HEMOGLOBIN VARIATIONS FOR WOMEN ON IRON THERAPY FOR THIRTY-ONE MONTHS[1]

DOROTHY G. WIEHL AND H. D. KRUSE, M.D.

AN investigation of the effect of prolonged iron therapy on hemoglobin levels of women with slightly subnormal values at an initial examination was undertaken in April, 1943. The increase in the hemoglobin level and the individual variation in hemoglobin values shown by determinations at approximately six-month intervals over a two and one-half year period on therapy have been studied. A comparison is made of the results obtained with reduced iron and with feosol.

For a control group of women who received no iron therapy, hemoglobin values were obtained at the same intervals over the two and one-half years. The long-time and periodic individual changes in hemoglobin values for this group also are presented.

DESCRIPTION OF THERAPY AND CONTROL GROUPS

The women who participated in this investigation were nurses employed by a visiting nurse agency in New York City. Their ages ranged from 18 to 58 years, but about three-fourths of the group were between ages 25 and 39 years at the initial examination in October, 1942. Of 107 women examined initially, eleven were colored; and data for the two groups are considered separately in the following analysis.

On the basis of the hemoglobin values obtained in October, 1942, the therapy subjects were selected. All persons with values less than 11.5 gms. were placed in the therapy group. These included seven white and three colored women, of whom three white and two colored women were given reduced iron and the others were given feosol. Another ten subjects for therapy were selected at random from those having hemoglobin values be-

[1] From the Milbank Memorial Fund, New York.

tween 11.5 and 11.9 gms. inclusive. The total group for reduced iron therapy included seven white and four colored women; the feosol group included eight white women and one colored woman.

Since approximately six months elapsed before therapy was distributed, the hemoglobin levels of some of the therapy subjects may have been considerably higher or lower at the time therapy was begun than at the initial examination. Thus, the therapy group may be described as comprising persons who at a previous examination had moderately or slightly subnormal hemoglobin values. Regardless of the spontaneous changes that may have occurred in the six-month interval before beginning therapy, the hemoglobin levels following one to two and a half years of therapy are of interest.

All the women used as "controls" in this Study of iron therapy and the total nontherapy group were subjects during the two and a half years for an investigation of the effect of vitamin therapy on selected specific signs. They were divided into five groups and subjects in each group received only one vitamin. An analysis of the hemoglobin values for the five groups indicated no significant difference among them with respect to hemoglobin values at any examination.

Methods of the Study

Subjects on reduced iron and those receiving feosol were given approximately the same amount of iron, 60 and 63 mgs. per day, respectively. The reduced iron was in 15 mg. tablets which were to be taken four times a day; the daily dosage of feosol was in a single tablet. Therapy for a two-month period was distributed to the subjects regularly. There was no way to observe whether the therapy was taken regularly, but the subjects had volunteered and were extremely interested. Although undoubtedly some occasionally missed taking their therapy, it is believed that any marked irregularity or interruption in taking therapy

would have been reported by those who remained in the Study and returned for reexaminations.

The first reexamination was made in July, 1943, or ten to twelve weeks after therapy was started and about nine months after the initial examination had been carried out. Later reexaminations were at approximately the following intervals after therapy started in April, 1943: 7 months, 12 months, 19 months, 25 months, and 31 months. Over so long a period it was inevitable that the number remaining in the Study would gradually diminish, especially since many of these nurses left to enlist in military service. Details of the changes in numbers will be given in the analysis, but it may be mentioned here that only one of the twenty persons on either form of iron therapy left the Study before completing one year of therapy.

The hemoglobin determinations were made on 20 cu. mm. of finger-prick blood by the Evelyn method for photoelectric determination of oxyhemoglobin. The same Evelyn photoelectric colorimeter was used for all determinations. Average hemoglobin values for two blood samples, one from the left and one from the right hand, are used throughout the Study except for the initial examination when only one determination was made for each person. Two technicians made all the hemoglobin determinations and, with a very few exceptions, one technician made all determinations for a given examination period. Hemoglobin values were computed to the nearest 0.1 gm.

Initial Hemoglobin Findings

The initial hemoglobin levels of the 107 women examined in October, 1942 are shown in Table 1 by color and age of the subjects. The average hemoglobin value for the ninety-six white women was 12.67 gms. and for the eleven colored women was 11.87 gms. Two white women and no colored women had less than 10.0 gms. of hemoglobin per 100 ml. of blood; seven white

women (7.3 per cent) and three colored women (27.3 per cent) had less than 11.5 gms. of hemoglobin. The range for hemoglobin values was wide and two white women had values over 15 gms. (15.2 and 15.3 gms.), but the maximum for the colored women was 13.6 gms. The average hemoglobin level for the eleven colored women is significantly lower than would be expected if these women were a random sample of the total 107 women examined.

There is an apparent upward trend in the average hemoglobin values as age increased for the white women, with the striking exception of the age group 40-49 for which the average hemoglobin value is the lowest of any age group although only very

Table 1. Hemoglobin values for ninety-six white women according to age and for eleven colored women at initial examination in October, 1942.

Hemoglobin Level Gms.	Number of White Women by Age Groups						Colored Women
	All Ages	18-29[1] Years	30-34 Years	35-39 Years	40-49 Years	50-59 Years	All Ages[2]
Total	96	30	23	26	13	4	11
8.18	1	1					
9.39	1		1				
10.0-10.9	3	2			1		2
11.0-11.4	2	1	1				1
11.5-11.9	20	10	3	4	3		4
12.0-12.4	16	4	6	3	3		2
12.5-12.9	16	3	4	6	3		
13.0-13.4	12	3	2	5	1	1	1
13.5-13.9	15	2	3	5	2	3	1
14.0-14.4	6	2	3	1			
14.5-14.9	2	1		1			
15.0+	2	1		1			
Mean	12.665	12.360	12.625	13.059	12.331	13.705	11.871
St. Error	±.118	±.258	±.229	±.176	±.243	±.126	±.296
St. Deviation	1.15	1.41	1.10	.897	.875	.252	.981

[1]Five women were under 25 years of age and of these one had a hemoglobin level less than 12.0 gms. (11.7 gms.).

