IV. RESULTS

I. PHYSICAL CALIBRATION OF THE MEASURING EQUIPMENT

1. Calibration of the Auto Gamma Well Counter:

Fig. 5 (Table II) shows the setting of the pulse height analyzer for the optimal operation of autogamma well counter to measure the gamma energy radiation emissions from ^{59}Fe, i.e. the base of 200 volts and the window width of 300 volts. The gamma energy of ^{59}Fe falls within the operating base of 200 to 500 volts the band of which efficient countings of ^{59}Fe by gamma well counter may be operated; otherwise the background and emissions from other source of energy may be included. The gamma measurement by means of such operating pulse height analyzer will hold true for any other gamma sources and offer the advantages of reducing the background and other detrimental counts, thus increasing the efficiency of gamma counting by means of combined scaler-spectrometer.

2. Calibration of Liquid Scintillation Counter:

Setting operations of liquid scintillation counter for simultaneous counting of ^{55}Fe and ^{59}Fe source are shown in Fig. 6 (Table III). The settings were started for ^{55}Fe first by varying the maximal efficiency of ^{55}Fe and ^{59}Fe was 5.2 % and 86.2 % respectively, and the cross counting ratio of ^{59}Fe were moderately sensitive to varying the strength of HCl[14]. The normality of the acid was chosen at 1.2 due to rapid and complete dissolution of the precipitate with an acceptable degree of quenching. Sufficient amount of ascorbic acid was used to produce complete reduction (and hence decolourisation) of ferric iron with little effect of counting rates. The amount of added iron as carrier in the sample was also adequate and optimal. However, some quenching still occured and the method of internal standardisation for quenching correction has to be used.

Quenching correction by internal standard was carried as follows: Adding a specific activity of 0.18 µCi in both the sample vials and the standard ^{55}Fe scintillant vials. A series of counts were obtained from the measurement in ^{55}Fe region. Now the procedures were repeated using instead 0.0105 µCi ^{59}Fe and recounting in ^{59}Fe region. The real count rates of each sample will be the difference of the recounting rates from the initial ones which were not internally standardised.

Measurements of iron absorption were obtained after such quenching correction by multiplying the added activities by a factor to normalise the values of the countings of administered doses and by this means all values were corrected for quenching.

TABLE II. COUNT RATES FROM AUTO-GAMMA WELL AT POSITION SENSITIVE FOR ^{59}Fe

Base(v)	Count Rate	Base(v)	Count Rate	Base(v)	Count Rate
170	7444	370	8275	580	1182
190	7299	390	7542	590	1180
210	7257	410	7017	600	1016
230	6910	430	6028	620	848
250	6864	450	5088	640	717
270	6577	480	3552	650	861
290	6792	500	2794	660	1830
310	7461	530	2139	670	1576
330	8149	550	1722	680	167
350	8493	570	1287		

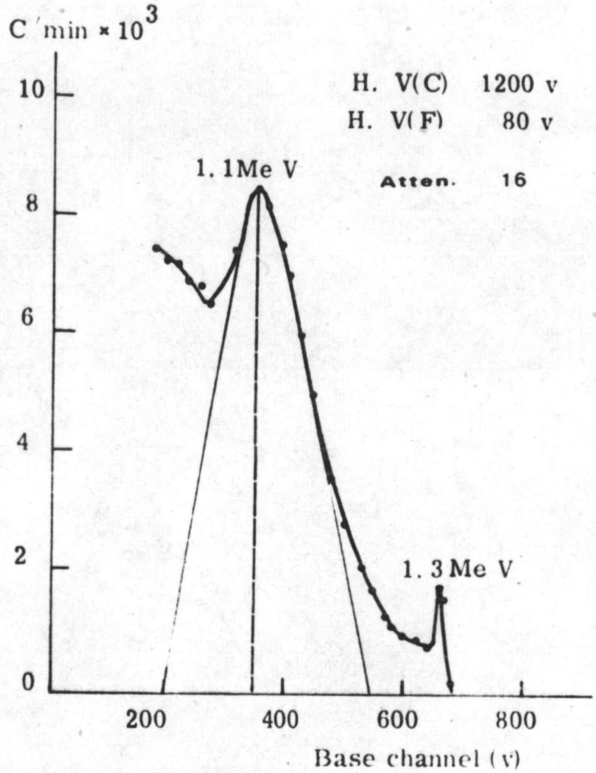

Fig. 5. The optimal operating voltage for gamma counting of ^{59}Fe.

