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Disappearance of Radioactive Iron from Plasma by Day and by Night. (23403)

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It has been shown that the diurnal variation of the plasma iron level is less evident in aplastic anemia, pernicious anemia in relapse, and in iron-deficiency anemia, and that full treatment of the 2 last conditions restores a normal diurnal variation. The diurnal variation was also found to be diminished and the plasma iron levels low in various conditions associated with increased erythropoiesis; polycythemia vera, congenital hemolytic anemia, nocturnal hemoglobinuria, pernicious anemia at the height of the reticulocyte response, iron-deficiency anemia treated with a limited quantity of iron(1) and in sickle-cell anemia (2). In similar conditions of increased erythropoietic activity, Huff *et al.*(3) found an increased plasma iron turnover. Under these circumstances the plasma iron level is low and remains relatively constant throughout the 24 hours. There is little or no rise in the level during sleep, possibly because the bone marrow is actively taking up iron during sleep. If this postulate be true it is reasonable to infer that the normal diurnal variation is brought about by diminished bone marrow activity during sleep and hence an increasing plasma iron level. In this respect it was especially interesting to find that the plasma iron level remained low throughout the 24 hours in the 2 patients with paroxysmal nocturnal hemoglobinuria, the hemolytic process in each case being compensated by increased erythropoietic activity.

Laurell(4) on the other hand noted the similarity between the diurnal plasma iron and bilirubin levels and explained both on the assumption that rate of destruction of hemoglobin is slightly greater during the night than during the day. It therefore seemed important to determine the fate of radioactive iron injected into the plasma by day and by night.

Method. The method adopted was similar to that of Wasserman *et al.*(5) except that the radioactive iron was converted to siderophilin by addition to the individual's plasma *in vitro*(6) and not by addition to Fraction IV-7 protein. Radioactive iron (^{59}Fe), in the form of ferric chloride, was neutralized with sterile NaOH and added to the plasma which was then allowed to stand for several hours before injection into the blood stream. After injection of radioactive iron, blood samples were collected by means of an in-dwelling needle in an antecubital vein and the subjects (except Subject 1) slept comfortably during the night experiments. Observations were made by day and by night (within a 24-hour period) on 3 normal individuals, one patient suffering from iron deficiency anemia and one from sickle-cell anemia. Diurnal plasma iron levels were determined by methods described previously(7).

Values for plasma iron turnover and the fraction of radio-iron removed per hour were calculated as in the paper by Huff *et al.*(3).

Results. For the 3 normal individuals T½ by day was found to be 96, 101 and 88 minutes and T½ by night 128, 126 and 126 minutes, respectively. The time-courses for Subject No. 3 are shown graphically in Fig. 1. In the patient with iron-deficiency anemia the clearance rates were almost identical by day and by night. Only one curve could be fitted, and T½ by day and by night was found to be 56 minutes (Fig. 2). In the patient with sickle-cell anemia the clearance rates were

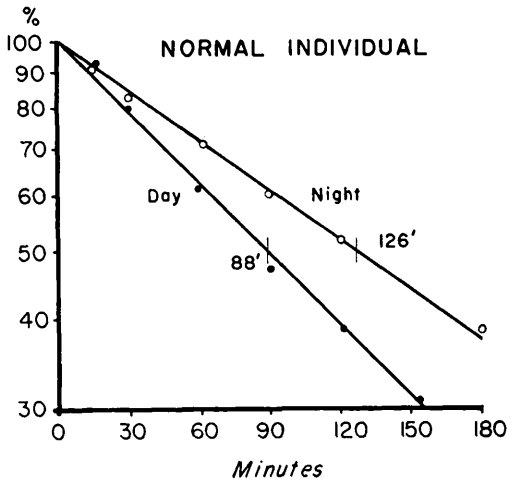

FIG. 1. Time-courses showing disappearance of radioactive iron from plasma by day and by night in normal individual.

found to be very rapid and were reversed so that the clearance by night was more rapid than the clearance by day; T½ by night was 33 minutes and T½ by day was 43 minutes (Fig. 3). Despite this patient's tall stature the measured blood volume exceeded the calculated volume. This finding has been noted by Smith(8) in patients with sickle-cell anemia during remission.