[2]Numbers of colored women by age were: 2 under 30 years; 2 aged 30-39 years; 3 aged 40-49 years; and 2 aged 50-59 years.

slightly lower than at ages 18 to 29 years. However, in each age group the variation in hemoglobin values is great and an analysis of variance for the three age groups under 40 years indicates that differences in the means for these three groups are within limits expected as a result of random sampling. Also, the average for the thirteen persons in the age group 40-49 years does not differ significantly from the total group under 40 years of age. Thus, these data give no evidence of significant age variations in hemoglobin values.

Adjustments in Reexamination Values for Technical Variations in Hemoglobin Determinations

Before the reexamination data were analyzed for evidence of effects of iron therapy, technical and error variation in the determinations were studied. Results of this study of the accuracy of the determinations have been reported (1) in detail. Comparison of the hemoglobin values obtained for nontherapy subjects at the first six of the seven examination periods indicated approximately constant average values at four periods and higher average values at two periods, the second and sixth examinations. Both of the higher averages were obtained by one of the technicians (C). On the basis of these findings and a special comparative study of the technicians made since the publication of the report, it was concluded that this technician had not maintained a constant technique and had a tendency to obtain higher values than the other technician. Presumably this is due to a slightly different standard of measuring the blood sample. This technical bias introduced into the serial examinations has necessitated some adjustment in the data for purposes of evaluating individual changes.

To adjust the relatively higher determinations obtained at the second and sixth examinations so that the average difference would be eliminated, a deduction of 0.5 gm. was made from

every individual determination in these periods. Although this adjustment removes the average difference, which, it is believed, was a fairly constant bias[2] in these periods, the periodic changes in individual values undoubtedly are affected by a residual variation due to the fact that the bias was not entirely constant.

A slight adjustment in individual determinations obtained in the third examination period by the other technician (B) was made in order to bring the average for the period more closely into line with the constant average used as a base line. Values in the third period were increased 0.1 gm. This adjustment makes allowance for significantly lower values obtained on left than on right-hand blood specimens in this period.[3]

All individual hemoglobin values in the seventh period were adjusted by reducing each value by 0.24 gm. The average for non-therapy subjects in this period was higher by this amount than that for the same subjects examined previously by the same technician. No explanation for a shift in the level of the determinations in the seventh period (made by technician B) has been identified, but it has seemed best to maintain the general average for this period.

In summary, the values used for the first, fourth, and fifth examinations are the original determinations obtained by the technicians. Adjusted values[4] are used for the other four examinations as follows: values at the second and sixth examinations are *reduced* 0.5 gm.; determinations at the third examination are *increased* 0.1 gm.; and those at the seventh examination are *re-*

[2] In the periods in which the technician obtained relatively high values, the error variation for his duplicate determinations (right and left hands) was about the same as in the period in which he obtained lower values; and the error variation for this technician was very similar to that for the other technician. *See* (1).

[3] In the previous report (1), this bias is discussed. The mean difference for the right and left-hand specimens was .16 gm. and it is thought to be due to imperfect drying of the pipet between specimens. The mean difference in other periods varied from .02 to .05 gm.

[4] For computation of the amounts to adjust values at the different periods, average values at different periods for the *same* individuals were used. Mean values for selected groups or for different individuals continue to vary somewhat at various periods.

duced 0.24 gm. It is unfortunate that these adjustments were necessary, and it may seem that they have complicated the analyses presented in this evaluation of hemoglobin changes. However, the arbitrary elimination of periodic changes resulting from a technical bias permits the changes due to other factors to be seen more clearly. Since the adjustment is constant for every individual in any period, measurements of variation within the period are not affected, and means for special groups of subjects are affected equally so that differences between them are not affected.

Hemoglobin Changes After Iron Therapy

Comparison of Reduced Iron and Feosol. Data on the hemoglobin values obtained at various intervals from 10-12 weeks to 31 months after beginning therapy are shown in Table 2 for the seven white women taking reduced iron and the eight white women taking feosol. For each group the average hemoglobin level and standard error of the average and the range of values

Table 2. Hemoglobin values (grams per 100 ml.) for white and colored women on iron therapy for two and a half years.

Color Group and Period of Therapy	Reduced Iron			Feosol			Iron or Feosol	
	Number	Mean and St. Error	Range	Number	Mean and St. Error	Range	Number	Mean and St. Error
White								
I. Initial Exam.	7	11.16±.353	9.4-11.9	8	10.93±.444	8.2-11.9	15	11.03±.280
II. 10-12 Weeks	5	12.60±.444	10.9-13.5	8	12.58±.209	11.8-13.7	13	12.58±.202
III. 7 Months	6	12.55±.565	11.1-14.4	8	12.99±.264	11.9-14.1	14	12.80±.279
IV. 12 Months	6	12.30±.137	11.9-12.7	8	12.69±.330	11.3-14.4	14	12.52±.199
V. 19 Months	5	12.72±.271	11.9-13.6	4	12.68	12.5-12.9	9	12.70±.147
VI. 25 Months	4	12.48	11.1-13.6	3	12.10	11.5-12.5	7	12.31±.309
VII. 31 Months	3	12.73	11.9-13.3	3	12.80	12.6-13.0	6	12.77±.198
Colored								
I. Initial Exam.	4	11.13	10.0-11.9	1	11.2		5[1]	11.14±.336
II. 10-12 Weeks	4	11.73	10.8-13.0	1	10.6		5	11.50±.456
III. 7 Months	3	12.53	11.8-13.2	1	11.1		4	12.18±.459
IV. 12 Months	4	11.93	11.3-12.3	1	12.6		5	12.06±.220
V. 19 Months	3	12.53	12.0-13.5	1	12.7		4	12.58±.345
VI. 25 Months	3	12.43	11.7-13.7	1	10.9		4	12.05±.591
VII. 31 Months	3	12.43	10.9-13.3	1	12.3		4	12.40±.545

[1]For the four women who continued therapy for 31 months, the average hemoglobin was 11.43±.229 gms. at the initial examination.

is shown. It will be noted that not every case was reexamined at each period. After 10 to 12 weeks of therapy, the two groups had almost identical average values, the means being 12.60 and 12.58 gms.[5] The maximum value obtained for any person also was about equal for the two groups, being 13.5 gms. for those on reduced iron and 13.7 gms. for those on feosol; but the minimum values differed and one subject taking reduced iron (this person had an initial value of 9.4 gms.) still had only 10.9 gms. of hemoglobin per 100 ml. compared with a minimum finding of 11.8 gms. among the feosol cases which included one with an initial value of 8.2 gms. There was, however, no real difference between these two therapy groups after 10-12 weeks of therapy.

