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THE ORAL MUCOSA IN IRON DEFICIENCY

Volume 2 of Two Volumes

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THESIS

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C O N T E N T S

VOLUME 2

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FIGURES AND TABLES

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ABBREVIATIONS

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APPENDIX 2

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Species	Iron
Human (male)	50 mg
Rat	60 mg
Dog	66 mg
Heifer	61 mg
Horse	66 mg

Table 1.1 Iron per kilogram of body weight for different species. Adapted from Moore et al (1962).

Compound	% of Total Body Iron
Haemoglobin	70.5
Myoglobin	3.2
Storage	26.0
Plasma and Enzyme Iron	0.3

Table 1.2 The distribution of body iron. Adapted from Moore et al (1962).

Age and Sex	Absorbed iron required (mg)	Recommended intake according to type of diet		
		Animal Foods below 10% of calories (mg)	Animal Foods 10-25% of calories (mg)	Animal Foods over 25% of calories (mg)
Infants *				
0- 4 months	0.5			
5-12 months	1.0	10	7	5
Children				
1-12 years	1.0	10	7	5
Boys				
13-16 years	1.8	18	12	9
Girls				
13-16 years	2.4	24	18	12
Menstruating women +	2.8	28	19	14
Men	0.9	9	6	5

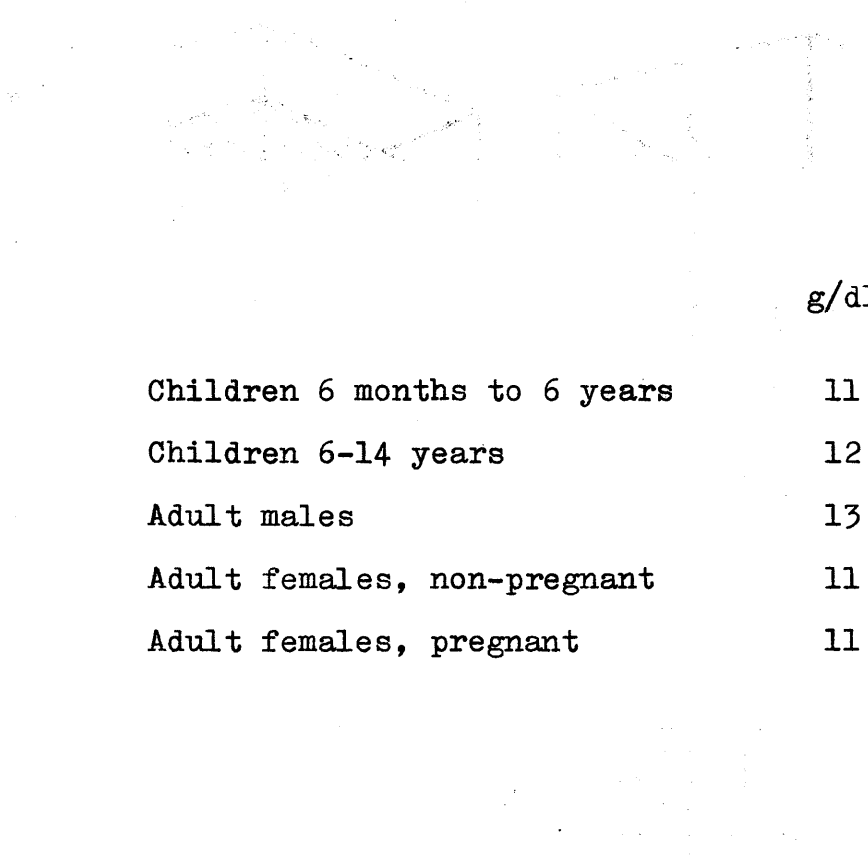
*Breast-feeding is assumed to be adequate

+ For non-menstruating women the recommended intakes are the same as for men

Table 1.3 Recommended daily intakes of iron (WHO, 1970).

Stage	Iron Stores	Serum Iron	Transferrin Saturation	Haemoglobin	Film
Iron depletion	Absent or reduced	Normal	Normal	Normal	Normal
Iron deficiency without anaemia	Absent	Low	16%	Normal	Normal
Iron deficiency anaemia	Absent	Low	16%	Low	Abnormal

Table 1.4 Stages of iron deficiency.



	g/dl
Children 6 months to 6 years	11
Children 6-14 years	12
Adult males	13
Adult females, non-pregnant	11
Adult females, pregnant	11

Table 1.5 Haemoglobin concentrations below which anaemia is likely to be present at sea level (WHO, 1972).

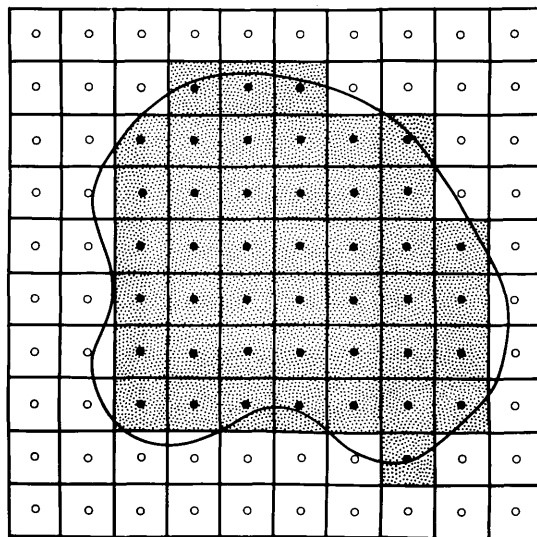
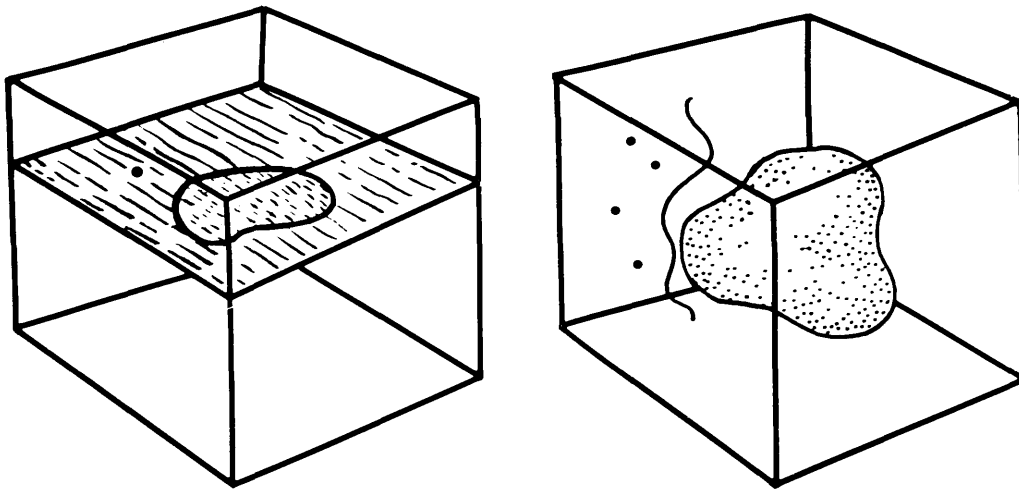


Figure 2.1 Three dimensional structures represented on a two dimensional section. Lattice of squares with marked centre points superimposed on the shape to be examined.

Number of Counts	Mean Values
1-10	a
1-20	b
1-30	c
1-40	d
1-50	e

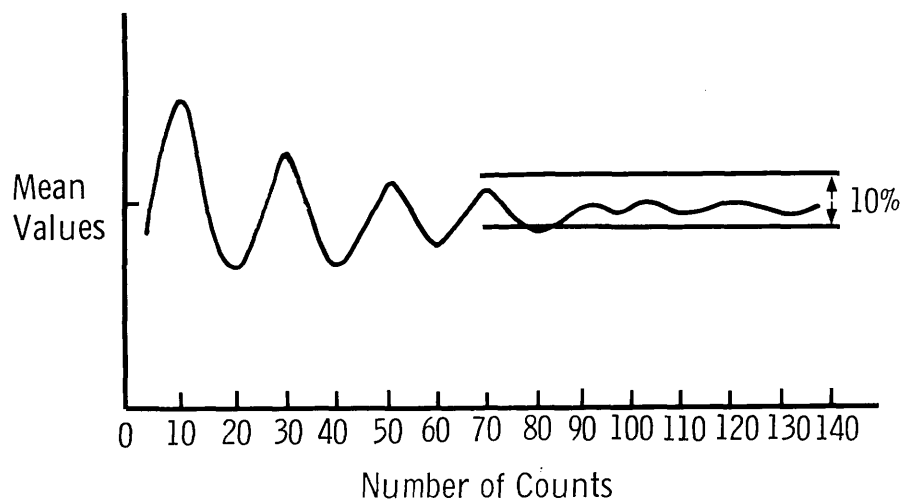


Fig. 2.2 Accumulative means test for determination of minimal sample size.

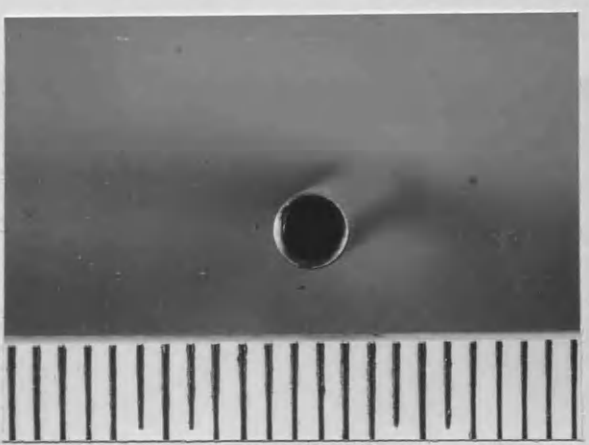
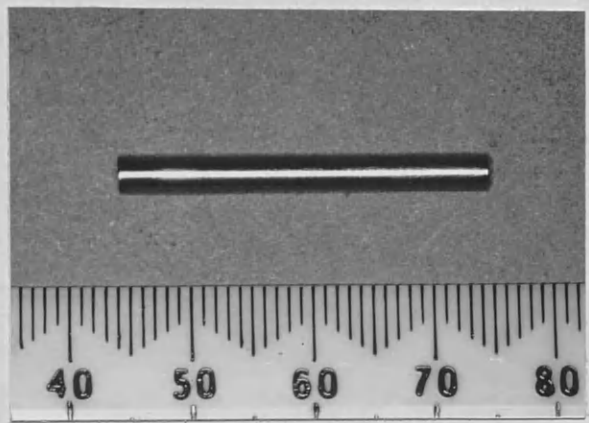


Figure 2.3 3 mm diameter trephine type biopsy punch and a mucosal punch biopsy.

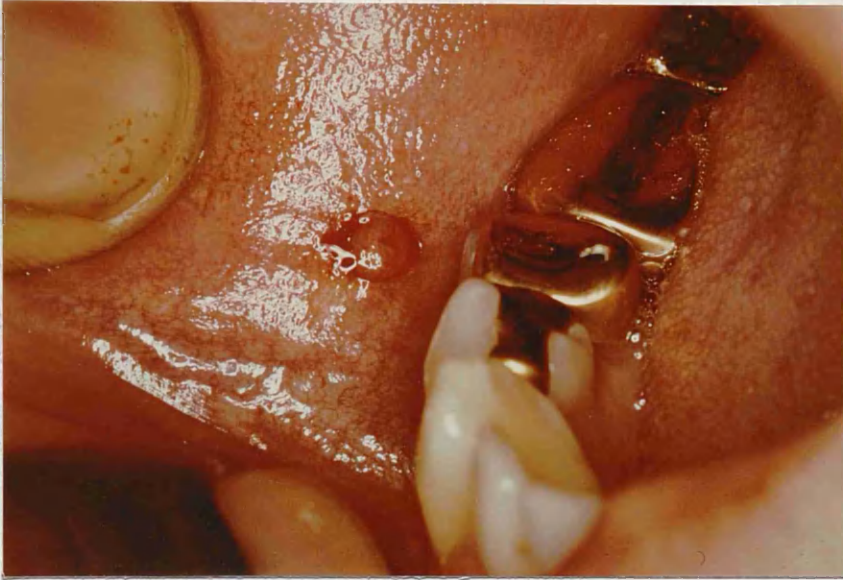


Figure 2.4 Biopsy site, incision and wound.

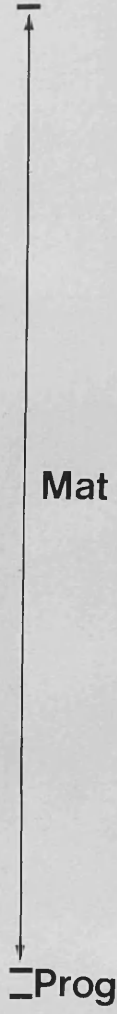
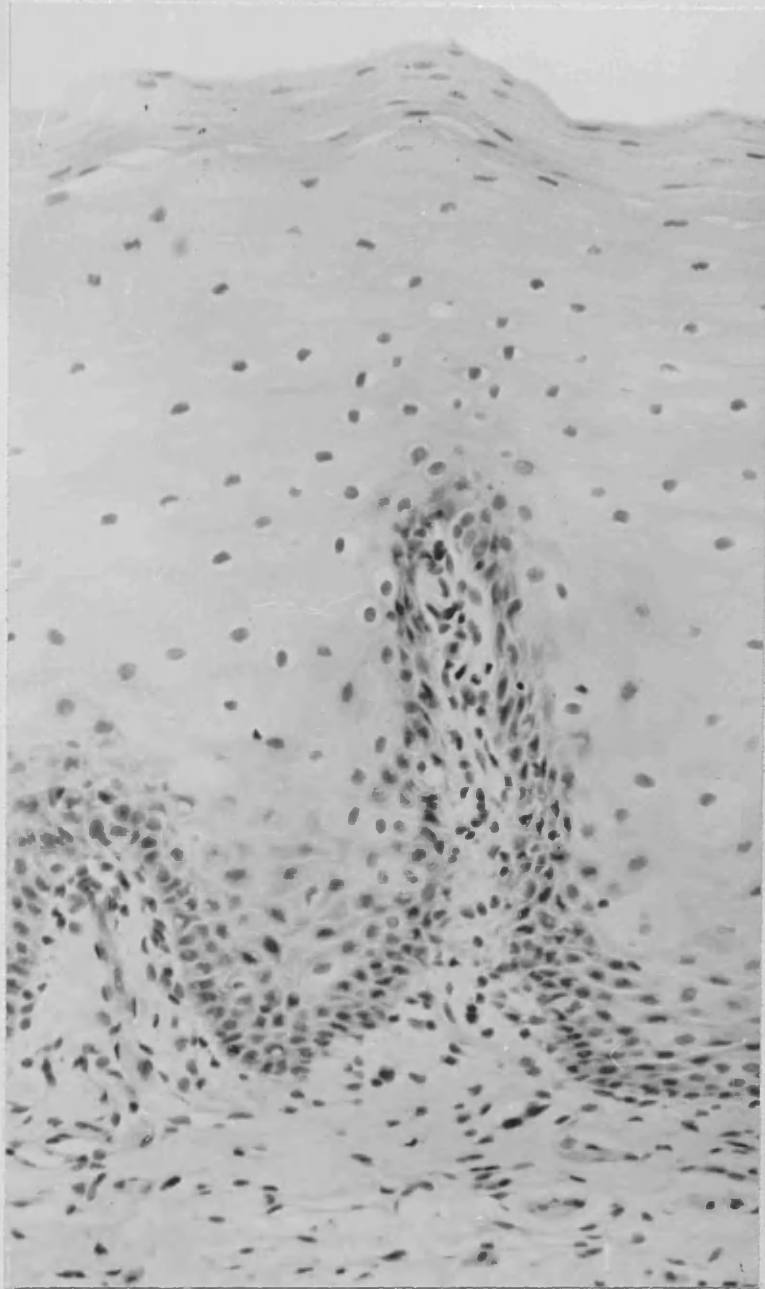


Figure 2.5 Normal buccal epithelium showing the distribution of the cell compartments.x368



Figure 2.6 The projection head of a Leitz Ortholux microscope with a superimposed 0.5- μ m grid for point counting. The red vertical lines define the column of epithelial cells.

Figure 2.6 Leitz Ortholux microscope and projection screen.



Figure 2.7 The projection head of a Leitz Ortholux microscope with a superimposed 0.5 cm grid for point counting. The two vertical lines define the column of epithelium used for stereological analysis.