H.V. of the data photomultiplier at the knob labelled " data photo " scale (between L_3-L_5 or 0.5-9.9 volts) the maximal cpm was obtained at 10 volts (an optimal operating level for data photomultiplier) at which the high voltage supplied with that level will give the most efficient counting of ^{55}Fe. Now for the optimal working of the gate high voltage supply, the gate was varied for its voltage until the maximal cpm was again obtained, i.e. at about 15 volts. With the combined operating settings of both high voltage supplies and with minimal attenuation, the countings were set for ^{55}Fe.

Now for ^{59}Fe, the same high voltages were applied while the attenuation was varied until maximal cpm for ^{59}Fe was obtained, i.e. in the spectral region of 0.5-9.9 volts. The equipment was made ready in good operating condition.

II. MEASUREMENT OF SAMPLE ACTIVITIES

1. Background or Blank Counting:

The background countings should be run first. This blank measurement has had a tendency to be erroneously high for about two hours when the blank sample consists of newly prepared emulsion which may create initial autofluorescence. The background countings will be stabilised after this period. These measurements

TABLE III. COUNT RATE AT VARIOUS DATA HIGH VOLTAGE

HIGH VOLTAGE	COUNT RATE
C_7	133762
C_8	200663
C_9	233508
C_{10}	250687
C_{11}	245928
C_{12}	203573
C_{13}	146622
C_{14}	101715

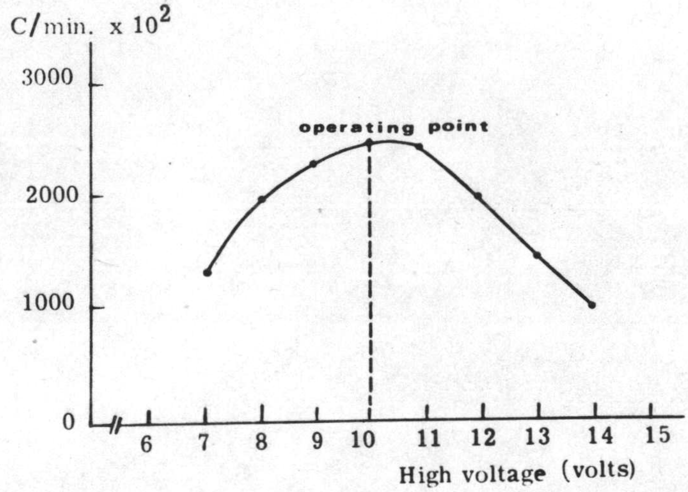

Fig. 6. The operating point of Data H.V.

TABLE IV. COUNT RATES OF VARYING GATES OF HIGH VOLTAGE

GATE HIGH VOLTAGE	COUNT RATE
C_6	125551
C_7	203157
C_8	290398
C_9	329674
C_{10}	349097
C_{11}	402883
C_{12}	430906
C_{13}	452818
C_{14}	467759
C_{15}	481054
C_{16}	496358

