A survey of hemoglobin values in Canada

Summary: A compilation of hemoglobin values has been made from submissions from laboratories in Canada using the cyanmethemoglobin standard prepared and distributed by the Canadian Communicable Disease Centre (formerly the Laboratory of Hygiene). From 84 participating laboratories 21,580 values were analyzed statistically by age and sex. "Medical referrals", exclusive of blood dyscrasias, were included but were documented separately from "well persons". In most age groupings no significant difference in these two categories was found.

Values for boys and girls were similar up to 12 years of age. For adult women from 18 to 84 years the mean value was 13.0 g. per 100 ml. (95% confidence limits 10.8-15.2 g.); for pregnant women 19 to 44 years the mean value was 12.2 g. per 100 ml. (9.7-14.6 g.). For men aged 17 to 24 years the mean value was 15.0 g. per 100 ml. (12.8-17.3 g.); 25-49 years 14.6 g. per 100 ml. (12.4-16.9 g.); 50-69 years 14.3 g. per 100 ml. (11.8-16.8 g.). It is noteworthy that for the most part the mean values were slightly lower than those frequently quoted as "normal" and that the mean values, particularly for the male, were lower with increasing age.

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Determination of the concentration of hemoglobin in the blood is one of the most frequently performed estimations in the clinical laboratory. The significance of this measurement for decisions regarding blood transfusions, blood donations and the diagnosis and prognosis of anemia is so well known that it does not require comment. Fundamental to each decision is the knowledge of the value, or range of values, which should be considered normal for the person concerned. "Normal values" for hemoglobin, as for many body constituents, have frequently been determined from testing only a small number of persons in a single laboratory. The values for hemoglobin have often been determined by methods of hemoglobinometry which are subject to considerable error. After the recommendation that a cyanmethemoglobin solution should be adopted as a photometric standard for hemoglobinometry,[1] the Laboratory of Hygiene began the distribution of such a solution in Canada.[2] It was believed that a realistic assembly of "normal" hemoglobin values in this country could be obtained by compilation of hemoglobin values from laboratories using this standard.

Plan of survey

A preliminary canvass of opinion utilizing a questionnaire sent to 21 laboratory directors of four types of establishments in eight provinces indicated that response would be sufficiently encouraging to warrant proceeding with the survey and that data processing would be the most efficient means of handling the returns. Accordingly, the Research and Statistics Directorate of the Department of National Health and Welfare was consulted early in the planning stage to enlist their aid in designing forms which could be coded readily for machine processing. A descriptive folder of general instructions and forms for recording data were sent to all laboratories which were on the mailing list to receive cyanmethemoglobin standard from the Laboratory of Hygiene (in 1963). Most of these laboratories were in hospitals. Aside from the practical advantage in being able to make use of "normal" values from hospital staff, it was believed that information about the hospitalized population would be worth while. It was requested that such "medical referrals" should be documented clearly and should not include any person known to have or suspected of having anemia or any condition affecting the liver, spleen or bone marrow. Also to be excluded were persons who had lived at an altitude higher than 5000 feet above sea level within six months previous to the measurement of hemoglobin, and persons who had given a blood donation within the previous four months. Because this study was concerned with a realistic approach to hemoglobin values which are being accepted as normal in everyday practice, there was no attempt to standardize various conditions such as the time of day, season of the year, posture, the interval since a meal and physical activity before measurement. Although it is generally recognized that such factors do have a bearing on the concentration of hemoglobin in any

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A more comprehensive report including detailed tables and a classified bibliography listing is available on request: National Health and Welfare Report, "A Survey of Hemoglobin Values in Canada". Address all correspondence to Mrs. M. W. Weatherburn, Canadian Communicable Disease Centre, Department of National Health and Welfare, Ottawa 3, Ontario.

