XX. STUDIES IN ANAEMIA. I.

THE INFLUENCE OF DIET ON THE OCCURRENCE OF SECONDARY ANAEMIA FOLLOWING REPEATED HAEMORRHAGES IN RATS.

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Aims.

It seems a very obvious theorem that repeated haemorrhages would produce a chronic anaemia much more easily in an animal fed on a poor diet which lacks food factors essential for the formation of haemoglobin, such for instance as iron, than in one fed on a satisfactory and complete diet, and the first part of the following research was undertaken to demonstrate this. In practice several limitations of this principle are found. Thus one of the unpurified diets poorest in iron, white bread and whole milk, contains some materials for blood formation. Moreover there is a marked tendency even on a comparatively iron-poor diet to storage of a reserve of blood forming materials. Further, a limitation in the contrary direction is that a diet which is adequate in a fully grown animal to prevent an anaemia is inadequate in a similarly bled growing animal, presumably because it has not only to maintain its blood mass but also to increase it. These points are brought out in the following work.

Preliminary work on cats.

When this work was begun cats were chosen in preference to rats because it was easy to withdraw blood repeatedly from them by cardio-puncture under an anaesthetic. This avoided open operation and the animals were perfectly well in 24 hours. Five cats were fed on bread, milk and fat and were subjected every week to bleedings amounting each to 1 % of the body weight. Controls were fed on the same diet but not subjected to bleeding. Other controls were given meat in addition to the bread and milk and were subjected to similar haemorrhages. The idea underlying the research was to find out if the first set of cats could be brought to a stage where they exhibited a chronic anaemia characterised by a fall in the colour index of the corpuscle. This was the case. The colour index of the cats fed on bread and milk and bled maintained itself or even increased at first, but then fell to sub-normal. The cats which were fed on bread and milk maintained their colour index. The controls which were to be given meat and bled gave considerable trouble, as an infectious gastro-intestinal disease broke out amongst them and the mortality amongst them and the difficulty of stamping it out led eventually to my abandoning the use of these animals and substituting rats. The difficulty of bleeding rats repeatedly, which had previously been an obstacle to their use, was solved by having recourse to leeches, as will be described later.

Outline of work on rats.

Rats are suitable animals for this work, because so much research on diets has been done on them. They were bred from mothers which had been fed before and during pregnancy and weaning on a liberal mixed diet.

In the inception of the work unpurified diets were used, the basal diet being white bread and whole milk and the ration the ample one of 20 g. white bread and 75 cc. whole milk per 100 g. rat per day. In addition certain controls were given from the time of weaning a liberal supply of green stuff, cabbage, etc., to the amount of 10 g. per 100 g. rat per day. The actual amount of green food eaten is not known but it was certainly one-half and may have been as much as three-quarters of the green food given. The rats exhibited marked preference for the tender parts of plants, *e.g.* the heart leaves of cabbage. Thus the two diets differed only in the presence or absence of green stuff but the difference in the result might be assigned to one or more of several factors:

1. *Differences in iron content.* The results of different observers taken from Sherman [1907] are given in tabular form.

Bread.

Observer	Iron per 100 g. mgm.	
Stockman	0·6	Fine bread
„	0·4	Common bread
Sherman	0·7	
Bunge	0·7	Minimum
„	1·0	Maximum
	Average 0·7	

Milk.

Bunge (1875)	0·24	
„ (1904)	0·30	
Abderhalden	0·23	
Stockman	0·33	Average of 3 estimations
Sherman	0·24	„ 5 „
	Average 0·27	

Thus there is fairly general agreement about the iron content of bread and milk. Taking the averages given above this would make in the bread and milk diet 0·35 mgm. iron per 100 g. rat per day if the whole of the bread and milk was eaten (which was not the case).

There is no such convincing similarity between the figures given for the iron content of green vegetables by different observers.

Green vegetables.

