = 1979)




Time (Local Clock Hour: Midpoint of 3-hour collection interval)

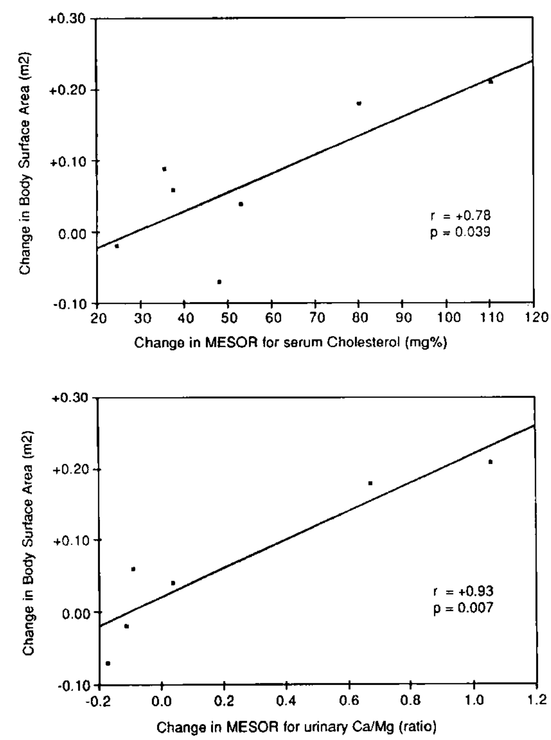
Figure 36
Chronograms of Urinary Zinc
(□ = 1969, ■ = 1979)



within the aging process (mid-20's and mid-30's) may be sub-optimal for conclusions about aging, very interesting trends definitely appear worth comment. There is some evidence in these data that the flattening of circadian rhythms may really accompany advancing age. In grouped data, this fall in amplitude may be secondary to an isolated fall in predictable swing around the mesor or a combination of this and increased variability of the acrophase with or without amplitude changes. The data are not robust enough to be sure of the relative contribution of these two components. In any event, the circadian amplitude of each and every physiologic variable studied demonstrated a tendency to fall between the mid-20's and mid-30's. This tendency toward a flattening of circadian variability is also a very prominent property of many of the serum chemistries which were measured. The circadian patterns of excretion of substances in the urine change much less between the mid-20's and mid-30's in our subjects. These findings may indicate a separate effect of aging especially upon metabolic hepatic variables and upon nephrologic circadian rhythms. Cardiovascular rhythms seem to change more in parallel with hepatic metabolic rhythms in contradistinction to the kidney-related serum and urinary rhythms.

Further, ongoing statistical analyses may hopefully turn up interesting and relevant cross-correlations among the individual data themselves in each study year and between the 10-year span, as well as with rhythm (mesor, amplitude and acrophase) and other physiologic characteristics of each subject. Planned re-observation of what happens to the circadian time structure of these seven individuals in their mid-40's will prove invaluable to further sorting out of the effects of aging upon circadian time structure.

Figure 37:
Statistically-significant correlation of change in Body Surface Area with change in Circadian MESOR for Serum Cholesterol (top, n =7) and Urinary Ca/Mg Ratio (bottom, n=6) for men studied in 1969 and again in 1979



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