PRELIMINARY OBSERVATIONS ON DIURNAL AND OTHER VARIATIONS IN HEMOGLOBIN LEVELS[1]

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IN the course of making hemoglobin studies on large numbers of individuals, primarily school children and teachers, we have carried out therapeutic tests with certain minerals and vitamins on persons having low hemoglobin levels. Adequate control groups always have been set up.

In the first such study set up in Florida, which included 810 school children, we were surprised to find that one subgroup which had received iron for several months showed an average decrease in hemoglobin level of cutaneous blood while the corresponding control group showed an increase. This was rather disconcerting and caused us to check and recheck our procedures rather critically to convince ourselves that these variations were not due to alterations in the technique.

In view of these peculiar results, we rechecked the hemoglobin levels of these two subgroups a few days later, duplicating the previous situation in every way in so far as possible. Results of this test were quite different. As compared with the initial tests done several months before, the control group was essentially unchanged while the group that received iron showed a small average increase in hemoglobin level. No explanation was at hand for this apparent shift in hemoglobin levels of these two groups within a few days.

As part of our attempts at analysis of this situation we tested one small group twice during the *same* day as a preliminary check on duplicability of our technique. The results were still discomforting because differences of as much as 2.5 gms. per 100 cc. were found on some individuals. However, after careful study of the data, it was noted that the lower values, in practically

[1] From Nutrition Investigations and Services, Florida State Board of Health.

every case, were obtained during the afternoon, while the higher values were found in the morning. In view of this apparent instability in hemoglobin levels, with a tendency toward lower values in the afternoon, we reexamined our data on the above-mentioned subgroup receiving iron and its corresponding control group. We found that the initial test on the subgroup receiving iron had been done in the morning and the first recheck in the afternoon while the control group had been tested in the reverse order. The second recheck, however, had been done at the same time of day as the initial test several months before.

Obviously such variations, if disregarded, potentially could have a marked effect on conclusions drawn from therapy in private practice or from therapeutic testing of groups. It seemed incredible that if such instability of hemoglobin values is usual that it was not generally known and was not given prominent consideration in the medical literature on hemoglobin. Also, why was it not mentioned in numerous textbooks on hematology, physiology, and biochemistry? However, Peters and Van Slyke (1) in their QUANTITATIVE CLINICAL CHEMISTRY I, page 549, give two references (2) and (3), and state that the authors of these two papers, after studies on very small groups, "agree in concluding that in a given subject the difference between the lowest and highest hemoglobin content observed during the same day may amount to 20 to 30 per cent of the average content." Peters and Van Slyke conclude: "The fact that a difference of 10 per cent between maximum and minimum hemoglobin content in a day is common, and that greater variations can readily occur, indicates the caution that must be used in interpreting single observations of minor changes or deviations from usual normal levels."

McCarthy and Van Slyke (4) in 1939 studied eighteen young men for hemoglobin changes during the day using the carbon monoxide capacity method. The greatest change reported was 11 per cent of the mean for the day. They state that values were

usually lower during the P.M. than the A.M., but that changes were inconstant. This is in accord with our initial findings.

In 1945 Mole (5) reported an analysis of the data published by McCarthy and Van Slyke. He showed an average fall of about 4 per cent in hemoglobin between 9 A.M. and 11 P.M., but that the changes which occurred between 9 A.M. and 5 P.M. (the working day) were not statistically significant. All of our tests, with the exception of one small group, have been made between 9 A.M. and 5 P.M.

In 1945 Johnson, *et al.* (6) reported that the average hemoglobin value for twelve men was 0.6 gm. lower at 7:30 P.M. than at 7 A.M. on one day, but on two other days the averages at 1 P.M. and 3 P.M. were about the same as at 7 A.M.

The studies mentioned above were on very small groups. The apparent instability of hemoglobin level, if true, could markedly interfere with our program of therapeutic testing unless better understood by us. Therefore, in order to get more specific information on large groups, we made it routine to test each individual both morning and afternoon. On a few individuals we have made tests throughout the course of the day. The results of these tests are described herein.

