The significance of folic acid, tissue iron stores, and tissue viability in determining iron uptake from serum by thyroid tissue slices

W. M. BUCHANAN

From the Department of Pathology, University College of Rhodesia, Harari Hospital, Salisbury

SYNOPSIS This paper describes an attempt to measure *in vitro* iron uptake from serum by human thyroid slices and to relate the uptake to tissue iron stores, folic acid status, and tissue viability. It is an extension of work previously reported (Buchanan, 1969).

Thyroids were obtained from patients undergoing partial thyroidectomy for colloid goitre and serum from clinically normal healthy adults. The haemoglobin, serum iron, and folic acid levels of both thyroid and serum donors were measured and thyroids examined histologically for the presence of stainable iron. Viable and non-viable tissue slices were incubated in sera treated with radioactive iron so as to produce high and normal levels of transferrin saturation.

Iron was taken up both from sera with normal and high transferrin saturation but the amount was, in almost all cases, greater from the more highly saturated.

The uptake by non-viable tissue was appreciable but did not vary to any great extent from one serum to the next, and was attributed to simple diffusion of ionic iron into the tissue. There was, however, marked variation in uptake from different sera by viable tissue. It was concluded therefore that viability is a factor affecting the uptake. As the variation in uptake by viable tissue incubated in a single serum was significantly less than tissue incubated in a number of different sera it was further concluded that there was also a factor in the serum itself affecting iron uptake. The nature of the factor was not elucidated but neither folic acid nor levels of iron stores appeared to influence uptake because no correlation was found between iron uptake and iron stores or folic acid

In iron overload due to haemochromatosis heavy deposits of iron are invariably present in a wide variety of epithelial tissues (Sheldon, 1935), while in Bantu siderosis epithelial iron deposits are usually restricted to the liver despite there being comparable amounts of excess iron in each condition (Higginson, Gerritsen, and Walker, 1953).

The factors which determine the uptake of iron by tissues from the blood have not yet been completely elucidated. It has been shown that when various samples of human tissue obtained at operation are incubated in autologous serum the iron uptake is partly dependent on the degree of transferrin saturation (Buchanan, 1969). It was found that the uptake of iron by all the tissues examined was greater from sera whose transferrin was 90% saturated than from that in which the transferrin was only 50% saturated. A high level of transferrin

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saturation was not the only determining factor because there was a considerable variation in uptake between samples of the same tissue taken from different individuals. The reason for this variation is not clear. It was noted that in one liver sample containing very heavy deposits of haemosiderin the uptake was small while in another from a patient with iron-deficiency anaemia the uptake was pronounced, suggesting that the variation in uptake might in some way be related to the level of tissue iron stores. Elsewhere, animal experiments have indicated that serum folic acid levels are important in determining iron uptake by tissues (MacDonald, Jones, and Pechet, 1965).

Material and Methods

Thyroid was used in this investigation because of its ready availability and because it is known to take up

iron well (Buchanan, 1969). In all cases partial thyroidectomy had been performed for colloid goitre. Samples were taken from areas which grossly most closely resembled normal thyroid while foci of degeneration and haemorrhage were avoided. Areas adjacent to those used for the tests were examined histologically. The sections were stained by haematoxylin and eosin and Perls's method for iron (Pearse, 1961).

Measurements were made of the haemoglobin (cyanmethaemoglobin method, Dacie and Lewis, 1968), serum iron (S.I.) (Bothwell and Mallet, 1955), unsaturated iron-binding capacity (Bothwell, Jacobs, and Kamener, 1959), and serum folic acid (Waters and Mollin, 1961). The normal range for folic acid in this laboratory has been found to be 3-15 ng/ml.

The serum was prepared by pipetting 8 ml aliquots into polythene tubes in pairs. The procedure followed then was exactly as described in the previous paper (Buchanan, 1969), that is, solutions containing ^{59}Fe were added to the sera to produce 50% saturation of the transferrin in one tube and 90% saturation in the second.

The tissue specimens were cut at a thickness of 0.5 mm and incubated in the sera for three hours. After washing in Krebs-Ringer solution the specimens were dried on blotting paper and weighed to the nearest milligram. They were then digested in nitric acid and the radioactivity of each was measured. The iron uptake was calculated and expressed as micrograms iron per 100 mg wet weight of tissue.

Thyroids from seven patients were examined. From the thyroid of each patient 20 aliquots of tissue each weighing between 90 and 110 mg were prepared. Ten of the aliquots were placed in tubes with transferrin 50% saturated and 10 in tubes with transferrin 90% saturated.

The first thyroid was used to test the accuracy of the method by incubating the aliquots of tissue in tubes all containing serum from the same donor. Thereafter, specimens of each thyroid were incubated in sera from 10 different donors.

