Serum iron, total iron-binding capacity, and percentage saturation in normal subjects

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SYNOPSIS The serum iron was determined in 60 normal subjects (38 males and 22 females) with a mean age of 32.0 and 36.2 years respectively. The plasma total iron-binding capacity (TIBC) was also determined in 54 of these subjects (32 males and 22 females). Serum iron and TIBC determinations were performed between 8.45 am and 9.15 am.

The mean serum iron concentration is $138.3 \pm 4.3 \mu\text{g}/100 \text{ ml}$ ($\text{SD} \pm 32.9 \mu\text{g}/100 \text{ ml}$), and the mean TIBC is $470.1 \pm 6.8 \mu\text{g}/100 \text{ ml}$ ($\text{SD} \pm 50.1 \mu\text{g}/100 \text{ ml}$); the mean plasma siderophilin saturation is 30.0%. The mean values for serum iron, TIBC, and siderophilin saturation are similar in both sexes. They are normally distributed, and there is no significant correlation of serum iron and TIBC with age in either males or females.

There have been many published reports giving the figures for the normal and pathological ranges of both plasma iron and iron-binding capacity. There is still disagreement about normal values. The mean serum iron concentrations reported vary from 100 to 182 $\mu\text{g}/100 \text{ ml}$ in males, and 88.5 to 160 $\mu\text{g}/100 \text{ ml}$ in females. Some of the earlier surveys had been made with methods giving low results, but Ramsay (1958) noted that even with newer and more accurate methods, no increased trend in serum iron levels had been seen. Several workers have found a sex difference in the serum iron after puberty, but others have reported little or no difference. This study was done to establish values for serum iron and iron-binding capacity in apparently healthy normal subjects resident in the Belfast area of Northern Ireland.

MATERIAL AND METHODS

SELECTION OF SUBJECTS Sixty subjects were selected from all social classes and were composed of doctors, nurses, laboratory personnel, secretarial assistants, domestic servants, motor mechanics, salesmen, and housewives. Normal values for serum iron were determined in these 38 male and 22 female subjects, and the plasma TIBC was established in 54 of them (32 males and 22 females). There was no evidence of anaemia, and all the control subjects were apparently healthy and free from organic disease.

The haemoglobin in the males was $>13.5 \text{ g}/100 \text{ ml}$ and in the females $>11.5 \text{ g}/100 \text{ ml}$. Their ages ranged from 18 to 62 years; the mean age in the males was 32.0 years (range 18-55 years) and in the females it was 36.2 years (range 18-62 years).

METHOD OF COLLECTING SERUM Samples of approximately 10 ml of blood were taken between 8.45 am and 9.15 am. All-plastic, sterile, disposable syringes with stainless steel needles were used for collecting blood. After standing for three hours at room temperature, the specimen was centrifuged and the serum separated immediately. The tubes used for collecting blood samples were washed with 1% nitric acid, and then with tap water, followed by glass-distilled water.

SERUM IRON AND TOTAL IRON-BINDING CAPACITY These were measured by the method for the automatic determination of serum iron as described by Young and Hicks (1965). This method eliminates the need to render the apparatus or reagents iron free. Its precision, as we found, was comparable with other accepted methods for the determination of serum iron. We analysed 48 serum samples, with iron values ranging from 26 to 344 $\mu\text{g}/100 \text{ ml}$ on two occasions and found the standard deviation to be 2.9 $\mu\text{g}/100 \text{ ml}$.

By using standard components of the AutoAnalyzer system of Technicon Instruments Co. Ltd, both serum iron and TIBC were determined colorimetrically.

PRINCIPLE Iron is simultaneously released from protein and reduced by hydrochloric acid and ascorbic acid. The reduced iron is separated from the protein by dialysis and allowed to react with tripyridyl-triazine.

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The complex is measured colorimetrically at a controlled pH.

TOTAL IRON-BINDING CAPACITY The procedure of Ramsay (1957) was used to determine the iron-binding capacity; 2 ml of a solution containing approximately 50 µg ferric iron per 100 ml was added to 1 ml of serum in a centrifuge tube, and the solutions were mixed. After five minutes approximately 200 mg 'light' magnesium carbonate was added. The tube was shaken at intervals for the next 30 to 60 minutes. The tube was then centrifuged at 3,000 rpm for five minutes, and a portion of the supernatant fluid was decanted into an AutoAnalyzer cup, and estimated as for serum iron. The total iron-binding capacity is three times the value calculated from the AutoAnalyzer recorder chart.