Comparison of Morning and Afternoon Hemoglobin Values

The results of morning and afternoon hemoglobin determinations on 651 individuals are shown in Table I for six different groups. Most of the tests were done during the usual school day, *i.e.,* between 9 A.M. and 3 P.M. Hemoglobin determinations on these six groups were done with four different "reading" instruments, including two photoelectric colorimeters of the alternating current type, one of the battery type, and one color block with prismatic glass standard. All were calibrated. Within a given group, the same colorimeter was used for the morning and afternoon tests. The same calibrated 20 cu. mm. pipet was used for

A.M. and P.M. tests. The A.M. and P.M. readings of each individual were made in the same glass tube, and were made under as nearly identical conditions as possible. Details of the method have been described by the authors (7).

For each of the six groups the average hemoglobin level is lower in the afternoon than in the morning. This is true for both males and females, and there is little difference by sex for those in the same group. The average differences between morning and afternoon levels vary from 0.5 gm. to 1.0 gm.

Individual differences between morning and afternoon vary widely. While the majority have shown a *drop* in the afternoon value, some individuals have shown a *gain* of as much as 1.6 gms. The numbers in each group with an increase in the afternoon, with no change, and with a decrease in the afternoon are shown

Table 1. Mean hemoglobin values in the morning and in the afternoon for several groups of children and of adults examined in Florida.

Group	Colorimeter Used	Age Range	No. of Subjects		Males			Females		
					Mean Hb.		Diff. A.M.-P.M.	Mean Hb.		Diff. A.M.-P.M.
			M	F	A.M.	P.M.		A.M.	P.M.	
Group A	Battery Lumetron	11-16	72	57	12.9	11.9	1.0	12.4	11.5	0.9
Group B	Glass Standard	6-18	133	129	12.7	12.2	0.5	12.1	11.6	0.5
Group C	Battery Lumetron	6-14[1]	41	56	11.5	11.0	0.5	11.8	11.2	0.6
Group D	Klett-Somerson	Adults	5	27	16.2	15.6	0.6	13.8	13.2	0.6
Group E	A.C. Lumetron	Adults	16	49	14.2	13.2	1.0	12.7	12.0	0.7
Group F	Battery Lumetron	12-19	66		13.4	12.9	0.5			

[1]In addition to eighty-nine children, there are eight adult women in this group.

in Table 2, and the mean difference and standard error of the difference are given. For every group the mean difference is very significant statistically.

Readings for Group A, which is composed of 129 seventh and eighth grade children, were made with a battery type photoelectric colorimeter. Five children showed no change, 11 showed an increase in the afternoon, and 113 showed an afternoon decrease. The greatest increase was 1.6 gms. and the average gain was 0.4 gms. for those who showed an afternoon gain. The greatest afternoon drop was 4.5 gms. Fifty-one children, or 40 per cent, showed a decrease of 1 gm. or more.

Table 2. Distributions of differences between morning and afternoon hemoglobin values for individuals in six different groups examined in Florida.

Difference A.M.-P.M. Grams	Total Subjects		Number of Subjects					
	Number	Per Cent	Group A	Group B	Group C	Group D	Group E	Group F
Total	651	99.9	129	262	97	32	65	66
Higher P.M.								
−1.6-2.0	1	0.2	1					
−1.1-1.5	3	0.5	1	2				
−0.6-1.0	13	2.0	2	7	1		1	2
−0.1-0.5	55	8.4	7	30	8	2	1	7
No Change	38	5.8	5	11	10	2	3	7
Lower P.M.								
+0.1-0.5	198	30.4	19	89	31	14	24	21
+0.6-1.0	204	31.3	50	67	41	8	21	17
+1.1-1.5	95	14.6	22	42	6	5	10	10
+1.6-2.0	27	4.1	9	12			4	2
+2.1-2.5	12	1.8	10	1			1	
+2.6-3.0	2	0.3		1		1		
+3.1-3.5	1	0.2	1					
+4.1-4.5	2	0.3	2					
Mean Difference	+0.63		+0.91	+0.54	+0.45	+0.59	+0.70	+0.48
Standard Error	±0.026		±0.077	±0.038	±0.042	±0.105	±0.068	±0.070
St. Deviation	0.668		0.869	0.609	0.418	0.595	0.546	0.569

The 262 children in Group B were in a consolidated school including grades one through twelve. Finger blood was used and the hemoglobin determinations were made by diluting 20 cu. mm. of blood in a calibrated tube to match a glass standard. As previously reported (7) we have found this method quite satisfactory. We have been primarily interested in testing this method since many health departments throughout the country are using it. On this group the findings are essentially the same as with other groups tested by more refined methods. Eighty per cent of the children in this group showed an afternoon drop in hemoglobin level, the mean drop for the whole group being 0.5 gm. per 100 cc.