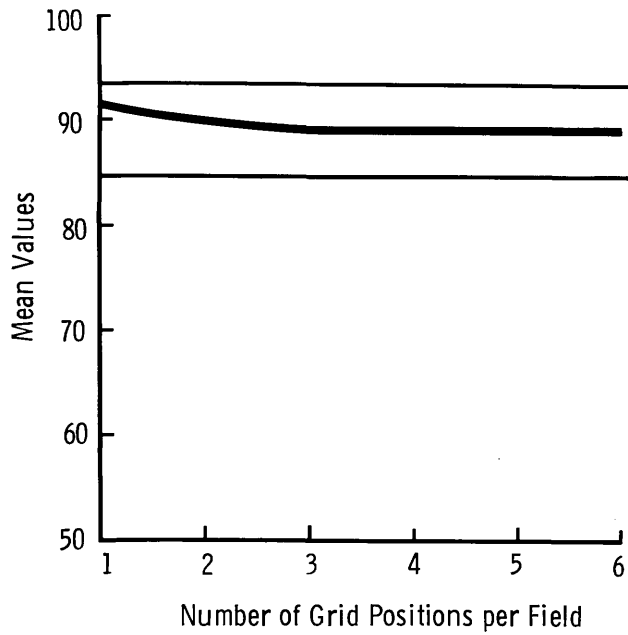


Figure 2.8 Accumulative means test for the number of point counts required per field to give values within 10 per cent of the mean.

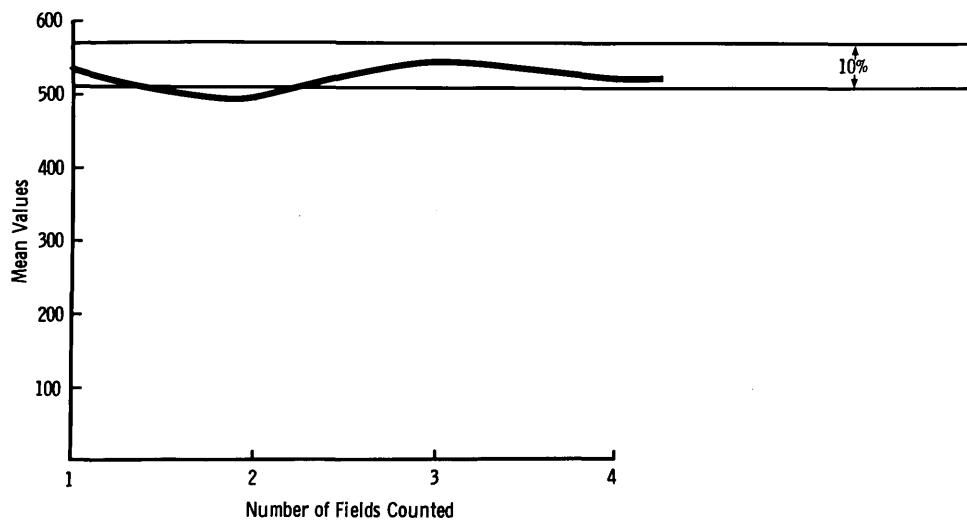


Figure 2.9 Accumulative means test for the number of fields per biopsy required to give values within 10 per cent of the mean.

$$\begin{aligned} \text{Magnification} &= 12.5 \times 25 \\ &= 312.5 \end{aligned}$$

$$\begin{aligned} \text{Points counted} &= P \\ \text{Column width} &= .27 \mu\text{m} \end{aligned}$$

$$\begin{aligned} \text{Unit area on screen represents} & \frac{10 \times 10}{312.5 \times 312.5} \text{ sq mm of section} \\ &= 0.001024 \text{ sq mm} \end{aligned}$$

$$\text{Area of compartment} = P \times 0.001024 \text{ sq mm}$$

$$\text{Thickness} = \frac{\text{Area}}{\text{Column Width}}$$

$$= \frac{P \times 0.001024}{.27} \times 1000 \mu\text{m}$$

$$= \underline{P \times 3.793} \mu\text{m}$$

Figure 2.10 Conversion of point count to area measurement and then to absolute thickness.

Subject	Sex	Age	Haemoglobin g/dl	Blood Film
1	M	40	15.2	normal
2	M	31	15.8	normal
3	M	38	15.4	normal
4	M	37	16.5	normal
5	M	33	15.6	normal
Mean		33.8	15.7	

Table 2.1 Age, sex and haematological values for control subjects.

Subject	Serum Iron $\mu\text{mol/l}$	T.I.B.C. $\mu\text{mol/l}$	% Saturation	Folate $\mu\text{g/dl}$	Vit B ₁₂ ng/l
1	12.9	62.3	20.7	116	295
2	18.3	67.7	27.0	272	315
3	24.7	75.2	32.8	189	400
4	37.9	88.1	43.0	229	510
5	16.6	69.8	23.8	250	450
Mean	28.1	76.6	29.5	211.2	394.0

Table 2.2 Biochemical values for control subjects.

<u>Patient</u>	<u>Sex</u>	<u>Age</u>	<u>Aetiology of Iron Deficiency</u>
1	M	70	Chronic blood loss; carcinoma of colon
2	M	56	Chronic blood loss; peptic ulcer
3	M	74	Dietary deficiency
4	M	45	Chronic blood loss; haemorrhoids
5	F	17	Chronic blood loss; menorrhagia
6	F	39	Chronic blood loss; peptic ulcer
7	F	60	Chronic blood loss; peptic ulcer
8	F	45	Chronic blood loss; menorrhagia
9	F	68	Chronic blood loss; diverticular disease
10	F	34	Chronic blood loss; peptic ulcer
11	F	72	Chronic blood loss; diet and aspirin ingestion
12	F	19	Chronic blood loss; menorrhagia
13	F	39	Chronic blood loss; menorrhagia

Table 2.3 Patients' age and sex. The aetiology of iron deficiency.

Patient	Haemoglobin g/dl	Serum Iron $\mu\text{mol/l}$	TIBC $\mu\text{mol/l}$	% Saturation	Folate $\mu\text{g/dl}$	Vit B ₁₂ ng/dl
1	8.7	6.8	74.8	9.1	347	411
2	7.1	4.3	96.7	4.4	190	200
3	7.0	3.6	84.3	4.3	366	460
4	8.1	0.5	94.5	0.5	278	-
5	10.2	4.5	85.9	5.2	121	430
6	6.1	4.3	89.1	4.8	normal	normal
7	4.4	3.6	73.0	4.9	normal	normal
8	9.6	4.5	65.5	6.9	364	750
9	5.9	4.8	94.6	5.1	normal	normal
10	9.9	6.8	65.5	10.4	287	489
11	8.3	4.7	81.6	5.6	normal	normal
12	9.2	4.5	85.5	5.3	392	315
13	10.2	3.0	78.6	3.8	272	450
Mean	8.05	4.29	82.01	5.41		

Table 2.4 Haematological and biochemical values of anaemic patients.

Subject	Progenitor	Maturation	Total
1	11.5	100.7	112.2
2	4.5	85.4	89.9
3	7.0	106.3	113.3
4	6.5	124.4	130.9
5	7.6	122.8	130.4
Mean	7.4	107.9	115.3

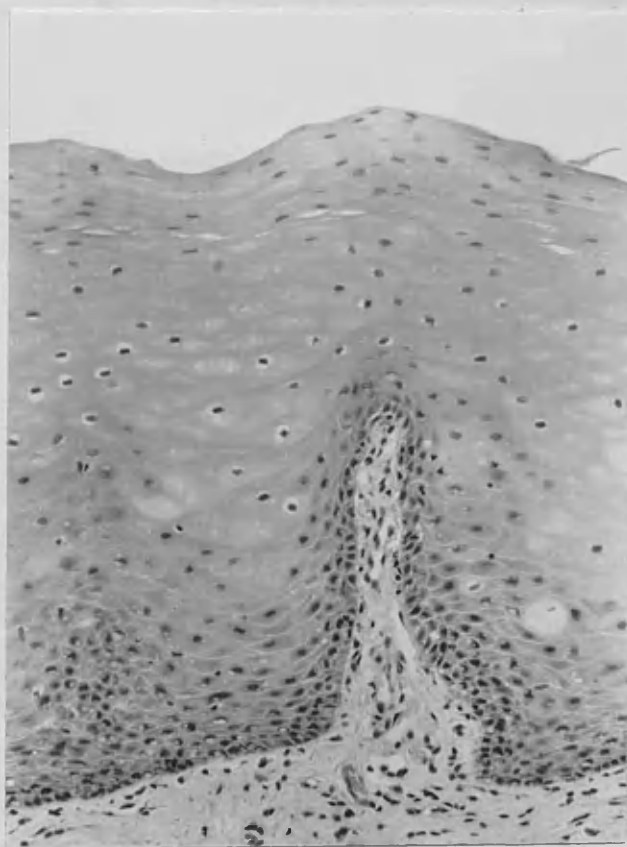
Table 2.5 Mean point count per grid position for normal subjects.

Subject	Progenitor	Maturation	Total
1	43.6	382.0	425.6
2	17.1	323.9	341.0
3	26.6	403.2	429.8
4	24.7	471.8	496.5
5	28.8	465.8	494.6
Mean	28.2	409.3	437.5

Table 2.6 Total epithelial and compartment thickness in μm for normal subjects.

Subject	Progenitor	Maturation	Prog/Mat x 100
1	10.2	89.8	11.4
2	5.0	95.0	5.3
3	6.2	93.8	6.6
4	5.0	95.0	5.3
5	5.8	94.2	6.2
Mean	6.4	93.6	7.0

Table 2.7 Percentage of the epithelium formed by each compartment in normal subjects and the progenitor/maturation compartment ratio.



Mat 93.6%

Prog 6.4%

Figure 2.11 Compartment analysis of buccal epithelium from normal subjects. X 220

Patient	Progenitor	Maturation	Total
1	7.5	93.5	101.0
2	9.2	67.2	76.4
3	7.0	76.5	83.5
4	8.8	50.4	59.2
5	6.8	42.2	49.0
6	13.7	62.9	76.6
7	9.4	53.3	62.7
8	9.7	41.4	51.1
9	9.0	42.6	51.6
10	7.0	63.5	70.5
11	5.4	53.1	58.5
12	6.3	22.9	29.2
13	6.4	30.5	36.9
Mean	8.2	53.9	62.0

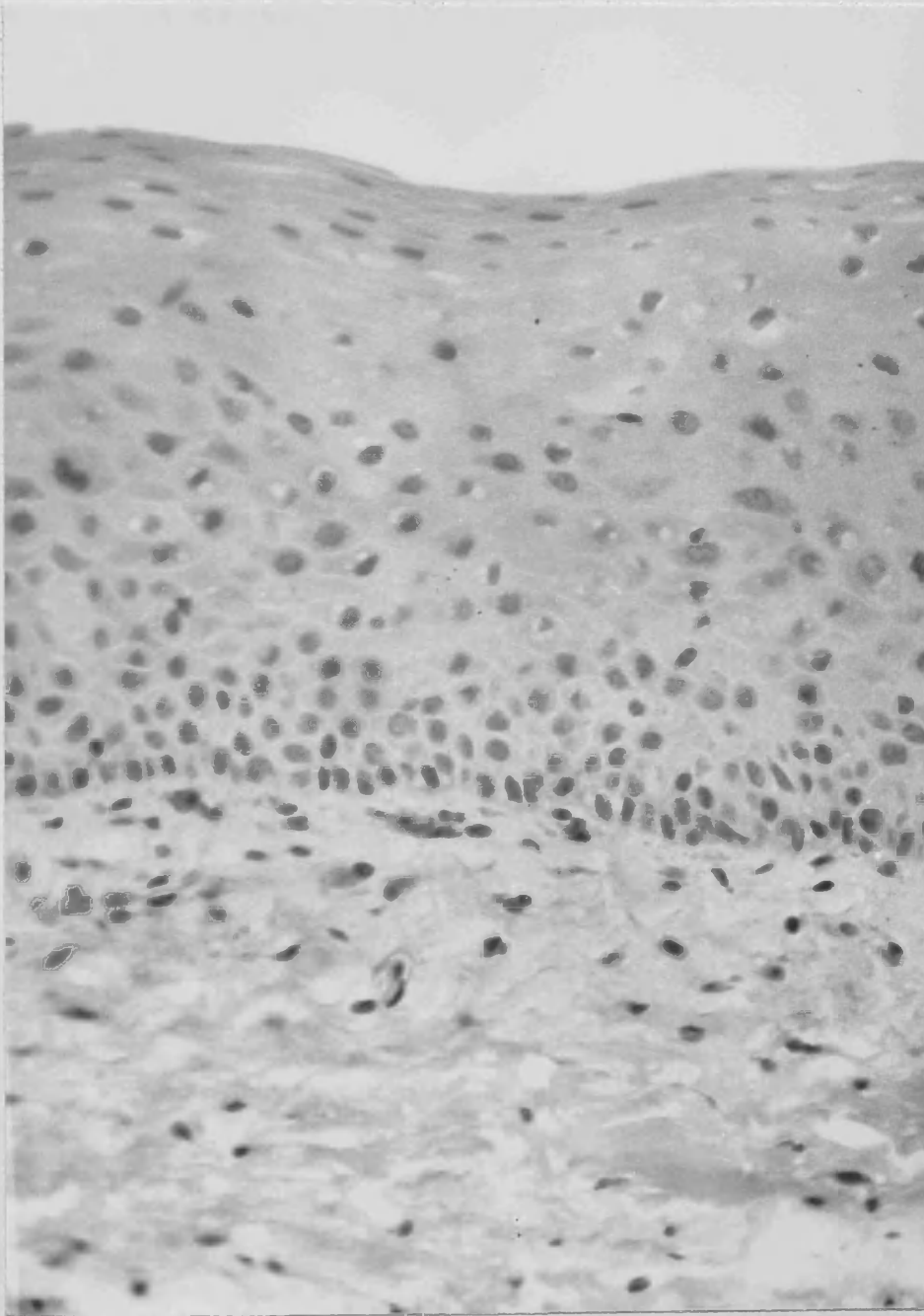
Table 2.8 Mean point count per grid position for anaemic patients.

Patient	Progenitor	Maturation	Total
1	28.4	354.7	383.1
2	34.9	254.9	289.8
3	26.5	290.2	316.7
4	33.4	191.2	224.6
5	25.8	160.1	185.9
6	52.0	238.6	290.6
7	35.7	202.2	237.9
8	36.8	157.0	193.8
9	34.1	161.6	195.7
10	26.5	240.9	267.4
11	20.5	201.4	221.9
12	23.9	86.9	110.8
13	24.3	115.7	140.0
Mean	31.0	204.3	235.3

Table 2.9 Total epithelial and compartment thickness in μm for anaemic patients.

Patient	Progenitor	Maturation	Prog/Mat x 100
1	7.4	92.6	8.0
2	12.0	88.0	13.6
3	8.4	91.6	9.2
4	14.9	85.1	17.5
5	13.9	86.1	16.1
6	17.9	82.1	21.8
7	15.0	85.0	17.6
8	19.0	81.0	23.4
9	17.4	82.6	21.1
10	9.9	90.1	11.0
11	9.2	90.8	10.2
12	21.6	78.4	27.5
13	17.3	82.7	21.0
Mean	14.1	85.9	15.3

Table 2.10 Percentage of the epithelium made up by each compartment in patients with anaemia and the progenitor/maturation compartment ratio.



10
14
7.5
12

Mat
85.9%

Prog
14.1%

Figure 2.12 Compartment analysis of buccal epithelium from anaemic patients.x600

Progenitor Cell Compartment Thickness

Normal Subjects	Rank	Anaemic Patients	Rank
43.6	17	28.4	10
17.1	1	34.9	14
26.6	9	26.5	7.5
24.7	5	33.4	12
28.8	11	25.8	6
	<u>43</u>	52	18
		35.7	15
		36.8	16
		34.1	13
		26.5	7.5
		20.5	2
		23.9	3
		24.3	4
			<u>128</u>

$$u = n_1 \times n_2 + n_2 \frac{(n_2 + 1)}{2} - R_2$$

$$= 5 \times 13 + 13 \frac{(13 + 1)}{2} - 128$$

$$u = 28$$

$$\underline{P > .1}$$

n_1 = number of values in the smaller group

n_2 = number of values in the larger group

R_2 = sum of the ranks of the group of sample size N_2

Figure 2.13 An example of the use of Mann-Whitney U Test.