Material	Observer	Iron per 100 g. mgm.
Spinach	Boussingault[1]	4·5
„	Sherman	2·8[2]
„	Bunge	3·2[2]
„	Baldoni	2·0
Cabbage	Boussingault[1]	3·9
„ (outer green) ...	Hausermann	1·4
„ (inner yellow) ...	„	0·4
„ ("edible portion")	Sherman	0·9
Lettuce[3]	„	0·3

[1] This observer's figures are regarded by Stockman as too high.

[2] Corrected as advised by Sherman "Iron in Food."

[3] Presumably the edible portion, *i.e.* the heart.

It would appear unsound to calculate from any average of these data the iron content of the mixed green stuffs given, but it is certain that the diet with green stuffs contains additional iron and it is probable that the iron is in some different form.

2. *Differences in vitamin content.* Bread and milk is usually recognised as containing a bare sufficiency of vitamins but green stuff contains a liberal supply of them.

3. *Differences in other substances.* Other mineral salts, organic groupings such as might be contained in chlorophyll, etc.

From a few days after weaning the experimental animals were fed on the basal diet alone and shortly after puberty, *i.e.* at the age of 90 days, they were subjected to a series of eight bleedings, each of about 1% of the body weight, at intervals of a week. The question was whether these animals would develop a more or less chronic anaemia characterised by a low colour index.

The controls were: (*a*) animals fed on the basal diet plus green stuff, these animals being subjected to an identical series of bleedings; (*b*) animals fed on the basal diet alone and not subjected to bleeding.

In consequence of preliminary experiments it was found necessary to start differential feeding at a very early age because indefinite results were obtained if the rats were fed on a mixed diet (with green stuff) till puberty and differentiation in the diet was delayed till then. The rats which were started on bread and milk only after puberty required a considerable number of these small bleedings (12 or more) before they showed any fall of colour index.

Preliminary Discussion.

At this early stage attention may be called to two papers which appeared while this work was in progress. It will be noted that the blood drawn on each occasion in this work was equal to 1% of the body weight; Geiling and Green [1921] showed that after a haemorrhage of double this size regeneration was complete in 7–10 days. It was therefore reasonable to expect that in this experiment between haemorrhages regeneration would take place to a considerable extent if not completely. The same authors, using purified diets, showed that a diet deficient in protein, vitamin or mineral matter appreciably delayed blood regeneration after two haemorrhages on successive days.

Jencks [1922] showed that protein causes more rapid regeneration than fat or carbohydrate; vitamin-rich food is specially valuable for blood regeneration.

The work which is detailed below bears out these results but also an intrinsic value may be claimed for it inasmuch as the problem is approached from a slightly different point of view; these observers noted the rate of recovery from an acute experimental anaemia on different diets; this work demonstrates the complementary proposition that a series of haemorrhages insufficient to cause an anaemia (as judged by the lowering of the colour index) on the rich diet is sufficient to cause a moderately persistent anaemia on the poor diet provided growing animals have been kept long enough on it to reduce their reserve stores of blood forming materials to a minimum.

Details of experimental procedure.

Method of bleeding. As fatty changes had been observed in the liver and kidneys in the experiments on cats it was thought wise to avoid anaesthetics, which themselves cause these changes. If the animals were bled from the tail it was a little difficult to control the amount abstracted and resort was made to the use of leeches. The rat was clipped with artery forceps in a piece of sacking; an area on the flank was shorn and a snick was made in the skin with a pair of scissors. To this the leech was applied. This proved an admirable way of extracting blood—for by this means a large number of profuse bleedings can be made on the same rat without trouble. A little experience enabled one to judge approximately from the degree of swelling of the leech how much blood had been abstracted and the exact quantity was determined by the difference in its weight before application and after removal.

Samples of blood, which flowed freely from the incision until stopped by pressure, were taken and examined by the haemocytometer and haemoglobinometer, the apparatus used being those of Bürker and Gowers respectively.

Attention was paid to the following points in the use of the haemocytometer.

1. A fair sample of blood was taken. The blood was allowed to flow freely and the haemocytometer and haemoglobinometer blood was taken so far as possible from the same drop.

2. Pipettes and counting chamber were standardised.

3. The cover glass and Bürker slide were polished till they adhered over parts of the areas in contact, Newton's rings being obliterated there.