Only small differences which are not significant statistically are found for the mean hemoglobin values for the two therapy groups at every reeexamination period throughout the 31 months. After the first 12 months, the numbers of persons continuing on therapy decreased and at the examination after 31 months of therapy only three cases remained in each group. No significant further increase in hemoglobin values is indicated after the first 12 weeks for either group. The general level remained static and there was no difference between the groups in the occurrence of high individual hemoglobin values or of low values. For these few subjects the response to reduced iron and to feosol over the 31 months was nearly identical.

Periodic Variation. Although the average level remained fairly constant, individual values varied to a considerable degree from one period to another. Some examples of individual periodic variation while on therapy may be cited. The subject with the lowest initial hemoglobin value in the series (8.2 gms.) had reached 12.0 gms. after 12 weeks of therapy and at the five later examinations her hemoglobin values varied from 12.3 to 12.9 gms. which

[5] These averages after 10-12 weeks of therapy are significantly higher than the averages for the initial examination, but the increase cannot be attributed to the therapy since the initial values were obtained six months before therapy started.

probably is within the limits of accidental variation. The subject with the next lowest initial hemoglobin value (9.4 gms.) showed a much slower increase in hemoglobin values; at the second, third, and fourth examinations her values were 10.9, 11.1, 11.9 gms., respectively, and at the fifth examination (19 months on therapy) the value rose to 13.6 gms. but six months later it had dropped again to 11.1 gms. Another case with an initial hemoglobin value of 10.9 had a value of 13.7 gms. after 12 weeks of therapy and 14.1 gms. after 7 months of therapy but after 12 months of therapy her hemoglobin value declined to 12.1 gms. A subject with a hemoglobin value of 14.4 gms. after 7 months of therapy had a value of 11.9 gms. at the next two examinations. Thus, the changes in hemoglobin values obtained at intervals over many months suggest that even on continuous therapy a number of these women failed to maintain moderately good hemoglobin levels.

A statistical measure of this variation in individual hemoglobin values from period to period is given in Table 3. For eleven women who were reexamined in the second, third, and fourth periods, *i.e.,* after 10-12 weeks, 7 months, and 12 months of therapy, the variation in the three hemoglobin determinations from their individual mean levels for these periods gives an average standard deviation of .70 gm. For six women who continued therapy for the entire 31 months, the standard deviation for their individual variation in values obtained after 19, 25, and 31 months is .67 gm. Of the six women who remained on therapy for 31 months, five received all six reexaminations. The standard deviation for their individual periodic variation for six examinations is .62 gm.

The analysis in Table 3 also shows that the eleven women on therapy for one year who were reexamined three times in this year did not differ with respect to their mean hemoglobin levels more than might be expected from chance in view of their large

individual periodic variation. The average hemoglobin level for this group for these three reexaminations was 12.65 gms., and the individual mean values ranged from 11.30 to 13.30 gms. Similarly, the six women on therapy for 31 months did not differ significantly in the last three reexaminations. Their mean hemoglobin level after 19 to 31 months of therapy was 12.64 gms., and the range in individual means was 12.20 to 13.07 gms. However, the individual mean values for the five women with all six post-therapy reexaminations did differ significantly and suggest that there was some tendency for the women to differ in their response to therapy. For the six examinations in a 28-month period, the average hemoglobin level of the five women was 12.50 gms. and the range in individual means was 11.75 to 12.95 gms.

Hemoglobin Changes for Colored Women on Therapy. Hemoglobin values for the five colored women who received therapy are shown in Table 2. Since only one colored woman received feosol and since there was no difference in the therapeutic re-

Table 3. Analysis of variance for white therapy subjects showing mean periodic individual variation in hemoglobin values and significance of differences among individual mean levels for several reexaminations.

Examination Periods and Source of Variation	Degrees of Freedom	Variance	Variance Ratio (F)	Probability	Standard Deviation for Individual Variation
Periods II, III, and IV					
Among Person Means	10	.9882	2.00	>.05	
Individual Periodic Var.	22	.4936			.703
Periods V, VI, and VII					
Among Person Means	5	.3286	*		
Individual Periodic Var.	12	.4483			.670
Periods II-VII					
Among Person Means	4	1.3320	3.42	.01-.05	
Individual Periodic Var.	25	.3897			.624

*Individual periodic variation greater than variance for person means.

sponse to reduced iron and to feosol for white women, only the results for the total colored group will be considered. After 10 to 12 weeks of therapy, the average hemoglobin value for the five colored women was 11.50 gms. and not significantly higher than the average value at the initial examination about 9 months previous. After 7 months of therapy, the average hemoglobin value for the four colored women who were reexamined had risen to 12.18 gms. and thereafter the average value for this group showed no consistent increase although at 19 months after therapy a maximum average of 12.58 gms. was obtained. Only the average for this period is significantly higher than the initial average of 11.43 gms. for the same four women. Thus, there is evidence of only slight improvement among the colored women.

At every examination after therapy, the average hemoglobin value for the four or five colored women was somewhat lower than the average value for white women at the same period. This difference is statistically significant[6] at the second examination (10 to 12 weeks after beginning therapy) but it is not significant at any other period. The experience with this small group of colored women suggests that their improvement in hemoglobin levels on therapy was less than that for whites. However, the colored women, as the white women, varied greatly in their response and a definite conclusion that the difference observed here is characteristic or typical is not warranted. Experience with larger numbers of women is needed.