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TABLE I
Participation in survey – Geographical distribution of laboratories and persons

Location	Laboratories receiving cyanmethemoglobin standard, January 1963		Laboratories contributing hemoglobin values*		persons from whom hemoglobin values were submitted		Category				
							Well		Medical referral		Female, pregnant, at delivery, etc.
	No.	%**	No.	%**	No.	%**	Male	Female	Male	Female	
Newfoundland	2	0.5	–	–	–	–	–	–	–	–	–
Prince Edward Island	1	0.3	–	–	61	0.3	28	33	–	–	–
Nova Scotia	20	5.1	4	4.8	608	2.8	167	120	145	176	–
New Brunswick	15	3.8	4	4.8	103	0.5	8	37	35	10	13
Quebec	98	24.8	15	17.9	1,609	7.5	460	515	299	257	78
Ontario	124	31.4	37	44.0	12,489	57.9	2,537	3,165	2,963	3,092	732
Manitoba	26	6.6	6	7.1	188	0.9	49	95	23	20	1
Saskatchewan	19	4.8	3	3.6	1,172	5.4	430	413	165	164	–
Alberta	51	12.9	9	10.7	3,521	16.3	570	1,074	686	771	420
British Columbia	37	9.4	6	7.1	1,829	8.5	584	1,049	98	83	15
Northwest Territories	1	0.3	–	–	–	–	–	–	–	–	–
Yukon	1	0.3	–	–	–	–	–	–	–	–	–
Total	395	100.0	84	100.0	21,580	100.0	4,833	6,501	4,414	4,573	1,259

* In addition three laboratories submitted values undocumented for age and hence could not be included.
**Percentages may not total exactly because of rounding.

individual at a specific time, it was believed that such variation would be slight in comparison with the overall range of normal for any one age group. There was no attempt to record the racial or ethnic origin unless the persons were from a group with a special socio-economic environment, current opinion being that any differences found in persons of different racial origin are the result of nutritional habits rather than racial origin itself.

Response

Response of participating laboratories is shown in Table I. Eighty-four laboratories out of the total of 395 on the mailing list contributed acceptable values, i.e. values from persons adequately documented by age, sex and, where applicable, medical diagnosis. Geographical distribution of the participating laboratories is roughly comparable to that of the laboratories receiving the standard, the largest numbers being in Ontario and Quebec. The total number of values, 21,580, represents a ratio of about 1:1000 of the total population. As outlined above, the project was not planned to provide a controlled sample of the population of the country; hemoglobin values were submitted voluntarily from the case-load of participating laboratories.

As expected, laboratories in hospitals composed the largest group of contributors, 65 out of 84; 39 of these were from hospitals with less than 300 beds. The total number of values from small hospitals was not disproportionately large because many of these laboratories returned a single data page listing 15 values or less. Distribution of values classified by category of persons showed that many values for "well" persons were submitted from laboratories in industry, government and universities. These were primarily from routine medical check-up of employees and from surveys conducted by the Nutrition Division of the Department of National Health and Welfare, mostly of children of school-age and of elderly persons. Breakdown of the hemoglobin values by residence showed a fairly even distribution of values among healthy and medical referrals, both male and female, in several provinces. The proportion of healthy persons in British Columbia, Alberta, Quebec and Prince Edward Island was higher than medical referrals because of surveys conducted by the Nutrition Division. With the exception of Prince Edward Island, from which all of the values were from persons over 60 years of age, the values from each province covered a range of age groups. Thus 92% of the various age categories were from five or more provinces.

Medical referrals were classified according to the system outlined by the World Health Organization;[3] diseases of the blood and blood-forming organs, Group IV of this classification, were excluded from this study. Several diagnostic categories were represented in every age group; most frequently there were nine or more such groupings.

Methodology

The photometric instruments and pipettes for measuring blood and diluent used in the survey are listed in Table II. Recorded in use were 36 spectrophotometers by laboratories reporting 7129 values and 50 colorimeters by laboratories reporting 14,451 values. Coleman Jr. spectrophotometers were used in 30 laboratories from which approximately 31% of the values were submitted. Fisher hemophotometers were used in 14 laboratories submitting almost 32% of the values. Two laboratories which did not perform hemoglobin measurements routinely submitted values from Beckman DU spectrophotometers.