Group C is composed of eighty-nine rural school children and eight women teachers and cafeteria workers in the school. The determinations were made with a battery type Lumetron photoelectric colorimeter and 80 per cent of the group showed a decrease in afternoon hemoglobin levels. The mean afternoon drop was 0.5 gm. The mean hemoglobin levels both in the morning and afternoon were rather low according to any standards. About 50 per cent of these children were infested with hookworms.

For Group D, composed of thirty-two school teachers including five men and twenty-seven women, morning and afternoon levels on capillary blood were made with a Klett-Somerson photoelectric colorimeter which had been calibrated to allow thirty minutes for acid hematin color development. All the men except one showed an afternoon drop in hemoglobin level; and their mean drop was 0.7 gm. Two of the women showed no change between morning and afternoon, one showed an increase, and twenty-four showed a decrease. The mean afternoon drop for the entire twenty-seven women was 0.6 gm.

This group differed from the others in that the first test was done one afternoon between two and three o'clock, and the morning test was done on the *following* morning between nine

and ten o'clock. This sequence was used since there had been some question in our minds as to whether the higher levels usually obtained in the morning might not be due partially to the greater apprehension or excitement which most likely would occur at the first test. However, in spite of having done the afternoon test first, the majority of this group showed a lower capillary blood hemoglobin level in the afternoon.

Group E included sixty-five teachers, forty-nine women and sixteen men. The greatest afternoon drop among the men was 2.2 gms. and the mean difference was 1.0 gm. Every man in the group had a lower hemoglobin value in the afternoon. Of the forty-nine women, two showed an increase during the afternoon, three showed no change, and forty-four showed a decrease, the greatest decrease being 2.0 gms. and the mean 0.7 gm. The morning tests were done between 10 and 12 o'clock and the afternoon tests between 2:30 and 4:40. The determinations were made on an alternating current type Lumetron photoelectric colorimeter which was calibrated for a five-minute color-development period for acid hematin.

The sixty-six high-school boys who comprise Group F were members of three football teams and they were tested with a battery type photoelectric colorimeter, as were Groups A and C. These boys were used as subjects for special tests to obtain data on the effect of exercise on hemoglobin levels and these data are discussed below. Values used for Tables 1 and 2 are from determinations made before exercise in the morning and in the afternoon. This Group shows results similar to those obtained for the other Groups.

Hemoglobin Changes Throughout the Day

All of the tests discussed above were done once in the morning and once in the afternoon, the actual times being quite variable. We felt that a small group should be tested several times during

the day using both venous and capillary blood. For this purpose, twenty-four college girls, aged 18 to 26, volunteered for five venipunctures and five finger pricks during a single day. Data for three girls are omitted because one girl missed the late afternoon test, another missed the evening test, and the third fainted immediately after the venous blood sample was drawn at the two o'clock test and her hemoglobin level at this test was much lower than that for any of the other tests. Duplicate or triplicate hemoglobin tests were done on each venous and capillary blood sample. The acid hematin method was used and the readings were made with a battery-type Lumitron photoelectric colorimeter.

Table 3. Average hemoglobin values at different hours of the day for college girls tested five times during one day on both a venous and capillary blood specimen at each test.