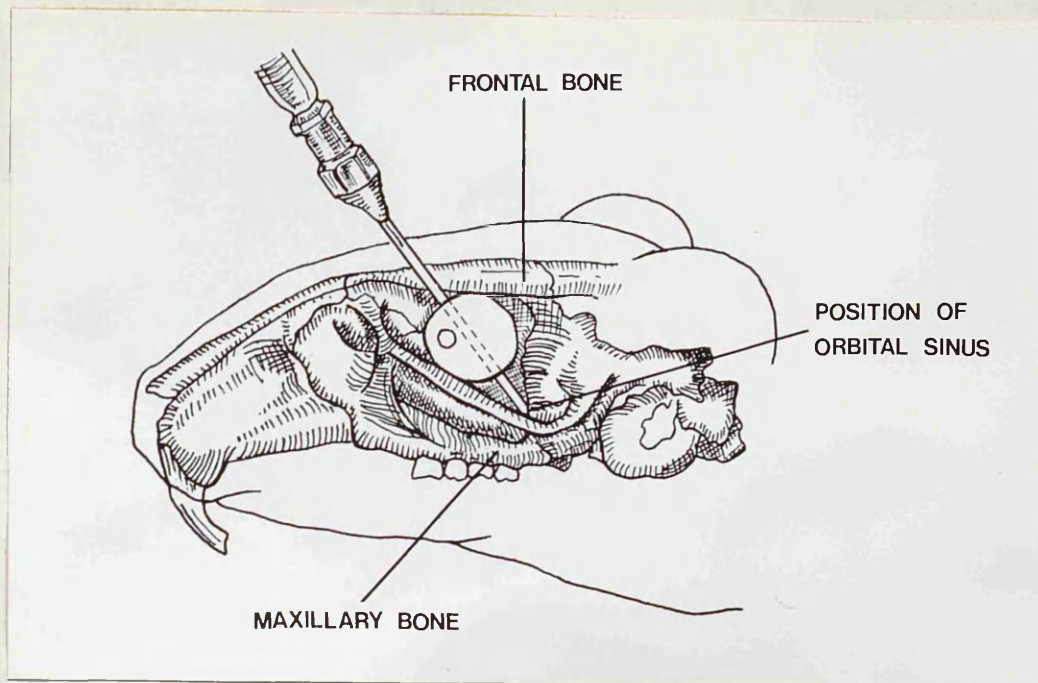


Figure 3.1 The position of the retro-orbital venous sinus in the hamster. Needle in situ (Pansky et al, 1961).



Figure 3.2 Needle position for removal of blood from the retro-orbital venous sinus.

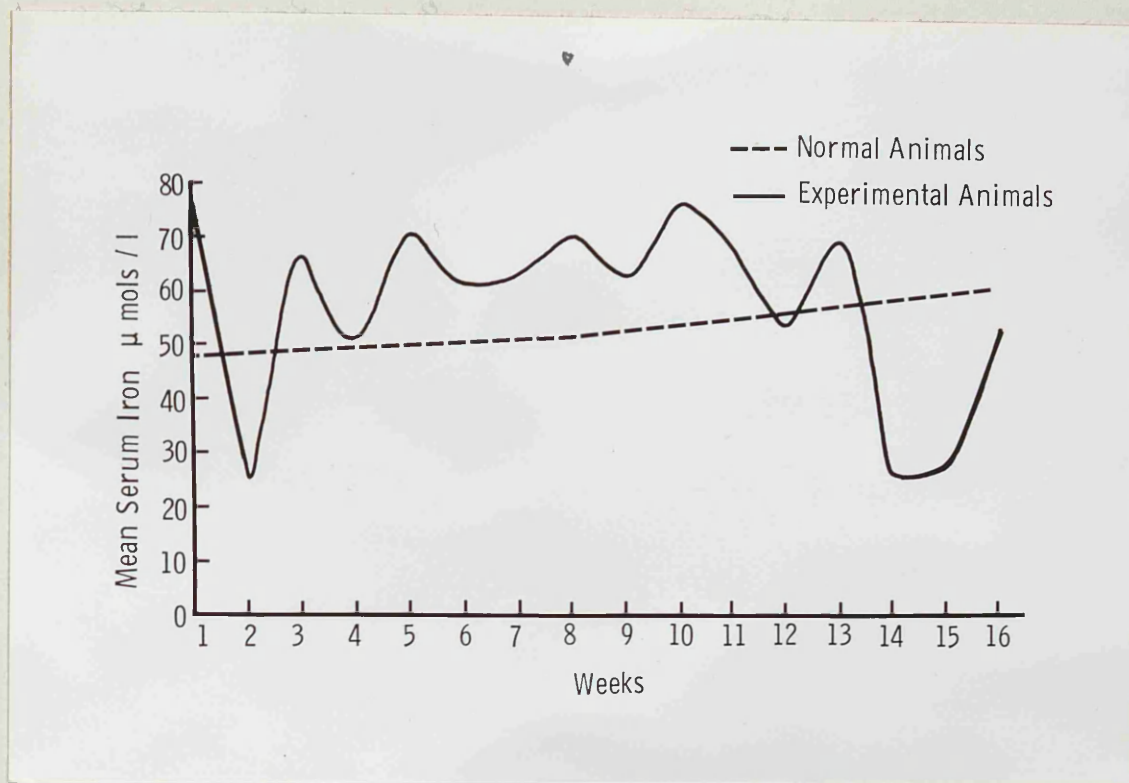


Figure 3.3 The serum iron of experimental animals over the course of the experiment.

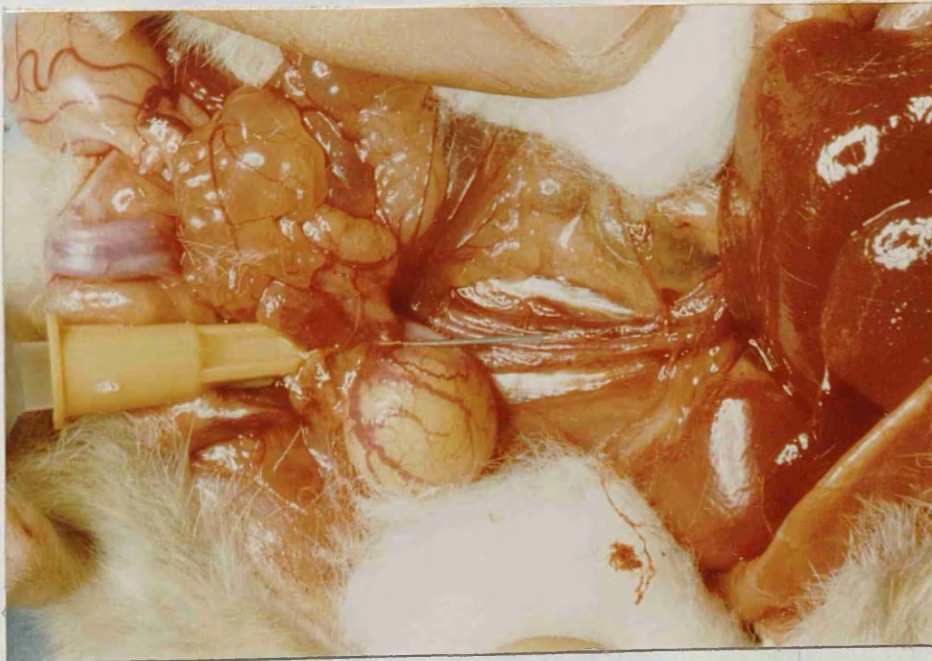


Figure 3.4 Collection of blood from the inferior vena cava of the hamster.

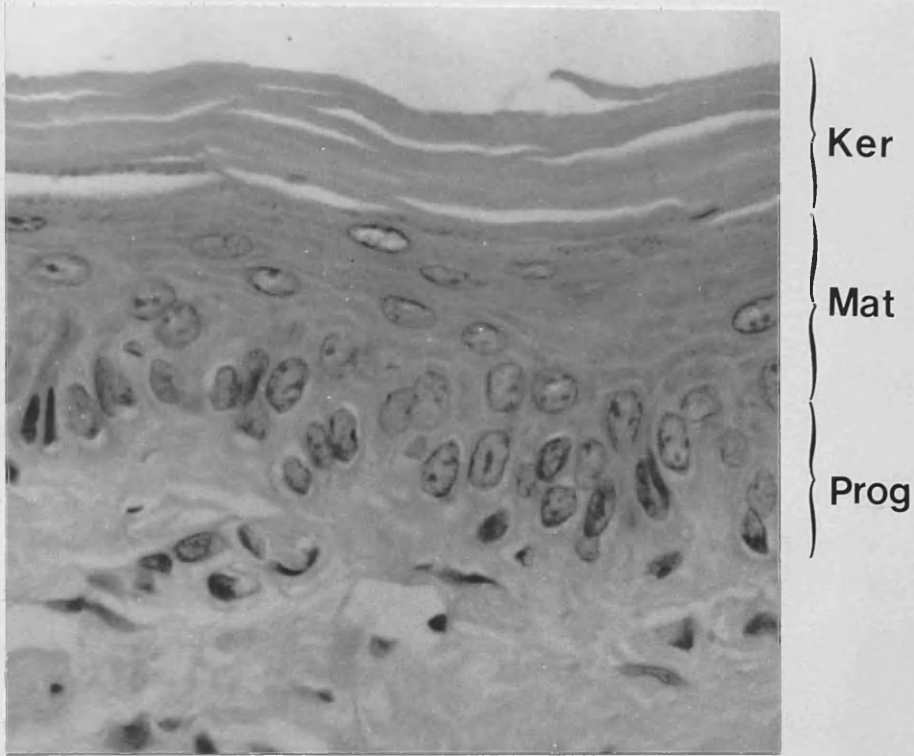


Figure 3.5 Compartments of normal hamster ventral tongue epithelium.X770

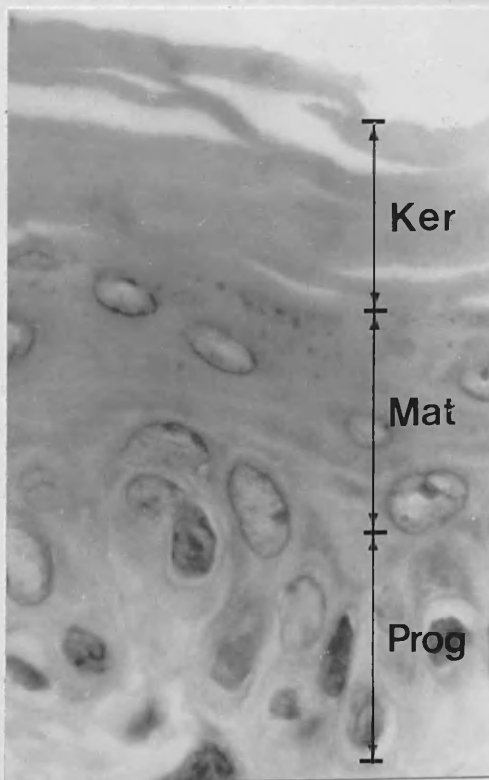


Figure 3.6 Compartments of hamster ventral epithelium.X1100

Week	Normal Animals			Iron Deficient Animals				Iron Deficient Diet and Bleeding Animals								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0	105	130	117	119	95	94	106	127	105	100	110	110	110	114	125	106
1	113	123	124	135	105	100	115	133	105	104	112	108	115	115	125	108
2	107	125	125	135	100	93	115	138	112	105	120	101	106	115	122	107
3	106	125	126	131	99	90	115	138	105	104	113	105	111	120	125	112
4	106	128	126	134	104	94	118	138	105	105	114	105	113	122	126	115
5	101	124	119	136	108	95	119	146	102	103	115	105	115	124	126	115
6	108	130	125	136	99	111	124	151	107	107	117	108	121	130	130	121
7	105	124	130	140	115	100	125	152	105	105	115	102	120	130	130	120
8	110	125	132	141	120	100	125	150	98	103	114	100	120	122	127	118
9	110	135	129	145	117	98	126	155	100	102	111	96	119	125	124	117
10	110	132	136	145	120	95	128	153	105	105	114	96	118	128	125	120
11	109	132	125	148	123	95	130	156	112	112	126	110	126	134	135	125
12	108	135	125	150	120	96	128	157	109	115	124	109	119	127	130	120
13	112	135	130	156	122	98	128	156	110	117	127	111	125	127	133	120
14	115	138	134	159	124	100	125	157	101	114	129	114	122	125	134	119
15	116	139	133	158	121	99	128	157	103	109	126	111	115	123	126	116
16	116	140	131	154	123	99	131	159	107	107	130	114	118	120	126	115
Weight gain	11	10	14	35	28	5	25	32	2	7	20	4	8	6	1	9

Table 3.1. Animal weights in grams. The data from animals dying during the experiment has been excluded.

Animal	Week 1	Week 8	Week 16
1	13.5	18.0	17.5
2	14.7	19.0	18.3
3	15.5	17.8	16.8

Table 3.2 Haemoglobin values in g/dl for normal animals.

Animal	Week 1	Week 8	Week 16
1	65.9	34.4	63.4
2	21.5	65.0	65.5
3	-	54.2	66.0

Table 3.3 Serum iron values in $\mu\text{mol/l}$ for normal animals.
(Missing value due to haemolysis of the blood sample).

Animal	Week 1	Week 8	Week 16
4	16.7	18.8	18.1
5	16.5	16.0	17.1
6	16.9	18.3	17.7
7	17.3	18.8	19.9
8	15.7	18.8	18.0

Table 3.4 Haemoglobin values in g/dl for animals in the iron deficient diet group.

Animal	Week 1	Week 8	Week 16
4	72.9	69.3	60.1
5	89.1	58.5	63.5
6	77.0	34.4	46.2
7	83.8	77.9	62.8
8	53.8	-	58.5

Table 3.5 Serum iron values in $\mu\text{mol/l}$ for animals in the iron deficient diet groups. (Missing value due to haemolysis).

Animal	Week 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
9	16.9	16.5	19.1	17.3	17.2	14.6	13.3	17.8	16.8	17.4	17.7	20.1	19.5	17.9	17.8	-	26.9
10	16.5	-	19.7	16.6	17.2	17.1	15.6	17.3	16.1	19.3	20.3	20.5	18.1	18.5	16.7	-	20.1
11	-	17	16.7	15.6	14.3	15.1	16.6	15	16.8	18.9	21.2	19.6	23.2	19.1	18.4	-	19.8
12	17.1	15.5	16	14.6	17.1	15.5	14.8	18.5	15.8	17.4	18.2	18.5	17.3	18.2	18.8	-	19.4
13	16.4	16.2	16.6	15.3	14.3	15.3	16.8	14.5	16.5	-	19.7	18.5	17.4	19.3	17.9	-	17.2
14	14.5	16.5	18.5	14.3	16.4	17.5	15.6	15.8	17.3	20.1	20.5	18.7	18.8	19.3	17.9	-	18.3
15	16.5	15	16.6	16.8	17.5	-	15.6	16.8	16.8	14.2	21.3	21	17.7	18.2	16.9	-	22.4
16	17.5	14.9	18.2	14.3	15.5	14.6	12.3	17.8	17.3	-	21.7	20.3	18.5	17.5	17.1	-	17.6

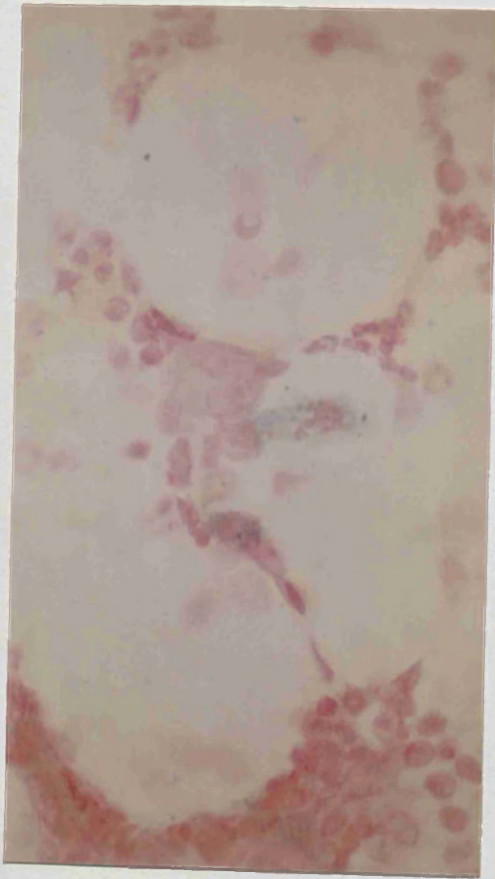
Table 3. 6. Haemoglobin values in g/dl for animals in the iron deficient diet and bleeding group.

(Missing values due to clotting of sample)

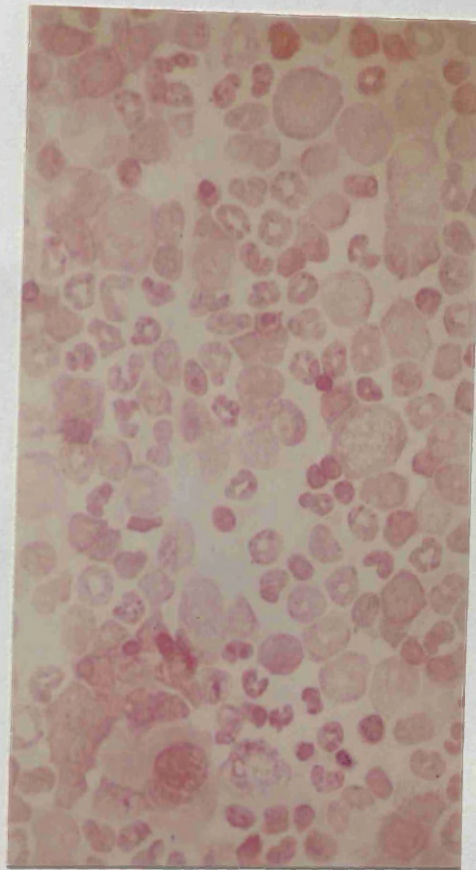
Animal	Week																
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
9	90.6	27.4	45.1	48.9	64.4	83.2	49.2	65.5	50.5	80.0	72.0	-	80.0	23.6	56.4	-	59.1
10	93.3	-	76.6	40.8	68.7	45.1	58.5	72.5	-	72.5	65.5	-	63.9	25.8	27.4	-	69.8
11	67.5	25.8	54.1	51.6	58.0	46.2	49.9	66.2	58.0	62.3	70.9	42.4	49.4	22.0	27.4	-	66.1
12	82.3	19.9	81.6	61.2	55.8	59.1	55.8	81.6	90.2	92.4	61.2	-	74.6	25.8	16.6	-	48.9
13	71.6	15.6	62.3	40.8	64.4	57.5	75.2	62.3	52.6	70.9	62.3	65.5	49.4	23.6	25.8	-	40.8
14	53.7	24.2	73.0	46.7	70.9	89.7	62.7	-	71.4	90.8	74.6	-	74.6	29.0	16.6	-	43.0
15	60.7	34.9	73.0	47.8	63.7	51.9	72.5	-	60.0	62.3	72.4	-	74.4	32.8	27.4	-	56.9
16	83.8	32.8	-	62.8	83.2	68.7	91.3	-	78.9	100.2	80.0	-	80.0	36.5	10.7	-	60.7

Table 3.7. Serum iron values in $\mu\text{mol/l}$ for animals in the iron deficient diet and bleeding group.