Sahli pipettes were used in 79 laboratories accounting for 93.4% of the values, three laboratories used combination Newcomer pipettes for measuring blood and diluent; and two used precalibrated capillary tubes. A variety of measurement techniques was used for measuring the diluent, including many repetitive or automatic types.

Two laboratories, reporting 77 values, used the oxyhemoglobin procedure, standardized by cyanmethemoglobin standard.

Hemoglobin values

Hemoglobin values were analyzed separately for male and female, well and medical referral, in age groupings by day up to 30 days, by month up to 23 months, by year from 2 to 19 years and by five-year groupings from 20 to the oldest, 96. The mean, standard deviation and 95%

confidence limits (±1.96 s) were calculated for each age category. In the category of "well" were persons who were not suspected of having any illness, i.e. persons whose hemoglobin had been measured in the conduct of a routine medical examination, in a survey by the Nutrition Division, or specially for this survey. Medical referrals, as mentioned previously, had been screened so that persons with known or suspected disorders of the blood would not be included.

Testing for significance of difference between well and medical referral mean values was done by the t test,[4] based on the actual values as recorded to the nearest tenth gram per 100 ml. of blood. For the female aged 2 years or over, few significant differences were found, and only for ages under 18 years. For the male, as well as at some of the earlier ages, significance was found at several of the five-year groups between 20 and 59 years. When the means were considered in terms of nearest whole grams, however, many of the differences themselves became zero. In addition, it was doubtful if any real categorical difference existed between many of the survey cases which had been coded as "medical referral" and those which had been coded as "well". Since practical application of the data for present purposes did not require greater refinement than expression of the results in whole grams, it was considered permissible to combine the two sets of data. No overall lowering of trend in mean values resulted, since means for medical referrals were often higher, particularly for the male in the 20-59 years age span, than for well persons.

Application of the statistical test for significance of difference between the mean values for male and female showed that, except at age 10, there was no difference each year from 2 to 12 years and thus it was possible to combine the values at these ages. In age categories younger than 2 years, i.e. by month and by day, the numbers of values were too small for statistical interpretation. Because little difference between the sexes in children from 2 to 12 years was found in this study and because there has never been any reported in infants and young children in other studies, it seemed valid to combine the values for male and female infants and children up to 23 months of age also.

Actual hemoglobin values by day for infants of less than one month are plotted in Fig. 1. Values for less than one day, 19 in all, were widespread, ranging from 13.8 to 27.6 g. per 100 ml.; five of these values were stated to be from cord blood. Values for the first week ranged from 10.8 to 25.8 g. per 100 ml., the mean value being 19.2 g. per 100 ml. for 114 val-