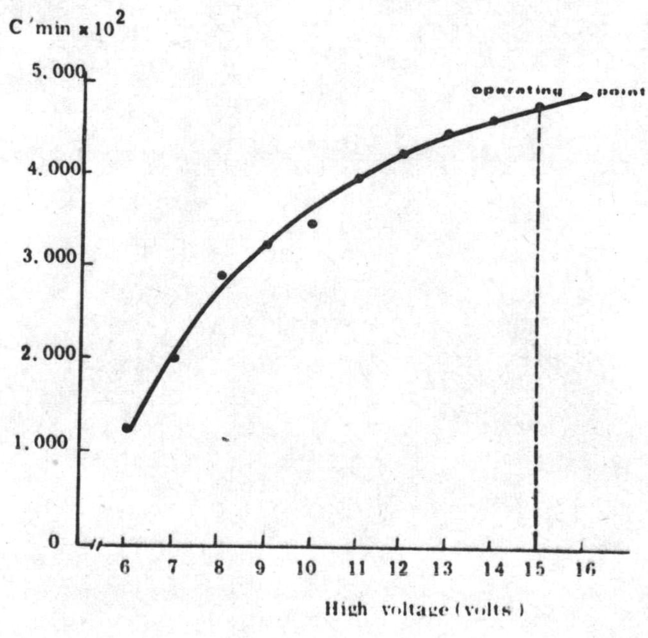

Fig. 7. The operating point of Gate H.V.

were made in both channels and the results were almost the same (57 and 61 cpm in ^{59}Fe and ^{55}Fe channels respectively).

2. Sample Counting, Quenching Effects and the Correction:

From I-2), roughly speaking, the settings for ^{55}Fe will be at data H.V. 10 and gate H.V. at 15 with minimum attenuation. For ^{59}Fe the attenuation was varied to 16.1.

Fig. 8 (Table V) shows the count rates of both ^{59}Fe and ^{55}Fe in the spectral region of ^{59}Fe , i.e. H.V. settings 10.1 and 15.16, and data attenuation at 16.1, it can be seen that at the pulse height of 2-9.9 volts, the activity of ^{59}Fe only (and none of ^{55}Fe) was measured solely and completely. Now from Fig. 9 (Table VI), when the attenuation was reduced down to 1 in the spectral region of ^{55}Fe, the sample containing both ^{55}Fe and ^{59}Fe will give activities of ^{59}Fe in the ^{55}Fe region. To obtain only ^{55}Fe countings, one has to determine a common factor (k) which is the ratio of ^{59}Fe counted in ^{55}Fe region over that in ^{59}Fe region. The real counts for ^{55}Fe will be $(\text{ cpm })_A - k(\text{ cpm })_B$ (14).

The Quenching Effect of the System and Its Correction:

The iron precipitate in the form yellowish powder has to be dissolved in different kinds of solvents creating several factors to be carefully studied for the resulting quenching effects.

3. Calculations:

$$\text{per cent efficiency} = \text{cpm/dpm} \times 100$$

$$\text{per cent absorption} = \frac{\text{cpm/10 ml} \times \text{BV} \times 100}{\text{cpm administered dose}}$$

where the corresponding blood volume (BV) can be obtained from the Table XI.

4. Reproducibility of Countings:

Table VII. shows the good reproducibility of the countings. The duplicate values give the critical value of degree of freedom at 7 and at level of 5 per cent significance ($X^2_{0.95} = 14.1$). All of the X^2 of the individual pairs of measurements appear very much smaller indicating the stability of the measuring equipment.

TABLE V . COUNT RATES OF ^{59}Fe AND ^{55}Fe AT OPTIMUM POSITION FOR ^{59}Fe

PULSE HEIGHT (v)	COUNT RATE OF ^{59}Fe	COUNT RATE OF ^{55}Fe
0.5	57620	600
1.0	44538	53422
1.5	37084	2409
2.0	30666	193
2.5	25437	20
3.0	21444	
3.5	17353	
4.0	14547	
4.5	11933	
5.0	9752	
5.5	7892	
6.0	6308	
6.5	4848	
7.0	3764	
7.5	2733	
8.0	2025	
8.5	1164	
9.0	498	
9.5	-	