A control plasma was analysed with each batch of specimens. The coefficient of variation for serum iron was 4.3% at a value of 108 µg/100 ml; the coefficient of variation for TIBC was 8.3% at a value of 380 µg/100 ml.

RESULTS

Table I shows the values for serum iron, total iron-binding capacity, and percentage saturation of siderophilin in the male and female subjects. The mean value for serum iron is 138.3 ± 4.3 µg/100 ml (SD ± 32.9 µg/100 ml) with a range of 78 to 229 µg/100 ml. The mean serum iron in the males is 139.0 ± 5.4 µg/100 ml (SD ± 33.3 µg/100 ml), and in females 136.6 ± 7.0 µg/100 ml (SD ± 33.1 µg/100 ml). The mean value for the total iron-binding capacity is 470.1 ± 6.8 µg/100 ml (SD ± 50.1 µg/100 ml). In the male subjects it is 467.9 ± 7.8 µg/100 ml (SD ± 44.0 µg/100 ml), and in females 473.3 ± 12.5 µg/100 ml (SD ± 58.8 µg/100 ml).

The mean saturation of siderophilin is 30.0% (range 15-55%). There is no appreciable difference in the mean levels of serum iron, total iron-binding capacity, and percentage saturation of siderophilin in the male and female subjects.

FIG. 1. Frequency distribution of serum iron in normal subjects.

FREQUENCY DISTRIBUTION OF SERUM IRON IN MALES The frequency distribution of serum iron in the 60 normal subjects is shown in Fig. 1, and the histogram in Fig. 2 illustrates the distribution of serum iron in the male subjects. The histograms do not appear to show any marked skewness. A statistical analysis was done to determine if there was a normal distribution of serum iron in the male subjects. The results for skewness and kurtosis with 36 degrees of freedom were $0.1 > P > 0.05$ and $0.6 > P > 0.5$ respectively, which are not significant. Thus, the serum iron was found to be normally distributed in the male subjects.

FREQUENCY DISTRIBUTION OF SERUM IRON IN FEMALES Figure 3 is a histogram showing the frequency

TABLE I
SERUM IRON AND TIBC IN NORMAL SUBJECTS

Subjects	Serum Iron (µg/100 ml)						TIBC (µg/100 ml)					
	No. of Observations	Age (years)	Range	Mean	SD	SE of Mean	No. of Observations	Age (years)	Range	Mean	SD	SE of Mean
Males	38	32.0 (18-55)	78-229	139.0	33.3	5.4	32	33.4 (18-55)	384-540	467.9	44.0	7.8
Females	22	36.2 (18-62)	78-190	136.6	33.1	7.0	22	36.2 (18-62)	352-586	473.3	58.8	12.5
Total	60	33.5 (18-62)	78-229	138.3	32.9	4.3	54	34.2 (18-62)	352-586	470.1	50.1	6.8

FIG. 2. Frequency distribution of serum iron in normal male subjects.

FIG. 3. Frequency distribution of serum iron in normal female subjects.

FIG. 4. Scattergram showing the relation of serum iron to age in normal males.

distribution of serum iron in female subjects. The value for measuring skewness with 20 degrees of freedom was $0.4 > P > 0.3$, and for kurtosis it was $0.3 > P > 0.2$. So, there was a normal distribution of serum iron in the females, as in the males.

CORRELATION OF SERUM IRON RELATED TO AGE IN MALES Figure 4 is a scattergram showing the relation of serum iron to age in the male subjects.

On statistical analysis, the correlation coefficient of $r = -0.02$, and at 36 degrees of freedom $P > 0.2$. Thus, there seems to be no association between age and serum iron levels in the male subjects.

CORRELATION OF SERUM IRON RELATED TO AGE IN FEMALES The correlation of serum iron and age in the female subjects is shown in Figure 5. The correlation coefficient of $r = +0.328$, and at

FIG. 5. Scattergram showing the relation of serum iron to age in normal females.

FIG. 6. Frequency distribution of total iron-binding capacity (TIBC) in normal subjects.

