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# Diurnal-Nocturnal Variations of Certain Blood Constituents in Normal Human Subjects: Plasma Iron, Siderophilin, Bilirubin, Copper, Total Serum Protein and Albumin, Haemoglobin and Haematocrit

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THE iron content and total iron-binding capacity of the plasma are subjects of wide scientific and medical interest. Evidence is accumulating that certain diseases have a characteristic effect not only on the concentration of plasma iron and its rate of turnover (Laurell, 1952; Brendstrup, 1953) but also on the concentration of the plasma iron-binding protein, siderophilin. Iron-deficiency anaemia, malignant neoplastic disease and acute and chronic infections, with the exception of viral hepatitis, are characterized by the level of plasma iron being lower than normal. The circulating siderophilin concentration is abnormally high in cases of iron-deficiency anaemia, sharply lowered in acute infections and somewhat less markedly depressed in chronic infections and malignancy. Several investigators, e.g., Vahlquist (1941), Høyer (1944) and Hemmeler (1951), have reported data on the variations of plasma iron levels in normal subjects over a 24-hour period. Relatively little study has been made of the possible daily variations of their total iron-binding capacities. It was primarily with the intention of examining normal subjects in this respect that the present studies were undertaken.

In addition to the plasma iron and total siderophilin levels of blood samples taken from twenty normal subjects over a 24-hour test period, we estimated other blood constituents whose concentrations might be related. Total serum protein and albumin concentrations were determined in order to associate any observed variation in siderophilin, a $\beta_1$ globulin, with possible changes in total globulin concentrations. The plasma samples were analysed for their copper content to determine any possible interrelationship between plasma iron and copper levels in normal subjects, as reported by Heilmeyer, Keiderling and Stüwe (1941) in their studies of clinical and experimentally induced infections. Bilirubin estimations were made on a number of sera to extend Laurell's (1953) observations of parallel variation of morning and evening serum iron and bilirubin concentrations in normal subjects. Haemoglobin concentrations and haematocrit values were also determined.

## MATERIALS AND METHODS

The subjects were normal adults, ten female and ten male, ranging in age from 22 to 53 years. Except for a 30-minute bed-rest period before each day and evening blood sampling, the subjects were allowed to follow their normal eating, working, and rest schedules. The 30-minute bed-rest period was intended to obviate variations in circulating blood volume because of postural changes.

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Venous blood samples (18 ml.) were drawn at 8 a.m., 12 noon, 4 p.m., 8 p.m., 11 p.m., 2 a.m., 5 a.m. and 8 a.m. 3.0 ml. of each specimen were allowed to clot to provide serum for protein and bilirubin determinations; the remainder was rendered incoagulable with heparin. Haemoglobin and haematocrit determinations were made immediately from the heparinized specimens.