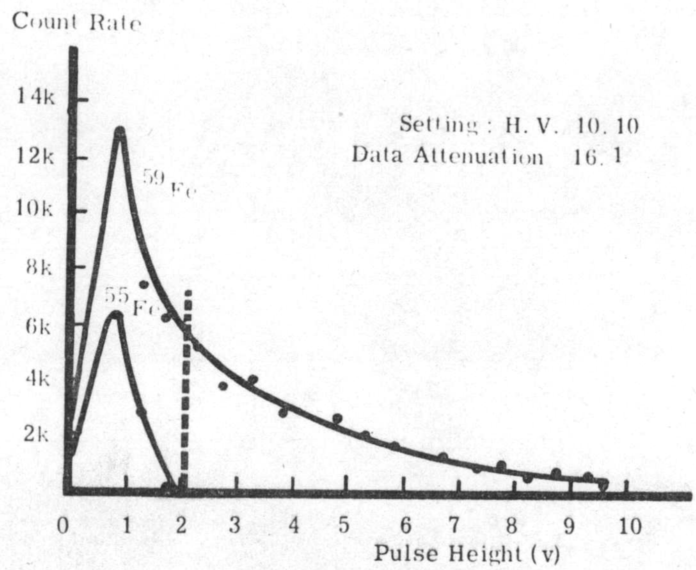

Fig. 8. Spectrum of ^{59}Fe and of ^{55}Fe in the position sensitive for ^{59}Fe.

TABLE VI . COUNT RATE OF ^{59}Fe AT OPTIMUM POSITION FOR Fe-59 AND AT OPTIMUM POSITION FOR Fe-55

PULSE HEIGHT (v)	COUNT RATE AT ^{59}Fe REGION	COUNT RATE AT ^{55}Fe REGION
0.5	57620	14088
1.0	44538	13093
1.5	37084	12432
2.0	30666	11789
2.5	25437	11028
3.0	21444	10214
3.5	17353	9370
4.0	14547	8512
4.5	11933	7475
5.0	9752	6684
5.5	7892	5756
6.0	6308	5240
6.5	4848	4312
7.0	3764	3571
7.5	2733	2662
8.0	2025	2032
8.5	1164	1302
9.0	498	593
9.5	-	10

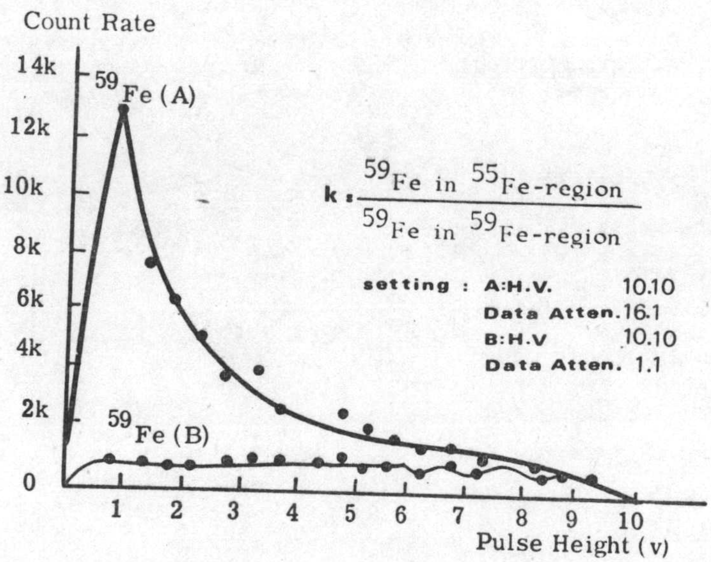

Fig. 9. Spectrum of ^{59}Fe in the position sensitive for ^{59}Fe and that for ^{55}Fe.

TABLE VII . REPRODUCIBILITY OF THE METHOD

No.	COUNT RATE x_1	COUNT RATE x_2	X^2
1	11689	12489	0.0756
2	7637	6230	0.0019
3	1229	1377	0.1174
4	10457	11396	0.0521
5	6860	5336	0.0229
6	2899	2802	0.1222
7	500	750	0.2535
8	6022	4027	0.2465