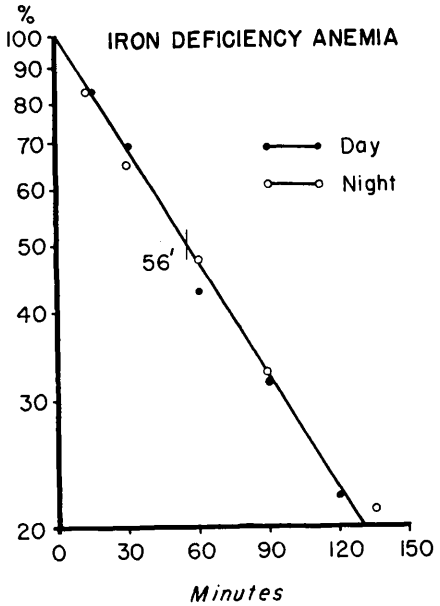

FIG. 2. Time-courses showing disappearance of radioactive iron from plasma by day and by night in iron-deficiency anemia.

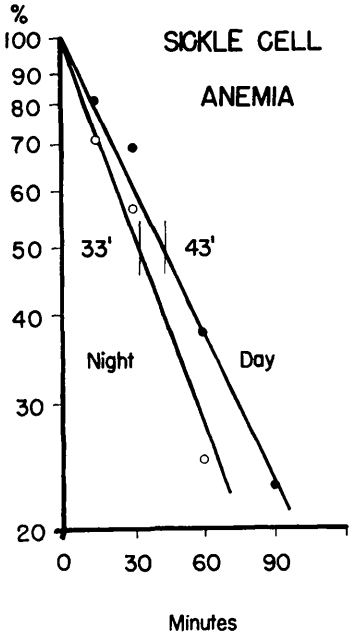

FIG. 3. Time-courses showing disappearance of radioactive iron from plasma by day and by night in sickle-cell anemia.

In neither of the last 2 cases was there any marked diurnal variation of the plasma iron level, whereas in the 3 normal subjects the diurnal variation followed the usual pattern of a rising level by night and a falling level by day (Fig. 4).

The calculated plasma iron turnover was

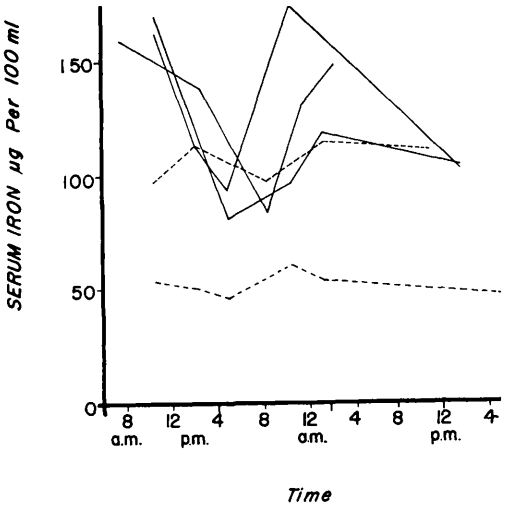

FIG. 4. Diurnal plasma iron levels. Solid lines—normal individuals (Subjects 1-3); dotted lines—upper, sickle-cell anemia (Subject 5), lower, iron-deficiency anemia (Subject 4).

TABLE I. Calculated Values following Injection of Radio-Iron by Day and by Night.

Subject and diagnosis	Sex and age	Time*	Plasma vol, ml	Plasma iron,† μg/100 ml	T½, min.	Iron fraction removed per hr	Plasma iron turnover, μg/hr
1. Normal	♂ 37	2 Day	3215	162 f	96	.432	2256
		1 Night		174	128	.325	1818
2. "	♀ 42	2 Day	2280	170 f	101	.412	1596
		1 Night		96 r	126	.330	722
3. "	♂ 19	1 Day	3240	142 f	88	.473	2174
		2 Night		129 r	126	.330	1380
4. Iron-deficiency anemia	♀ 23	2 Day	2630	50 f	56	.743	977
		1 Night		60 f	56	.743	1172
5. Sickle-cell anemia	♂ 18	1 Day	4690	105 r	43	.967	4762
		2 Night		105 s	33	1.26	6206

* Figures 1 and 2 refer to order in which experiments were carried out.
† Plasma iron levels at time of Fe^{59} admin. Determinations were also made 2-4 hr later, and the level was found to be falling (f), rising (r) or showing no change (s).

found to be decreased by night in the normal individuals with normal diurnal plasma iron variation, and increased by night in the anemic subjects who showed little or no diurnal variation. The findings are summarized in Table I.