(Missing values due to haemolysis)



NORMAL



EXPERIMENTAL

Figure 3.7 Marrow from normal animals and animals in the iron deficient diet and bleeding group. Perls prussian blue. x 1000

Animal	Progenitor	Maturation	Keratin	Total
1	436	1331	490	2257
2	632	1650	980	3262
3	474	1527	693	2694

Table 3.8 Total numbers of points counted per compartment for normal animals.

Animal	Progenitor	Maturation	Keratin	Total
1	10.1	30.9	11.3	52.3
2	14.6	38.3	22.7	75.6
3	11.0	35.4	16.1	62.5

Table 3.9 Epithelial and compartment thickness in μm for normal animals.

Animal	Progenitor	Maturation	Keratin
1	19.4	59.0	21.6
2	19.4	50.6	30.0
3	17.6	56.6	25.7

Table 3.10 Percentage of the epithelium made up by each compartment for normal animals.

Animal	Progenitor	Maturation	Keratin	Total
4	483	1775	565	2823
5	538	1371	823	2732
6	498	1401	636	2535
7	670	2118	1083	3871
8	533	1563	902	2998

Table 3.11 Total number of points counted per compartment for animals in the iron deficient diet group.

Animal	Progenitor	Maturation	Keratin	Total
4	11.2	41.1	13.1	65.4
5	12.5	31.7	19.1	63.3
6	11.6	32.5	14.7	58.8
7	15.5	49.1	25.1	89.7
8	12.3	36.2	20.9	69.4

Table 3.12 Epithelial and compartment thickness in μm for animals in the iron deficient diet group.

Animal	Progenitor	Maturation	Keratin
4	17.1	62.8	20.1
5	19.8	50.1	30.1
6	19.7	55.3	25.0
7	17.3	54.7	28.0
8	17.7	52.2	30.1

Table 3.13 Percentage of the epithelium made up by each compartment for animals in the iron deficient diet group.

Animal	Progenitor	Maturation	Keratin	Total
9	447	939	657	2043
10	502	1307	484	2293
11	492	1119	647	2258
12	557	1361	653	2571
13	608	1457	482	2547
14	510	1171	789	2470
15	655	1771	883	3309
16	599	1520	749	2868

Table 3.14 Total number of points counted per compartment for animals in the iron deficient diet and bleeding group

Animal	Progenitor	Maturation	Keratin	Total
9	10.3	21.7	15.2	47.2
10	11.6	30.3	11.2	53.1
11	11.4	25.9	15.0	52.3
12	12.9	31.5	15.1	59.5
13	14.1	33.7	11.2	59.0
14	11.8	27.1	18.3	57.2
15	15.2	41.0	20.5	76.7
16	13.9	35.2	17.3	66.4

Table 3.15 Epithelial and compartment thickness in μm for animals in the iron deficient diet and bleeding group.

Animal	Progenitor	Maturation	Keratin
9	21.9	45.9	32.2
10	21.9	57.0	21.1
11	21.8	49.5	28.7
12	21.7	52.9	25.4
13	23.9	57.1	19.0
14	20.6	47.4	32.0
15	19.8	53.5	26.7
16	20.9	53.0	26.1

Table 3.16 Percentage of the epithelium made up by each compartment for animals in the iron deficient diet and bleeding group.

Week	Animal	1	2	3	4	5	6	7	8	9	10	Mean
1	98	86	103	99	106	110	93	107	102	95	99.9	
2	101	90	83	96	100	103	100	102	111	86		
3	107	105	94	99	104	110	110	98	118	81		
4	111	109	107	100	104	114	114	104	115	84		
5	119	111	110	100	107	118	117	106	116	86		
6	123	115	116	101	107	115	119	117	117	85		
7	126	118	119	101	104	116	119	115	120	80		
8	126	123	120	104	102	115	118	118	113	86		
9	122	115	120	106	108	111	108	116	113	87		
10	123	112	120	105	107	109	115	115	113	89		
11	123	112	120	105	108	108	116	116	115	110		
12	121	112	119	101	109	109	115	115	115	111		
13	123	111	118	103	108	113	117	117	117	117	114.2	
14	120	110	120	100	105	115	120	115	115	115		
15	118	111	118	97	108	111	116	111	111	114		
16	118	110	115	100	110	110	120	108	110	114		
17	118	108	114	95	107	109	116	91	104	112		
18	120	110	114	92	105	109	116	91	99	114		
19	123	110	114	99	100	113	120	98	101	112		
20	126	114	116	105	106	114	125	102	105	115		
21	128	120	118	95	108	118	125	100	102	113		
22	126	115	117	94	105	112	125	109	100	110		
23	125	115	117	95	105	117	125	109	105	112		
24	124	116	113	92	105	114	124	101	115	108		
25	124	116	117	95	105	116	124	108	122	106		
26	121	115	119	91	104	115	120	112	120	106		
27	120	105	115	90	104	109	119	111	118	102	109.3	

Table 4. 1. Weekly weights in grams for control animals

Week	Animal	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Mean
1	106	104	102	102	90	113	105	72	104	115	95	105	100	100	108	112	92	105	85	94	98	119	106	101.4
2	108	106	104	104	111	116	97	80	105	116	103	107	89	89	114	117	101	110	80	98	102	101	104	101.4
3	114	105	115	115	121	120	107	93	104	119	117	118	99	120	108	118	114	116	88	108	107	107	109	104
4	113	106	114	114	126	124	109	98	101	120	120	114	102	119	108	118	118	123	92	108	108	112	114	104
5	120	105	119	119	131	129	115	98	100	121	126	118	109	124	112	120	123	122	93	110	106	114	120	104
6	122	106	117	117	132	129	117	99	100	124	132	121	118	123	115	125	128	117	90	115	103	120	121	104
7	119	109	120	120	138	126	120	100	99	120	132	123	120	123	114	126	130	124	90	114	103	119	124	104
8	123	110	118	142	142	121	118	100	89	121	133	117	120	125	119	121	131	127	93	113	101	116	120	104
9	123	113	120	140	140	125	120	100	85	124	133	117	118	124	119	116	130	126	90	107	95	112	119	104
10	123	116	118	142	142	125	119	99	85	120	136	112	120	121	115	119	127	133	93	100	103	113	118	104
11	123	116	115	138	138	124	116	103	83	123	136	115	120	125	116	120	130	139	95	97	106	111	119	104
12	124	112	115	138	138	128	120	104	87	123	139	104	119	125	116	120	123	138	98	94	108	112	116	104
13	123	119	113	138	138	130	127	102	88	125	138	112	115	128	117	119	141	141	100	94	110	111	117	104
14	125	120	120	115	115	130	125	100	82	125	140	115	135	125	120	115	140	140	100	95	110	120	120	104
15	125	120	115	112	112	129	121	97	84	120	138	114	138	126	115	118	134	134	95	94	108	112	116	104
16	125	120	111	111	111	128	121	96	87	121	135	116	138	130	119	120	140	140	100	95	105	111	117	104
17	125	118	113	112	112	129	119	93	85	117	136	117	138	130	119	111	121	135	91	106	105	112	112	104
18	121	110	109	109	109	129	120	94	83	119	137	118	138	127	112	111	122	136	93	93	99	105	112	104
19	125	115	116	116	110	131	119	98	81	123	141	124	133	126	120	105	125	139	90	101	99	114	114	104
20	124	121	120	112	112	132	121	98	80	125	143	120	136	134	124	106	131	141	90	105	100	110	117	104
21	124	110	118	119	119	132	120	93	81	123	140	124	138	136	123	108	130	140	91	108	94	110	115	104
22	128	104	105	122	122	134	121	94	76	125	134	119	133	132	118	109	130	132	90	112	93	105	112	104
23	120	102	104	120	120	128	109	90	72	115	129	124	127	133	120	115	131	135	90	113	95	109	105	104
24	121	98	106	117	117	120	101	82	67	109	124	120	125	129	123	109	129	128	89	119	92	111	105	104
25	120	85	105	119	119	120	100	85	65	110	120	120	120	128	124	111	124	134	93	115	95	96	105	104
26	105	89	95	114	114	111	94	80	69	110	116	115	120	124	115	97	120	128	90	112	95	90	105	104
27	105	89	88	110	110	111	88	80	65	106	115	106	111	131	103	91	111	85	68	95	80	86	80	95.6

Table 4.2. Weekly weights in grams for experimental animals.

Animal	Week 1	Week 13	Week 27
1	16.6	17.5	13.9
2	16.8	15.6	15.2
3	16.8	15.5	13.3
4	16.8	17.5	16.5
5	14.5	16.2	15.2
6	15.6	16.5	15.6
7	13.4	16.1	16.3
8	13.4	15.4	14.1
9	16.1	16.8	16.1
10	16.3	16.2	14.2
Mean	15.6	16.3	15.0

Table 4.3 Haemoglobin values in g/dl for normal animals.

Animal	Week 1	Week 13	Week 27
1	57.5	72.5	37.1
2	56.9	57.5	63.9
3	66.2	-	31.1
4	60.7	67.7	-
5	62.7	43.5	55.5
6	39.0	68.9	37.1
7	68.6	66.1	48.9
8	54.1	59.6	58.0
9	56.7	34.2	43.9
10	55.5	57.5	39.0
Mean	57.8	58.6	46.1

Table 4.4 Serum Iron values in $\mu\text{mol/l}$ for normal animals. (Missing values are the result of haemolysis of occasional blood samples).

Animal	Serum Iron	T.I.B.C.	% Saturation
1	37.1	-	-
2	63.9	119.4	53.5
3	31.1	81.1	38.3
4	-	-	-
5	55.5	-	-
6	37.1	81.1	45.7
7	48.9	-	-
8	58.0	101.3	57.3
9	43.9	109.2	40.2
10	39.0	135.1	28.9
Mean	46.1	104.5	44.0

Table 4.5 Total Iron binding capacity in $\mu\text{mol/l}$ and transferrin saturation for normal animals.

Week	Animal	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Mean
1	18.1	17.2	18.1	14.3	14.5	16.6	16.2	16.6	16.8	17.3	16.4	15.0	16.3	15.5	16.5	15.9	17.2	15.7	-	16.3	15.2	16.3	16.3	16.3
2	15.9	15.6	15.2	16.8	16.6	15.4	15.7	16.4	15.3	15.8	17.5	17.6	16.6	14.2	17.6	15.7	17.4	17.2	16.3	14.7	16.2	16.2	15.9	16.3
3	18.1	17.5	16.9	15.8	16.4	17.2	15.8	17.3	17.2	16.8	16.6	14.7	14.7	16.1	15.8	14.3	17.8	15.4	17.5	16.3	15.8	17.5	17.5	16.3
4	17.0	17.5	17.5	16.8	16.4	16.4	14.3	17.1	16.6	16.8	16.0	15.6	16.0	15.5	15.5	-	17.5	17.2	17.2	16.3	16.9	15.8	15.8	16.3
5	16.0	16.7	16.7	16.7	16.4	15.7	15.4	15.3	16.7	16.5	16.0	16.7	16.6	16.0	16.4	14.5	14.5	17.1	16.7	16.7	16.3	16.9	15.1	15.1
6	16.4	16.4	16.2	16.0	16.0	16.0	14.4	15.7	16.2	15.8	17.3	17.1	16.6	16.2	17.5	16.9	16.8	-	16.6	15.8	16.9	15.5	15.5	16.9
7	17.6	20.4	18.0	15.3	18.1	17.0	17.0	17.0	17.0	16.8	16.6	16.7	15.8	15.5	17.0	15.7	15.7	17.2	18.5	17.9	17.0	16.9	17.7	17.0
8	16.8	16.4	16.8	15.7	16.8	16.6	14.7	15.1	16.8	16.2	17.5	16.6	16.2	16.5	16.9	15.4	16.0	18.0	17.9	17.9	16.2	16.2	16.4	16.4
9	16.1	16.9	16.8	16.1	16.4	15.6	15.1	-	15.1	16.0	17.3	16.0	15.5	16.2	16.2	15.6	17.1	17.2	17.7	16.2	16.5	16.0	16.0	16.0
10	16.7	16.4	16.4	15.5	16.6	15.6	14.7	15.6	16.4	16.9	17.1	16.0	16.1	15.5	16.6	16.8	16.4	16.8	16.7	16.7	16.2	16.5	16.0	16.2
11	15.5	17.1	16.9	16.8	16.9	15.1	14.5	16.9	16.4	16.4	17.3	16.5	16.0	16.2	16.4	17.0	16.4	17.5	18.5	16.4	17.7	17.9	16.0	16.1
12	16.0	16.8	16.6	16.2	17.5	14.4	14.4	16.8	15.8	17.2	16.8	16.8	16.8	17.0	16.6	17.0	16.0	17.0	15.4	15.4	16.6	16.8	16.1	16.1
13	-	15.8	16.9	16.0	15.6	14.5	13.6	14.9	16.4	16.6	16.6	15.7	16.1	15.1	15.6	17.0	15.3	15.7	16.8	15.8	13.8	16.4	15.8	15.8
14	16.9	16.4	16.8	15.2	16.2	14.8	13.9	16.2	15.7	15.7	14.3	14.8	14.5	16.5	17.1	17.6	15.1	17.0	14.9	14.9	17.0	17.8	15.3	15.3
15	-	15.1	16.2	15.7	15.3	14.0	12.3	15.7	15.1	14.9	15.9	14.3	14.8	15.6	15.9	15.7	13.8	15.9	15.6	14.5	15.7	15.9	15.3	15.3
16	15.9	15.1	14.7	15.2	14.2	12.5	12.6	12.6	15.1	14.9	14.7	16.2	17.9	17.9	16.5	16.2	15.8	17.3	15.8	15.8	17.5	16.2	15.0	15.0
17	15.6	13.4	15.4	16.1	14.8	-	12.3	12.3	14.8	15.2	16.1	14.7	16.2	14.3	15.2	14.9	15.1	16.2	15.9	14.5	15.4	15.2	15.0	15.0
18	15.0	16.1	3	15.0	14.8	12.0	12.5	12.0	14.8	14.8	13.8	13.8	15.0	14.3	16.6	15.6	16.3	17.0	-	13.4	16.1	17.7	15.0	15.0
19	16.1	14.1	15.0	15.6	13.9	12.9	12.1	14.6	14.5	15.7	14.4	15.7	16.1	16.1	16.2	15.2	15.2	16.8	15.4	14.6	15.4	13.8	15.6	15.6
20	16.8	15.6	15.7	15.4	15.2	13.3	13.5	15.2	15.2	16.3	16.8	14.4	15.7	16.1	16.1	16.2	16.3	17.0	13.4	13.5	14.5	16.5	15.0	15.0
21	14.8	16.1	15.2	14.6	15.4	12.3	13.0	13.7	15.2	15.2	13.4	14.2	16.8	16.8	15.7	15.6	16.5	17.0	14.3	13.5	14.5	16.5	15.0	15.0
22	-	15.7	14.9	15.9	15.6	12.3	13.6	13.6	16.4	16.8	15.0	15.0	14.2	16.8	16.2	16.4	16.5	17.0	14.3	13.5	-	15.0	14.5	14.5
23	17.3	15.0	14.5	14.3	15.7	13.1	12.6	12.4	15.6	16.1	16.8	15.6	15.3	15.6	16.2	15.5	13.7	15.9	13.4	13.4	16.0	15.0	15.7	15.7
24	15.0	14.2	12.8	16.8	14.6	13.5	11.3	11.1	12.4	12.4	14.4	13.9	15.7	15.7	14.9	15.4	15.4	15.8	12.6	12.6	14.9	13.7	13.7	13.7
25	15.5	14.6	14.6	14.9	11.8	11.0	11.3	-	13.1	13.1	-	14.0	14.0	15.3	17.0	14.8	14.7	16.8	12.2	12.2	12.5	14.8	14.9	14.9
26	14.9	15.0	12.8	16.4	11.0	11.2	11.8	11.8	11.8	13.5	13.9	15.5	15.5	15.5	14.9	13.6	15.3	16.8	12.5	12.5	15.3	14.8	14.9	14.9
27	15.9	15.2	15.2	13.9	11.9	11.9	11.9	11.9	11.8	13.0	14.0	15.8	15.8	15.0	14.5	14.9	15.3	14.5	10.2	13.1	13.0	13.3	13.7	13.7