Reproducibility by using Chi-square Test

Level of significance = 5 %

Critical value of $X^2_{0.95}$ = 14.1

TABLE VIII. COUNT RATE OF DIGESTED BLOOD SAMPLES AFTER INTERNAL STADARDIZATION

Subject	Count Rate of ^{59}Fe	Count Rate of ^{55}Fe
Basal 5:		
J 1	21401	129362
J 2	20205	121785
J 3	21058	138409
J 4	20647	145095
D 5	27097	232397
D 6	22099	140920
P_n 7	20183	239471
P_n 8	19568	98759
Basal Asc:		
J 1	25128	169105
J 2	22063	140068
P_n 3	23586	150876
Basal 100:		
N 1	27349	199347
N 2	28842	232853
N 3	31826	189337
N 4	28843	207493
J 5	28883	206632
J 6	29579	156242
J 7	27335	191698
P_n 8	25571	158049
P_n 9	26824	230633
P_n 10	27583	188412
B 100:		
N 1	25918	202678
N 2	26975	221829
J 3	27376	199471
J 4	28437	201144
P_n 5	24842	182341
P_n 6	20209	174395
C 100:		
N 1	27732	187682
N 2	27363	162492
J 3	27197	217392
J 4	28643	204074
P_n 5	18654	215971

TABLE IX. PER CENT ABSORPTION OF IRON FROM REFERENCE DOSE OF 181 SUBJECTS

% absorption	No.	subjects	consisting of
0 - 10	33	J(0.2),N(1.7),J(1.9),J(2.1),D(2.4),D(2.8),J(2.9),N(3.0),N(4.9) J(5.4),J(5.5),J(5.8),J(6.0),J(6.2),J(6.4),N(6.4),J(6.7),J(7.1) N(7.4),N(8.1),N(8.5),J(8.5),P_n(8.5),D(8.5),J(8.6),J(8.7),P_n(8.7) N(8.8),P_n(9.2),J(9.3),D(9.3),N(9.6),N(9.7).	4D , 3P_n 10N , 16J
10 - 20	48	J(10.2),J(10.5),J(10.6),J(11.1),J(11.2),J(11.3),J(11.4),N(11.6) J(11.7),J(11.9),J(12.5),J(12.7),N(12.7),D(12.9),P_n(13.1),J(13.5) J(13.5),J(13.7),J(13.9),J(14.0),J(14.3),J(14.4),J(14.4),N(14.6) P_n(14.6),P_n(14.8),N(15.1),N(15.2),J(15.3),N(15.6),D(15.6),N(15.8) N(15.9),P_n(16.2),P_n(16.3),J(16.4),D(16.5),J(16.8),N(16.9),P_n(16.9) P_n(18.0),N(19.1),N(19.2),D(19.3),J(19.3),P_n(19.5),P_n(19.7),P_n(19.9).	4D , 11N 11P_n, 22J
20 - 30	34	N(20.1),P_n(20.3),N(20.6),N(20.8),J(21.1),N(21.4),D(21.4),P_n(21.7) N(22.3),P_n(22.4),J(22.7),D(23.1),P_n(23.6),N(23.9),N(24.2),J(24.3) J(24.7),P_n(24.8),D(25.0),N(25.5),P_n(25.6),D(25.7),J(26.1),D(26.2) J(26.4),N(26.8),N(27.3),P_n(27.3),N(27.5),J(27.8),J(28.3),N(28.8) N(28.9),P_n(29.3).	5D , 8J 8P_n,13N
30 - 40	24	P_n(30.3),D(30.7),P_n(31.7),J(31.9),D(31.9),D(32.1),J(32.2),J(32.9) N(32.9),P_n(33.1),P_n(33.7),P_n(33.7),D(33.8),P_n(35.3),P_n(36.0),D(36.1) N(37.2),J(37.2),P_n(37.3),N(37.5),P_n(38.0),J(38.8),P_n(38.9),P_n(39.2).	3N , 5D 5J , 11P_n
40 - 50	13	N(40.8),N(41.3),N(41.7),P_n(43.6),N(44.6),D(44.9),P_n(45.8),J(46.2) N(46.8),P_n(47.8),N(48.3),P_n(48.5),J(49.2).	1D , 2J 4P_n, 6N
50 - 60	6	D(51.8),P_n(52.3),D(55.6),D(55.9),D(55.9),D(59.4).	1P_n, 5D
60 - 70	8	P_n(60.6),D(60.9),P_n(61.3),P_n(61.7),P_n(63.3),J(63.9),P_n(66.4),P_n(69.8).	1D,1J,6P_n
70 - 80	3	D(74.1),D(74.3),P_n(77.9).	2D , 1P_n
80 - 90	5	D(80.3),P_n(83.9),D(86.5),D(89.2),P_n(89.5).	2P_n, 3D
90 - 100	7	D(90.7),N(91.3),P_n(92.7),D(93.3),D(94.2),D(98.8),D(99.6).	1N,1P_n,5D