Therapy and Control Subjects

In order to evaluate the improvement in hemoglobin values shown by the white[7] women taking iron therapy, the values ob-

[6] At the second examination, the difference between means (white-colored) is +1.08 gms., the standard error of the difference is ±.427, and P is .01-.05. The maximum difference at any other period (third) is +.625 ±.578 gm. and is not significant. Furthermore, analysis of variance for periods III and IV indicated no significant difference in means for color groups and a similar result was obtained when periods V-VII are combined.

[7] Comparison with a control group is limited to the white women although the colored

(Continued on page 384)

Examination Period and Group	Number	Mean Hb. Gms. and St. Error	Standard Deviation	Control-Therapy, Difference and St. Error
II Therapy 10-12 Weeks				
Therapy—Total	13	12.58 ±.202	.730	−.113 ±.220
" —Initial Hb. 11.5	6	12.37 ±.407	.997	+.105 ±.298
" " " 11.5-11.9	7	12.77 ±.146	.386	−.300 ±.172
Control " " 11.5-12.0	14	12.47 ±.097	.365	
III Therapy 7 Months				
Therapy—Total	14	12.80 ±.279	1.042	−.120 ±.425
" —Initial Hb. 11.5	7	12.27 ±.422	1.116	+.409 ±.517
" " " 11.5-11.9	7	13.33 ±.255	.675	−.649 ±.437
Control " " 11.5-12.0	10	12.68 ±.317	1.003	
IV Therapy 12 Months				
Therapy—Total	14	12.52 ±.199	.743	+.115 ±.357
" —Initial Hb. 11.5	6	12.55 ±.219	.536	+.086 ±.460
" " " 11.5-11.9	8	12.50 ±.320	.906	+.136 ±.460
Control " " 11.5-12.0	11	12.64 ±.315	1.044	
V Therapy 19 Months				
Therapy—Total	9	12.70 ±.147	.442	+.030 ±.201
" —Initial Hb. 11.5	4	12.88		
" " " 11.5-11.9	5	12.56		
Control	10	12.73 ±.137	.432	
VI Therapy 25 Months				
Therapy—Total	7	12.31 ±.309	.818	+.273 ±.416
Control	8	12.59 ±.279	.790	
VII Therapy 31 Months				
Therapy—Total	6	12.77 ±.198	.484	+.393 ±.269
Control	5	13.16 ±.175	.391	

Table 4. Comparison of hemoglobin values of white women taking iron therapy with those of a nontherapy control group initially having low hemoglobin values.

tained for this group over the two and one-half year period are compared in Table 4 with the hemoglobin values obtained at the

and white groups on therapy did not differ significantly with respect to their hemoglobin values except at the second examination. Since the averages for the colored group were consistently somewhat lower than those for the white group, inclusion of the colored therapy subjects would reduce the average values for therapy subjects and minimize any advantage shown for them. Comparison of the more homogenous groups of white women seems preferable.

same examination periods for a group of white women who did not receive any iron therapy and who had hemoglobin values at the initial examination of 11.5 to 12.0 gms. This latter group will be designated as a control group.

Since approximately half of the white women who received therapy had hemoglobin values of less than 11.5 gms. at the initial examination, data for these women and for those with 11.5-11.9 gms. are shown separately in Table 4. After 10 to 12 weeks of therapy and about 9 months after the initial examination, subjects with the lower initial hemoglobin values had, on the average, lower values than those with higher initial values but the difference (.40 gm.) is not statistically significant. At the third examination the superiority of the group with higher initial values increased to 1.06 gms. but again the difference is not significant. At later reexamination the differences in the average hemoglobin levels for these two therapy groups were small.

The difference between the average hemoglobin value for the control group and the therapy group at each of the six post-therapy examinations is shown in Table 4. At no examination is the difference statistically significant. The control group had an average hemoglobin value slightly lower than the therapy group at the second and third examinations (.11 and .12 gm.) and thereafter their average value was slightly higher (.03 to .39 gm.). Thus, there is no evidence that these women who took iron therapy for two and a half years improved their hemoglobin values any more than the spontaneous improvement that occurred among women not taking therapy during the same period.

Reexamination Hemoglobin Levels for Women Classified According to Initial Values

Since the average hemoglobin level for the control group (initial values 11.5-12.0 gms.) remained at a fairly constant level

Table 5. Comparison of hemoglobin values at seven examinations over a three-year period for three groups of white women whose hemoglobin values at first examination were: A, 11.5-12.4 gms.; B, 12.5-13.4 gms.; and C, 13.5-15.3 gms.

Examination and group by First Hb. Value	Number	Mean Hb. Gms. and St. Error	Standard Deviation
I October, 1942			
Total	79	12.94 ±.099	.877
A—Low Hb.	25	12.04 ±.053	.279
B—Intermediate Hb.	27	12.95 ±.064	.331
C—High Hb.	24	13.99 ±.106	.518
II 9 Months Later			
Total	72	12.95 ±.093	.792
A	24	12.46 ±.091	.446
B	24	12.87 ±.162	.794
C	24	13.53 ±.143	.703
III 13 Months Later			
Total	67	12.99 ±.122	.997
A	23	12.61 ±.174	.835
B	23	13.12 ±.189	.909
C	21	13.26 ±.253	1.161
IV 18 Months Later			
Total	67	12.97 ±.116	.947
A	22	12.56 ±.197	.924
B	24	12.97 ±.168	.824
C	21	13.39 ±.209	.957
V 25 Months Later			
Total	53	13.01 ±.078	.568
A	19	12.81 ±.094	.409
B	21	13.04 ±.119	.547
C	13	13.27 ±.197	.711
VI 31 Months Later			
Total	43	13.07 ±.091	.598
A	16	12.79 ±.172	.688
B	15	13.13 ±.134	.519
C	12	13.35 ±.120	.417
VII 37 Months Later			
Total	39	13.10 ±.101	.630
A	12	12.85 ±.210	.727
B	15	13.30 ±.158	.612
C	12	13.11 ±.143	.494

following an increase within the nine months between the initial and second examination, it is of interest to examine the changes in averages for women with higher initial hemoglobin levels. All nontherapy white women, including the "controls," were classified according to their initial hemoglobin value in one of three groups as follows: A, low initial value, 11.5-12.4 gms.; B, intermediate initial value, 12.5-13.4 gms.; and C, high initial value, 13.5-15.3 gms. For each group the mean and standard deviation of individual values at each of the seven examinations over approximately three years are shown in Table 5.