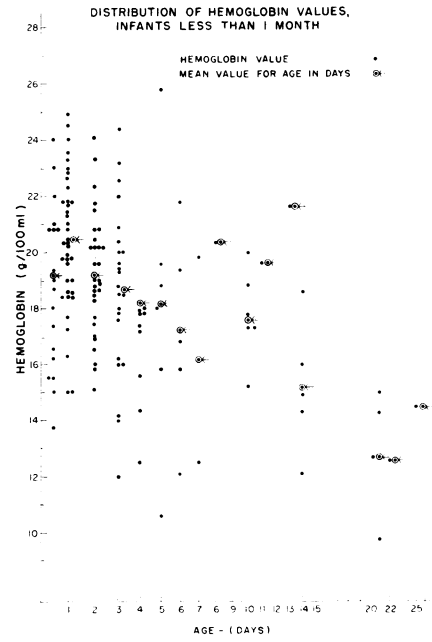

FIG. 1

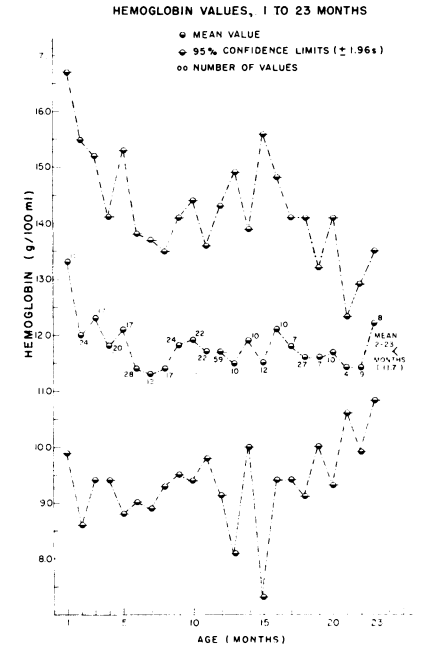

FIG. 2

TABLE II
Equipment used in survey

Equipment	Contributing laboratories No.*	Contributing laboratories %**	Hemoglobin values No.	Hemoglobin values %
Instruments				
Spectrophotometers:				
B & L Spectronic 20	3	3.5	95	0.4
Beckman B	1	1.2	228	1.1
Beckman DU	2	2.3	132	0.6
Coleman Jr.	30	34.9	6,674	30.9
Colorimeters:				
Beckman C	1	1.2	15	0.1
Cenco Sheard Sanford	2	2.3	2,190	10.1
EEL Portable	1	1.2	72	0.3
Fisher Electrophotometer	3	3.5	1,592	7.4
Fisher Hemophotometer	14	16.3	6,820	31.6
Hellige	4	4.7	463	2.1
Klett Summerson	11	12.8	1,714	7.9
Leitz	2	2.3	353	1.6
Leitz Rouy Photometer	10	11.6	1,022	4.7
Lumetron 401A	2	2.3	210	1.0
Total	86	100.0	21,580	100.0
Pipettes for measuring blood:				
Sahli	79	94.0	20,153	93.4
Combination (Newcomer)	2	2.4	535	2.5
Sahli and Combination	1	1.2	546	2.5
Capillary tubing, precalibrated	2	2.4	346	1.6
Total	84	100.0	21,580	100.0
Pipettes for measuring diluent:				
Transfer	24	27.6	5,276	24.4
Mohr	17	19.5	1,471	6.8
Serological	3	3.4	316	1.5
Combination (Newcomer)	2	2.3	535	2.5
Automatic pipette	15	17.2	6,337	29.4
Burette	3	3.4	253	1.2
Automatic burette	3	3.4	1,797	8.3
Automatic pipetting device	17	19.5	5,113	23.7
Automatic pipetting machine	2	2.3	476	2.2
Automatic syringe	1	1.1	6	<0.05
Total	87	100.0	21,580	100.0

* Total number of contributing laboratories = 84; a few laboratories used more than one type of instrument and diluent pipette.
**Percentages may not total exactly because of rounding.

ues. For the second week the values ranged from 12.5 to 20.0 g. with a mean of 18.2 g. for 11 values. For the third and fourth weeks the values ranged from 9.8 to 18.7 with a mean of 14.2 g. for 12 values.

Mean values and 95% confidence limits for ages 1 to 23 months are shown in Fig. 2. Values obtained for these ages were not numerous and the standard deviations ranged from 0.4 to 2.1. The mean hemoglobin for children aged 1 month but less than 2 months was 13.3 g. per 100 ml. For those aged 2 to 23 months the overall average was 11.7, varying by month between 11.3 and 12.3 g. per 100 ml. It has been well documented that hemoglobin values are high and variable at birth and for the first few days, and that they stabilize to a narrower range after the first few weeks. This trend is evident in the present study in spite of the small number of values reported at these ages.

The mean values and 95% confidence limits for ages 2 years and over are shown in Figs. 3, 4 and 5. For the male, mean hemoglobin values increased up to a maximum of 15.1 g. per 100 ml. at 18 years, then declined gradually with age, the change after 55 years being slightly more rapid. Values for elderly persons were few in number and generally the values for the well persons at these ages tended to be a little higher than those for medical referrals; these have been indicated separately in the illustrations. The mean hemoglobin for well males 65 to 69 years of age (eight values) is remarkably out of line and probably indicates poor sampling for this age group.