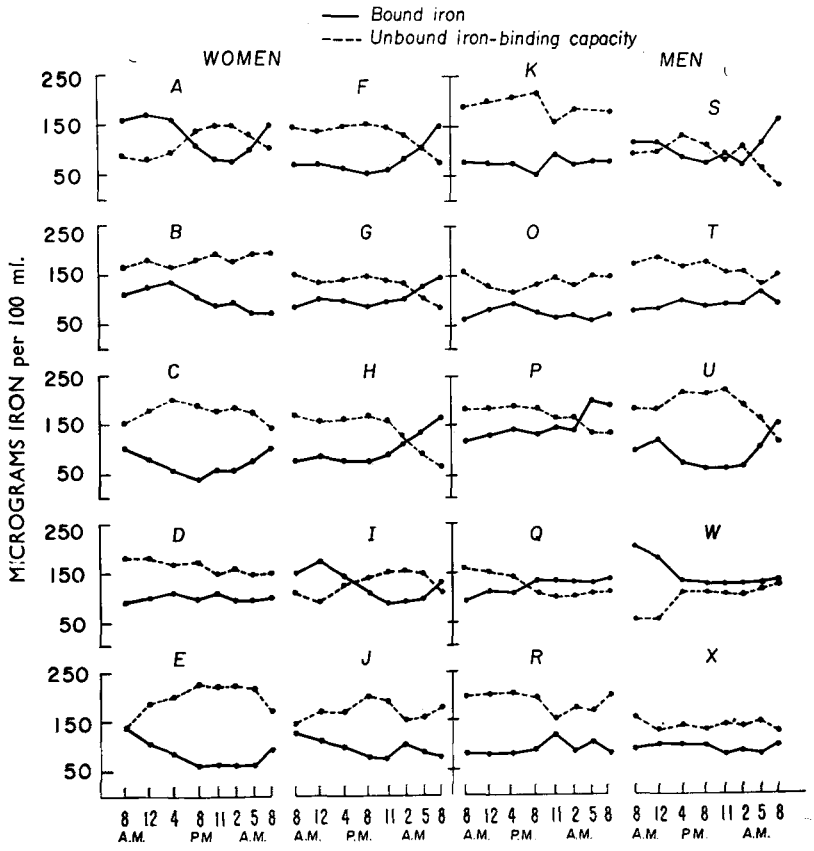


FIG. 1. *Intra diem* variations of the bound iron concentration and unsaturated (unbound) iron-binding capacity of the plasma of twenty normal subjects.

Haemoglobin concentration was measured as oxyhaemoglobin in a photoelectric colorimeter at 540 mμ.; the haematocrit was determined in a standard graduated tube centrifuged at 1200 *g* for 60 minutes. Plasma iron and total iron-binding capacity were determined by the methods of Schade, Oyama, Reinhart and Miller (1954) and plasma copper by the method of Nielsen (1944). Total serum protein was estimated by the biuret method and serum albumin by the method of Rutstein, Ingenito and Reynolds (1954). Bilirubin concentration was assayed by the procedure of Jendrassik and Gróf (1938) on each serum sample of nine of the subjects.

## RESULTS AND DISCUSSION

*Plasma Bound Iron and Unsaturated Iron-Binding Capacity*

In Fig. 1 are summarized the results of the plasma bound iron (BI) and unsaturated iron-binding capacity (UIBC) determinations for all twenty subjects over the 24-hour test period. It is apparent that each BI curve approximates to a mirror image of its associated UIBC curve. The variation of these two factors is inversely related so that the total iron-binding

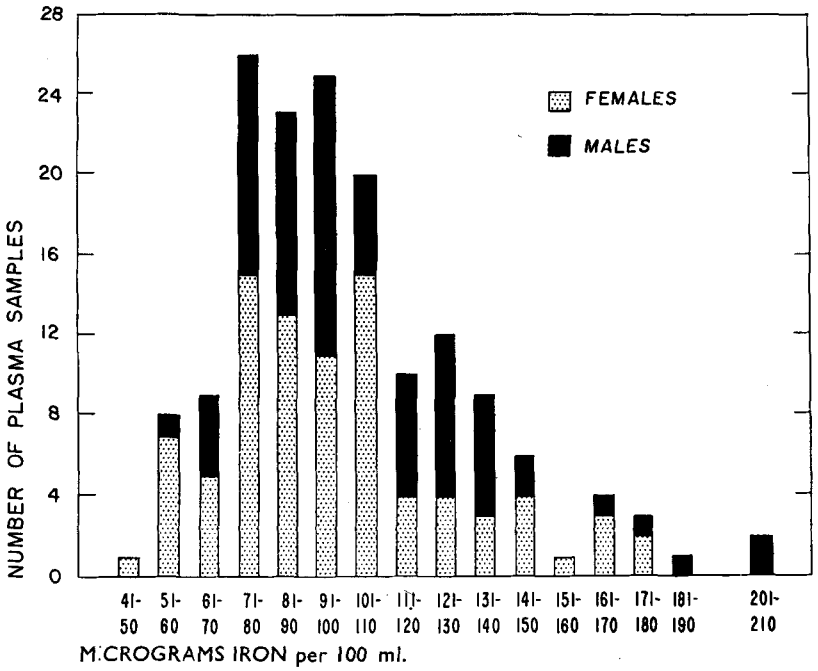


FIG. 2. Distribution curve of plasma bound iron concentrations derived from determinations of eight blood samples taken at intervals over a 24-hour period from each of twenty normal subjects.

capacity (TIBC), or siderophilin level, remains nearly constant throughout the 24-hour period in all cases. If, in fact, the TIBC is a relatively constant factor, the determination of the UIBC affords a check on the validity of the bound iron values.

