STUDIES IN THE ANAEMIAS OF INFANCY AND EARLY CHILDHOOD*

PART XIII.—EXPERIMENTS ON BLOOD REGENERATION AND THEIR SIGNIFICANCE FOR THE LIFE SPAN OF ERYTHROCYTES

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The problem of the length of time for which the red cell normally survives in the circulation has been investigated for many years by a great variety of methods and the literature on this subject is enormous. Much of it has been reviewed by Robschey-Robbins (1929), Isaacs (1937), Schiødt (1938b) and by Dekkers (1939). Study of these reviews reveals a remarkable diversity of estimates, the time allotted by different authors varying from ten to two hundred days in one species of experimental animal and a life span of as much as a year having been suggested by one observer in the case of the erythrocyte in man. The results of many investigations involve the acceptance of a purely hypothetical mechanism for the formation and destruction of red cells; here will be discussed only those which have a direct relation with the present investigation.

Hunter (1884-86) noticed that the time required for the blood to return to normal after bleeding is almost exactly the same as that required for the removal of excess blood corpuscles in artificial plethora. 'This similarity in the results is probably more than a mere coincidence, and can only be explained by assuming that after loss of blood the return to the normal is effected partly by increased formation, but mainly through a diminished destruction of blood corpuscles; and the time taken for complete restoration probably thus represents approximately the average duration of life of newly-formed red corpuscles.' Boycott and Douglas (1910) were the first to approach the problem by making a thorough investigation of the rate of blood regeneration and their experiments are of particular interest for the total haemoglobin was estimated by the CO-method in their animals. They found that rabbits bled to about two-fifths of their total haemoglobin regenerate their loss at a regular rate of about 0.3 c.c. oxygen capacity per day. 'If P = the normal daily production to make good D , the normal daily loss, and p = the excess production in response to the haemorrhage then $P - p = \frac{3}{5} D = 0.3$ c.c.; assuming that P is still equal to D , $\frac{2}{5} P - p = 0.3$ c.c., from which we can get a definite outside

limit that P or D cannot be greater than 0.75 c.c. per day.' The assumption made in this calculation, that the destruction after haemorrhage decreases in proportion to the diminution of haemoglobin is not correct, however, as will be shown later. Schiødt (1937), who has published a series of papers dealing with the regeneration of red cells following blood loss from gastric or duodenal ulcer, gives a more precise but at the same time more one-sided idea as to the mechanism of regeneration and the possibility of estimating the life span of red cells by the 'regeneration time.' If, for example, a life span of twenty-five days is assumed for the R.B.C. this means that 'out of five million cells, every day approximately 200,000 are destroyed to be replaced by new-formed cells. If now a patient with a normal count of five million R.B.C. in one day loses half his blood (and has no subsequent bleeding) he will—after correction of volume—have 2.5 millions left. The haemorrhage has taken place without any selection of the blood lost: thus the 2.5 millions left behind represent as well corpuscles formed the day before as those standing upon the threshold of destruction. The 2.5 millions therefore will disappear in the course of twenty-five days, every day 100,000 will be destroyed. But if the normal production of 200,000 still goes on, there will be a surplus of 100,000 corpuscles daily, in twenty-five days 2.5 millions. Thus the normal count of five millions will be reached at this date.'

From this consideration, which implies that a loss of blood is repaired by the organism merely by a reduction of the number of erythrocytes destroyed daily in proportion to the number actually present at the time immediately following the loss of blood and without any change in the blood formation, Schiødt identifies the life span of the red cell with the time required to repair a loss of blood. Schiødt's idea is supported by the close relation which he and Gram (1936) found between the lowest red cell count and the daily rise in red cells and by the course of recovery. In Schiødt's observations the rise of the erythrocyte count from the lowest point was a straight line and a constant level was reached (in cases treated with Meulengracht's diet) after thirty-three days. If the daily rise in R.B.C. was plotted against the lowest count a practically straight line was obtained 'the equation of which may be expressed as daily rise (in millions) $\times 33 = 4.54$ — lowest count of R.B.C. (in millions).'

* Part XII appeared in the March 1943 issue: Part XIV will appear in the September issue. References will be given at end of Part XIV.