Except at age 10, the mean values for the female were the same as for the male up to 12 years and then reached a maximum of 13.5 g. per 100 ml. at 13, 14 and 17 years. By age 19 the mean hemoglobin level had dropped slightly—to 13.0 g. per 100 ml.; it then remained remarkably constant close to that level up to 84 years of age. A tendency towards decrease after ages 60-64 is very slight; values for the 843 females 65 to 84 years of age maintained an average of 12.9 g. per 100 ml.

Combinations of age groups were made when there appeared to be a levelling of values. A summary of these data is given in Table III. In general these groupings are indicated by significances found for the differences between means for consecutive ages and age groups, and thus are intrinsic in the data, but they should not be interpreted as indicative of sudden changes in hemoglobin values at certain ages. Frequency distributions of the grouped data are presented in Fig. 6 (a, b, c and d). Generally these appear to be fairly normal distribution curves, except for those 85 years and over, which are definitely skewed toward low values.

Values for 295 pregnant women aged 19 to 44 years averaged 12.2 g. per 100 ml.; values at delivery for 471 women averaged 12.0 g. per 100 ml. The postpartum mean values were slightly lower, 11.1 g. per 100 ml. for 131 values up to the sixth day post partum. These averages and also the mean values found for each month of pregnancy and for a few days after delivery are given in Table IV. In a study in Trinidad it was found that there was a distinct drop in hemoglobin values as the pregnancy progessed.[5] In the present study there was a definite lowering of hemoglobin values but the change was not progressive. The actual values compare quite closely with those from two groups of pregnant women receiving iron therapy, rather than from the control group which did not, in a study by de Leeuw, Lowenstein and Hsieh.[6] It seems possible that some of the pregnant women in the present study were taking increased iron in the diet or in fact supplemental iron, although values from persons known to be on iron therapy were supposed to be indicated so that these could be analyzed separately. For pregnant women "on iron" the 58 values averaged 11.8 g. per 100 ml. and for 203 women at delivery the average was 12.1 g. per 100 ml.

From the results of this survey two facts of extreme interest emerge. Firstly, the mean hemoglobin values arrived at were, for the most part,