Time of Test (Approximate)	Capillary Hemoglobin Mean Gms.	Venous Hemoglobin Mean Gms.
11 Subjects:		
7 A.M.	12.73	12.43
10 A.M.	12.80	12.67
2 P.M.	11.87	11.87
4 P.M.	11.68	11.89
8 P.M.	12.01	11.93
Average for Day	12.22	12.16
10 Subjects:		
8 A.M.	13.15	12.77
12 Noon	12.79	12.83
3 P.M.	12.13	12.12
5 P.M.	11.79	11.74
9:30 P.M.	12.27	12.26
Average for Day	12.43	12.34

The twenty-one girls for whom average hemoglobin values are shown in Table 3 are divided into two groups. For one group, the hours at which tests were made were as follows: between 6:30 and 7:30 A.M. except for one test at about 8 o'clock, between 9:30 and 10:30 A.M., between 1:30 and 2:30 P.M., between 3:30 and 4:30 P.M., and between 7:30 and 8:30 P.M. except for one test at about 9 o'clock; for the second group, the tests were made at the following hours: between 7:30 and 8:30 A.M., between 11:30 A.M. and 12:45 P.M. except for one test at 11 o'clock, be-

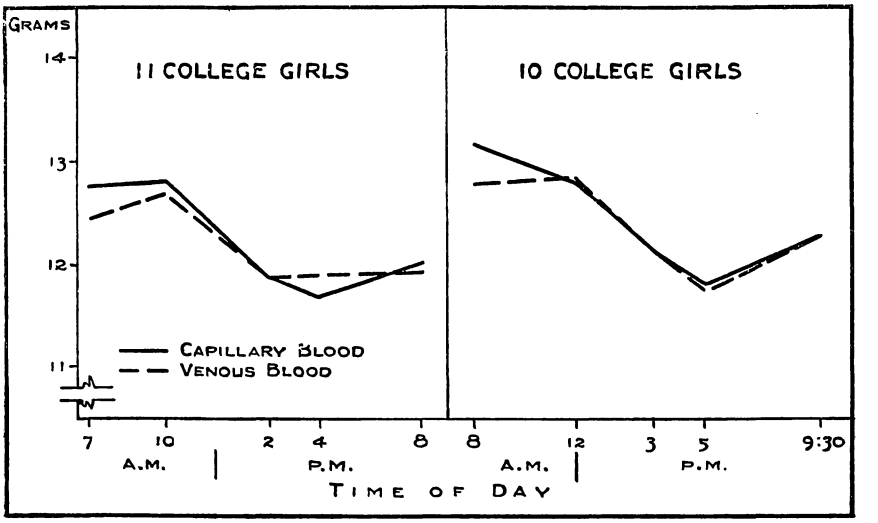

Fig. 1. Diurnal variation in hemoglobin levels of twenty-one college girls tested five times in one day. Average grams of hemoglobin per 100 cc. of whole blood for eleven girls tested at approximately the same hours and for another group of ten girls tested at approximately the same hours.

tween 2:45 and 3:30 P.M., between 4:45 and 5:45 P.M., and between 9 and 10 P.M. except for one test at 8 o'clock. Average values for each group for both venous and capillary blood at the five examinations are shown in Figure 1.

The curves for venous and capillary blood show a distinct tendency to be parallel. However, early in the morning, mean hemoglobin levels for capillary blood were somewhat higher[2] than those for venous blood. The principal drop in hemoglobin values, in both capillary and venous blood, occurred between the 10 and 2 o'clock tests and between noon and 3 P.M. The values continued low or showed a further slight decline in the late afternoon. The average evening values at 8 o'clock on venous blood are the same as in the afternoon, but on capillary blood a slight but significant increase over the 4 o'clock average is shown

[2] For the 105 hemoglobin determinations on both venous and capillary blood, the mean difference is 0.07 ± 0.04 gm., and the difference is not significant. At the early morning test, much higher capillary than venous values were obtained for three subjects, the differences being 1.3, 1.4, and 1.8 gms.; no other differences exceeded 0.9 gm.

(P. is .02-.05). The average values at 9:30 P.M. on both venous and capillary blood are significantly higher than at 5 o'clock.

Most of the individual curves corresponded in general with the group pattern. Six of the eleven girls had their minimum capillary value at 4 o'clock, four at 2 o'clock, and one had the same minimum value at 2 and also at 4 o'clock; on venous blood, nine of the eleven girls had a minimum value at either 2 or 4 o'clock but two of them had a minimum value at 8 o'clock. In the other group of ten girls the capillary value was at a minimum at 5 o'clock for eight and at 3 o'clock for two; the venous value was at a minimum at 5 o'clock also for eight, at 3 o'clock for one girl and at 8 A.M. and 10 P.M. for one girl.