There are, however, a number of observations in the literature which are hard to bring into accord with Schiødt's simple conception and Schiødt himself in a later paper (1938b) was less decided on the mechanism of blood regeneration. Thus the time of repair is not independent of the amount of blood lost. Hühnerfauth (1879) and Lyon (1881) found that in dogs a blood loss of 4.42–4.5 per cent. of the body weight was repaired in thirty-one to thirty-four days, a loss of 3.58–4.27 per cent. of body weight in nineteen to twenty-five days, and of 1.14–2.86 per cent. in seven to fourteen days. In man the blood count became normal after small losses of blood in two to five days, after a haemorrhage of medium degree (1–3 per cent. of body weight) in five to fourteen days and after large losses (about 4 per cent. of body weight) in fourteen to thirty days. Boycott and Douglas (1909a) found that by 'repeated bleedings the rate of regeneration may be increased several times over that which is induced by a single bleeding though the percentage of total oxygen capacity may have been reduced to a lower level by the single bleeding.' Geiling and Green (1921) observed recovery from a single haemorrhage in rats in seven to ten days but from a double haemorrhage in ten to fourteen days. The increase in the number of R.B.C. does not always follow a straight line. Leichsenring and Höring (1931) found little change between two to three days and one week after repeated bleeding of dogs; the most rapid increase occurred between one and two weeks and this was followed by a slower rise. Similarly those curves of Ritz (1909) representing recovery from phenylhydrazine anaemia resemble more an S-shaped curve whereas those of haemorrhagic anaemia approach a straight line.

It must be realized that a curve representing the rise of haemoglobin or of R.B.C. does not indicate the real regeneration but is the result of two factors, the formation (or, more exactly, the release from the bone marrow) of R.B.C. and their destruction. It was decided therefore to separate these two factors in experimental anaemia, calculating the real regeneration or the daily haemoglobin intake from the number of reticulocytes and their maturation time determined *in vitro*, and sometimes *in vivo*, and estimating the destruction subtracting the observed rise of haemoglobin from the intake determined in this way. Among eighteen experiments only four rabbits and two guinea pigs were followed for a sufficiently long time and these only will be reported here with one animal as a control for each species. Anaemia was produced by subcutaneous injections of phenylhydrazine hydrochloride. Rabbit 15 was followed for 128 days, rabbits 17 and 18 for 126 days, rabbit 20 for 79 days and the two guinea pigs for 77 days. Haemoglobin determinations and reticulocyte counts were made three times a week, erythrocyte counts irregularly. Haemoglobin determinations were sometimes made with Haldane's haemoglobinometer, sometimes with Evelyn's photoelectric step-photometer, both standardized by determinations of oxygen capacity with van Slyke's method. In the first week of the experiments only the photometric method was used. At this time the blood of animals poisoned with phenylhydrazine

shows a brownish colour which renders visual methods for haemoglobin determinations unreliable. This brown colour was first reported by Domarus (1908) who describes the blood in post-mortem findings as 'chocolate-brown.' It has been studied in greater detail by Price Jones (1911–12). The error in the photoelectric method due to the extinction by the brown pigment in the spectral region of filter 540 is negligible.

The results are recorded in tables 1–6 and in charts I–VI. The charts show the figures for haemoglobin in gm. per cent. and the reticulocyte concentration in 10,000 per c.mm.

It will be seen that the time interval between the lowest and highest haemoglobin level was 12, 13, 19 and 23 days respectively for the four rabbits, 13 and 16 days respectively for the two guinea pigs. The points representing the haemoglobin levels during the recovery from phenylhydrazine anaemia lie on a practically straight line for the rabbits 15 and 17 and for the guinea pigs, whereas in rabbits 18 and 20 a distinct decrease in the speed of recovery will be noticed. For a detailed analysis of the regeneration the following figures are given in tables 1–6 (p. 70). In column 2 the daily regeneration of haemoglobin (=intake) per 100 c.c. blood is noted for the two- or three-day periods indicated in column 1. This figure is calculated by taking the arithmetic mean of two subsequent reticulocyte counts (in per cent.), dividing by 100 and multiplying by 24 over the maturation time in hours. The maturation time was determined during the period of high reticulocyte counts for rabbits both *in vitro* and *in vivo*, for guinea pigs *in vitro* only. These figures, which were 10 hours for rabbits and 8 hours for guinea pigs, were used for the whole experimental period. The regeneration per 100 c.c. blood is therefore given by the formula $\frac{\text{Hb} \times \text{R}}{100} \times 2.4$ for