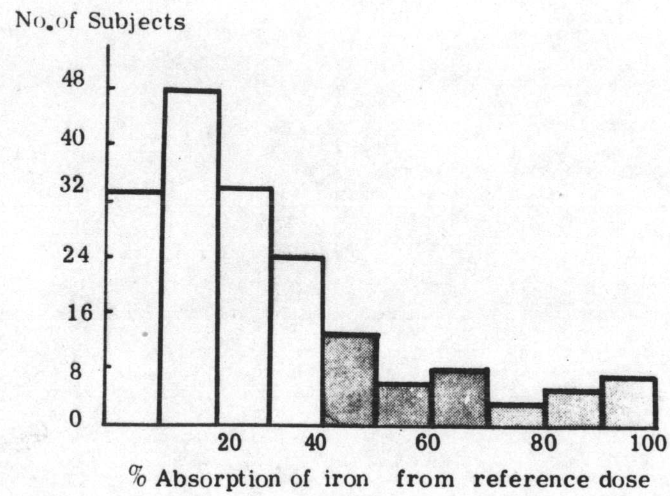

Fig. 10. Distribution of iron absorption from reference dose of 181 subjects.

TABLE X . ABSORPTION OF IRON FROM REFERENCE DOSE

No.	%Absorption from Auto-Gamma well	%Absorption from liq. scint. counter	No.	%Absorption from Auto-Gamma well	%Absorption from liq. scint. counter
1	8.49	20.94	16	5.41	13.40
2	20.82	20.90	17	32.96	36.01
3	15.58	26.37	18	14.00	23.44
4	26.10	26.15	19	24.80	37.24
5	15.38	19.80	20	14.36	11.13
6	6.36	8.70	21	8.55	8.84
7	60.66	59.84	22	38.76	28.33
8	8.53	5.98	23	15.58	9.83
9	33.09	26.25	24	13.98	15.98
10	41.26	55.26	25	13.70	14.93
11	4.92	12.60	26	32.24	37.14
12	0.22	3.37	27	16.84	19.17
13	11.87	26.24	28	6.66	6.44
14	37.23	55.09	29	38.93	21.28
15	1.74	7.81	30	19.51	23.86
		Mean value		19.62	22.74
		S.D.		14.76	19.11
		Standard error		2.53	3.28

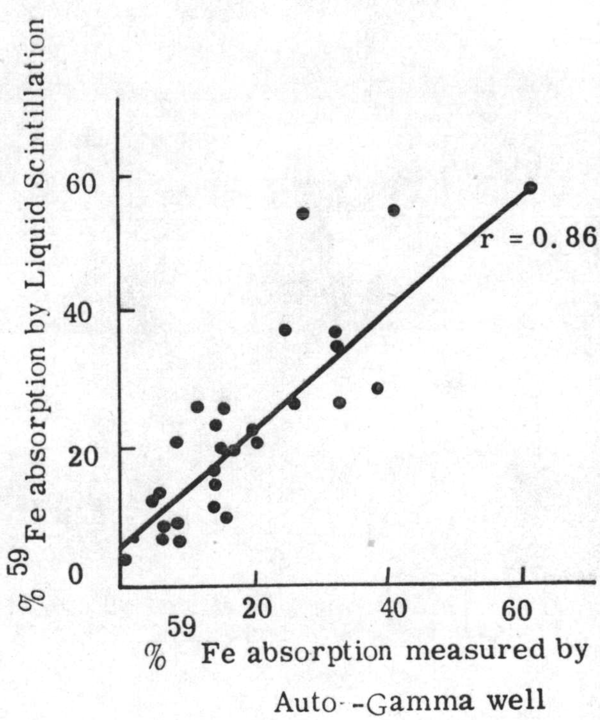

Fig. 11. Comparison of per cent absorption ^{59}Fe ferrous sulfate dose between measurement by Auto-Gamma Well and that by Liquid Scintillation system.