The five greatest differences in capillary blood during the day were 1.6, 2.0, 2.0, 2.1, and 2.9 gms. The five greatest drops in venous blood hemoglobin were 1.5, 1.6, 1.7, 1.8, and 2.1 gms. All represented differences between morning and afternoon. These changes in both capillary and venous blood show very definitely that, under the conditions of this experiment, blood hemoglobin is quite variable during the day. One hemoglobin determination on an individual, even when carefully and accurately made, may give results quite different from another determination made at some other time.

Five determinations during the day, of course, greatly increase the chance of getting blood samples near the maximum and minimum hemoglobin levels as compared with taking only two samples during the day. This would account for the higher percentage of the twenty-one college girls showing greater changes in hemoglobin levels during the day than did any other group. It seems likely that actual differences between the morning maximum and the afternoon minimum may be greater than shown by the mean differences *observed* when only two tests are done, since the chances are against getting the two samples at the exact times that these extremes occur in the blood stream.

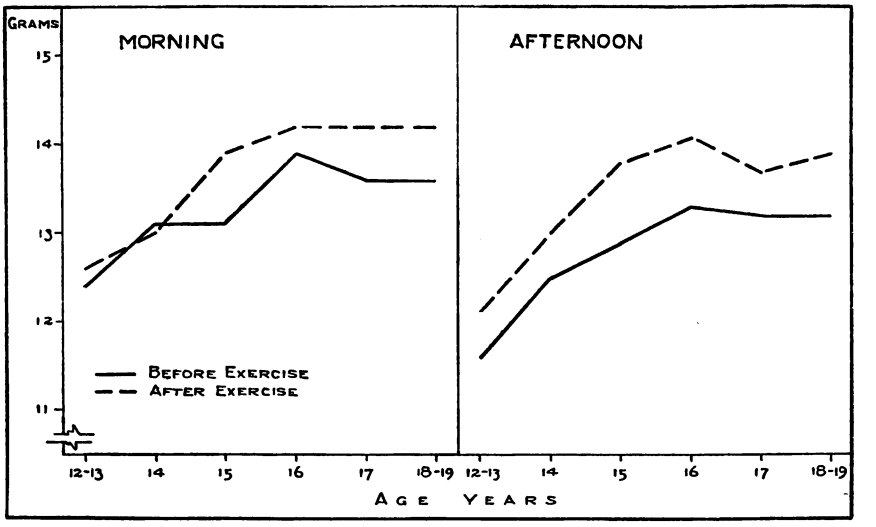

Fig. 2. Average hemoglobin levels before and after exercise for tests made in the morning and again in the afternoon on sixty-six high school boys classified according to age.

Effect of Several Factors on Hemoglobin Level

In order to check certain factors which are thought or known to produce fleeting changes in hemoglobin level, on limited groups we have recorded pulse rates at the time the blood was taken, made tests before and after exercise, and tested individuals who skipped the mid-day meal.

"Excitement." We have observed repeatedly that individuals coming up for blood tests, although attempting to appear calm, actually may be quite excited. Excitement is said to increase the blood hemoglobin level ((1), p. 557). To check this on a larger scale, we took pulse rates on several hundred children and adults at the time the morning and afternoon blood specimens were being taken. In a typical group of 361 children, pulse rates averaged 104 both in the morning and in the afternoon. There seemed to be no correlation between pulse rate as such and temporary hemoglobin level.

"Exercise." Figure 2 shows the finger blood hemoglobin levels

of sixty-six high school boys before and after exercise, both morning and afternoon, by age groups. There was an increase in the "resting" hemoglobin level with age, which is to be expected for boys of high school age. As previously shown (Group F, Tables 1 and 2), resting levels are lower in the afternoon and this is consistent for the different age groups. The exercise consisted of a ten-minute trot on a cinder track followed by a one-minute rapid sprint.

After exercise, the average hemoglobin level was higher than before exercise both for the morning tests and the afternoon tests. The mean difference for the total group in the morning is 0.39 gm. and in the afternoon is 0.66 gm.; both of these differences are statistically significant. A greater elevation in hemoglobin after exercise for the afternoon tests is consistent for each age group, and the greater increase in the afternoon than in the morning is statistically significant.