TABLE II

SIGNIFICANT DIFFERENCE IN SERUM IRON LEVELS BETWEEN FEMALES AGED 40 YEARS AND OVER AND 39 YEARS AND UNDER DETERMINED BY *t* TEST

Mean Serum Iron ($\mu\text{g}/100\text{ ml}$)		Mean Difference ($\mu\text{g}/100\text{ ml}$)	SE of Difference	<i>t</i>	Degrees of Freedom	P
40+ Years	- 39 Years					
150.6 (10) ¹	123.8 (12) ¹	26.8	13.0	2.06	20	0.05 > P > 0.10

¹There were 10 female subjects who were aged 40 years and over and 12 aged 39 years and under.

FIG. 7. Frequency distribution of total iron-binding capacity (TIBC) in males.

FIG. 8. Frequency distribution of total iron-binding capacity (TIBC) in females.

20 degrees of freedom, $P > 0.1$, which is not significant. This suggests that there is no association between age and serum iron in females.

The female subjects were divided into two groups: 40 years and over and 39 years and under. This was to separate the female subjects into those who were in the postmenopausal stage or in the latter part of their reproductive lives, and those in their active reproductive years. A *t* analysis was done in these

two groups of female subjects, and the results are shown in Table II. The *t* test showed that at 20 degrees of freedom $0.10 > P > 0.05$. The difference between the mean plasma iron levels in the females aged 40 years and over and 39 years and under is $26.8 \mu\text{g}/100 \text{ ml}$; the plasma iron concentration varies from 78 to $190 \mu\text{g}/100 \text{ ml}$. As in the males, there is no significant correlation between serum iron and age in females.

FREQUENCY DISTRIBUTION OF TIBC RELATED TO SEX Figure 6 shows the distribution of TIBC for the whole group, and Figs. 7 and 8 that respectively for males and females separately. These figures suggest that there may be a normal distribution of TIBC in both sexes.

The frequency distribution of TIBC in males and females was analysed statistically. In males it was found that for skewness, $0.7 > P > 0.6$, and for peak or kurtosis, $0.9 > P > 0.8$, figures which are not significant. In the females it was found that for skewness $0.95 > P > 0.90$, and for kurtosis $0.5 > P > 0.4$, results which are not significant. Thus, there is a normal distribution of TIBC in the subjects of both sexes.

CORRELATION OF TIBC AND AGE IN MALES In the 32 normal male subjects, the values of plasma TIBC were statistically analysed with regard to age. The correlation coefficient of $r = +0.20$, and at 30 degrees of freedom $P > 0.1$. So, there is no direct relationship between plasma TIBC and age in the male subjects.

CORRELATION OF TIBC AND AGE IN FEMALES In the

22 normal female subjects, the plasma TIBC and ages were analysed to determine if there was a relationship between them. On statistical analysis the correlation coefficient of $r = +0.28$, and with 20 degrees of freedom, $P > 0.1$. Again, this study shows that there is no correlation between age and plasma TIBC in the female subjects.

CORRELATION BETWEEN SERUM IRON AND UNSATURATED IRON-BINDING CAPACITY To determine if there was a direct relationship between the serum iron concentration and the unsaturated iron-binding capacity (UIBC), a correlation coefficient statistical analysis was carried out.

In the 54 male and female subjects, the correlation coefficient of $r = -0.83$, and with 52 degrees of

TABLE III
MEAN VALUES FOR SERUM IRON, TIBC, UIBC, AND PERCENTAGE SATURATION IN 54 NORMAL SUBJECTS

	Mean	SD	SE
Serum iron ($\mu\text{g}/100\text{ ml}$)	138.1	31.2	4.2
TIBC ($\mu\text{g}/100\text{ ml}$)	470.1	50.1	6.8
UIBC ($\mu\text{g}/100\text{ ml}$)	332.0	74.1	10.1
% saturation	30.0		

TABLE IV

COMPARISON OF SERUM IRON RESULTS WITH VALUES IN OTHER PUBLISHED REPORTS

Males and Females					Males				
No. of Observations	Range	Mean	SD	SE	No. of Observations	Range	Mean	SD	SE
54		130		5.2	17	112-255	171	37	—
					25	—	126.2	21.4	4.3
					50	—	142.0	43.0	6.1
					35	—	143.0	24.0	4.1
					—	125-238	182	—	—
					15	94-175	122	26	—
					50	—	131.2	30.0	4.2
—	28-210	104.7	32.8	—	49	43-210	105	—	—
					43	56-183	119.7	35.6	5.4
					18	59-185	106	—	—
					10	56-206	100	—	—
	20-265	106.2	61.3	—	89	20-265	111.2	67.9	—
60	78-229	138.3	32.9	4.3	38	78-229	139.0	33.3	5.4