Table 4. 6. Haemoglobin values in g/dl for experimental animals.
(Missing values are as a result of clotting of occasional samples)

Week	Animal	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Mean
1	57.5	58.0	66.1	-	72.1	60.7	52.6	48.5	50.7	64.1	62.1	69.3	56.9	54.1	58.7	52.6	58.7	62.7	54.1	-	54.6	45.8	62.1	57.5
2	36.3	49.9	40.5	65.2	46.0	60.7	56.6	37.1	48.5	40.5	62.7	55.3	59.1	60.7	59.1	61.6	60.0	60.7	47.6	71.2	49.4	39.9	61.2	
3	52.1	66.8	59.1	65.2	60.7	53.9	53.9	52.1	53.9	51.2	44.4	39.0	47.8	54.6	64.3	65.2	64.3	64.3	53.9	61.6	47.8	47.8	65.2	
4	56.4	60.7	58.2	72.1	65.2	63.7	24.2	60.9	46.9	77.0	-	39.0	47.8	-	49.4	65.2	-	69.5	53.9	72.5	37.2	59.1	63.4	
5	65.9	77.0	63.7	78.2	63.7	63.7	24.2	60.9	49.9	77.0	-	-	74.6	80.6	69.5	38.7	38.7	76.3	62.5	45.8	45.8	67.3	-	
6	72.7	70.7	74.6	68.7	76.1	80.6	80.6	49.8	55.7	75.2	56.2	27.2	59.1	60.1	64.8	51.7	61.6	60.1	30.8	20.9	36.5	16.5	33.3	
7	72.0	82.7	65.5	76.8	67.3	-	41.7	59.1	58.4	77.0	57.3	28.3	53.9	75.9	65.3	45.8	67.5	60.7	47.1	31.7	43.1	45.1	72.5	
8	75.5	74.1	58.5	77.3	74.1	41.7	55.7	48.5	50.5	78.2	78.2	24.2	44.8	-	50.5	43.9	61.8	78.0	-	33.7	72.3	57.8	70.2	
9	74.1	72.3	58.5	72.1	74.1	74.1	55.7	42.2	-	41.5	52.1	21.8	70.0	70.0	63.0	27.9	72.9	67.1	57.5	26.9	36.3	72.9	52.8	
10	85.4	85.4	78.4	24.2	75.5	34.9	34.9	44.8	29.4	84.5	71.4	72.9	14.5	24.2	20.0	13.6	31.3	13.6	49.9	30.8	17.9	-	71.8	
11	64.4	64.4	40.3	60.0	49.9	26.9	26.9	24.2	24.2	23.3	43.5	45.1	41.9	47.3	34.5	17.7	68.4	36.2	-	19.7	16.8	63.0	60.3	
12	-	56.9	53.7	68.4	56.9	28.6	28.6	22.7	25.1	23.8	57.3	43.0	77.0	48.7	78.0	17.0	71.4	75.2	69.6	-	16.8	56.4	51.0	
13	60.9	62.3	53.7	-	62.3	30.4	30.4	26.9	39.4	23.3	-	41.7	33.8	-	26.7	13.2	64.4	-	50.1	35.8	40.1	-	41.6	
14	48.7	55.5	36.2	54.8	55.5	20.4	20.4	19.7	29.7	36.9	82.7	53.7	60.1	63.7	60.9	18.1	56.9	65.3	63.0	16.5	27.6	-	27.7	
15	-	48.2	23.6	72.9	48.2	23.6	23.6	47.3	27.0	34.2	57.5	38.8	79.8	44.9	34.7	33.5	58.7	53.7	-	41.3	-	40.1	20.6	
16	81.6	-	64.8	36.2	-	11.3	11.3	74.3	16.1	28.6	-	33.5	31.7	46.7	54.2	24.2	48.3	37.1	58.0	20.6	-	25.8	87.5	
17	22.6	38.7	19.3	46.7	38.7	38.7	23.1	37.1	29.5	51.0	71.4	60.1	24.2	32.2	-	18.3	-	38.7	-	29.5	-	48.3	66.1	
18	60.7	66.1	56.4	73.0	66.1	-	-	33.3	15.0	37.1	-	20.4	78.4	74.6	72.5	67.7	61.2	94.0	33.1	54.8	-	34.4	50.1	
19	56.4	18.3	31.1	15.6	18.3	19.3	19.3	14.5	25.2	32.8	71.4	44.0	63.4	74.1	70.3	68.7	38.7	76.8	-	17.7	44.6	62.8		
20	27.9	-	81.1	21.5	-	14.5	14.5	81.4	27.9	74.6	66.6	102.6	14.5	29.0	36.5	51.0	29.0	51.0	14.5	26.3	60.7	44.6	56.4	
21	62.8	32.2	16.6	51.0	32.2	23.6	23.6	29.5	8.1	65.5	25.2	8.1	58.0	21.5	58.0	21.5	58.0	58.0	14.5	26.3	-	-	56.4	
22	-	-	50.5	65.5	-	20.6	20.6	-	15.0	46.7	70.9	52.1	65.5	58.0	-	-	58.0	58.0	29.0	9.7	-	21.5	57.5	
23	70.3	68.7	29.5	66.6	68.7	73.0	73.0	24.7	40.3	67.7	61.2	40.3	66.6	61.2	30.6	70.3	85.4	75.2	29.0	27.9	43.5	14.5	48.3	
24	66.1	18.3	27.4	61.8	18.3	-	-	14.5	7.0	43.5	58.0	36.9	63.4	59.1	64.4	-	60.7	73.0	44.6	20.4	-	47.3	12.9	
25	58.0	36.5	43.5	55.3	36.5	7.0	7.0	7.0	14.5	7.0	29.0	-	66.1	32.2	33.3	10.7	36.5	48.3	18.8	7.0	14.5	17.2	29.0	
26	25.2	53.7	10.2	-	53.7	12.4	12.4	17.7	12.4	35.4	20.4	17.7	24.2	11.8	41.9	21.5	24.2	18.8	21.5	5.9	34.9	8.6	18.8	
27	33.3	26.9	11.8	36.7	26.9	22.0	22.0	29.5	35.4	6.6	7.0	16.1	31.1	25.8	44.0	31.7	31.7	16.3	21.5	16.6	12.9	16.1	23.6	

Table 4. 7. Serum iron values in $\mu\text{mol/l}$ for experimental animals
(Missing values due to haemolysis)

Animal	Serum Iron	T.I.B.C.	% Saturation
11	33.3	-	-
12	26.9	-	-
13	11.8	69.1	17.1
14	36.7	113.1	32.4
15	26.9	61.6	43.7
16	22.0	104.5	21.1
17	29.5	60.1	49.1
18	35.4	98.6	35.9
19	6.6	81.3	8.1
20	7.0	109.2	6.4
21	16.1	84.8	19.0
22	31.1	96.1	32.4
23	25.8	86.1	30.0
24	44.0	-	-
25	31.7	97.9	32.4
26	31.7	97.9	32.4
27	16.3	96.3	16.9
28	21.5	83.6	25.7
29	16.6	88.4	18.8
30	18.3	-	-
31	12.9	85.2	15.1
32	16.1	71.0	22.4
Mean	23.6	88.1	25.5

Table 4.8 Total Iron binding capacity in $\mu\text{mol/l}$ and transferrin saturation for experimental animals.

Animal	Haemoglobin	Serum Iron	T.I.B.C.	% Saturation
15	11.9	26.9	61.6	43.7
16	11.9	22.0	104.5	21.1
17	11.9	29.5	60.1	49.1
18	11.8	35.4	98.6	35.9
28	10.2	21.5	83.6	25.7
Mean	11.5	27.1	81.7	35.1

Table 4.9 Anaemic animals.

Animal	Haemoglobin	Serum Iron	T.I.B.C.	% Saturation
13	15.2	11.8	69.1	17.1
19	13.0	6.6	81.3	8.1
20	14.0	7.0	109.2	6.4
21	13.2	16.1	84.8	19.0
27	14.5	16.3	96.3	16.9
29	13.1	16.6	88.4	18.8
31	13.3	12.9	85.2	15.1
Mean	13.8	12.5	87.8	14.5

Table 4.10 Iron deficient but not anaemic animals.

Animals	Haemoglobin	Serum Iron	T.I.B.C.	% Saturation
11	15.9	33.3	-	-
12	15.2	26.9	-	-
14	13.9	36.7	113.1	32.4
22	15.8	31.1	96.1	32.4
23	15.0	25.8	86.1	30.0
24	14.5	44.0	-	-
25	14.9	31.7	97.9	32.4
26	15.3	31.7	97.9	32.4
30	13.0	18.3	-	-
32	13.4	16.1	71.0	22.4
Mean	14.7	29.6	93.7	30.3

Table 4.11 Iron depleted animals.

Animal 1

Points counted for each compartment.

Progenitor cell compartment = 950
 Mature cell compartment = 1814
 Keratinised compartment = 1062
 Total = 3826

Number of fields used = 8

Grid positions per field = 6

Mean number of points per grid position = $\frac{\text{Total Points}}{48}$

Mean epithelial point count = $\frac{3826}{48} = 9.7$

Column Width = .18 μm

Magnification = 12.5 x 40 = 500

Unit area on screen represents $\frac{5 \times 5}{500 \times 500}$ sq mm of section

= .0001 sq mm

Area measured = Number of points x 0.0001 sq mm

Thickness = $\frac{\text{Area}}{\text{Column Width}} = \frac{\text{Points} \times 0.0001}{.18} \times 1000 \mu\text{m}$

= $\frac{79.7 \times .0001 \times 1000}{.18} \mu\text{m}$

Epithelial Thickness = 44.3 μm

Figure 4.1 Conversion of total point count to thickness measurement.

PERCENTAGE OF THE EPITHELIUM
FORMED BY EACH COMPARTMENT

THICKNESS IN μm

Animal	Progenitor	Maturation	Keratin	Total	Progenitor	Maturation	Keratin
1	11.0	21.0	12.3	44.3	24.8	47.4	27.8
2	15.5	28.1	14.1	57.7	26.9	48.7	24.4
3	11.6	21.3	14.6	47.5	24.4	44.8	30.8
4	13.9	19.9	13.6	47.4	29.3	42.0	28.7
5	13.6	20.4	11.0	45.0	30.2	45.3	24.5
6	18.5	26.7	20.6	65.8	28.1	40.6	31.3
7	13.1	20.6	13.4	47.1	27.8	43.7	28.5
8	13.8	20.5	13.4	47.7	28.9	43.0	28.1
9	21.1	29.4	19.6	70.1	30.1	41.9	28.0
10	18.7	28.4	16.8	63.9	29.3	44.4	26.3
Mean	15.1	23.6	14.9	53.7	28.0	44.2	27.8

Table 4.12 Epithelial thickness, compartment thickness and the proportion of the epithelium formed by each compartment in normal animals

Animal	THICKNESS IN μm				PERCENTAGE OF THE EPITHELIUM FORMED BY EACH COMPARTMENT		
	Progenitor	Maturation	Keratin	Total	Progenitor	Maturation	Keratin
15	15.1	17.5	19.7	52.3	28.9	33.5	37.6
16	13.0	17.5	17.5	48.0	27.0	36.5	36.5
17	15.5	18.9	18.6	53.0	29.2	35.7	35.1
18	16.1	19.8	20.9	56.8	28.3	34.9	36.8
28	13.3	16.5	14.3	44.1	30.2	37.4	32.4
Mean	14.6	18.0	18.2	50.8	28.7	35.6	35.7

Table 4.13 Epithelial thickness, compartment thickness and the proportion of the epithelium formed by each compartment in anaemic animals.

Animal	THICKNESS IN μm				PERCENTAGE OF THE EPITHELIUM FORMED BY EACH COMPARTMENT			
	Progenitor	Maturation	Keratin	Total	Progenitor	Maturation	Keratin	
13	18.1	27.6	20.4	66.1	27.4	41.7	30.9	
19	15.1	19.9	18.5	53.5	28.2	37.2	34.6	
20	15.1	21.8	16.0	52.9	28.5	41.4	30.3	
21	13.2	16.9	15.1	45.2	29.2	37.4	33.4	
27	19.1	25.4	19.2	63.7	30.0	39.9	30.1	
29	14.1	21.0	16.3	51.4	27.4	40.9	31.7	
31	15.4	17.8	15.5	48.7	31.6	36.6	31.8	
Mean	15.7	21.5	17.3	54.5	28.9	39.3	31.8	

Table 4.14 Epithelial thickness, compartment thickness and the proportion of the epithelium made up by each compartment in animals with iron deficiency without anaemia.

Animal	THICKNESS IN μm				PERCENTAGE OF THE EPITHELIUM FORMED BY EACH COMPARTMENT			
	Progenitor	Maturation	Keratin	Total	Progenitor	Maturation	Keratin	
11	19.5	22.3	18.0	59.8	32.6	37.3	30.1	
12	14.2	15.3	11.9	41.4	34.3	37.0	28.7	
14	20.5	31.8	18.9	71.2	28.8	44.7	26.5	
24	18.8	17.1	15.5	51.4	36.5	33.3	30.2	
25	20.5	20.9	20.5	61.9	33.1	33.8	33.1	
26	17.5	22.9	17.4	57.8	30.3	39.6	30.1	
27	15.4	18.9	16.1	50.4	30.6	37.5	31.9	
28	16.3	22.3	14.2	52.8	30.9	42.2	26.9	
33	15.5	17.4	13.3	46.2	33.5	37.7	28.8	
35	16.3	24.5	12.4	53.2	30.6	46.1	23.3	
Mean	17.5	21.3	15.8	54.6	32.1	38.9	29.0	

Table 4.15 Epithelial thickness, compartment thickness and the proportion of the epithelium made up by each compartment in iron depleted animals.

PROPORTION OF THE EPITHELIUM
FORMED BY EACH CATEGORY

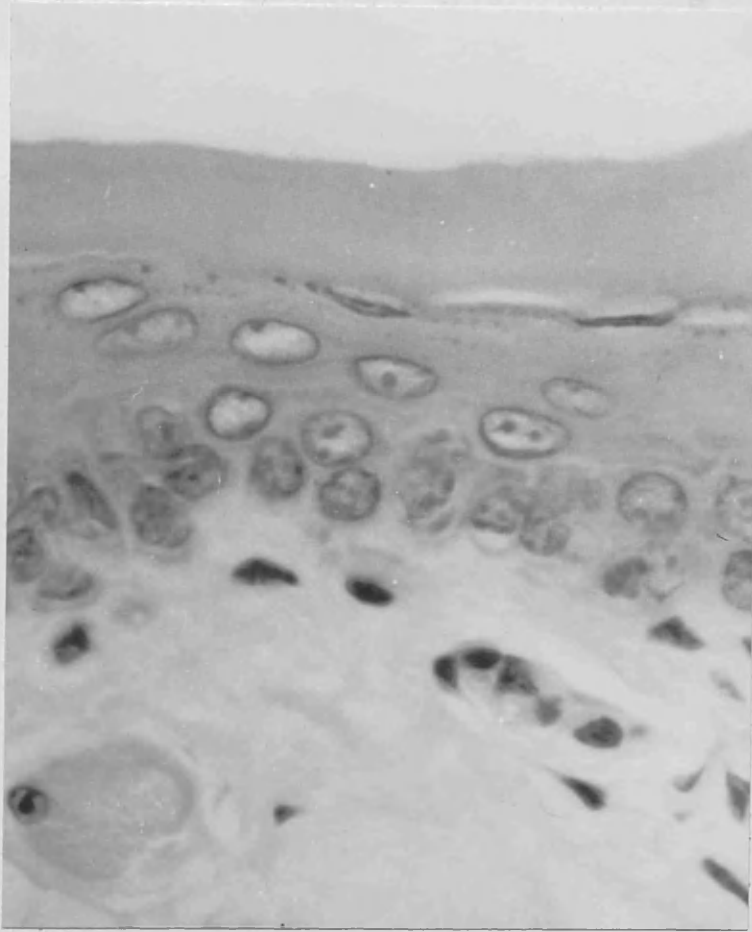


Figure 4.2 Ventral tongue epithelium from an animal with iron deficiency anaemia. x 860

STAGE OF IRON DEFICIENCY	COMPARTMENT CHANGES	PROPORTION OF THE EPITHELIUM FORMED BY EACH COMPARTMENT
Iron Depletion	Nil	Progenitor larger proportion Maturation smaller proportion
Iron Deficiency Without Anaemia	Nil	Maturation smaller proportion Keratin larger proportion
Iron Deficiency Anaemia	Maturation reduced Keratin increase	Maturation smaller proportion Keratin larger proportion

Table 4.16 The alterations in epithelial structure in the three stages of iron deficiency.