For the low group (A), average hemoglobin values at each examination after the first vary from 12.46 to 12.85 gms. and are about the same as those for the control group which forms approximately one-half of Group A. There is a definite increase from the initial average value of 12.04 gms. to 12.61 gms. at the third examination about thirteen months after the initial examination, and thereafter changes are not significant. At every examination the average for this group is lower than the averages for groups B and C.

For the intermediate group (B) the average hemoglobin level continues to be in between the average for group A and the average for group C at every examination except the last when it is slightly higher, but not significantly higher, than the average for the high initial value group. At the initial examination the average hemoglobin for group B is 12.95 gms. and for the other six examinations it ranges from 12.87 to 13.30 gms. The average value for group C, high initial values, decreased from 13.99 gms. at the first examination to 13.53 gms. at the second and 13.26 gms. at the third and thereafter remained fairly constant.

In Table 6 the significance of the variation among mean hemoglobin levels for the three groups at each examination period is shown. The means, of course, are very significantly different at the first examination on which the groups are selected. The

Table 6. Significance of variation in [illegible] nations over a period of three years for three groups of white women [illegible] initial examination into those with low, intermediate, and high hemoglobin levels.

Examination Periods and Source of Variation	Degrees of Freedom	Variance	Variance Ratio (F)	Probability
I Initial Examination				
Means of Groups	2	24.4357	167.02	<.001
Within Groups	76	.1463		
II 9 Months Later				
Means of Groups	2	7.0588	16.01	<.001
Within Groups	69	.4409		
III 13 Months Later				
Means of Groups	2	2.5831	2.73	>.05
Within Groups	64	.9446		
IV 18 Months Later				
Means of Groups	2	3.6732	4.53	.01-.05
Within Groups	64	.8102		
V 25 Months Later				
Means of Groups	2	.8461	2.81	>.05
Within Groups	50	.3014		
VI 31 Months Later				
Means of Groups	2	1.1109	3.47	.01-.05
Within Groups	40	.3198		
VII 37 Months Later				
Means of Groups	2	.6753	1.77	>.05
Within Groups	36	.3816		
	ANALYSIS OF VARIANCE FOR LOW GROUP COMPARED WITH INTERMEDIATE AND HIGH GROUPS COMBINED			
III 13 Months Later				
Between Means	1	4.9648	5.32	.01-.05
Within Groups	65	.9332		
IV 18 Months Later				
Between Means	1	5.4130	6.54	.01-.05
Within Groups	65	.8275		
V 25 Months Later				
Between Means	1	1.2810	4.22	.01-.05
Within Groups	51	.3035		
VI 31 Months Later				
Between Means	1	1.9087	5.97	.01-.05
Within Groups	41	.3197		
VII 37 Months Later				
Between Means	1	1.1057	2.93	>.05
Within Groups	37	.3779		

groups continued to differ very significantly at the second examination but were not significantly different at the third. For the remaining four examinations the means differ significantly at two examinations and do not differ significantly at two examinations.

Since the difference between averages for those with intermediate and with high initial values is small after the second reexamination, these two groups were combined and compared with the low hemoglobin group. As shown in Table 6, the low group had a significantly lower average at each examination except the last.

In general, therefore, these data indicate that women with relatively low hemoglobin values at one examination showed a definite tendency to continue over a three-year period to have relatively low values although some improvement in their hemoglobin occurred. Women with relatively high hemoglobin values at one period continued, on the average, to have higher values for about nine months, but over a longer period their superiority diminished and their values became similar to those for a group with intermediate or slightly lower hemoglobin values at the initial examination.

These results at successive examinations for women in the low third of the distribution of hemoglobin values at an initial examination indicate that in the majority of cases lower than average hemoglobin was a chronic or intermittently recurring condition over a period of three years.

Periodic Individual Variation in Hemoglobin Values

Although there was a significant tendency for women with relatively low hemoglobin values to continue to have low values, changes of considerable magnitude in individual values from one examination to another occurred for many persons in this group as well as for those in the higher initial level groups. An analysis

Table 7. Periodic individual variation in hemoglobin values obtained at approximately six-month intervals over a two and a half year period for white women and for three subgroups according to hemoglobin values at a previous initial examination. Analysis of variance for variation among individual means and for individual values from their means.

Examination Periods and Source of Variation	Number of Cases	Mean Hb. (Gms.)	Analysis of Variance: Degrees of Freedom	Analysis of Variance: Variance	Analysis of Variance: Variance Ratio (F)	Analysis of Variance: Probability	St. Deviation for Intra-Individual Variation
TOTAL NONTHERAPY GROUP							
Periods II, III, and IV	54	13.03					
Among Person Means			53	1.4024	3.00	<.01	
Within Persons			108	.4674			.684
Periods V, VI, and VII	33	13.10					
Among Person Means			32	.4472	1.67	.01-.05	
Within Persons			66	.2670			.517
Periods II-VII	26	13.15					
Among Person Means			25	1.1823	2.68	<.01	
Within Persons			130	.4411			.664
INITIAL HEMOGLOBIN VALUE 13.5 GMS. OR MORE							
Periods II, III, and IV	20	13.36					
Among Person Means			19	1.2686	1.88	.05	
Within Persons			40	.6740			.821
Periods V, VI, and VII	9	13.26					
Among Person Means			8	.2232			
Within Persons			18	.3322	*		.576
INITIAL HEMOGLOBIN VALUE 12.5-13.4 GMS.							
Periods II, III, and IV	18	13.12					
Among Person Means			17	.9040	2.40	.01-.05	
Within Persons			36	.3767			.614
Periods V, VI, and VII	14	13.24					
Among Person Means			13	.3054	1.11	>.05	
Within Persons			28	.2760			.525
INITIAL HEMOGLOBIN VALUE LESS THAN 12.5 GMS.							
Periods II, III, and IV	16	12.50					
Among Person Means			15	.9600	3.08	<.01	
Within Persons			32	.3113			.558
Periods V, VI, and VII	10	12.76					
Among Person Means			9	.4107	2.10	>.05	
Within Persons			20	.1957			.442