rabbits and $\frac{\text{Hb} \times \text{R}}{100} \times 3$ for guinea pigs, where Hb is haemoglobin in gm. per cent. and R the reticulocyte count per cent. of red cells. If this figure be multiplied by the blood volume in decilitres the total daily intake of haemoglobin is obtained. Taking the midpoint between two counts not only is the average of a continually changing regeneration approached but errors in haemoglobin determinations and reticulocyte counts are also minimized. In the third column the actual rise or fall of haemoglobin in gm. per 100 c.c. observed in the corresponding period is given. If the drop be added and the rise subtracted from the regeneration figure the destruction of haemoglobin in gm. per 100 c.c. blood is obtained (column 4). The regeneration has been also calculated on the basis of a hypothetical maturation time of twenty-four hours and the figures for destruction thus obtained are given in column 4 in brackets. This calculation gives a new support to the conclusions on the maturation time reached in the previous paper (part XII). It is obvious that the lowest possible limit for haemoglobin destruction is 0, it can never have a negative sign. Column 4

CHART I.—Rabbit No. 15. ——— Haemoglobin in gm. per cent., - - - - reticulocytes in 10,000 per c.mm. Arrows indicate phenylhydrazine injections.

CHART II.—Rabbit No. 17. ——— Haemoglobin in gm. per cent., - - - - reticulocytes in 10,000 per c.mm. Arrows indicate subcutaneous injections of phenylhydrazine hydrochloride.

CHART III.—Rabbit No. 18. ——— Haemoglobin gm. per cent., - - - - reticulocytes in 10,000 per c.mm. Arrows indicate injections of phenylhydrazine hydrochloride.

CHART IV.—Rabbit No. 20. ——— Haemoglobin gm. per cent., - - - - reticulocytes in 10,000 per c.mm.

CHART V.—Guinea pig No. 21. — Haemoglobin in gm. per cent., - - - reticulocytes in 10,000 per c.mm. Arrows indicate subcutaneous injections of phenylhydrazine hydrochloride.

CHART VI.—Guinea pig No. 22. — Haemoglobin in gm. per cent., - - - reticulocytes in 10,000 per c.mm. Arrows indicate subcutaneous injections of phenylhydrazine hydrochloride.