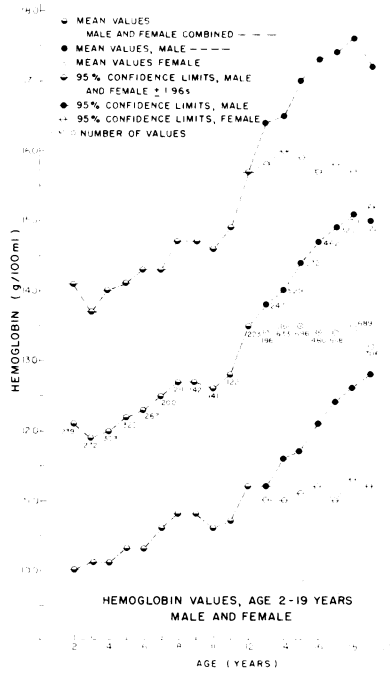

FIG. 3

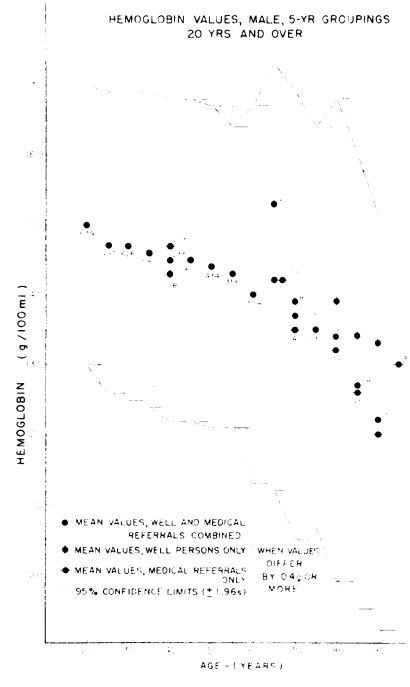

FIG. 4

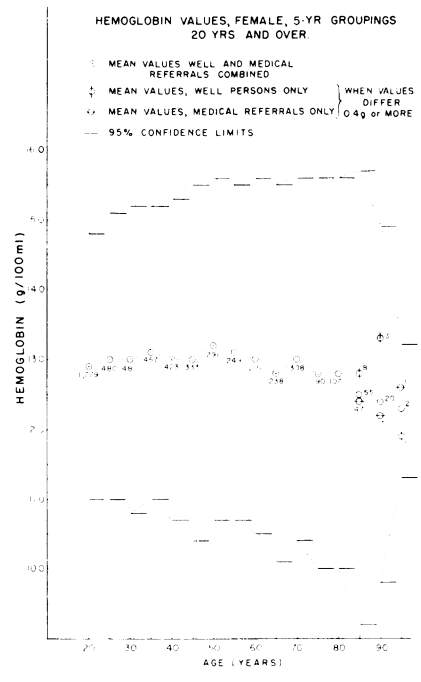

FIG. 5

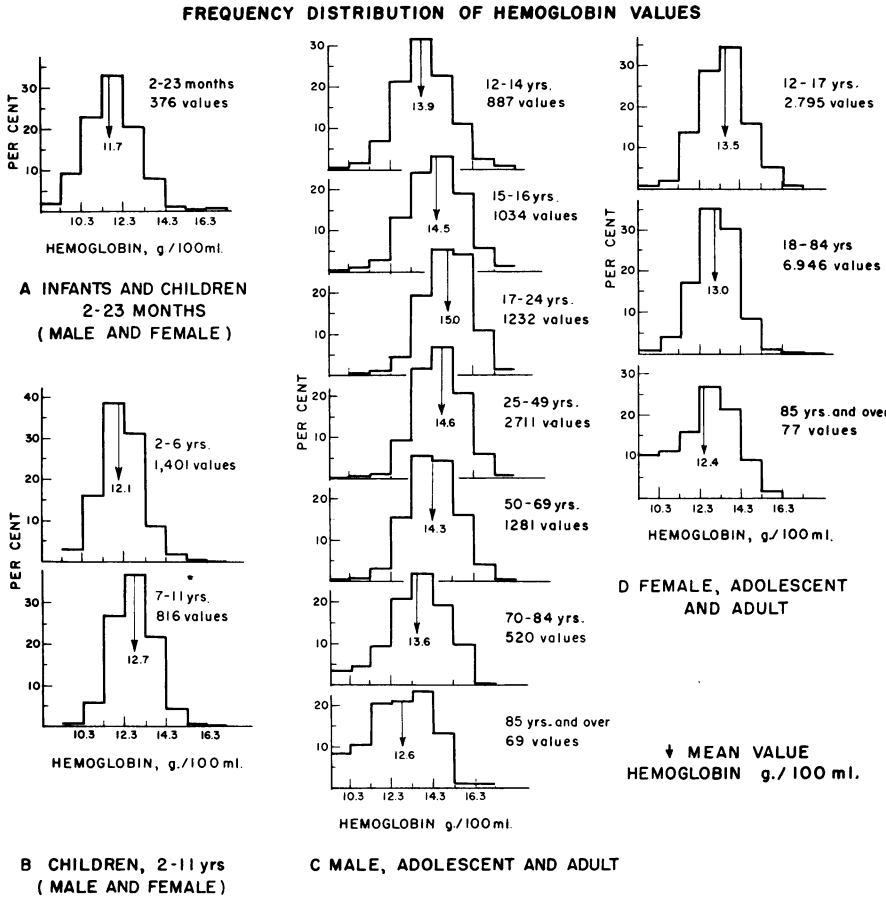

FIG. 6

lower than values frequently reported as "normal", and secondly, for adults, they decreased with increasing age, particularly in the male. Most surveys have been concerned with a specific group of persons rather than with a wide range of age groups. Possibly it is for this reason that the change with age has not been stressed even though a few other surveys such as one in this country by Hawkins, Speck and Leonard[7] in 1954 and in Norway in 1963 by Natvig[8] have pointed out this decrease in the male. It is of interest that the hemoglobin values reported by Hawkins and his colleagues in the Halifax area are very similar to the values in the present Canada-wide survey, the only exception being the values for children 6 to 12 years, which were about 0.5 g. per 100 ml. higher in the Halifax survey. From an analysis of some records of the American Red Cross Society, Hervey, Adashek and Gibson[9] concluded that the hemoglobin level must decline with age because the rate of rejection of older men was higher even though these prospective donors felt physically fit.

In specifying "normal" hemoglobin values, texts usually record only a single value and range of values for the adult male and a single value and range of values for the adult female. The most widely quoted text, Wintrobe,[10] specifies 16.0 ± 2.0 g. per 100 ml. for the male and 14.0± 2.0 g. per 100 ml. for the female. Obviously these values are unrealistic as applied to a large segment of the population in the present survey. Surveys of hemoglobin levels are frequently conducted to determine the incidence of anemia in a segment of the population. Such surveys must of course be based on a preliminary decision as to the cut-off level below which anemia would be suspected. The cut-off level suggested by the World Health Organization[11] is 14 g. per 100 ml. for the adult male and 12 g. per 100 ml. for the adult female. If these figures were to be applied to the results of the present survey, one might conclude that approximately 40% of the middle-aged men, 60 to 70% of the elderly men and 20% of