4. Solution of corpuscles in the counting fluid (which occurs in Toison and sodium citrate solutions in the case of anaemic bloods) was avoided by using instead mammal Ringer to every 100 cc. of which 3 cc. formalin were freshly added.

5. The mixed fluid was run quickly and evenly through the Bürker chamber. Delay leads to the count being high, as also does any great overflow into the trough.

6. At first 256 squares in each Bürker chamber were counted, but later it was found sufficient to count two sets of 64 squares each in each chamber.

As regards the haemoglobinometer, Gower's standard was finally selected in preference to Haldane's as it was found that its correction constant when tested against a blood of known oxygen capacity remained the same, being 100/83·3 at the beginning of the experiments and 100/83·5 at the end. In the same time the correction constant for the Haldane standard fell from 100/86·5 to 100/92·3, indicating that the standard had faded.

The colour index is expressed in terms of the human standard, this being taken as 1. It is obtained by dividing the percentage of haemoglobin by twice the number of red blood corpuscles.

Tables and a chart showing the results of the experiment follow.

Table I. *Showing the blood condition of normal adult rats on mixed diet.*

Haemoglobin %	Red blood corpuscles in millions per mm.^{3}	Colour index (human index = 1)
81·5	7·34	·56
103	8·74	·59
96	8·57	·56
117	10·6	·55
100	9·03	·55
103	9·27	·56
103	9·27	·56
93	8·40	·55
114	9·63	·59
105	9·53	·55
99	8·90	·56
72	6·50	·55

Average colour index = ·56.

Table II. *Showing the blood condition of control rats put on a diet of bread and milk after weaning and not subjected to bleedings.*

Age in weeks	Haemoglobin %	R.B.C. in millions per mm.^{3}	Colour index (human = 1)	Average C.I.
14	100	9·06	·55	
	112	10·08	·56	
	102	8·32	·61	
	100	8·85	·56	·56
	100	9·16	·54	
	105	9·56	·55	
18	95	8·70	·55	
	107	9·26	·58	
	95	8·90	·53	·54
	90	8·75	·51	
22	93	8·70	·53	
	100	9·48	·53	
	102	8·78	·58	·53
	107	10·76	·50	
26	95	9·25	·51	
	98	9·80	·50	·51
	102	9·80	·52	

Table III. *Showing blood condition of rats put on a diet of bread and milk at weaning and at the age of* 13 *weeks subjected to a series of* 8 *weekly haemorrhages.*

Rat 1.

Age in weeks	Bled cc.	Haemoglobin %	R.B.C. in millions per mm.^{3}	Colour index (human = 1)
14	1·2	90	7·63	·59
15	1·2	76	5·91	·64
16	1·0	67	6·42	·52
17	1·4	54	5·92	·46
18	1·3	39	4·75	·41
19	1·1	51	7·80	·33
20	1·6	51	7·35	·35
21	1·3	—	—	—
22	Not bled	51	7·30	·35
23	,,	56	8·07	·35
24	,,	—	—	—
25	,,	88	11·55	·38
26	,,	88	9·68	·46
27	,,	102	9·65	·53

Average weight during period of bleeding 116 g.
Average amount of blood taken 1·3 cc. = 1·12 % of body weight.

Rat 2.

14	1·4	93	8·16	·57
15	1·5	78	6·26	·62
16	1·3	88	8·04	·55
17	1·8	54	4·86	·56
18	1·1	49	5·98	·41
19	1·3	44	5·24	·42
20	1·3	26	4·22	·31
21	1·4	42	5·56	·37
22	Not bled	54	8·03	·34
23	,,	59	7·48	·40
24	,,	—	—	—
25	,,	83	10·64	·39
26	,,	90	10·36	·44
27	,,	100	9·85	·51

Average weight during period of bleeding 129 g.
Average amount of blood taken 1·4 cc. = 1·08 % of body weight.

Rat 3.