Thyroid tissue was used either immediately after operation or after it had been allowed to stand for 24 hours at +4°C. It was assumed that the thyroid incubated immediately remained viable for the period of the experiment while that which had been stored in the refrigerator was not viable.

Localization of absorbed iron in the tissues was sought by making autoradiographs of three pairs of samples following incubation in sera. After tissues had been incubated, rinsed, and dried small portions were set aside for histology while the balance was digested as described. Histological sections were cut at 4 μ in a cryostat. The sections were fixed in dried formalin vapour for 48 hours. They were then rinsed in distilled water and autoradiographs made using Ilford G 5 liquid emulsion with an exposure of three weeks.

Results

The values for haemoglobin, serum iron, and serum

Thyroid Number	Sex	Age	Hb (g/100 ml)	Serum Iron ($\mu\text{g}/100\text{ ml}$)	Folic Acid (ng/ml)
1	M	32	13.3	126	3.8
2	M	24	14.6	112	5.7
3	M	25	14.8	141	6.6
4	F	60	12.8	123	8.8
5	F	24	14.1	139	4.2
6	F	25	13.4	109	9.7
7	F	32	15.1	112	3.9

Table I Clinical data of thyroid donors

Serum Number	Serum Donor			Uptake of Iron from Transferrin		Difference in Uptake
	Hb (g/100 ml)	SI ($\mu\text{g}/100\text{ ml}$)	FA (ng/ml)	Approximately 50% Saturated	Approximately 90% Saturated	
1	15.8	120	3.2	0.042	0.301	0.259
				0.045	0.330	0.295
				0.048	0.312	0.264
				0.043	0.310	0.267
				0.049	0.390	0.341
				0.040	0.356	0.316
				0.041	0.290	0.349
				0.039	0.381	0.342
				0.044	0.322	0.278
				0.045	0.364	0.319
				Range 0.039-0.049	0.290-0.390	0.259-0.349
				Mean 0.044	0.330	0.293
				Standard deviation 0.003	0.034	0.034

Table II Iron uptake by a viable thyroid (no. 1) from 10 samples of a single serum¹

¹Results expressed in $\mu\text{g Fe}/100\text{ mg}$ wet weight of tissue.

folic acid of thyroid donors are given in Table I. No thyroid donor was suffering from iron-deficiency anaemia or folic acid deficiency. Histological examination of the thyroids in no case revealed any stainable iron. It is unlikely that appreciable iron overload was present in any of the thyroid donors, except possibly in the woman of 60 years, as in Rhodesian Africans marked siderosis is uncommon in males under 30 and females under 40 years (Buchanan, 1966 and 1967).

Table II gives the iron uptake by viable thyroid samples incubated in aliquots of a single serum indicating a high degree of reproducibility of the method.

In Table III the iron uptake by a non-viable thyroid from 10 different sera is shown. The particularly small standard deviation of the differences in uptake compared with the standard deviations at 50% and 90% saturation considered separately suggest that a factor within the different sera is exerting a con-

siderable effect on the variation in uptake at the different levels.

Table IV shows the iron uptake by five different viable thyroids, aliquots of each being incubated in 10 different sera. Except for a very few cases the uptake of iron by the tissues was greater from transferrin 90% saturated than from transferrin 50% saturated. Each thyroid showed a fairly wide range in uptake from the various sera with a 50% saturation of transferrin, though in cases 3, 5, and 6 this range was much greater than in cases 4 and 7. In three instances the uptake at this degree of saturation was notably high, being more than 0.200 $\mu\text{g}/100 \text{ mg}$ tissue. Each of these high uptakes involved different thyroids.

Autoradiographs showed radioactivity in sections from both degrees of transferrin saturation and that the ^{59}Fe was largely concentrated in the epithelial cells. The distribution was patchy throughout the sections, some follicles showing much

Serum Number	Serum Donor			Uptake of Iron from Transferrin		Difference in Uptake
	Hb (g/100 ml)	SI ($\mu\text{g}/100 \text{ ml}$)	FA (ng/ml)	Approximately 50% Saturated	Approximately 90% Saturated	
2	13.7	102	4.1	0.037	0.147	0.110
3	12.2	68	8.1	0.050	0.170	0.120
4	10.7	68	7.4	0.044	0.150	0.106
5	13.2	70	3.8	0.084	0.194	0.110
6	14.1	108	9.4	0.051	0.155	0.104
7	14.7	70	6.2	0.011	0.118	0.107
8	15.2	71	6.6	0.057	0.163	0.106
9	16.0	122	5.3	0.081	0.187	0.106
10	14.8	90	2.6	0.089	0.196	0.107
11	16.3	71	7.1	0.041	0.140	0.099
Range				0.011-0.089	0.118-0.196	0.099-0.120
Mean				0.054	0.162	0.107
Standard deviation				0.024	0.025	0.006

Table III Iron uptake by a non-viable thyroid (no. 2) from 10 different sera¹

¹Results expressed in $\mu\text{g Fe}/100 \text{ mg}$ wet weight of tissue.