TABLE 1
REGENERATION AND DESTRUCTION OF HAEMOGLOBIN IN PHENYLHYDRAZINE
POISONING. RABBIT NO. 15

Date	Regeneration gm. per cent. Hb per day	Gain or loss per day gm. per cent. Hb	Destruction gm. per cent. per day	$\sum \left(\frac{\text{Hb} \times \text{R}}{100} \times 2.4 \right)$
29-31.7.40	1.392	-1.45	2.842 (2.03)	2.784
31.7-2.8.40	2.56	-1.038	3.598 (2.103)	7.89
2-5.8.40	3.90	-0.69	3.21 (0.95)	19.6
5-7.8.4	6.48	-0.069	6.41 (2.769)	32.3
7-9.8.40	6.97	-0.415	6.56 (2.485)	46.2
9-12.8.40	4.42	-0.46	3.96 (1.38)	59.5
12-14.8.40	1.04	-0.069	1.11 (0.364)	61.6
14-16.8.40	1.24	-0.62	0.62 (-0.104!)	64.3
16-19.8.40	1.17	-0.1	1.07 (0.388)	67.8
19-21.8.40	0.83	-0.138	0.69 (0.208)	69.5
21-23.8.40	1.59	-0.415	2.0 (1.078)	
23-24.8.40	2.10	-1.1	1.0 (-0.225 !)	
24-26.8.40	1.68	-0.14	1.54 (0.56)	
26-28.8.40	0.985	-0.14	1.125 (0.376)	
28-30.8.40	1.05	-0.965	2.015 (1.403)	
30.8-2.9.40	1.22	-0.595	0.625 (-0.085 !)	
2-4.9.40	1.12	-0.484	1.604 (0.95)	
4-6.9.40	0.99	+0.28	0.71 (0.095)	
6-9.9.40	0.86	-0.1	0.76 (0.28)	
9-11.9.40	0.93	-0.21	0.72 (0.178)	
11-13.9.40	0.87	-0.83	1.70 (1.192)	
13-16.9.40	0.705	-0.23	0.475 (0.063)	
16-18.9.40	0.98	-0.069	0.91 (0.338)	
18-20.9.40	1.53	-0.35	1.18 (0.288)	
20-23.9.40	1.45	-0.097	1.547 (0.701)	
23-25.9.40	1.17	-1.11	2.28 (1.598)	
25-27.9.40	2.16	-2.06	4.22 (2.96)	
27-30.9.40	2.78	-0.47	2.31 (1.16)	
30.9-2.10.40	3.38	-0.69	2.69 (0.72)	
2-4.10.40	3.44	-0.62	2.82 (0.815)	
4-7.10.40	2.54	-0.65	1.89 (0.405)	
7-9.10.40	1.5	-0.41	1.91 (1.034)	
9-11.10.40	0.97	-0.14	0.83 (0.247)	
11-14.10.40	0.74	-0.18	0.92 (0.488)	2.22
14-16.10.40	1.03	+0.83	0.20 (-0.401!)	4.28
16-18.10.40	1.34	-0.28	1.62 (0.138)	6.96
18-21.10.40	1.96	-2.35	4.31 (3.167)	12.84
21-23.10.40	1.60	-0.41	1.19 (0.255)	16.04
23-25.10.40	2.18	-0.41	1.77 (0.50)	20.40
25-28.10.40	2.80	-0.73	2.07 (0.43)	28.8
28-30.10.40	1.99	-0.69	1.30 (0.137)	32.78
30.10-1.11.40	1.02	-0.41	0.81 (0.015)	34.82
1-4.11.40	0.59	-0.18	0.41 (0.066)	36.59
4-6.11.40	0.63	-0.14	0.49 (0.122)	37.85
6-8.11.40	0.50	-0.225	0.725 (0.433)	
8-11.11.40	0.50	-0.225	0.275 (-0.017)	
11-13.11.40	0.372	-0.70	1.072 (0.855)	
13-15.11.40	0.465	+0.85	-0.385 (-0.656)	
15-19.11.40	0.198	-0.15	0.346 (0.232)	
19-21.11.40	0.61	-0.25	0.36 (0.004)	
21-25.11.40	0.985	-0.38	0.605 (0.03)	
25-27.11.40	0.58	-0.125	0.750 (0.367)	
27-29.11.40	0.314	-0.40	-0.086 (-0.269)	
29.11-2.12.40	0.286	0.0	0.286 (0.119)	
2-4.12.40	0.59	-1.25	1.84 (1.496)	

Injections of phenylhydrazine hydrochloride: 29.7.40 -0.02 gm., 31.7.40 -0.02 gm., 24.9.40 -0.02 gm. subcutaneously.

Regeneration is calculated on the basis of a maturation time of reticulocytes of 10 hours $\left(\frac{\text{Hb} \times \text{R}}{100} \times 2.4 \right)$. The figures in brackets indicate the destruction if regeneration is calculated on the basis of a maturation time of 24 hours. The figures in the last column represent the total regeneration for the indicated period up to the second date in column 1.