14	1·3	85	7·00	·60
15	1·2	78	5·84	·67
16	1·5	93	7·92	·59
17	1·3	58	5·53	·53
18	1·0	44	5·40	·41
19	·9	41·5	5·28	·39
20	1·7	49	6·46	·38
21	1·3	44	5·65	·39
22	Not bled	49	6·62	·37
23	,,	54	7·54	·36
24	,,	—	—	—
25	,,	99	10·62	·46
26	,,	—	—	—
27	,,	100	9·07	·55

Average weight during period of bleeding 137 g.
Average amount of blood taken 1·3 cc. = 1 % of body weight.

Rat 4.

Age in weeks	Bled cc.	Haemoglobin %	R.B.C. in millions per mm.^{3}	Colour index (human = 1)
14	1·1	98	8·40	·58
15	1·5	—	—	—
16	1·5	83	6·05	·68
17	1·3	68	6·40	·56
18	1·2	73	6·27	·58
19	1·7	54	5·00	·54
20	1·5	49	5·64	·43
21	1·8	59	6·55	·43
22	Not bled	60	8·12	·37
23	"	63·5	9·82	·32
24	"	—	—	—
25	"	93	11·56	·41
26	"	—	—	—
27	"	98	9·38	·52

Average weight during period of bleeding 144 g.
Average amount of blood taken 1·5 cc. = 1·04 % of body weight.

Rat 5.

14	1·0	98	8·35	·59
15	1·1	—	—	—
16	1·0	—	—	—
17	1·2	73	6·63	·55
18	1·5	49	5·00	·49
19	1·3	61	6·10	·50
20	1·4	66	8·45	·39
21	1·4	46	6·15	·37
22	Not bled	41·5	5·94	·35
23	"	44	7·35	·30
24	"	—	—	—
25	"	100	10·70	·47
26	"	—	—	—
27	"	100	9·55	·52

Average weight during period of bleeding 112 g.
Average amount of blood taken 1·3 cc. = 1·16 % of body weight.

Table IV. *Showing the blood condition of control rats fed on bread and milk and green stuffs and at the age of* 13 *weeks subjected to a series of* 8 *weekly haemorrhages.*

Rat A.

Age in weeks	Bled cc.	Haemoglobin %	R.B.C. in millions per mm.^{3}	Colour index (human = 1)
14	1·0	98	8·46	·58
15	1·1	107	8·52	·63
16	1·1	84	7·36	·57
17	1·3	88·5	8·20	·54
18	1·2	84	7·62	·55
19	1·6	84	7·78	·54
20	1·5	94	8·40	·56
21	1·9	98	8·92	·55
22	1·9	90	8·06	·56

Average weight during period of bleeding 143 g.
Average amount of blood taken 1·4 cc. = 1 % of body weight.

Rat B.

Age in weeks	Bled cc.	Haemoglobin %	R.B.C. in millions per mm.³	Colour index (human = 1)
14	1·1	110	9·33	·59
15	1·1	91	7·35	·62
16	1·3	98	8·42	·58
17	1·2	86	7·56	·57
18	1·1	89	8·26	·54
19	1·3	95	8·66	·55
20	1·9	93	8·20	·57
21	1·9	91	8·22	·55
22	2·0	91	8·32	·55

Average weight during period of bleeding 140 g.
Average amount of blood taken 1·4 cc. = 1 % of body weight.

Rat C.

14	·9	108	9·33	·58
15	1·3	93·5	9·16	·51
16	1·4	84	8·42	·50
17	1·4	103	9·38	·55
18	1·2	98	9·06	·54
19	1·3	95	8·80	·54
20	1·8	93	8·48	·55
21	1·7	98	9·07	·54
22	1·5	98	9·00	·54

Average weight during period of bleeding 140 g.
Average amount of blood taken 1·4 cc. = 1 % of body weight.

Rat D.

14	·8	105	8·74	·60
15	·9	103	7·98	·65
16	1·4	98	8·40	·58
17	1·5	95	7·68	·55
18	1·2	98	8·64	·57
19	1·4	93	8·76	·53
20	1·7	88	7·96	·55
21	1·9	88	8·10	·54
22	1·3	86	8·08	·53

Average weight during period of bleeding 139 g.
Average amount of blood taken 1·3 cc. = 1 % of body weight.

