fusion out of the vascular system of mercury attached to proteins(13) nor can the disappearance of the mercury be accounted for in extracellular fluid obtained from various sites(4). Within the first minute or so after injection, mercurial dilutions of 1:500 and less exist(1). At these dilutions, there should be appreciable quantities of nonprotein bound mercury. It would appear, therefore, that much of this initially "free" mercury becomes bound to structures of or immediately adjacent to these blood vessels.

Summary and conclusion. The experiments herein reported corroborate the observations of other investigators that the mercury of an organic mercurial diuretic may be bound by the proteins of the plasma. The extent of this binding is a function of mercurial concentration; at dilutions of the diuretic of 1:1000 or higher the mercury is over 90% bound; at dilutions of 1:100 or lower, the mercury is over 50% free. This may well explain certain phenomena observed in the tracer studies of the mercurial diuretics.

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Diurnal Variation in the Plasma Iron Level of Man.* (18102)

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During a study of factors affecting the level of iron in the plasma of man, it became apparent that the plasma iron level normally undergoes a regular diurnal variation. Reference to the literature shows that several continental investigators have described such a variation. Anglo-American workers have either failed to observe this, taken their samples at a constant time, or ignored the variation in their studies on plasma iron.

Methods. In this study 19 adult male subjects, without demonstrable organic disease which would affect the plasma iron, were used. Samples of approximately 8.0 ml of blood were taken at intervals of 2 to 4 hours throughout a 24-hour period from 12 subjects beginning at 9:00 a. m. and from 7 subjects beginning at 5:00 p. m. Glassware used for the collection, storage and estimation of plasma iron was carefully cleansed by first rinsing with water and then immersing in potassium dichromate and sulfuric acid cleaning solution for a minimum of one hour. It was then rinsed thoroughly with tap water and this was followed by three washings in double distilled water prepared in an all glass distillation apparatus. The blood was taken with all glass syringes and placed into 15 ml tubes to which 2 drops of 20% potassium oxalate had been added previously. The plasma was then separated by centrifugation and the iron estimation made by a modification of the method of Barkan and Walker(1).

Reagents. 1. Hydrochloric acid, 1.2% solution in double distilled water. 2. Trichloroacetic acid (redistilled), 20% solution in double distilled water. 3. Thioglycolic acid (Eastman Kodak-Practical). 4. O-phenanthroline monohydrate, 0.1% solution in double distilled water. 5. Sodium acetate, saturated solution in double distilled water.

Procedure. To 1.5 ml of plasma or serum, 0.75 ml of 1.2% hydrochloric acid is added. The plasma is then incubated at 37°C for

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1. Barkan, G. and Walker, G., *J. Biol. Chem.*, 1940, v37, 135.

1 hour, after which time 0.75 ml of 20% trichloroacetic acid is added, the resulting precipitate being stirred vigorously. The mixture is then allowed to stand at room temperature for 1 hour. The supernatant is separated by centrifugation and an aliquot of 1.80 ml is taken. To this are added in the following order, with mixing after each addition, 0.05 ml of thioglycolic acid (2 drops), 0.4 ml of 0.1% O-phenanthroline, 0.4 ml of saturated sodium acetate solution and 0.35 ml of water, making a total of 3.0 ml. A blank is prepared by adding the same reagents to 0.45 ml hydrochloric acid, 0.45 ml trichloroacetic acid and 1.25 ml water. The test and blank solutions are then allowed to stand at room temperature for 30 minutes, which is adequate for the full color reaction of the ferrous iron with O-phenanthroline to develop. In this modification of the Barkan and Walker method, thioglycolic acid has been found to be a more satisfactory reducing agent than hydrazine sulfate. The addition of the O-phenanthroline before the sodium acetate facilitates the more rapid development of the color reaction. Absorption is measured in the Beckman spectrophotometer at 510 mμ with a slit width of 0.032 mm. Under these conditions, using cuvettes with a light path of 1.0 cm, a constant K ($K = \frac{\text{Concentration}}{\text{Density}}$) of 14.87 was obtained using standard iron solutions where the concentration was expressed in micrograms of iron per 3.0 ml.