TABLE 2

REGENERATION AND DESTRUCTION OF HAEMOGLOBIN IN PHENYLHYDRAZINE POISONING. RABBIT NO. 17

Date	Regeneration gm. per cent. Hb per day	Gain or loss per day gm. per cent. Hb	Destruction gm. per cent. per day	$\sum \left(\frac{\text{Hb} \times \text{R}}{100} \times 2.4 \right)$
31.7-2.8.40	0.663	-1.45	2.113 (1.726)	1.32
2-5.8.40	2.08	-0.276	2.356 (1.141)	7.54
5-7.8.40	4.55	-0.138	4.688 (2.038)	16.74
7-9.8.40	5.30	+0.76	4.54 (1.45)	27.34
9-12.8.40	3.26	-0.51	2.75 (0.85)	37.12
12-14.8.40	1.34	-0.415	0.925 (0.149)	39.80
14-16.8.40	0.71	-0.415	1.125 (0.711)	41.22
16-19.8.40	0.59	-0.825	-0.235 (-0.579)	42.99
19-21.8.40	0.63	-0.965	1.595 (1.227)	44.25
21-23.8.40	1.11	-0.55	0.56 (-0.083)	46.47
23-24.8.40	1.35	-1.65	3.0 (2.213)	47.82
24-26.8.40	1.12	-0.825	0.295 (-0.352)	50.60
26-28.8.40	0.75	-0.485	1.235 (0.798)	52.1
28-30.8.40	0.41	-0.346	0.064 (-0.175)	52.92
30.8-2.9.40	0.354	-0.276	0.630 (0.423)	53.98
2-4.9.40	0.338	+0.138	0.200 (0.003)	54.68
4-6.9.40	0.595	0.0	0.595 (0.248)	
6-9.9.40	0.63	-0.138	0.867 (0.400)	
9-11.9.40	0.334	-0.276	0.058 (-0.137)	
11-13.9.40	0.405	-0.62	1.025 (0.789)	
13-16.9.40	0.47	-0.138	0.332 (0.058)	
16-18.9.40	0.58	-0.138	0.442 (0.104)	
18-20.9.40	0.77	-0.138	0.908 (0.457)	
20-23.9.40	0.69	+0.276	0.414 (0.011)	
23-27.9.40	0.57	-0.069	0.639 (0.307)	
27.9-2.10.40	0.57	-0.028	0.542 (0.210)	2.84
2-4.10.40	0.775	-2.07	2.845 (2.393)	4.39
4-7.10.40	0.87	-0.276	0.594 (0.086)	6.99
7-9.10.40	0.87	0.0	0.870 (0.362)	8.73
9-11.10.40	1.14	-0.48	0.66 (-0.005)	11.01
11-14.10.40	1.66	-0.76	0.90 (-0.067)	16.01
14-16.10.40	1.16	-0.346	1.506 (0.829)	18.33
16-18.10.40	0.65	0.0	0.65 (0.271)	19.63
18-21.10.40	0.91	0.0	0.91 (0.379)	22.35
21-23.10.40	0.75	0.0	0.75 (0.312)	23.85
23-25.10.40	0.53	-0.485	0.045 (-0.264)	24.91
25-28.10.40	0.50	0.0	0.50 (0.209)	
28-30.10.40	0.385	-0.207	0.178 (-0.101)	
30.10-1.11.40	0.42	-0.276	0.144 (-0.101)	
1-4.11.40	0.288	-0.097	0.191 (0.023)	
4-6.11.40	0.202	-0.276	0.478 (0.360)	
6-8.11.40	0.177	-0.138	0.039 (-0.064)	
8-11.11.40	0.196	-0.20	-0.004 (-0.118)	
11-13.11.40	0.135	-0.50	0.635 (0.556)	
13-15.11.40	0.190	-0.05	0.24 (0.085)	
15-19.11.40	0.498	-0.275	0.223 (-0.671)	
19-21.11.40	0.298	-0.15	0.448 (0.278)	
21-25.11.40	0.254	-0.20	0.054 (-0.094)	
25-27.11.40	0.635	0.0	0.635 (0.264)	
27-29.11.40	0.567	-0.25	0.817 (0.487)	
29.11-2.12.40	0.322	-0.167	0.155 (-0.033)	
2-4.12.40	0.35	-0.95	1.20 (1.096)	

Injections of phenylhydrazine hydrochloride: 31.7.40 -0.02 gm., 5.8.40 -0.02 gm., subcutaneously.

Regeneration is calculated on the basis of a maturation time of reticulocytes of 10 hours $\left(\frac{\text{Hb} \times \text{R}}{100} \times 2.4 \right)$. The figures in brackets indicate the destruction if regeneration is calculated on the basis of a maturation time of 24 hours. The figures in the last column represent the total regeneration in the indicated period up to the second date in column 1.

in tables 1-6 shows that when a maturation time of 24 hours is assumed 44 such impossible figures occur and that even with a maturation time of 10 and 8 hours respectively a few figures with a negative sign are obtained. This suggests that the maturation time determined during the period of high reticulocyte counts is somewhat too long for the period of normal or low reticulocyte counts.