The distribution of individual differences between resting

Table 4. Individual differences between hemoglobin values obtained before and after exercise from tests made in the morning and the afternoon on sixty-six boys ages 12-19 years.

Difference (After Ex.-Resting) Hb. Gms.	Morning Tests		Afternoon Tests	
	Number	Per Cent	Number	Per Cent
Total	66	99.9	66	100.0
-0.6-1.0	2	3.0	1	1.5
-0.1-0.5	8	12.1	2	3.0
No Change	10	15.2	4	6.1
+0.1-0.5	22	33.3	17	25.8
+0.6-1.0	20	30.3	33	50.0
+1.1-1.5	2	3.0	7	10.6
+1.6-2.0	0		1	1.5
+2.1-2.5	2	3.0	1	1.5
Mean Difference	+0.39		+0.66	
St. Error	±0.070		±0.059	
St. Deviation	0.566		0.479	

hemoglobin values and after exercise values is shown in Table 4. In the morning ten subjects showed a drop after exercise and ten showed no change, leaving forty-six, or 70 per cent, that increased. In the afternoon, only three cases showed a decrease after exercise, four showed no change, and fifty-nine, or 89 per cent, showed an increase. The maximum increase in the morning was 2.4 gms. and in the afternoon was 2.5 gms.

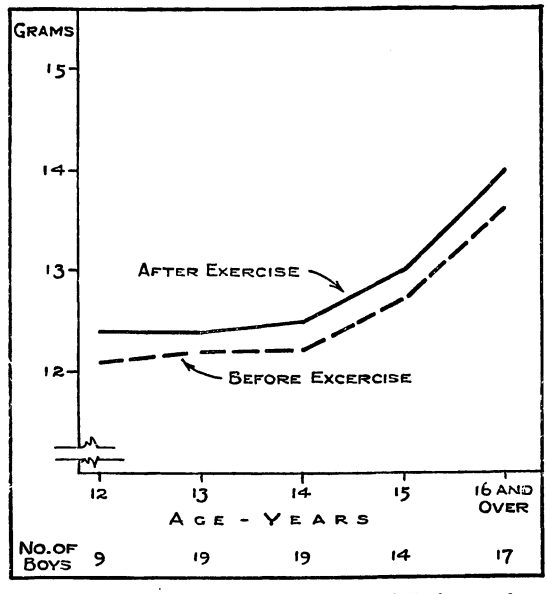

Fig. 3. Average hemoglobin levels before and after exercise for seventy-eight high school boys classified according to age. Grams of hemoglobin per 100 cc. of venous blood.

Hemoglobin elevations of *venous* blood following the type of exercise mentioned above are shown in Figure 3 for seventy-eight boys 12 years of age or older. At each age, the average hemoglobin level after exercise is higher than before exercise. The increase with exercise is 0.2 to 0.5 gm. for the different age groups.

"Effect of Mid-Day Meal." We have not as yet studied this factor on a large scale but have merely observed that individuals who skipped the mid-day meal, for one reason or another, showed no trend in hemoglobin values different from those who had a large one. This is now being studied on larger groups.

"Temperature." Most of our work has been done either in school buildings or in a trailer-laboratory. Thus it has not been possible to control the temperature. Study of our data seems to show a slight trend toward less afternoon drop in hemoglobin

level when the weather was unusually cool in the afternoon. This, however, is a very preliminary observation. Plans are under way to study this more specifically on a group of college students. We have arranged to use the facilities of a cold storage plant having large rooms maintained at different temperatures.

Summary

Data on morning and afternoon hemoglobin determinations are presented for 651 adults and children. These show appreciable variation during the day and the majority of individuals tested show lower levels in the afternoon. This was found for determinations on venous and on capillary blood.

Twenty-one college girls were tested five times during the day from 7 A.M. to 10 P.M. for hemoglobin values on both venous and capillary blood. The highest values obtained were in the morning for every subject and the lowest were between 2 and 5 o'clock in the afternoon with the exception of three subjects for whom venous values, but not capillary, were lowest at 8 to 10 P.M. There was a significant tendency for values to rise in the evening above the afternoon minimum value.

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