Many authors have described a diurnal-nocturnal variation in the concentration of the bound iron in the plasma of normal subjects. In each series reported there is general agreement that the mean value is highest at some time in the morning or early part of the day and lowest in the evening. Our mean values follow this pattern. Yet it seems timely to point out, in view of the increasing clinical use of the plasma iron determination, that this observation is not sufficiently regular in occurrence to be of value in predicting any individual's pattern. Inspection of Fig. 1 reveals that only in subjects A, C, E, I, S and U did the bound iron variation clearly conform to the described pattern. The plasma iron in some subjects (B, J, O and W), while found at its highest level in the early part of the test day, fell in the evening but failed to show any tendency to rise again to a high morning value. Others (F, G, H, P and Q) started the test period with low morning levels which rose towards the end of the 24-hour observation time. Finally, certain subjects (D, K, R, T and X) showed very little quantitative variation in plasma bound iron during the entire test period.

Our figures show no consistent difference in plasma iron levels between normal males and females and our average normal levels are somewhat lower than those of previous authors. Hemmeler (1951) summarized the mean values recorded by various authors. They vary for adult men between 118 and 142 μg. per 100 ml. and for adult women between 90 and 123 μg. per 100 ml. Our mean values based on all samples are 98 μg. per 100 ml. for females

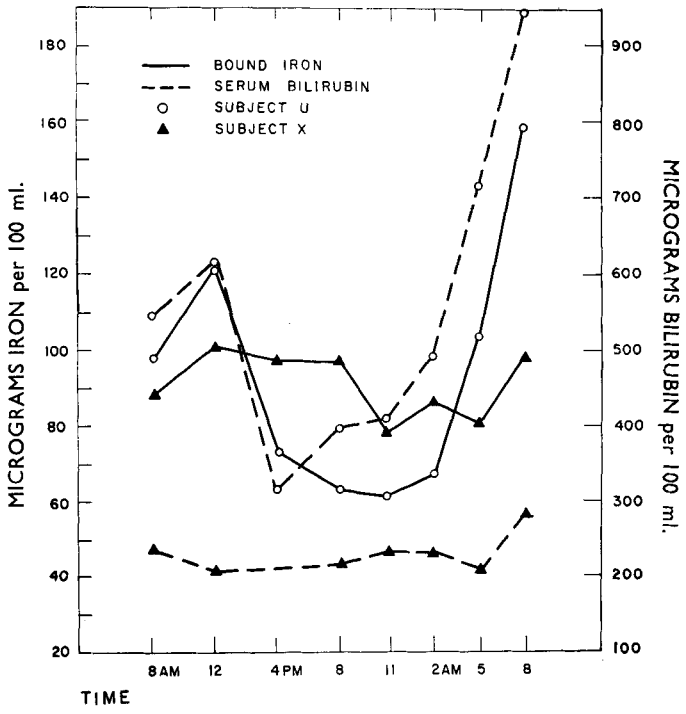


FIG. 3. *Intra diem* variations of serum bilirubin and plasma bound iron concentrations to illustrate the parallelism between these two factors found in all of the nine normal subjects studied.

with a spread from 43 to 175, and for males 100 μg. per 100 ml. with a spread from 56 to 206. In Fig. 2 is shown the distribution of the BI values for both sexes. The male subjects are more responsible for the extension of the curve to the right, while the females extend it slightly to the left. Calculation shows that 84 per cent of all values fall between 60 and 140 μg. per 100 ml.

The mean total iron-binding capacity level of the female subjects was found to be 250 μg. per 100 ml. with a spread from 213 to 306, and for males 253 μg. per 100 ml. with a spread from 184 to 337. The mean figures are somewhat lower than those found by other investigators (Hagberg, 1953) and may reflect the difference in methods employed. Like previous authors, however, we found no difference between the sexes in their level of siderophilin.