KEYS TO TABLES ON CLINICAL RESULTS

F O O D

Basal 5	=	Basal diet (rice,vegetable,spice)
Basal Asc	=	Basal diet plus 50 mg ascorbic acid
Basal 100	=	Basal diet plus 100 mg ferrous sulphate
B 100	=	Diet B (rice, vegetable, spice, fish) plus 100 mg ferrous sulphate
C 100	=	Diet C (rice, veg., spice, fish, fruits) plus 100 mg ferrous sulphate

S U B J E C T S

N	=	Nurses
P_n	=	Practical nurses
J	=	Janitors
D	=	Blood donors

TABLE XI . COUNT RATE OF DIGESTED BLOOD BY LIQUID SCINTILLATION SYSTEM

Subject	Count Rate of ^{59}Fe	Count Rate of ^{55}Fe	Predicted B.V.
Basal 5:			
J 1	2242	142	4073
J 2	4799	268	4468
J 3	2825	233	5183
J 4	897	98	4232
D 5	1540	151	4944
D 6	2180	229	4628
P_n 7	3457	435	3550
P_n 8	3758	291	3550
Basal Asc:			
J 1	1420	105	4468
J 2	3500	167	5102
P_n 3	2250	126	3332
Basal 100:			
N 1	12089	92	3185
N 2	5542	152	3113
N 3	6458	19	2943
N 4	6934	268	3477
J 5	5077	27	4310
J 6	3343	35	3994
J 7	1303	86	5183
P_n 8	10926	274	3915
P_n 9	1318	181	3477
P_n 10	6098	136	3404
B 100:			
N 1	13240	312	3477
N 2	2851	198	3770
J 3	625	150	4628
J 4	5025	231	3624
P_n 5	14976	458	3697
P_n 6	11249	166	2828
C 100:			
N 1	19126	733	3550
N 2	1922	125	3477
J 3	2515	77	4547
J 4	5120	284	4073
P_n 5	8219	268	3843

TABLE XII . SHOWING THE CLINICAL RESULTS OF IRON ABSORPTION FROM REFERENCE DOSE AND FROM FOOD

Subject	Hct. (%)	Auto-Gamma Well Counter %absorption Ref. dose	Liquid Scintillation Counter %absorption Ref. dose	%dietary absorption
Basal5:				
J 1	46.50	13.696	14.934	5.63
J 2	50.50	32.236	37.143	12,93
J 3	46.50	16.841	19.169	10,99
J 4	35.50	6.655	6.435	3.39
D 5	37.35	15.580	9.830	4.05
D 6	48.75	13.980	15.980	9.48
P_n 7	43.50	38.930	21.280	8.13
P_n 8	42.50	19.510	23.860	10.65
Basal Asc:				
J 1	50.50	8.550	8.840	4.12
J 2	54.50	38.760	28.330	7,18
P_n 3	38.50	14.360	11.130	3,92
Basal 100:				
N 1	40.50	27.500	47.650	1.86
N 2	43.00	8.490	20.940	3,33
N 3	46.50	20.820	20.900	0,46
N 4	40.80	15.580	26.370	7,25
J 5	48.00	26.100	26.150	0,91
J 6	48.50	15.380	19.800	1.29
J 7	53.50	6.360	8,700	2,99
P_n 8	40.75	60.660	59.840	9,14
P_n 9	43.00	8.530	5.980	3,44
P_n 10	44.75	33.090	28.310	2.73
B 100:				
N 1	46.50	41.260	55.260	7.65
N 2	41.00	4.920	12.600	4,19
J 3	48.50	0.220	4.050	4,38
J 4	47.50	11.860	26.440	4,72
P_n 5	39.00	39.120	57.590	12,23
P_n 6	42.50	37.320	55.090	3,28
C 100:				
N 1	42.50	48.310	78,660	17,47
N 2	45.50	1.740	7.810	3.91
J 3	49.30	5.410	13.400	2,60
J 4	49.00	13.990	23.440	5,27
P_n 5	42.50	24.800	37.240	7,74