*Within persons variation greater than variance for individual means.

of this periodic or intra-individual variation is presented in Table 7. The standard deviation for intra-individual variation is shown for the total nontherapy group and for each of the three subgroups. Since there was a marked decrease in the number of persons in the Study after the fourth examination, intra-individual variation is shown separately for the first three reexaminations which have an interval of about nine months with one examination at approximately the middle of the interval and for the last three reexaminations which have an interval of one year with a mid-year examination. For the total nontherapy group, individual periodic variation for all six reexaminations having a total interval of twenty-eight months also is shown.

The standard deviation for intra-individual variation for the entire nontherapy group at the second, third, and fourth examinations is .684 gm. and at the fifth, sixth, and seventh examination it is .517 gm. The standard deviation for the earlier series of examinations is significantly greater than for the later series. This may have been the result of greater error variation[8] in the earlier series of determinations or there may have been less individual variation in the last year of the Study.

When the intra-individual variation for the three subgroups according to a previous examination value is compared for the earlier and later series of examinations, a higher standard deviation is found for each subgroup in the earlier series but the difference is significant only for the group with initially high hemo-

[8] Differences between any two or more hemoglobin determinations are a composite of error variation and true physiological change in the hemoglobin level. If the former is known, the latter can be measured. The accidental error of determinations usually is based on a series of two or more determinations on independent blood samples taken in close succession. For independent duplicate determinations by the same technician, the standard deviation for error variation in these data was found to be .174 gm. (1) However, this is not a satisfactory measure of the total procedural or error variation, for, as previously discussed, additional variation believed to be procedural affected the values obtained at different periods. The adjustment of periodic values partially eliminated procedural periodic variation but, since this was not an absolutely constant factor, the residual variation, which is of unknown magnitude, would make the total error variation included in the intra-individual variation greater than the .174 gm. found in duplicates.

globin values. This latter group also shows significantly greater intra-individual variation for the second to fourth examinations than either of the lower hemoglobin groups. It seems probable, though not definitely proved, that individuals selected on the basis of a relatively high hemoglobin value did have greater intra-individual variation than those with medium or low hemoglobin values. This is consistent with the earlier finding that, on the average, the high hemoglobin group gradually lost its superiority over the intermediate group but the low hemoglobin group continued to be relatively low. In other words, unusually high hemoglobin values were, for the most part, temporary and those who had them showed wide periodic fluctuations.

Within each subgroup of the nontherapy subject, individuals differed significantly with respect to their mean hemoglobin levels for the second, third, and fourth examinations. In spite of high intra-individual variation for these examinations, average individual levels were different and none of the subgroups were really homogeneous. At the last three examinations, the groups became homogeneous, that is, individual means for the three examinations did not differ more than would be expected from their intra-individual variation. For the total nontherapy group, individuals continued to differ significantly for the later series of examinations but this is due entirely to the low hemoglobin group. If the middle and high groups are combined, individual means are not significantly different and these form a homogeneous group. Thus, two years after the initial examination, individuals with intermediate or high values originally were no longer characterized by different hemoglobin levels although they maintained individual differences for a year or more.

Other Studies of Intra-Individual Variation

Studies of individual variation over various time intervals have been made and it is of interest to compare the periodic variation

at approximately six-month intervals obtained in this Study with individual variation at shorter intervals.

Studies of diurnal variation have been reported by Rabinovitch (2) and McCarthy and Van Slyke (3). Rabinovitch made hemoglobin determinations on twenty men six times during the day, at two-hour intervals from 8 A.M. to 6 P.M., using the oxygen combining capacity method of Van Slyke. The mean standard deviation for intra-individual variation during the day is 5.2 per cent (computed by us). The 100 per cent value for hemoglobin is not reported, but 5.2 per cent is 0.7 to 0.8 gm. McCarthy and Van Slyke made six observations on hemoglobin at three-hour intervals from 9 A.M. to 11 P.M. using the carbon monoxide capacity method. An analysis of their data has been published by Mole (4) who reported that the standard deviation for the six daily values on twelve men was 0.54 volume per cent or 0.40 gm. of hemoglobin. Both studies indicate that diurnal variation may be very large. The daily variation found by Rabinovitch is as great as the highest periodic variation found for any group in our Study; that found by McCarthy and Van Slyke is similar to the minimum periodic variation shown for any group in our Study. On the other hand, apparently under some conditions variation within one day may be small; a standard deviation of 0.19 gm. is probably little larger than the blood sampling and accidental procedural error of the determinations.

Differences in hemoglobin estimates for college women made at intervals ranging from one week to six months have been reported by Donelson, Leichsenring, and Ohlson (5). All determinations were made on morning blood samples. The reported standard deviation for differences in hemoglobin values at one-week intervals is 0.79 gm., at two to six weeks is 0.87 gm., and at seven weeks to six months is 1.20 gm. For comparison with the standard deviations for periodic intra-individual variation shown in Table 7, these standard deviations should be divided by

$\sqrt{2}$ to obtain the standard deviation for variation from the mean of the two estimates used in obtaining differences. On this basis, the standard deviations for variation are: 0.56 gm. at weekly intervals, 0.62 at two to six weeks, and 0.85 at seven weeks to six months; and, as the groups examined were large (238, 137, and 92, respectively), these standard deviations are significantly different.