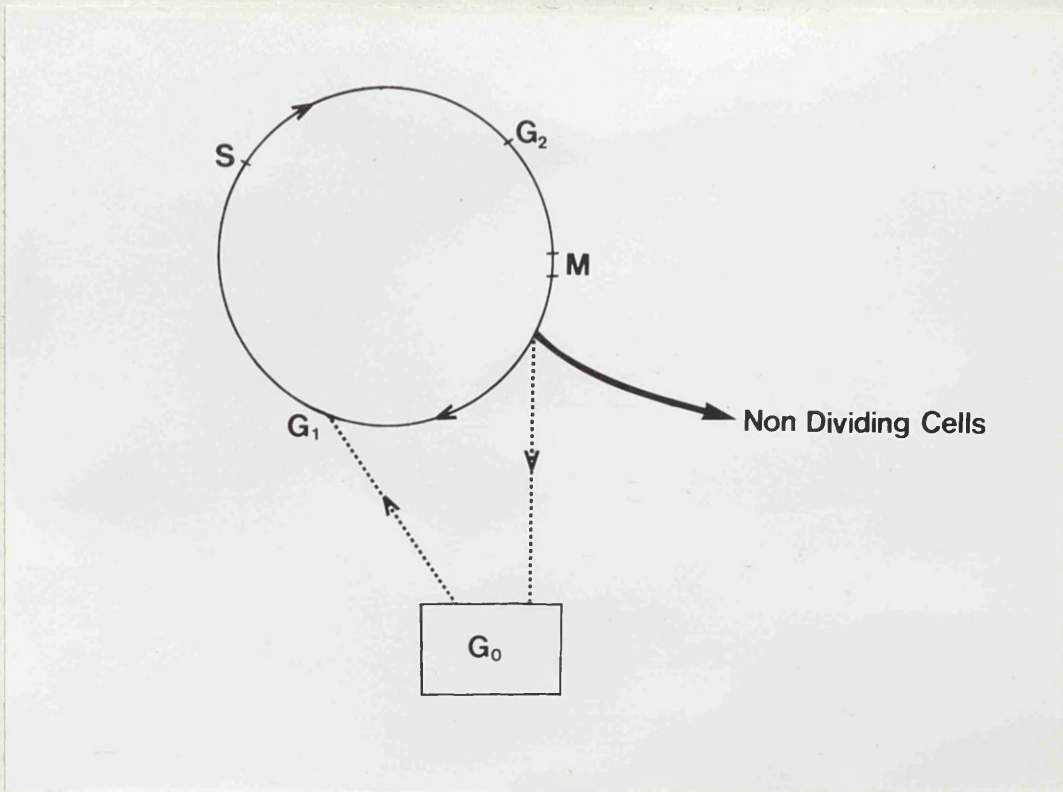


Figure 5.1 Diagrammatic illustration of the cell cycle. S - DNA Synthesis phase; G_1 - presynthetic gap; G_2 - postsynthetic gap; G_0 - non proliferating cells.

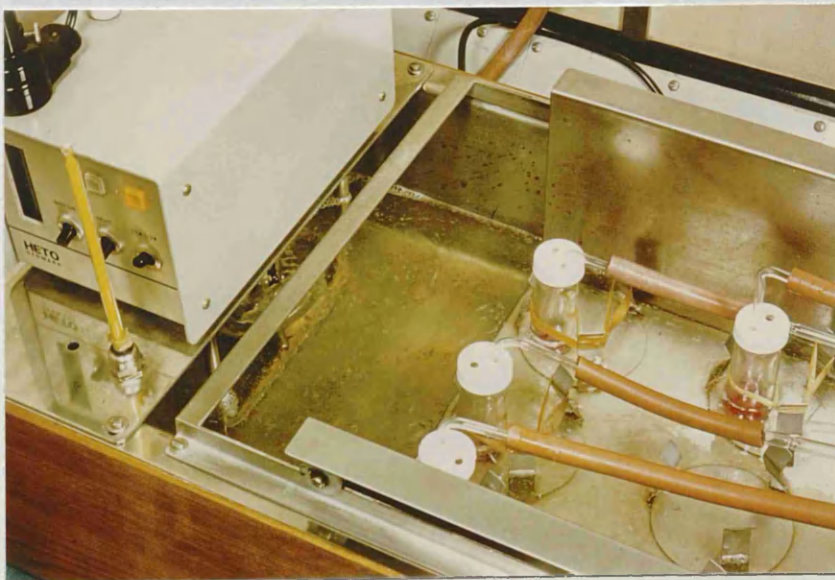


Figure 5.2 Apparatus used for in vitro ^3H thymidine labelling.

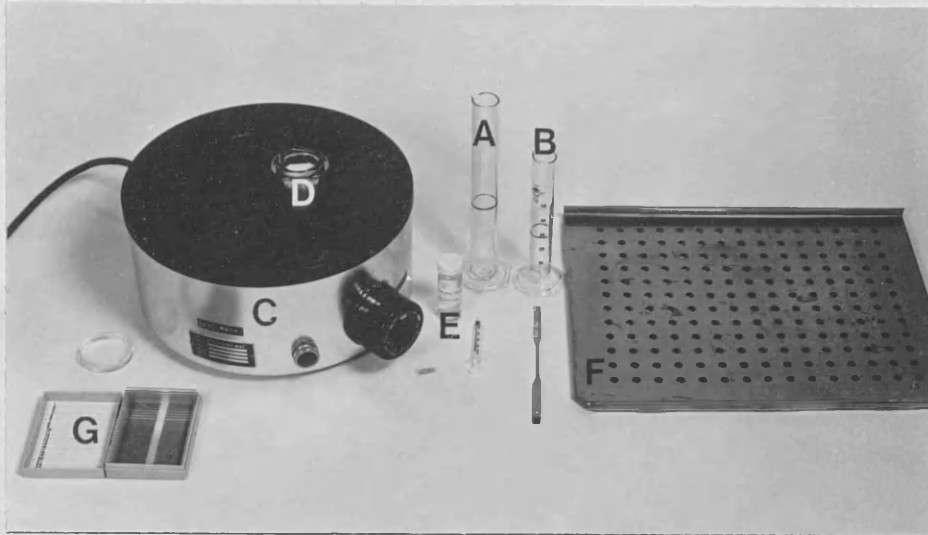


Figure 5.3 Equipment used for autoradiography. (A) and (B) measuring cylinders. (C) water bath. (D) Coplin jar with molten emulsion. (E) Glycerol. (F) Plate for drying slides. (G) Light proof plastic box for exposure.

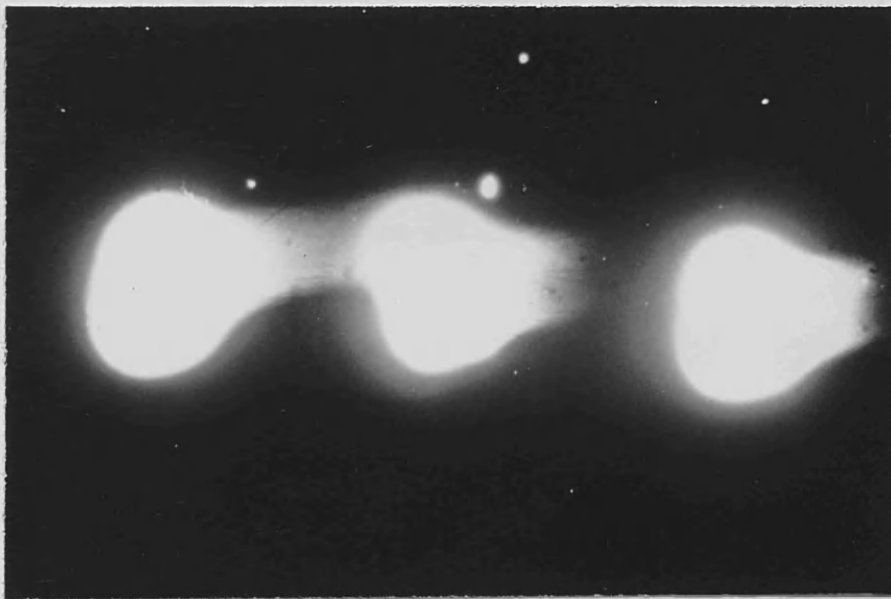


Figure 5.4 A hot control showing the effect of negative chemography. X100

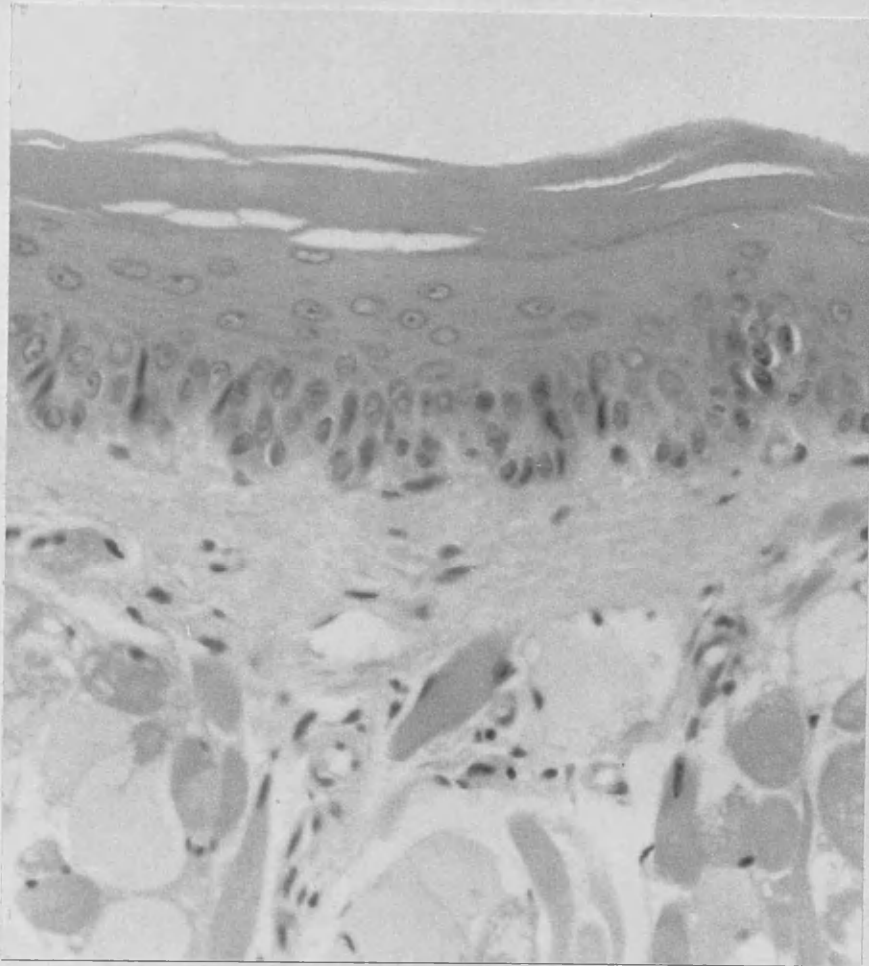


Figure 5.5 Hamster ventral tongue epithelium.x390

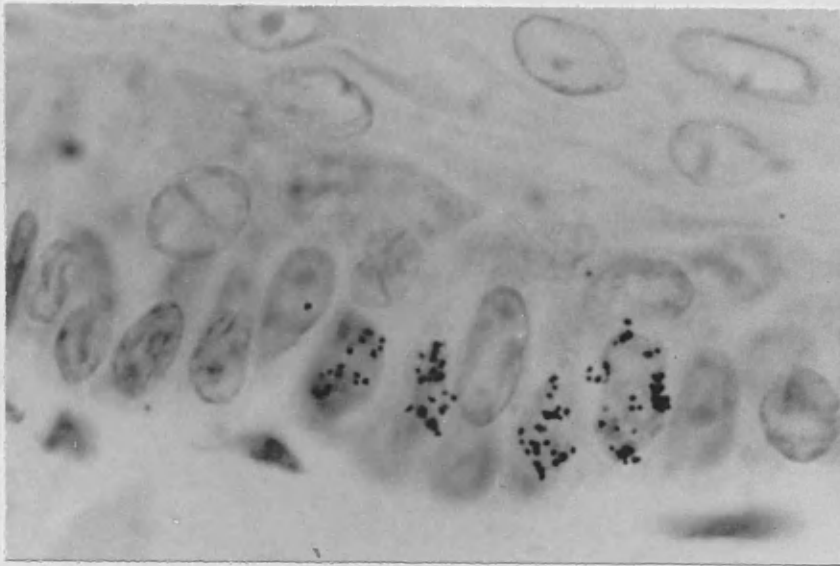


Figure 5.6 Autoradiograph of in vitro labelled hamster tongue epithelium showing labelled cells ($1 \mu\text{Ci/ml}$).x1150

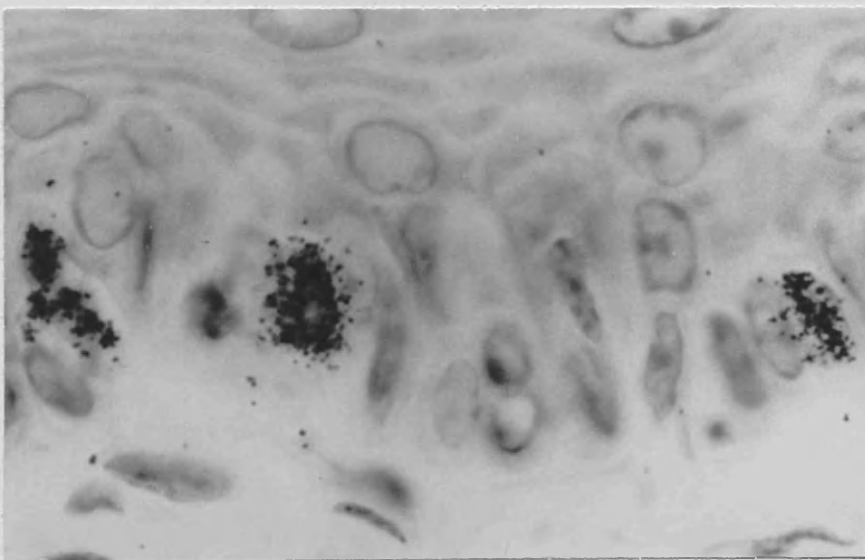


Figure 5.7 Autoradiograph of in vitro labelled hamster tongue epithelium showing heavily labelled cells.x1300

Animal	Nuclei Counted	Labelled Cells	Labelling Index per 100 viable nuclei	Fields	Labelling Index per field
2	2678	218	8.14	40	5.45
3	3650	339	9.29	79	4.29
4	4198	330	7.86	84	3.93
5	3694	287	7.77	85	3.38
6	3175	153	4.82	60	2.55
7	3561	229	6.43	75	3.05
Mean	3492.7	259.3	7.39	70.5	3.78

Table 5.1 Labelling index for control animals expressed in terms of total viable nuclear count and in terms of unit surface length.

Animal	Heavily Labelled Nuclei	Lightly Labelled Nuclei	T_s
1	94	20	4.70
2	68	25	2.72
3	111	16	6.94
4	111	42	2.64
5	40	19	2.10
6	109	46	2.37
7	130	64	2.03
8	137	61	2.25
Mean	100.0	36.6	3.22

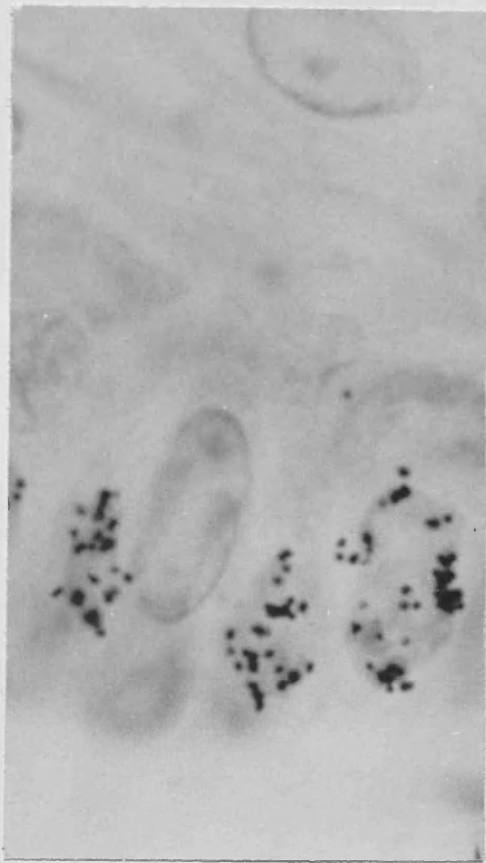
Table 5.2 T_s in hours for control animals.