Serum Number	Serum Donor			Uptake of Iron from Transferrin		Difference in Uptake
	Hb (g/100 ml)	SI ($\mu\text{g}/100 \text{ ml}$)	FA (ng/ml)	Approximately 50% Saturated	Approximately 90% Saturated	
12	16.0	140	7.5	0.012	0.014	0.002
13	12.6	144	4.4	0.111	0.280	0.169
14	12.0	45	5.7	0.330	2.260	1.930
15	13.4	140	6.6	0.017	0.177	0.160
16	13.0	106	9.0	0.019	0.019	0.000
17	15.2	97	4.0	0.029	0.020	-0.009
18	13.7	132	3.6	0.010	0.015	0.005
19	14.1	160	5.3	0.013	0.017	0.004
20	15.0	65	5.7	0.028	0.350	0.322
21	13.7	80	6.0	0.089	0.093	0.004
Range				0.010-0.330	0.014-2.260	0.009-1.930
Mean				0.066	0.325	0.259
Standard deviation				0.120	0.690	0.597

Table IVa Iron uptake by viable thyroid (no. 3) from different sera¹

¹Results expressed in $\mu\text{g Fe}/100 \text{ mg}$ wet weight of tissue.

activity while others showed none (Fig. 1). Activity in sections from the highly saturated transferrin was more extensive and intense than that from transferrin 50% saturated. Nevertheless, in localized areas of sections from the latter follicles showed fairly marked activity (Fig. 2).

Serum Number	Serum Donor			Uptake of Iron from Transferrin		Difference in Uptake
	Hb (g/100 ml)	SI (μg/100 ml)	FA (ng/ml)	Approximately 50% Saturated	Approximately 90% Saturated	
22	13.0	86	6.8	0.018	0.025	0.007
23	14.8	72	2.2	0.048	0.231	0.183
24	15.2	160	6.7	0.022	0.023	0.001
25	13.7	100	8.9	0.030	0.120	0.090
26	14.2	128	3.8	0.011	0.854	0.843
27	13.4	116	9.6	0.025	0.021	-0.004
28	12.6	50	5.1	0.026	0.035	0.009
29	13.4	80	3.4	0.028	0.140	0.112
30	12.8	80	4.8	0.035	0.240	0.205
31	12.8	146	7.4	0.025	0.025	0.000
Range				0.011-0.048	0.021-0.854	0.004-0.183
Mean				0.027	0.171	0.145
Standard deviation				0.010	0.255	0.257

Table IVb Iron uptake by viable thyroid (no. 4) from different sera

Serum Number	Serum Donor			Uptake of Iron from Transferrin		Difference in Uptake
	Hb (g/100 ml)	SI (μg/100 ml)	FA (ng/ml)	Approximately 50% Saturated	Approximately 90% Saturated	
32	12.7	100	2.8	0.005	0.043	0.038
33	13.0	104	7.4	0.043	0.056	0.013
34	11.8	82	5.0	0.003	0.003	0.000
35	14.5	104	6.0	0.240	0.254	0.014
36	12.6	151	5.8	0.012	0.020	0.008
37	14.0	96	3.4	0.005	0.007	0.002
38	12.9	108	9.4	0.030	0.057	0.027
39	13.7	122	9.8	0.009	0.010	0.001
40	13.0	100	5.7	0.050	0.140	0.090
41	11.4	92	3.8	0.050	0.094	0.044
Range				0.003-0.240	0.003-0.254	0.000-0.090
Mean				0.045	0.068	0.024
Standard deviation				0.071	0.104	0.028

Table IVc Iron uptake by viable thyroid (no. 5) from different sera

Serum Number	Serum Donor			Uptake of Iron from Transferrin		Difference in Uptake
	Hb (g/100 ml)	SI (μg/100 ml)	FA (ng/ml)	Approximately 50% Saturated	Approximately 90% Saturated	
42	12.2	60	2.2	0.042	0.103	0.061
43	11.0	111	2.6	0.012	0.440	0.428
44	15.2	140	4.2	0.020	0.053	0.033
45	12.1	44	5.8	0.041	0.136	0.095
46	13.0	60	3.8	0.042	0.190	0.148
47	11.8	32	3.4	0.042	0.213	0.171
48	12.6	112	3.5	0.053	0.130	0.077
49	13.7	59	5.4	0.504	1.141	0.637
50	14.8	136	4.0	0.023	0.051	0.028
51	14.8	84	4.6	0.041	0.083	0.042
Range				0.012-0.504	0.051-1.141	0.028-0.638
Mean				0.082	0.254	0.172
Standard deviation				0.148	0.332	0.202