*Serum Bilirubin*

Serial determinations of bilirubin were performed in nine subjects; two sets of representative

data are shown in Fig. 3. When the plasma iron level remained relatively stable throughout the 24-hour period (Subject X) the serum bilirubin also changed little. On the other hand, when marked changes occurred in the plasma iron level (Subject U) parallel changes were found in the serum bilirubin concentrations. The similarity of the fall and rise in the plasma iron level and the serum bilirubin concentration in our cases, and in those reported

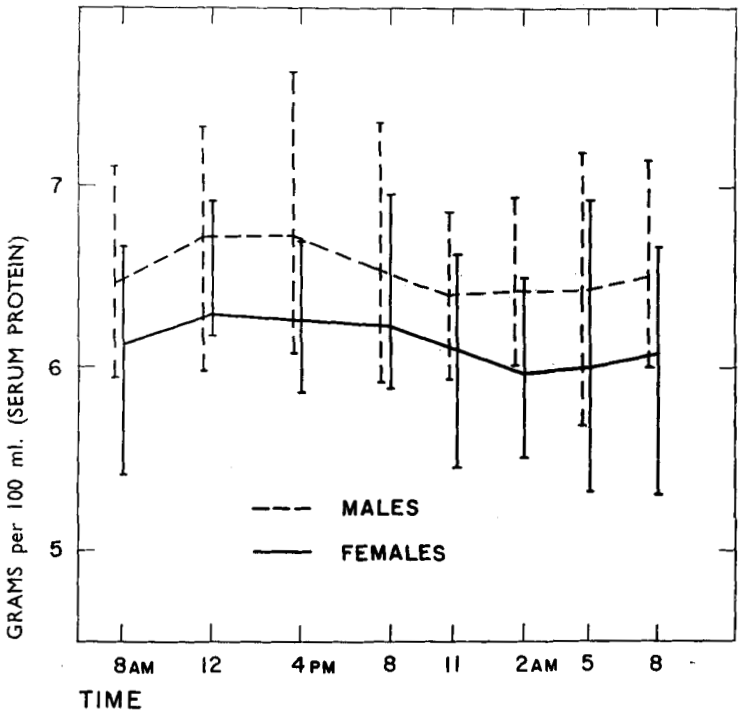


FIG. 4. Mean total serum protein concentrations and spread of values at intervals during a 24-hour period, derived from study of ten female and ten male subjects.

by Laurell (1953), supports the hypothesis that the levels of both factors depend largely upon a variation in the rate of haemoglobin catabolism.

*Plasma Copper*

The mean plasma copper level of all samples from female subjects was 115 μg. per 100 ml. (range, 93–168); in male subjects the mean value was 124 μg. per 100 ml. (range, 84–164). There was no regular diurnal-nocturnal variation, nor was there any evidence of a relationship, direct or inverse, between the plasma iron and copper levels in any individual. In fact, the plasma copper tended to remain at a nearly constant level in each subject over the entire 24-hour period. This constancy, in contrast to the large variations in plasma iron levels in these normal subjects strongly suggests that plasma copper and plasma iron concentrations are independent variables. Heilmeyer, Keiderling and Stüwe's (1941) observation of an inverse relationship between plasma iron and copper levels in acute infection, therefore, denotes independent effects of the infectious process on the metabolism of these two metals.

*Total Protein and Albumin*

The mean total serum protein levels for both male and female subjects at each time period are shown in Fig. 4. The ranges of values for each group are also recorded. Although inspection of the data suggests a possible regular diurnal-nocturnal variation in the concentrations of serum proteins for both sexes, statistical analysis does not support this conclusion. There