It is to be noted that the first bleeding was followed by a rise in the colour index instead of by a fall. This increase in colour index has been specially noticed by Hough and Waddell [1916].

Conclusion.

Rats fed from the time of weaning on white bread and whole milk and subjected to a series of eight bleedings each amounting to 1 % of the body weight show a fall in colour index which persists for 2–3 weeks after the bleeding is stopped; whereas controls fed from the time of weaning on this diet plus green stuff show a comparatively inappreciable fall of colour index. Controls fed on this diet but not bled also show a comparatively inappreciable fall of colour index.

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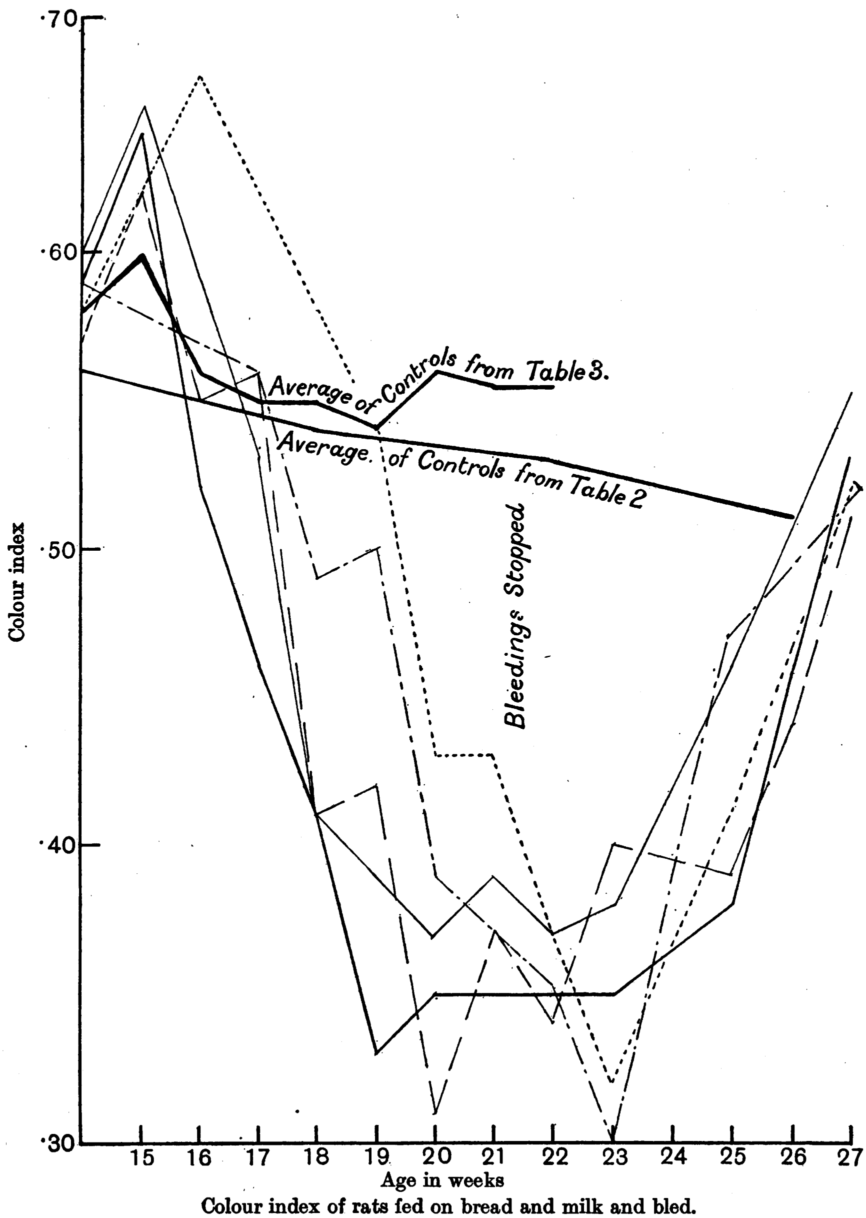

Colour index of rats fed on bread and milk and bled.

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