TABLE V

COMPARISON OF PLASMA TIBC AND SATURATION VALUES WITH OTHERS REPORTED

Males and Females					Males				
No. of Observations	Range	Mean	SD	SE	No. of Observations	Range	Mean	SD	SE
54	—	330	—	4.9	26		330		4.9
34	248-422	333	46	8			311		
	250-400	300-340							
	250-472	367							
	184-337	252							
54	352-586	470.1	50.1	6.8	32	384-540	467.9	44.0	7.8

It was found that when the serum iron concentration was increased, the UIBC was decreased proportionately. The mean values of serum iron, TIBC, and UIBC, with their standard errors and standard deviations, are shown in Table III.

The method used for the measurement of serum iron and total iron-binding capacity (TIBC) is rapid and easy to perform.

This is higher than the values reported by Heilmeyer and Plötnner (1937), Moore *et al* (1939), Høyer (1944), Powell (1944), Cartwright *et al* (1948), Card, Brown, and Valberg (1964), and others, as shown in Table IV, but the values of serum iron in females in this study are lower than those given by Kingsley and Getchell (1956).

In the present study, no significant difference in the serum iron concentrations between normal male and female subjects was seen. Similar findings were published by Cartwright *et al* (1948), Fowler and Barer (1952), Peters, Giovanniello, Apt, and Ross (1956), Stengle and Schade (1957), and Card *et al* (1964), but other studies show a difference in the serum iron levels between males and females. Ramsay (1958) stated that the plasma or serum iron concentration of the normal adult female is some 10 to 15% lower, but there was a great deal of overlap between the sexes. Lower values of serum iron in females were found by Heilmeyer and Plötner (1937), Moore *et al* (1939), Vahlquist (1941), Powell (1944), Høyer (1944), and Kingsley and Getchell (1956). The lower values of serum iron in the female subjects reported by these workers may have been due to iron deficiency. Høyer did

COMPARISON OF SERUM IRON RESULTS WITH VALUES IN OTHER PUBLISHED REPORTS

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COMPARISON OF PLASMA TIBC AND SATURATION VALUES WITH OTHERS REPORTED

<i>Females</i>					<i>Saturation (%)</i>		<i>Authors</i>
<i>No. of Observations</i>	<i>Range</i>	<i>Mean</i>	<i>SD</i>	<i>SE</i>	<i>Range</i>	<i>Mean</i>	
28		330		4·9		39·4	Hagberg (1953)
						35·7	Peters <i>et al</i> (1956)
		309					Brendstrup (1953)
					25-50	30-40	Ramsay (1958)
					14·4-46·8	29·4	Card <i>et al</i> (1964)
						39·3	Stengle and Schade (1957)
22	352-586	473·3	58·8	12·5	15-55	30·0	Present study

not use blood counts in the selection of his cases, and moreover, blood donors were used. The lower levels of serum iron in their female subjects may have been due to the selection of female subjects in their early reproductive years.

There was a wide variation in the plasma TIBC, ranging from 352 to 586 $\mu\text{g}/100\text{ ml}$, with a mean value of $470.1 \pm 6.8\ \mu\text{g}/100\text{ ml}$ ($\text{SD} \pm 50.1\ \mu\text{g}/100\text{ ml}$). The values obtained in this study are higher than those found by other workers (Table V), and it is difficult to find any reason related to the comparative methods used which would explain this. It might perhaps be of value to assess the absolute value for transferrin concentration in a series of normal controls in an attempt to obtain an independent measure of iron-binding capacity. Ramsay (1958) reported the mean values to lie between 300 and 340 $\mu\text{g}/100\text{ ml}$, and that individual figures less than 250 or more than 400 $\mu\text{g}/100\text{ ml}$ were rare in normal subjects.

The mean saturation of siderophilin in this study was found to be 30.0% (range 15-55%). There was no significant difference between males and females (Table I). This agrees with the results obtained by Peters *et al* (1956), Ramsay (1958), and Card *et al* (1964), who stated that the saturation of siderophilin with normal plasma usually ranges from 25 to 50%, and the mean lies between 30 and 40%, but higher values of saturation were noted by Hagberg (1953) and Stengle and Schade

(1957). These are summarized in Table V.

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