At one-week intervals the individual variation for these women is greater[9] than that for diurnal variation found by McCarthy and Van Slyke (3). It is about equal to the individual periodic variation (S.D., 0.52 gm.) found in our Study for three examinations at six-month intervals in the third year of the Study, but somewhat lower than the periodic variation (S.D., 0.68 gm.) found for three examinations in the earlier years of the Study. In the study of college women by Donelson, *et al.*, intra-individual variation for intervals of two to six weeks gave a standard deviation of 0.62 gm. and this is similar to the standard deviation of 0.66 gm. obtained in our Study for six observations over a total period of 28 months, and for the longer intervals of seven weeks to six months the standard deviation of 0.85 gm. is significantly higher than the periodic variation found in our Study.

Donelson, *et al.* also report that day-to-day determinations on four women for periods of 27, 28, 39, and 39 days had the following standard deviations: 0.81 gm., 0.54 gm., 0.69 gm., and 0.71 gm. The average standard deviation is 0.7 gm. Over a period of three months, Reich and Green (6) made hemoglobin determinations every three or four days for six young women, obtaining 28 values for each woman. The determinations were made on peripheral blood with a Sahli instrument; all are reported and

[9] Hemoglobin determinations were made on duplicate samples of finger-tip blood by the Newcomber method in the study of Donelson, Leichsenring, and Ohlson. This method has greater error variation than the CO capacity method used by McCarthy and Van Slyke and also has greater error variation than the photoelectric method of Evelyn used in the present Study. Therefore, some of the differences in intra-individual variation may be due to greater error variation.

are given in per cent of hemoglobin. The standard deviations for intra-individual variation over the three months have been computed and converted to grams using 14.0 gms. as 100 per cent, although the 100 per cent value was not reported. For these six women the standard deviations are: 0.70, 0.57, 0.69, 0.73, 0.70, and 0.64 gm., and the average standard deviation is 0.7 gm. Reich and Green were studying the effect of menstruation on hemoglobin values, but found no evidence of variation associated with the menstrual cycle. Thus, the day-to-day variation for about one month and the variation for three to four-day intervals over three months in these two studies are the same, and are as large or larger than the periodic variation shown for our subjects studied over a three-year period.

It is clearly evident that hemoglobin content of the blood is not stable. There is about one chance in three that two values obtained within one day will differ as much as 0.6 gm. or more, and one chance in twenty that the difference will be greater than 1.1 gms. on the basis of the McCarthy and Van Slyke (3) findings. From day to day and at longer intervals, equal or greater differences are to be expected. Therefore, in order to obtain an average hemoglobin level that is typical or representative for an individual, several values should be obtained, preferably on several days at different times of the day. Differences among persons shown by values obtained at a single examination reflect the intra-individual variability of the hemoglobin level and in large part are of no significance.

Summary and Discussion

Fourteen white women who had hemoglobin values at an initial examination from 8.2 to 11.9 gms. were given reduced iron or feosol for periods of 12 to 31 months, and reexaminations were made at intervals after therapy as follows: 10-12 weeks, 7 months, 12 months, 19 months, 25 months, and 31 months. At

no examination was there a significant difference between the mean levels for the iron and feosol subjects.

Three colored women who had initial values from 10.0 to 11.9 gms. took reduced iron and one with an initial value of 11.2 gms. took feosol for 31 months. Their average hemoglobin level was consistently below that for the white women, although at the initial examination (six months before therapy was begun) the average hemoglobin level for the colored and white women was approximately equal. However, after 7 months of therapy, the differences between the colored and white were not significant statistically.

The average hemoglobin level for the white women after 10-12 weeks of therapy was 12.6 gms. and it did not increase significantly at any later examination. The average hemoglobin for colored women after three months of therapy was 11.5 gms. and after 7 months was 12.2 gms; thereafter it did not increase significantly.

A "control" group of white women who had hemoglobin values ranging from 11.5 to 12.0 gms. at the initial examination was reexamined at the same intervals as the therapy group. At every examination period the average hemoglobin level for the "control" subjects was similar to that for therapy subjects. At no examination period did the average hemoglobin levels for the "control" and for the therapy subjects attain the average levels obtained for women who had had higher values at the initial examination and received the periodic reexaminations.

Both therapy and all nontherapy subjects showed large individual variations from one period to another. This intra-individual variation, which is found also within one day and from day to day, is so great that determinations at a single examination are unsatisfactory for classifying persons as having low, average, or high values, since any value obtained may be an extreme fluctuation from the representative, average level of the individual

as a result of accidental technical error, temporary physiological variation, or both. It is interesting, however, that in spite of this individual variation, and the fact that the therapy subjects and "control" subjects were selected on a single determination, both *groups* were characterized throughout the three years by relatively low hemoglobin values. Undoubtedly, there were some women included in each group whose usual or average levels at the beginning of the Study were really considerably higher than their initial values. These individuals cannot be identified and differentiated from those whose average levels initially were subnormal on the basis of the single initial determination.

Although the therapy subjects as a group failed to obtain higher levels than the "control" subjects, subjects in both groups exhibited several patterns of apparent change. But because of the considerable diurnal, and day-to-day variability, it is impossible to categorize them as real changes, on the basis of a few widely spaced examinations. Some exhibited an apparent increase only to be followed by a decline. Two in the therapy group with relatively low initial values showed a slow, steady advance; one of them declined at the last two examinations. The subject with the lowest initial value showed a sharp increase after three months of therapy to a moderate level and thereafter made no further gain. Among subjects with mild degrees of hypochromic anemia who had received iron therapy for sixty days, or continuously during the study to a maximum of twenty-six weeks; Fowler and Barer (7) found that, following a rise in hemoglobin levels for about ten weeks, the majority tended to return to the pretreatment level; others continued to have values somewhat above it. They suggested that the former group actually had only low normal values. This same interpretation could be applied to our therapy subjects whose average level for two and a half years remained below the normal average. Indeed, the over-all failure of the subjects receiving therapy to surpass in hemoglobin level those not

receiving it might also be attributed to the same reason; namely, that all of the subjects in the Study had only low normal hemoglobin values. Such a view connotes that the subject by his very nature possesses low values that cannot nor need not be elevated. Actually, this discrimination between two identically low values, calling the one normal and the other abnormal, is based upon the therapeutic test. Yet it is scarcely permissible to designate low values as normal because they fail to respond to iron; for it does not cover influences that might be preventing an increase, nor does it exhaust all possible therapeutic measures. Hence, designating the subjects as low normal is not the only interpretation.