Animal	Nuclei Counted	Labelled Cells	Labelling Index per 100 viable nuclei	Fields	Labelling Index per field
9	2421	161	6.65	48	3.35
10	2741	210	7.66	55	3.82
11	1785	106	5.94	40	2.65
12	1589	93	5.85	48	1.94
13	2853	156	5.47	43	3.63
14	2534	175	6.9	54	3.24
15	2837	119	4.19	60	1.98
16	2156	106	4.92	42	2.52
Mean	2364.5	140.8	5.95	48.8	2.89

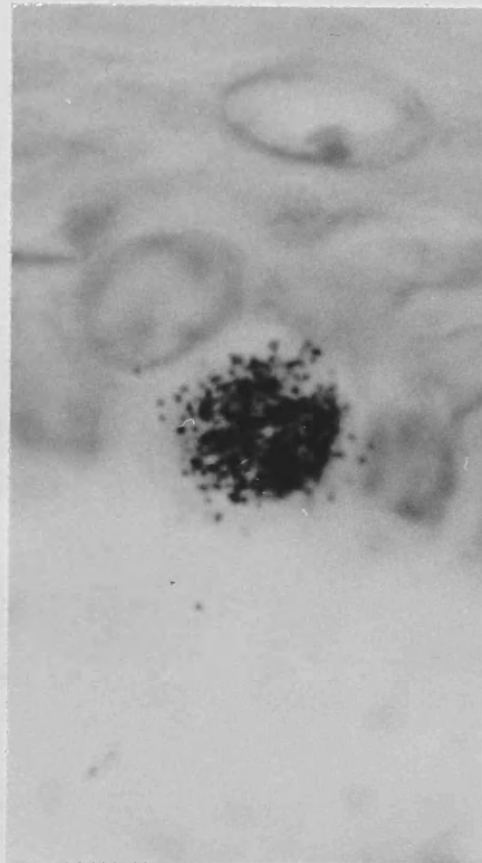
Table 5.3 Labelling index for experimental animals in terms of total viable nuclear count and in terms of unit surface length.

Animal	Heavy Label	Light Label	T_s
9	88	20	4.40
10	159	26	6.12
11	214	47	4.55
12	163	41	3.98
13	161	44	3.66
14	180	48	3.75
15	72	40	1.80
16	145	63	2.30
Mean	147.8	41.1	3.82

Table 5.4 T_s in hours for experimental animals



1 μCi



10 μCi

Figure 6.1 Autoradiographs from tissue labelled in vitro with a light (1 $\mu\text{Ci}/\text{ml}$) and a heavy (10 $\mu\text{Ci}/\text{ml}$) dose of ³H thymidine. x1900

$$N = n \times \frac{T}{T + d}$$

N = Corrected cell count

n = Actual cell count

T = Section thickness

d = Nuclear diameter

<u>Animal 1</u>	Progenitor cells counted	= 938
	Mature cells counted	= 343
	Labelled cells counted	= 56
	d progenitor	= 5.34 μm
	d maturation	= 8.67 μm
	T Section thickness	= 3.5 μm

$$\begin{aligned} \text{N. prog} &= 938 \times \frac{3.5}{3.5 + 5.34} \\ &= \underline{371.4} \end{aligned}$$

$$\begin{aligned} \text{N. Mat} &= 343 \times \frac{3.5}{3.5 + 8.67} \\ &= \underline{99.47} \end{aligned}$$

$$\begin{aligned} \text{N. Lab} &= 56 \times \frac{3.5}{3.5 + 5.34} \\ &= \underline{22.17} \end{aligned}$$

Figure 6.2 Correction of cell counts (Abercrombie, 1946).

Animal	Progenitor cell nuclear diameter in μm	Mature cell nuclear diameter in μm
1	5.34	8.67
2	5.12	7.79
3	5.74	8.57
4	5.48	8.79
5	4.69	7.44
7	4.86	7.91
8	5.09	8.51
9	5.55	7.58
10	5.99	8.46
Mean	5.31	8.13
S.D.	0.40	0.52

Table 6.1 Nuclear diameters of control animals.

Animal	Progenitor cell nuclear diameter in μm	Mature cell nuclear diameter in μm	
15	5.25	7.50	A N A E M I C
16	5.00	8.37	
17	4.81	8.14	
18	5.29	9.61	
28	5.34	8.98	
Mean	5.14	8.52	
S.D.	0.23	0.81	
13	5.39	8.36	D E F I C I E N T
19	4.79	8.19	
20	5.07	7.73	
21	4.90	7.69	
27	5.87	8.23	
29	4.48	8.39	
31	5.56	9.12	
Mean	5.15	8.24	
S.D.	0.48	0.48	
11	5.80	8.9	D E P L E T E D
12	6.03	9.63	
14	4.68	8.37	
22	4.52	7.79	
23	4.95	8.01	
24	5.73	8.34	
25	4.31	8.37	
26	5.22	9.02	
30	4.53	8.21	
32	5.42	8.66	
Mean	5.12	8.53	
S.D.	0.61	0.54	

Table 6.2 Nuclear diameters of experimental animals.

LABELLING INDEX

Animal	Prog.	Mat.	T.V.C.	L.C.	Columns	100 Progenitor cells	100 Total Viable cells	Unit Surface Length
1	938	343	1281	56	42	6.0	4.4	1.3
2	1100	371	1471	42	45	3.8	2.9	0.9
4	1082	362	1444	72	41	6.7	5.0	1.8
5	984	311	1295	32	39	3.3	2.5	0.8
7	1055	352	1407	86	43	8.2	6.1	2.0
8	819	289	1108	52	36	6.3	4.7	1.4
9	839	216	1055	27	23	3.2	2.6	1.2
Mean						5.36	4.03	1.34

Table 6.3 Labelling index for normal animals (1 μ Ci/ml)

LABELLING INDEX

Animal	Prog.	Mat.	T.V.C.	L.C.	Columns	100 Progenitor cells	100 Total Viable cells	Unit Surface Length
1	1451	455	1906	61	42	4.20	3.20	1.50
2	2155	603	2758	75	45	3.48	2.72	1.70
3	1945	617	2562	167	27	8.59	6.52	6.20
5	863	260	1123	33	39	3.82	2.94	0.90
7	1772	539	2311	111	43	6.26	4.80	2.60
8	1772	532	2304	110	36	6.21	4.77	3.10
9	929	300	1229	44	23	4.74	3.58	1.33
10	1752	416	2168	99	26	5.65	4.57	3.80
Mean						5.37	4.14	2.64

Table 6.4 Labelling index for normal animals (10 μ Ci/ml)

Animal	P _c	M _c	T _c	L.C.c	Cols	100 Progenitor cells	100 Total Viable cells	Unit Surface Length
1	375.20	99.47	474.67	22.40	42	5.97	4.72	0.53
2	451.00	115.01	566.01	17.22	45	3.81	3.04	0.38
4	421.98	101.36	523.34	28.08	41	6.65	5.37	0.69
5	423.12	99.52	522.64	13.76	39	3.25	2.63	0.35
7	443.10	109.12	552.22	36.12	43	8.15	6.54	0.84
8	335.79	83.81	419.60	21.32	36	6.35	5.08	0.59
9	327.21	69.12	396.33	10.53	26	3.22	2.66	0.41
Mean						5.34	4.29	0.54

Table 6.5 Corrected labelling index for normal animals
(1 μ Ci/ml)

LABELLING INDEX

Animal	P _c	M _c	T _c	L.C.c	Cols	100 Progenitor cells	100 Total Viable cells	Unit Surface Length
1	458.00	88.45	546.45	20.80	33	4.54	3.81	0.63
2	410.00	92.07	502.07	11.89	36	2.90	2.37	0.33
3	616.74	106.43	723.17	26.22	51	4.25	3.63	0.51
4	345.93	85.40	431.33	20.67	30	5.98	4.79	0.69
5	656.61	155.84	812.45	30.10	46	4.58	3.70	0.65
7	360.78	70.06	430.84	33.60	20	9.31	7.80	1.70
8	223.45	48.14	271.59	8.20	21	3.67	3.02	0.39
9	457.47	128.00	585.47	68.25	31	14.92	11.66	2.20
10	452.88	113.39	566.27	37.37	43	8.25	6.60	0.87
Mean						6.49	5.26	0.99

Table 6.6 Corrected cell counts and labelling index of normal animals obtained from double labelled material (1/10 μ Ci/ml)

Animal	P _c	M _c	T _c	L.C.c	Cols	100 Progenitor cells	100 Total Viable cells	Unit Surface Length
15	464.40	93.76	540.16	15.20	40	3.41	2.81	0.38
16	359.57	67.57	427.14	16.81	33	4.68	3.94	0.51
17	523.74	93.90	617.64	17.46	41	3.37	2.83	0.43
20	535.20	122.04	657.24	12.00	40	2.24	1.83	0.30
28	449.20	85.40	534.60	18.00	31	4.01	3.37	0.58
Mean						3.54	2.96	0.44

Table 6.7 Corrected labelling index for anaemic animals
(1 $\mu\text{Ci/ml}$)

LABELLING INDEX

Animal	Pc	Mc	Tc	L.C.c	Cols	100 Progenitor cells	100 Total Viable cells	Unit Surface Length
13	287.82	93.00	380.82	19.50	37	6.78	5.12	0.53
19	328.86	97.80	426.66	10.08	33	3.07	2.36	0.31
20	281.26	95.17	376.43	21.32	36	7.58	5.66	0.59
21	250.32	71.61	321.93	15.12	30	6.04	4.70	0.50
29	312.84	60.90	373.74	12.32	29	3.94	3.30	0.43
Mean						5.48	4.23	0.47

Table 6.8 Corrected labelling index for animals with iron deficiency without anaemia (1 μ Ci/ml)

LABELLING INDEX

Animal	P _c	M _c	T _c	I.C.c	Cols	100 Progenitor cells	100 Total Viable cells	Unit Surface Length
11	353.78	91.84	445.62	39.52	32	11.17	8.87	1.24
12	230.14	56.16	286.13	9.99	25	4.34	3.49	0.40
14	296.27	78.88	375.15	21.07	26	7.11	5.16	0.81
23	345.63	99.30	444.93	17.22	33	4.98	3.87	0.52
24	311.60	93.90	405.50	20.14	28	6.46	4.97	0.72
25	363.15	86.13	449.28	20.70	24	5.70	4.61	0.86
26	403.60	94.54	498.14	27.20	36	6.74	5.46	0.76
Mean						6.64	5.20	0.76

Table 6.9 Corrected labelling index for iron depleted animals (1 μ Ci/ml)

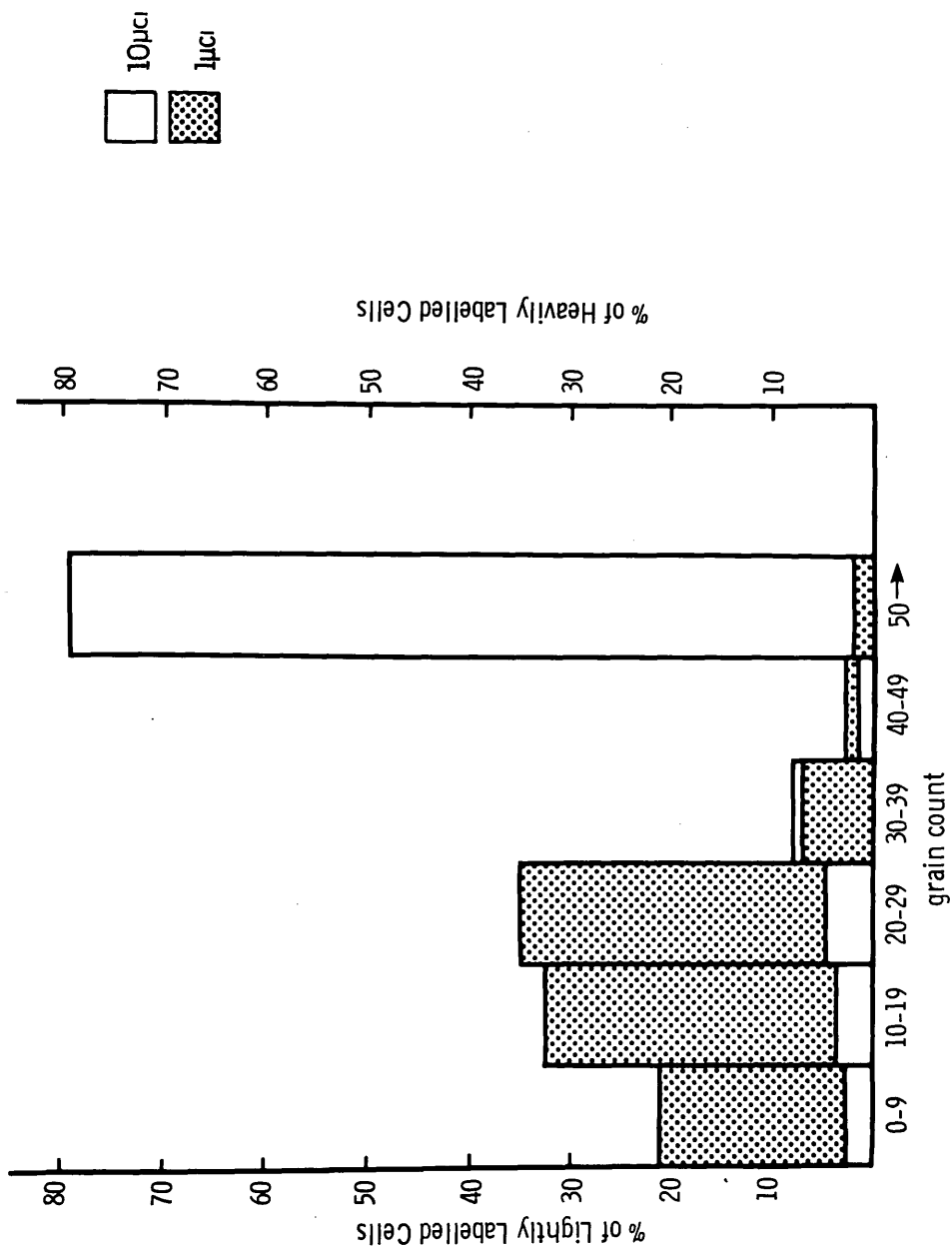


Figure 6. 3. Grain Distribution for 1μc and 10μc Material

Animal	L.L.C.	H.L.C.	T_s Hours
1	10	52	5.20
2	9	29	3.22
3	14	69	4.93
4	12	53	4.42
5	19	70	3.68
7	15	80	5.33
8	3	20	6.67
9	38	175	4.61
10	16	101	6.31
Mean	15.1	72.1	4.93

Table 6.10 T_s for normal animals.

Animal	L.L.C.	H.L.C.	T_s Hours
15	9	34	3.78
16	13	21	1.62
17	12	32	2.67
18	4	17	4.25
28	19	70	3.68
Mean	11.4	34.8	3.20

Table 6.11 T_s for anaemic animals.

Animal	L.L.C.	H.L.C.	T _s Hours
13	8	26	3.25
20	41	97	2.37
21	13	42	3.23
27	26	95	3.65
29	8	28	3.50
31	10	35	3.50
Mean	17.6	53.8	3.25

Table 6.12 T_s for iron deficiency without anaemia animals.

Animal	L.L.C.	H.L.C.	T _s Hours
11	32	173	5.38
14	12	53	4.42
23	7	44	6.29
24	9	50	5.56
25	7	29	4.14
26	10	85	8.50
32	8	34	4.25
Mean	12.1	66.8	5.32

Table 6.13 T_s for iron depleted animals.

Animal	Pc	Columns	Pc/Column	Progenitor Thickness	Cells/ 100 sq μ m
1	375.20	42	8.93	11.0	0.902
2	451.00	45	10.02	15.5	0.718
3	270.56	27	10.02	11.6	0.960
4	421.98	41	10.29	13.9	0.823
5	423.12	39	10.85	13.6	0.886
7	443.10	43	10.30	13.1	0.874
8	335.79	36	9.32	13.8	0.750
9	327.21	23	14.23	21.1	0.749
10	289.70	26	11.14	18.7	0.662
Mean					0.814
S.D.					0.099

Table 6.14 Number of progenitor cells per 100 sq μ m in normal animals.

Animal	Mc	Columns	Mc/Column	Maturation Compartment Thickness	Cells/ 100 sq μm
1	99.47	42	2.37	21.0	.125
2	115.01	45	2.56	28.1	.101
3	64.38	27	2.38	21.3	.124
4	101.36	41	2.47	19.1	.138
5	99.52	39	2.55	20.4	.139
7	109.12	43	2.54	20.6	.137
8	83.81	36	2.33	20.5	.126
9	69.12	23	3.01	29.4	.114
10	56.84	26	2.19	28.4	.086
Mean					.121
S.D.					.018

Table 6.15 Number of mature cells per 100 sq μm in normal animals.