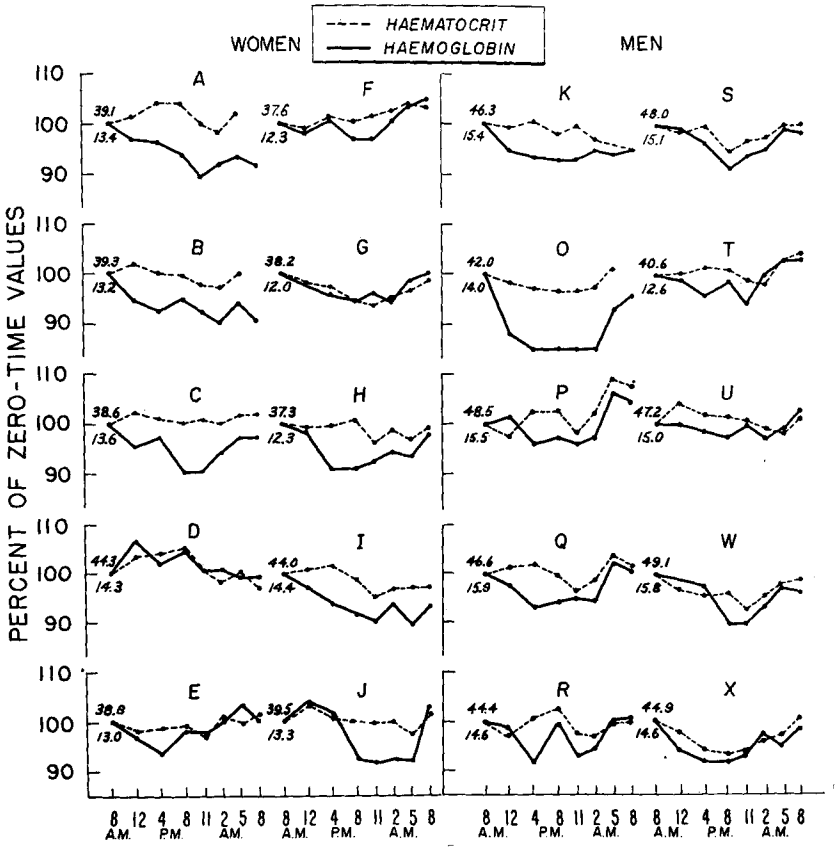


FIG. 5. *Intra diem* variations of haemoglobin and haematocrit values of twenty normal subjects. The upper figure at the initial point of each pair of curves represents the absolute haematocrit value as a percentage and the lower figure the absolute haemoglobin value in grams per 100 ml. These initial readings in each case are taken as 100 per cent and subsequent values are related to them.

is, however, statistically valid evidence that there is a difference in total protein levels between the sexes: the mean value for male subjects was 0.38 g. per 100 ml. higher than that of the females. This sex difference is further substantiated by the data of Gordon (1956 and personal communication). Gordon's subjects were normal adults, fifteen female and twenty-two male. Evaluation of the two sets of data, our's and Gordon's, by the analysis of variance shows that the sex difference is significant at the $P = 0.01$ level.

*Haemoglobin and Haematocrit*

The results of serial haemoglobin and haematocrit determinations are shown in Fig. 5. In

each case the initial reading (8 a.m.) was taken as 100 per cent and the subsequent values are related to it. While the series is considered too small for statistical analysis, examination of the graphs reveals that in the majority of subjects the haemoglobin level is highest in the morning and lowest at some time in the evening. In one instance this difference amounted to 15 per cent. The mean difference between the highest haemoglobin level and the lowest was 8.8 per cent in females and 8.4 per cent in males. These figures are quite in keeping with previous reports on the diurnal-nocturnal variation of haemoglobin level.

Inspection of the graphs shows that in about half the subjects the haematocrit curves paralleled more or less closely the haemoglobin curves; this suggests a change in red-cell number as the basic variant. In the rest of the subjects there is a disparity between the two curves. The haematocrit level remains relatively stable, while the haemoglobin concentration varies through the usual morning-high, evening-low cycle. This suggests that *intra diem* changes in both cell volume and cell numbers occur. Although such changes have been studied in the past (Price-Jones, 1920; Ponder, 1934), no general agreement exists as to the significance or even reality of diurnal-nocturnal variations in cell volume. Our findings underscore the desirability of obtaining more definitive measurements of red-cell number and volume through application of present-day techniques.

## CONCLUSIONS

In twenty normal adult human subjects from whom blood samples were taken at frequent intervals during a 24-hour period, we found that:

(1) The total iron-binding capacity of the plasma remained constant.

(2) In a minority (six) of the subjects the bound iron level traversed a diurnal-nocturnal pattern in which it was highest in the morning and lowest in the evening. The bound iron levels of the majority followed no such regular pattern.

(3) The mean bound iron levels of males (100 μg. per 100 ml.) and of females (98 μg. per 100 ml.) as well as their mean total iron-binding capacities (253 μg. and 250 μg. per 100 ml., respectively) showed no significant difference.

(4) The levels of plasma bound iron and of serum bilirubin followed a nearly parallel course.

(5) The mean level of plasma copper in female subjects was 115 μg. per 100 ml., and in males 124 μg. per 100 ml. Copper concentrations tended to remain constant and showed no relationship to variations in plasma iron.

(6) The total serum protein level was higher in male subjects than in female. This difference was statistically significant.

(7) In most subjects the haemoglobin level was highest in the morning and lowest in the evening. In about half of the subjects a disparity was found between the fluctuations of the haemoglobin concentration and the haematocrit level, the latter being the more stable.

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