Results. From the figure it can be seen that the level of plasma iron is highest in the early morning and that it falls during the day to its lowest level in the evening, and rises again during sleep.

Blood samples were taken throughout the 24 hour period at intervals beginning at 5:00 p.m. since it might be argued that the diurnal variation observed above was the result of repeated vena-puncturing and sampling. The mean plasma iron level at 5:00 p.m. was 66 ± 14 μg % and this rose during the night, while the samples were being taken, to an average of 148 ± 11 μg % in the early morning, a difference of 82 ± 15 μg %. This average maximal value compares well with the average early morning values found in the 12 subjects on whom bleeding began at 9:00 a.m. The declines in plasma levels from morning to evening, it will be noted, were also similar. Thus the variation is not related to the taking of blood samples.

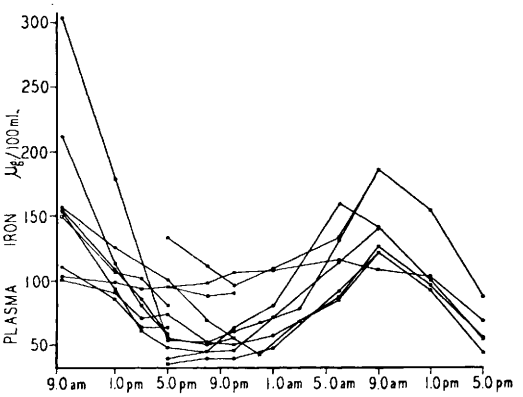

FIG. 1.

The diurnal variation in the plasma iron of 19 individuals.

Discussion. The above observations indicate that the plasma iron of man undergoes a definite diurnal variation. These observations are in agreement with the results of others(2-7) which have been summarized, analyzed statistically, and presented in Table I. In addition to the tabulated studies, those of Nilsson may be mentioned. He was reported by Hoyer(3) to have published a 24 hour curve for serum iron in a normal subject which showed a decrease at night, followed by an increase the next morning. Schäfer(8) also noted a similar diurnal variation in 10 healthy subjects with a mean decrease of 22 μg %. It can be seen that the diurnal variation was greater in our subjects and in those reported in Höyer's second paper(4) than is given by the other workers(2,3,5,6,7).

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6. Waldenström, J., *Acta Med. Scand.,* 1946, v170, 252.
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TABLE I. Diurnal Variations in the Plasma Iron of Normal Human Subjects.

Investigator	No. and sex of subjects	Plasma iron morning, μg%	Plasma iron evening, μg%	Difference, μg%
This investigation	12 males	155 ± 16	65 ± 5	89 ± 18
Hemmeler, '44	25 "	127 ± 6	82 ± 6	45 ± 7
" "	23 "	106 ± 6	74 ± 4	32 ± 7
Hoyer, '44, till 11 p.m.	6 "	129 ± 11	88 ± 7	41 ± 6
" " " "	6 females	114 ± 4	70 ± 4	44 ± 8
" " through night	9 males	131 ± 11	76 ± 6	56 ± 9
" " " "	11 females	130 ± 10	66 ± 5	64 ± 11
Valquist, '41	15 "	135 ± 11	99	36 ± 9
Waldenström, '46	15 "	113 ± 8	79 ± 4	34 ± 9
Heilmeyer, '37	1 male	93	72	21

This may be due to the fact that in our study, as in Höyer's second investigation, samples were taken at intervals throughout a whole 24 hour period and in this way lower levels of plasma iron may have been found.