Table IVd Iron uptake by viable thyroid (no. 6) from different sera

Serum Number	Serum Donor			Uptake of Iron from Transferrin		Difference in Uptake
	Hb (g/100 ml)	SI (μg/100 ml)	FA (ng/ml)	Approximately 50% Saturated	Approximately 90% Saturated	
52	11.4	74	2.8	0.055	0.145	0.090
53	14.5	76	4.2	0.085	0.574	0.489
54	12.2	86	9.3	0.055	0.391	0.336
55	15.1	86	3.0	0.048	0.203	0.155
56	12.2	121	0.4	0.029	0.785	0.756
57	13.7	80	5.6	0.052	0.069	0.017
58	14.5	110	3.6	0.043	0.090	0.047
59	14.1	82	5.6	0.073	0.180	0.107
60	14.8	120	2.0	0.036	0.166	0.130
61	13.8	136	1.6	0.060	0.126	0.066
			Range	0.029-0.085	0.069-0.785	0.017-0.756
			Mean	0.054	0.273	0.219
			Standard deviation	0.058	0.237	0.238

Table IVe Iron uptake by viable thyroid (no. 7) from different sera

Fig. 1 This is an autoradiograph of thyroid 4 from serum 30 with transferrin 90% saturated. It shows that ⁵⁹Fe is almost entirely confined to the epithelial cells of the follicles. The patchy distribution of the iron uptake is also clearly seen. (× 125)

Fig. 2 This is an autoradiograph of the same thyroid and serum as in Fig. 1 but with transferrin 50% saturated. There is appreciably less uptake of ⁵⁹Fe than from transferrin 90% saturated. It also is confined to the epithelial cells. (× 125)

Discussion

The results of this investigation confirm those already demonstrated that high percentage saturation of transferrin by iron is in some measure responsible for uptake of this element by thyroid tissue (Buchanan, 1969). While high degrees of transferrin saturation increase uptake in most cases it is clear that some uptake also occurs from transferrin only 50% saturated. In the previous study it was suggested that this uptake by tissues from transferrin 50% saturated was more apparent than real and might in fact be due to non-specific trapping of radioactive serum in the tissue during incubation (Buchanan, 1969). This explanation appears unlikely now, because if the uptake was only due to non-specific trapping, one would expect that the variation in uptake by viable thyroids would be unaffected by the incubation in different sera. That this is not the case is shown by the fact that the variance for each thyroid in Table IV is statistically significantly greater than the variance of the one thyroid incubated repeatedly in the single serum in Table II ($0.01 > p$). Further confirmation that there is uptake of iron by epithelial cells from transferrin 50% saturated is provided by the autoradiographs. These show that the little radioactivity seen is almost entirely confined to the epithelial cells and that scanty activity is present in the interstitial tissue.

Rat liver slices accumulate iron when incubated in Krebs-Ringer solution containing ionic iron (Saltman, Fiskin, and Bellinger, 1956) and this accumulation of iron does not require metabolic energy (Saltman, Fiskin, Bellinger, and Alex, 1956). Mazur, Green, and Carleton (1960) have suggested that at high levels of saturation some iron is not firmly bound to transferrin and may therefore be in the ionic form. It is possible that to some extent iron uptake by tissues from highly saturated transferrin is due to diffusion of ionic iron into the cells rather than the result of an active process. Some support is given to this argument in the present study by the fact that there was an appreciable uptake by non-viable thyroid slices. The method of uptake by viable tissues, however, cannot be entirely by simple diffusion of ionic iron because the variances of uptake at 50% saturation, at 90% saturation, and of the differences in uptake in Table IV are significantly higher than for the non-viable thyroid in Table III ($p < 0.01$). Viability of the tissue would, therefore, appear to play some part in regulating iron uptake from serum.

In addition there must be some element in the serum which affects uptake because samples of the same thyroid took up different amounts of iron from

different sera. Probably the viability factor and serum factor work in conjunction to depress uptake in some cases and enhance it in others.

No correlation could be shown between haemoglobin and serum iron values of serum donors and the uptake at either level of transferrin saturation or the difference in uptake. Also there was no evidence that any of the thyroid donors was suffering from iron deficiency or iron excess. This appears to indicate that it is unlikely that iron stores play any significant part in iron uptake by tissues from serum.

Some degree of folic acid deficiency was found in nine of the donors of sera. There was no correlation between folic acid levels of the incubation sera and the uptake of iron. Also none of the thyroid donors was folic acid deficient so it appears that iron uptake can still be considerable despite normal folic acid levels in recipient tissue. This is of particular interest, as, in rats, iron uptake by epithelial tissues other than liver occurs with high degrees of transferrin saturation only if there is concomitant folic acid deficiency (MacDonald *et al*, 1965).

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