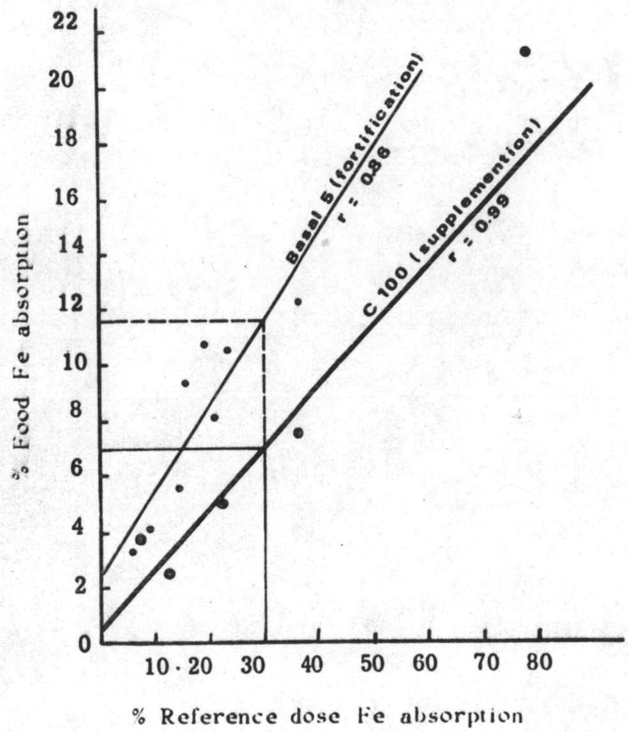

Fig. 12. Relation of per cent iron absorption from reference dose ferrous sulfate and that from food containing 5 and 100 mg Fe the amounts of iron from food absorption were identified at 30 % iron absorption of the reference dose.

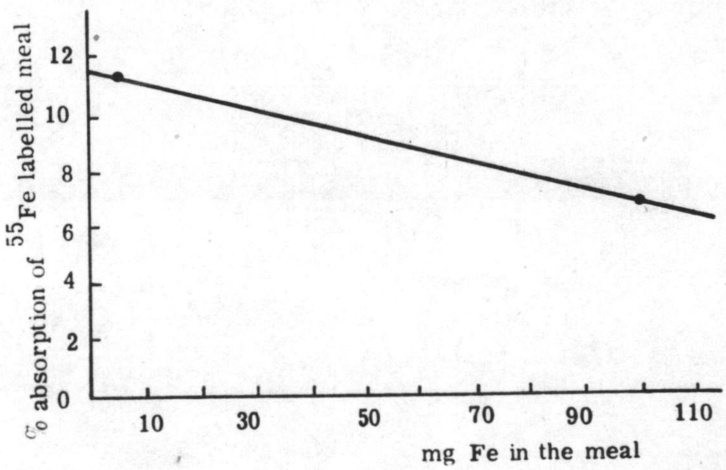

Fig. 13. Per cent absorption of iron from food of 5 and 100 mg Fe contents at 30 % iron absorption of the reference dose.

Clinical data

Absorption of iron from the reference dose:

Despite normal hematological findings, some of the subjects particularly those of blood donors and women with hypermenorrhea showed varying values higher than 20 which is the mean per cent absorption of iron from the reference dose, reflecting deficits of body iron store (Fig. 10).

The results of measurement (Fig. 11) by well counter agreed favourably with those by liquid scintillation counter ($r = 0.86$).

Absorption of iron from food:

Each of the values had to be related always to its individual absorption of iron from reference dose and both values were expressed as per cent of the administered dose and of the total amount of iron in the food in which extrinsic iron was added at 5 mg (fortification) and 100 mg (supplementation) levels respectively (Fig. 12)

The amounts of iron absorbed from the food at (chosen) 30 per cent absorption of iron from the reference dose were plotted at 5 and 100 mg iron content in food. This is the most wanted data most valuable for operation research for iron fortification and iron supplementation for specific areas of the country (Fig. 13).