the women included in the survey should be suspected of being anemic. Percentages for the general population would presumably be higher because the persons included in this survey had been limited to those who "felt well" and to those who, while medical referrals, were without suspicion of anemia and had actual hemoglobin values which were found to be comparable to those of well persons. A more valid conclusion and one which is in agreement with other workers[9,12] is that some of the widely quoted figures for normal hemoglobin values are unrealistically high and should be revised. Widespread use of automated techniques should make it possible to reassess normal values for hemoglobin, as for other constituents, making use of large numbers of values and also of improvements in methodology. In fact, a screening survey on a large scale, making use of automated equipment, has been described recently by Kaufman, Grant and Moorhouse.[13] It is noteworthy that the authors observed a downward trend of hemoglobin values in the male after 20 years of age, a change similar to the findings of the present interlaboratory report. For females the values in their study increased after the menopausal stage. This change seems to have some theoretical foundation and has been reported in some studies but not in others, including the present interlaboratory study.

In the preparation of cyanmethemoglobin reference solution the recommendations of the International Committee for Standardization in Haematology of the European Society of Haematology[14] have now been adopted in the United States[15] and in Canada. Thus since June 1967 the concentration of the cyanmethemoglobin standard prepared by the Laboratory of Hygiene has been calculated on the basis of a millimolar extinction coefficient of 44.0 for cyanmethemoglobin and a molecular weight of 64,458 for hemoglobin. In theory this could result in an increase of 2.0% in the hemoglobin values over those measured and recorded (as in this study) using the pre-1967 standard reference solution. In practice the allowed variation in the preparation of the reference standard, also 2.0%, and the laboratory error in the measurement of hemoglobin prevent an assumed prediction of a consistent increase of 2.0% in the values.