Moore, Minnich and Welch(9) failed to find such a variation. These workers took 6 or 7 blood samples at intervals throughout the day from an unspecified number of normal subjects and hospital patients. The fluctuations they found were not constant in direction. Wetzel is quoted by Hemmeler(2) as having found in a series of 11 subjects that the plasma iron increases during the day. These appear to be the only conflicting results in the literature although it may be noted that most workers report some variations from the general trend in a few individuals in their series. Thus, of Valquist's 15 men, 2 showed an increase of plasma iron level in the evening sample, and, in our series of 12 subjects, there was one whose iron level showed a decrease of only 10 μg/100 ml during the day.

The diurnal variation in the plasma iron would seem to be independent of the initial plasma iron level. Thus Hemmeler(10) found that in 16 cases of infectious hepatitis, a disease in which the plasma iron level is high, the morning mean plasma level was 218 ± 13 μg %. This fell to a level of 135 ± 13 μg % in the evening, a difference of 83 ± 10 μg %. Similarly in 20 cases of anemia of infection(11) in which the plasma iron level is usually low, the mean morning plasma iron level was 56 ± 6 μg % and the evening value 39 ± 4 μg %, representing a fall of 17 ± 4 μg %. The diurnal rhythm appears to be related to activity and sleep since it has been shown by both Höyer(4) and Waldenström(6) that the diurnal variation is shifted in night workers, so that the level of plasma iron in these subjects is highest in the afternoon or evening after waking, and falls during the night, to rise again during the day when they sleep. Likewise, we have found that there was no definite diurnal cycle in a group of 5 normal individuals who were leading irregular hours of activity and sleep.

There is little evidence that muscular effort is a factor influencing the decrease in plasma iron during the day. Vannotti and Delachaux(12) stated that the plasma iron content decreases after heavy muscular effort but this conclusion is not apparent from the data they presented. Furthermore, Biörck (13) has been unable to confirm these findings.

Recent studies from this laboratory have demonstrated that there is a relationship between adrenocortical function and the level of plasma iron in dogs(14) and rats(15). It has been shown that the decrease in the plasma iron following acute stress is associated with

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12. Vannotti, A., and Delachaux, A., Der Eisenstoffwechsel und seine Klinische Bedentung, Basel, 1942.

increased adrenocortical secretion. Since Romanoff *et al.*(16) have observed that the excretion of adrenocortical steroids in man is greater during the morning and the rest of the day than during sleep, and that this excretion of steroids undergoes a constant diurnal variation, it might be postulated that the decrease in the level of plasma iron during the day may be associated with the increase in adrenocortical activity and similarly that the increase in the plasma iron during sleep may be associated with the decrease in the secretion of cortical hormone. However, it has been observed that the levels of eosinophils and lymphocytes in the peripheral blood increase rather than decrease as the day progresses(17,18). Furthermore, two patients with Addison's disease whom we have studied showed a normal diurnal variation in the level of plasma iron. There is even less evidence to suggest that the decrease in plasma iron during the day is due to sympathetic tone and that its rise during the night is due to an increase in parasympathetic tone(8). Thus, the physiological mechanisms responsible for the diurnal variation in plasma iron of man remain obscure.

Summary. It has been shown that the plasma iron of man undergoes a regular diurnal variation. These results have been compared with those of other investigators.

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Conversion of 3-Phosphoglycerate to Phosphoenolpyruvate by Tissue Homogenates.* (18103)

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A series of recent observations(1-3) made it interesting to pursue the problem of regulatory mechanisms of enzyme reactions participating in the glycolysis of Harden-Young ester. The present paper is a part of this study and describes a method for the determination of phosphopyruvate formation from 3-phosphoglycerate, catalyzed by dilute tissue homogenates. Evidence is also presented suggesting that this enzyme reaction might be under endocrine influence.

Experimental. Two enzymes, phosphoglyceromutase and enolase(4-6), are necessary to bring about the formation of phosphoenolpyruvate from 3-phosphoglycerate. Both enzymes are present in tissue homogenates. The principle of the assay of these two consecutive enzyme reactions is the determination of

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