At least two other possible explanations should be considered in relation to the failure of the group receiving iron to attain higher hemoglobin levels than the nontherapy group. The one is the influence of nondietary conditions in the etiology of deficiency states. According to Heath (8), strong evidence indicates that a diet deficient in iron will not produce iron deficiency unless various stresses upon the body are likewise attendant. Gray and Wintrobe (9) state that chronic hypochromic anemia in women is the resultant of three influences: either faulty alimentary function or defective diet, or both; and excessive requirements for hemoglobin induced by such conditions as pregnancy, menstruation, and menorrhagia. Relapse is common in this type of anemia if increased requirements for hemoglobin persist; but it may often be prevented if the condition necessitating excessive requirements is removed or controlled. On the other hand, infection may retard response to therapy in hypochromic anemia (9, 10, 11). This mechanism should not be overlooked in a therapeutic failure or an incomplete response.

The second possible explanation for the failure of the group receiving iron to gain higher hemoglobin values than the "control" group embodies the evidence that multiple nutrients are necessary for the production of hemoglobin and maintenance of

a maximum level. Obviously iron is one, as reports of therapeutic response attest (7, 9, 12, 17). In 1934 Whipple and coworkers (13) studying anemia induced in dogs by blood withdrawal differentiated between iron and the organic factors in hemoglobin regeneration. Again, these investigators (14), pointing out the dissociation between the potency for hemoglobin production and the iron content of different fractions of liver, reaffirmed the view that not one but several nutrient factors have a potent influence on the regeneration of hemoglobin. Later, Whipple (15) enumerated several nutrient factors conducive to hemoglobin production, among them certain amino acids. Prompt elevation of a low hemoglobin level in a child by administration of amino acids has been reported (16). Obviously, a low hemoglobin level due mostly to protein deficiency, for example, and to a less extent to iron deficiency, would respond only moderately and incompletely to iron alone. Thus, failure to respond either fully or at all to iron could be due to nondietary conditions or a deficiency in hemoglobin-producing nutrients other than iron, or both.

Acknowledgments are made to Mead, Johnson and Company for the reduced iron supplied for the Study.

References

1. Wiehl, Dorothy G.: Accuracy of Hemoglobin Determinations on Finger-Tip Blood. Milbank Memorial Fund *Quarterly,* January, 1946, xxiv, 1, pp. 1-28.

2. Rabinovitch, I. M.: Variations of the Percentage of Hemoglobin in Man During the Day. *Journal of Laboratory and Clinical Medicine,* 1923-24, 9, pp. 120-123.

3. McCarthy, Earl F. and Van Slyke, Donald D.: Diurnal Variations of Hemoglobin in the Blood of Normal Men. *Journal of Biological Chemistry,* May, 1939, 128 pp., 567-572.

4. Mole, R. H.: Diurnal and Sampling Variations in the Determination of Haemoglobin. *Journal of Physiology,* June, 1945, 104, 1, pp. 1-5, London.

5. Donelson, Eva G.; Leichsenring, Jane M.; and Ohlson, Margaret A.: Variability of Certain Factors in the Blood Picture of Women. *American Journal of Physiology,* March, 1943, 138, 4, pp. 626-629.

6. Reich, Carl and Green, Dorothy: Red Cell Regeneration During the Menstrual Cycle. *Archives of Internal Medicine,* March, 1932, 49, pp. 534-538.

7. Fowler, W. M. and Barer, A. P.: Some Effects of Iron on Hemoglobin Formation. *American Journal of Medical Science,* 1941, 201, pp. 642-651.

8. Heath, E. W.: Iron in Nutrition. Chapter vii—Handbook of Nutrition. Chicago, 1943, American Medical Association.

9. Gray, L. A. and Wintrobe, M. M.: Chronic Hypochromic Anaemia in Woman. A Consideration of its Etiology and Treatment, With Special Reference to the Relationship of Gynecologic Disorders. *American Journal of Obstetrics and Gynecology,* 1936, 31, pp. 3-13.

10. Robscheit-Robbins, F. S. and Whipple, G. H.: Infection and Intoxication. Their Influence upon Hemoglobin Production in Experimental Anemia. *Journal of Experimental Medicine,* 1936, 63, pp. 767-787.

11. Cartwright, G. E.; Lauritsen, M. A.; Humphreys, S.; Jones, P. J.; Merrill, I. M.; and Wintrobe, M. M.: The Anemia Associated with Chronic Infection. *Science,* January 18, 1946, 103, 2664, pp. 72-73.

12. Abbott, O. D. and Ahmann, C. F.: Iron Deficiency Anemia in Children. *American Journal Diseases of Children,* October, 1939, 58, pp. 811-816.

13. Whipple, G. H. and Robscheit-Robbins, F. S.: Hemoglobin Production Factors in the Anemic Horse Liver. *American Journal of Physiology,* 1934, 108, pp. 270-278.

14. Robscheit-Robbins, F. S.; Walden, G. B.; and Whipple, G. H.: Blood Regeneration in Severe Anemia. Fractions of Kidney, Spleen and Heart Compared with Standard Liver Fractions. *American Journal of Physiology,* 1935, 113, pp. 467-475.

15. Whipple, G. H.: Protein Production and Exchange in the Body Including Hemoglobin, Plasma Protein, and Cell Protein. *American Journal of Medical Science,* 1938, 196, pp. 609-621.

16. Bass, M. H.: Deficiency Anemia in Infants. Report of two Cases with Associated Temporary Deficiency of Anti-Anemic Factor in one and Allergy and Abnormal Digestion of Protein in the Other. *American Journal Diseases of Children,* May, 1944, 67, pp. 341-347.

17. Widdowson, E. M. and McCance, R. A.: Iron in Human Nutrition. *Journal of Hygiene,* London, February, 1936, 36, 1, pp. 13-23.