The present study, involving interlaboratory results, was undertaken only because it was believed that the error in hemoglobinometry had been lessened in the past number of years. A detailed documentation of

TABLE III

Summary of hemoglobin values, combined well and medical referrals – arbitrary groupings by age

(Note: Medical referrals by definition excludes any known anemias and conditions affecting the liver, spleen or bone marrow)

Male and Female (number of values)	*Hemoglobin conc. mean (g./100 ml.)*	*Standard deviation(s)*	*95% Confidence limits (±1.96s)*
Under 1 day (19)	19.2	3.5	12.4-26.0
1st week (114)	19.2	2.9	13.5-24.8
2nd week (11)	18.2	2.6	13.1-23.3
3rd and 4th weeks (12)	14.2	2.3	9.8-18.7
1 month (15)	13.3	1.7	9.9-16.7
2 – 23 months (376)	11.7	1.3	9.1-14.3
2 – 6 years (1,401)	12.1	1.0	10.1-14.1
7 – 11 years (816)	12.7	1.0	10.7-14.6
Male			
12 – 14 years (887)	13.9	1.3	11.4-16.4
15 – 16 years (1034)	14.5	1.3	11.9-17.1
17 – 24 years (1232)	15.0	1.2	12.8-17.3
25 – 49 years (2711)	14.6	1.1	12.4-16.9
50 – 69 years (1281)	14.3	1.3	11.8-16.8
70 – 84 years (520)	13.6	1.6	10.4-16.7
85 and > (69)	12.6*	1.7	9.4-15.9
Female (non pregnant)			
12 – 17 years (2795)	13.5	1.2	11.1-15.8
18 – 84 years (6946)	13.0	1.1	10.8-15.2
(18 – 64 years (6103)	13.0	1.1	10.9-15.1)
(65 – 84 years (843)	12.9	1.4	10.2-15.6)
85 and > (77)	12.4**	1.6	9.4-15.5
Pregnant			
19 – 44 years (295)	12.2	1.3	9.7-14.6
* Well persons only (13)	13.4		
**Well persons only (12)	12.9		

TABLE IV

Hemoglobin values for women 19 to 44 years

	Number	*Mean Hb. g./100 ml.*	*S.D.*
Non-pregnant:	4326	13.0	1.0
Pregnant:			
Month 1	2	14.0	1.8
Month 2	11	12.5	1.1
Month 3	13	12.4	1.2
Month 4	11	11.3	0.5
Month 5	8	11.5	0.9
Month 6	13	11.9	0.7
Month 7	21	12.1	0.9
Month 8	38	12.1	1.3
Month 9	161	12.2	1.4
Month 10	1	12.1	–
Month not specified	16	12.4	1.1
Total	295	12.2	1.3
At delivery:	471	12.0	1.6
Post partum:			
Day 1	15	10.8	1.1
Day 2	12	10.1	1.4
Day 3	72	11.2	1.7
Day 4	20	11.3	1.8
Day 5	9	11.8	1.5
Day 6	3	10.9	1.2
Day 7	2	13.4	0.5
Day not stated	16	13.2	1.3
Total	149	11.4	1.8
On iron			
Pregnant	58	11.8	0.9
At delivery	203	12.1	1.2
Post partum	8	10.4	1.1

equipment, manufacturers' tolerances and checks of calibration was requested because it was realized that there are sources of error other than those at the photometric stage. Careful scrutiny of this information was made so that the results from a particular laboratory could be excluded if the anticipated error were exceptionally great, but in fact it was not necessary to exclude any laboratory. In analyzing the combined results from all laboratories it was found that for most age groups the standard deviations from the mean value were approximately 1.1 g. per 100 ml.; standard deviations were higher for the very young, under 2 years, and for persons over 60 years. These figures are comparable to those reported in many studies from single laboratories, and hence the 95% confidence limits in this study do not appear to be unreasonably large. It might be noted, however, that the condition of minimal error from combined sources was achieved in very few laboratories. In the future the more widespread use of hemoglobin pipettes having a manufacturer's tolerance of ±1% and greater care in the handling and matching of cuvettes could result in less error in the determination. Development and use of a satisfactory whole blood standard which would serve as a check on every stage of the determination rather than only the photometric stage would be ideal. The possible effect which factors such as these might have in lessening the error of the determination and ultimately in narrowing the limits of the range of normal for each age category would need to be studied separately.

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