Conclusion. These findings provide support for the hypothesis that the plasma iron level rises by night because of diminished utilization of iron for hemoglobin synthesis during sleep in the individual with normal erythropoietic activity. The conclusion is inferential in that no actual measurement of the newly formed hemoglobin was made but is clearly suggested by the findings of Wasserman and others(5) who interpret rate of removal of iron from the plasma as providing an index of erythropoietic activity. Bothwell and Mallett(9) found that plasma iron turnover varied throughout the day and was significantly affected by the plasma iron level. It is probable that an important consideration in assessing the findings of these authors concerns the state of the plasma iron level, *i.e.*, whether it is rising or falling at the time of plasma iron turnover determination. An interesting finding by Bothwell and Mallett was that the fraction of iron removed per hour from the plasma was uniformly large in both morning and evening determinations in anemic subjects.

The plasma iron reaches its lowest level just after sleep begins and rises during sleep (10). This diurnal pattern is reversed in night-workers who sleep by day(10-13). The usual rhythm, with morning levels higher than evening levels, is found only if the subject has slept during the night(13). The diminished plasma iron turnover and increased T½ by night suggest that diurnal variation of the plasma iron level is secondary to a diurnal variation in the rate of hemoglobin synthesis, which in turn is dependent upon the sleep rhythm. The high morning level of bilirubin in the plasma may be considered due to diurnal fluctuation in liver function rather than to increased hemolysis by night, as was held by Laurell(4). Josephson and Larsson(14) and Forsgren(15) studied diurnal excretion of bile by means of bile fistulae and found the quantity excreted by night less than that excreted by day.

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Anticonvulsant Activity of Some Substituted Ethylene Glycols. (23404)

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The clinical utility of diols such as mephenesin and 2,2-diethyl-1,3-propanediol (DEP) (I) has been limited by the short duration of action of these otherwise interesting drugs. Riley and Berger(1), and Berger and Ludwig(2) showed that rapid oxidation of the hydroxyl groups is responsible for this brief effect. Therefore it seemed desirable to synthesize structural variants less vulnerable to oxidative detoxication.

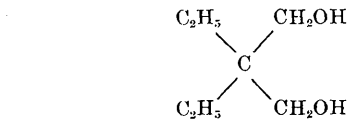
In this study a structural modification was considered favorable when the compound was effective against both electroshock and Metrazol convulsions at a dose below 100 mg/kg and had a duration of not less than 3 hours.

Methods. All compounds examined were administered orally as 5% gum acacia suspensions to Wistar-strain rats of either sex. The anticonvulsant activity of the compounds was measured at a predetermined time of peak activity by the maximal electroshock seizure test (M.E.S.) of Toman *et al.*(3), and by the subcutaneous Metrazol seizure test (Met.) described by Goodman *et al.*(4). The animals were observed before and after the convulsive challenges. Particular emphasis was placed on detection of motor impairment and other manifestations of central depression when present. Dose-response curves were established for active compounds whenever supply permitted. At least 3 dosage groups of 8 animals each were used to complete the median effective doses (ED_{50}) by the method of Bliss(5).

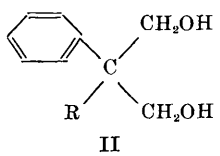
TABLE I. Effect of DEP Modification on Activity.

Compound	$ED_{50} \pm$ S.E. (mg/kg) Maximal electroshock seizure	Metrazol seizure	Peak action (min.)
I (DEP)	154.4 ± 7.7	155.2 ± 8.4	30
II a	159.0 ± 9.9	229.3 ± 17.2	30–60
II b	142.8 ±11.4	235.3 ± 30.6	30
III	120.7 ± 9.3	181.0 ± 15.4	30–90

Results. Table I shows the effect of modification of the DEP molecule upon anticonvulsant activity. Replacement of one ethyl group by phenyl (IIb) enhanced the depressant effect. A similar observation has been reported by Berger(6). In a further variation, the 1,3-diol arrangement of IIa was changed to 1,2-diol (III). Although this alteration diminished the depressant effect, the anticonvulsant activity of the new compound was superior to that of either I or II (Table I).

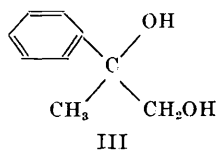


I (DEP)



II

a) R = CH_3
b) R = C_2H_5



III