Animal	Pc	Columns	Pc/Column	Progenitor Thickness	Cells/100 sq μ m	
15	446.04	40	11.16	15.1	0.821	A N A E M I C
16	359.57	33	10.09	13.0	0.932	
17	523.74	41	12.77	15.5	0.915	
18	535.20	40	13.38	16.1	0.923	
28	449.20	31	14.49	13.3	1.120	
Mean					0.942	
13	287.82	37	7.78	18.1	0.478	D E F I C I E N T
19	328.86	33	9.97	15.1	0.734	
20	281.26	36	7.81	15.1	0.575	
21	250.32	30	8.34	13.2	0.702	
29	312.84	29	10.79	14.1	0.850	
Mean					0.668	
11	353.78	32	11.06	19.5	0.630	D E P L E T E D
12	230.14	25	9.21	14.2	0.721	
14	296.27	26	11.04	20.5	0.618	
23	345.63	33	10.47	10.5	0.567	
24	311.60	28	11.13	17.5	0.707	
25	363.15	24	15.13	15.4	1.092	
26	403.60	36	11.21	16.3	0.764	
Mean					0.728	

Table 6.16 Number of progenitor cells per 100 sq μ m in experimental animals.

Animal	Mc	Columns	Mc/Column	Maturation Thickness	Cells/ 100 sq μ m	
15	93.76	40	2.34	17.5	0.149	
16	67.57	33	2.05	17.5	0.130	A
17	93.09	41	2.29	18.9	0.135	N
18	122.04	40	3.05	19.8	0.171	A
28	85.40	31	2.75	16.5	0.185	E
						M
						I
						C
Mean					0.154	
13	93.00	37	2.51	27.6	0.101	D
19	97.80	33	2.96	19.9	0.165	E
20	95.17	36	2.64	21.8	0.135	F
21	71.61	30	2.39	16.9	0.157	I
29	60.90	29	2.10	21.0	0.111	C
						E
						N
						T
Mean					0.134	
11	91.84	32	2.87	22.3	0.143	
12	56.16	25	2.25	15.3	0.163	D
14	78.88	26	3.03	31.8	0.106	E
23	99.30	33	3.01	20.9	0.160	P
24	93.90	28	3.35	22.9	0.163	L
25	86.13	24	3.59	18.9	0.211	E
26	94.54	36	2.63	22.3	0.131	T
						E
						D
Mean					0.154	

Table 6.17 Number of mature cells per 100 sq μ m in experimental animals.

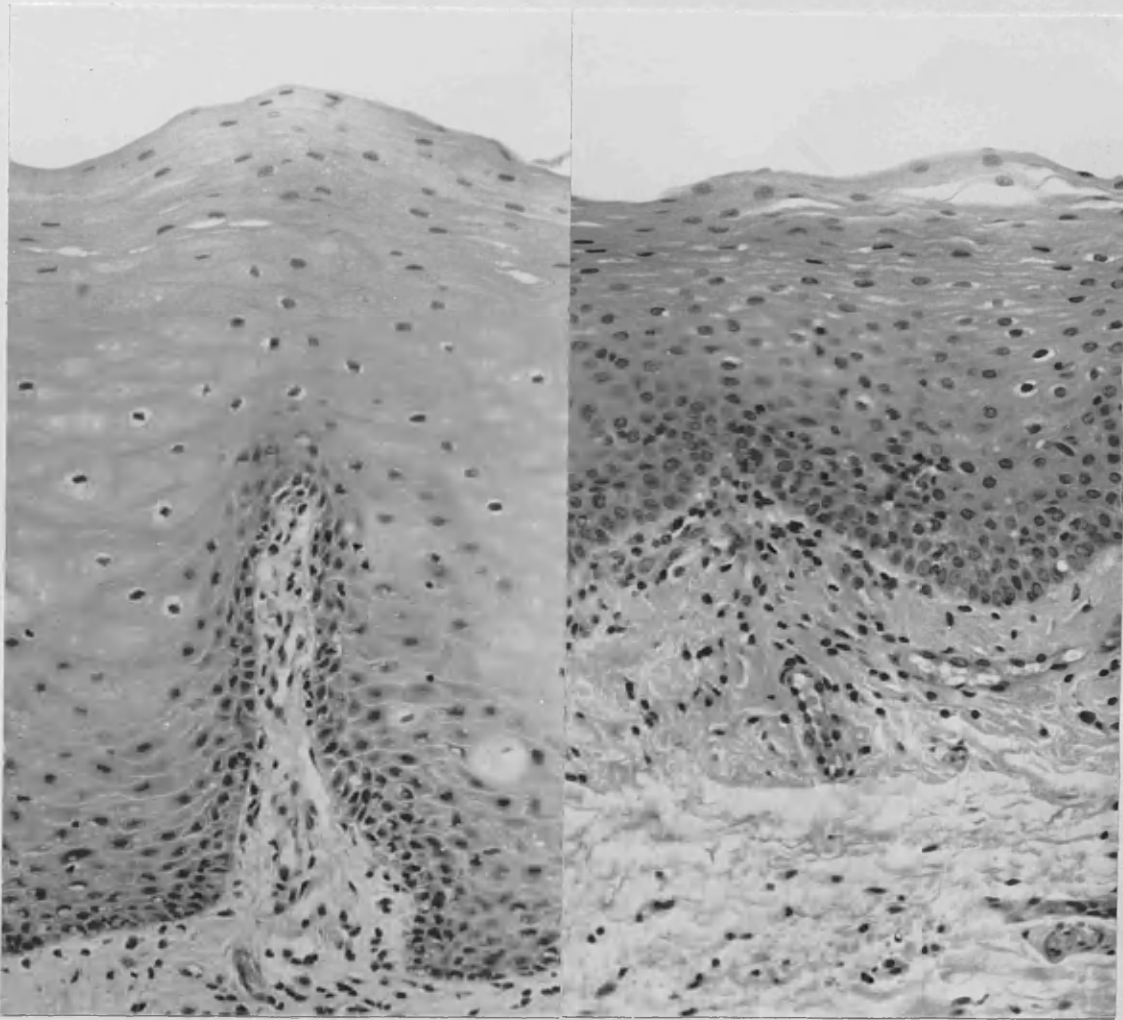


Figure 7.1 Normal buccal epithelium and epithelium from a patient with iron deficiency anaemia.x340

Angle from Vertical	Cosine	% Change in Thickness
5	.9962	0
10	.9848	2
15	.9659	3
20	.9397	6
25	.9063	9
30	.8660	13

Table 7.1 Change in epithelial thickness, expressed as a percentage, following alteration of the tissue orientation.

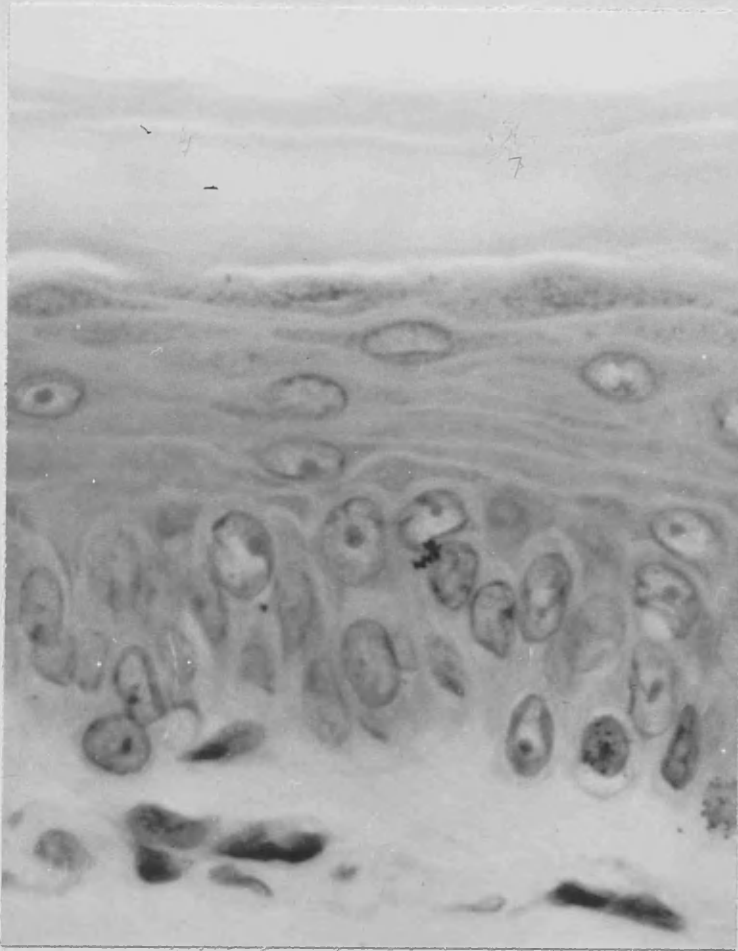


Figure 7.2 Epithelium from the ventral surface of hamster tongue in iron depletion. X1100

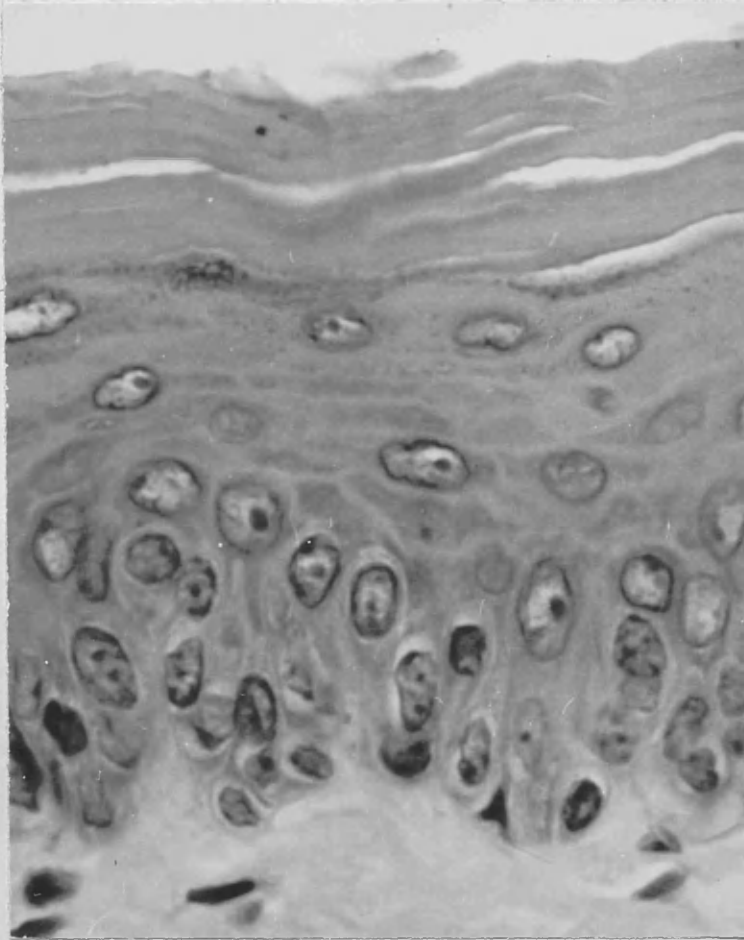


Figure 7.3 Epithelium from the ventral surface of hamster tongue, in iron deficiency without anaemia.X960

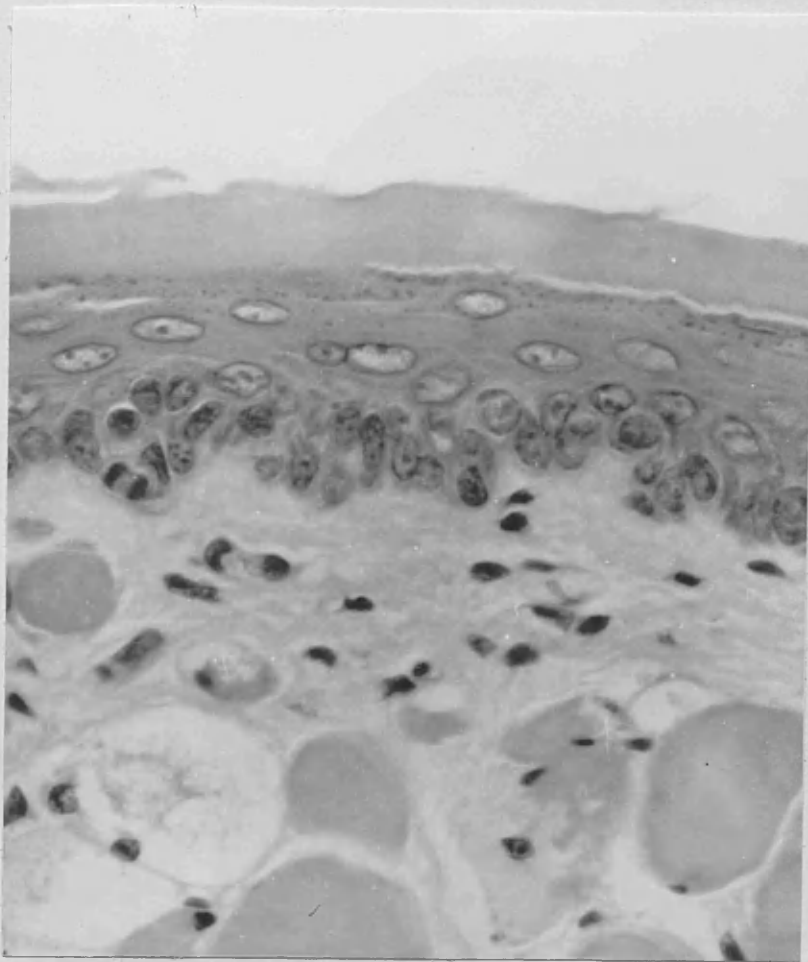


Figure 7.4 Epithelium from the ventral surface of hamster tongue in iron deficiency anaemia.X320

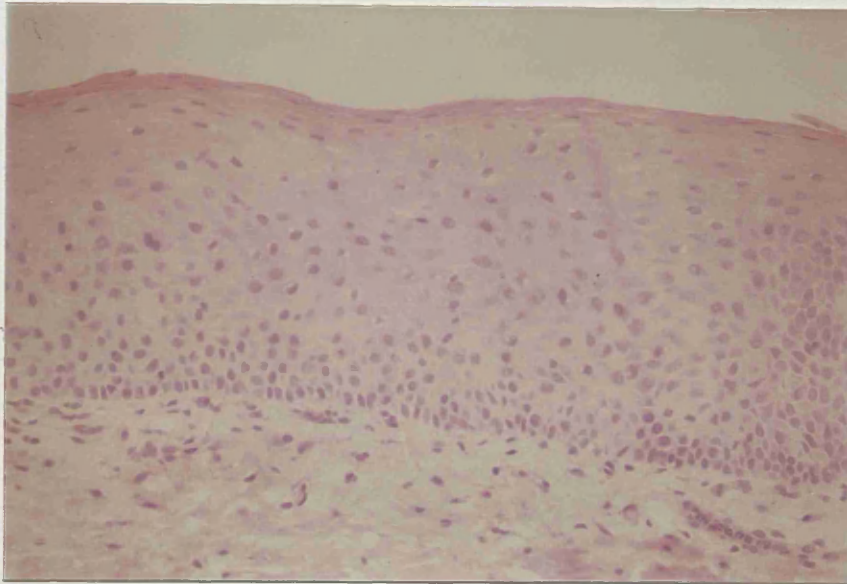


Figure 7.5 Keratinisation of the buccal epithelium in iron deficiency anaemia.X120

ABBREVIATIONS

Cols	-	epithelial columns
G ₁	-	postmitotic - presynthetic gap
H.L.C.	-	heavily labelled nuclei
Ker	-	keratinised compartment
L.C.	-	labelled nuclei
L.C. _c	-	corrected labelled nuclei
L.L.C.	-	lightly labelled nuclei
Mat	-	maturation compartment
Mc	-	corrected maturation compartment nuclei
mean	-	arithmetical mean
P _c	-	corrected progenitor compartment nuclei
Prog	-	progenitor cell compartment
S	-	DNA synthesis phase
S.D.	-	standard deviation
T _c	-	corrected total nuclear count
T.I.B.C.	-	total iron binding capacity
T _s	-	time taken for DNA synthesis
T.V.C.	-	total viable cell count

APPENDIX 1PROCESSING CYCLE

1.	50% spirit	30 minutes
2.	80% spirit	1 hour
3.	8% phenol in methylated spirit	2 hours
4.	8% phenol in methylated spirit	1 hour
5.	8% phenol in methylated spirit	2 hours
6.	Absolute alcohol	2 hours
7.	Absolute alcohol	3 hours
8.	Absolute alcohol chloroform	30 minutes
9.	Chloroform	3 hours
10.	Chloroform	4 hours
11.	Paraffin wax	2 hours
12.	Paraffin wax	2 hours

APPENDIX 2PERL'S PRUSSIAN BLUE REACTION

1. Bring sections to water
2. Transfer to a fresh solution of equal parts of 2% aqueous potassium ferrocyanide and 2% hydrochloride for 30 minutes at 57°C
3. Wash thoroughly for 10 minutes
4. Counterstain lightly with 1% neutral red for 10 - 15 seconds
5. Wash in water, dehydrate, clear and mount in Canada balsam

Results: Ferric - iron - containing pigments - Blue
nuclei - Red