For the study of the kinetics of haemoglobin

regeneration summation curves have been constructed (charts VII-XI). In these charts the time in days (for the period under consideration) is plotted against the total haemoglobin intake up to the time (t); $\sum \frac{\text{Hb} \times \text{R}}{100} \times 2.4$ for rabbits and $\sum \frac{\text{Hb} \times \text{R}}{100} \times 3$ for guinea pigs, obtained by addition of figures in column 2 of tables 1-6.

TABLE 3

REGENERATION AND DESTRUCTION OF HAEMOGLOBIN IN PHENYLHYDRAZINE POISONING. RABBIT NO. 18

Date	Regeneration gm. per cent. Hb per day	Gain or loss per day gm. per cent. Hb	Destruction gm. per cent. per day	$\sum \left(\frac{\text{Hb} \times \text{R}}{100} \times 2.4 \right)$
31.7-2.8.40	0.87	-1.38	2.25 (1.742)	1.74
2-5.8.40	1.92	-0.041	1.961 (0.841)	7.49
5-7.8.40	4.07	-0.346	4.416 (2.046)	15.63
7-9.8.40	4.85	+0.41	4.44 (1.61)	25.33
9-12.8.40	3.78	+0.46	3.32 (1.12)	36.63
12-14.8.40	2.16	+0.138	2.022 (0.762)	40.95
14-16.8.40	1.34	-0.276	1.064 (0.282)	43.63
16-19.8.40	1.07	-0.276	0.794 (0.169)	46.84
19-21.8.40	0.62	-0.138	0.482 (0.120)	48.08
21-23.8.40	0.585	-0.276	0.861 (0.520)	49.25
23-24.8.40	1.27	-0.83	2.10 (-0.295)	
24-26.8.40	1.38	-0.138	1.718 (0.713)	
26-28.8.40	0.92	+0.276	0.644 (0.107)	
28-30.8.40	0.665	+0.138	0.527 (0.139)	
30.8-2.9.40	0.48	-0.41	0.89 (0.61)	
2-4.9.40	0.352	-0.138	0.490 (0.285)	
4-6.9.40	0.463	-0.346	0.117 (-0.153)	
6-9.9.40	0.718	-0.46	1.178 (0.790)	
9-11.9.40	0.765	-0.207	0.558 (0.113)	
11-13.9.40	0.793	-0.276	0.517 (0.054)	
13-16.9.40	1.01	-0.184	1.194 (0.606)	
16-18.9.40	1.04	-0.276	0.764 (0.154)	
18-20.9.40	0.707	0.0	0.707 (0.294)	
20-23.9.40	0.60	0.0	0.60 (0.25)	
23-25.9.40	0.73	-0.138	0.592 (0.166)	1.46
25-27.9.40	0.813	-0.553	1.366 (0.891)	3.086
27.9-2.10.40	1.24	-0.331	1.571 (0.848)	9.286
2-4.10.40	1.72	-0.69	2.41 (1.406)	12.73
4-7.10.40	1.46	-0.316	1.124 (0.294)	17.11
7-11.10.40	1.04	-0.103	0.937 (0.330)	19.2
11-14.10.40	0.686	-0.553	1.239 (0.845)	20.56
14-16.10.40	0.736	-1.51	-0.774 (-1.201)	
16-18.10.40	1.80	-0.62	2.42 (1.37)	
18-21.10.40	2.05	-0.322	1.728 (0.533)	
21-23.10.40	1.35	-0.138	1.488 (0.701)	
23-25.10.40	0.80	-0.276	1.076 (0.610)	
25-28.10.40	0.638	-0.183	0.821 (0.449)	
28-30.10.40	0.607	-0.827	-0.220 (-0.574)	
30.10-1.11.40	0.527	0.0	0.527 (0.220)	
1-4.11.40	0.685	-0.276	0.961 (0.561)	
4-6.11.40	0.398	-0.276	0.122 (-0.110)	
6-8.11.40	0.53	-0.276	0.806 (0.496)	
8-11.11.40	0.95	-0.42	0.53 (-0.026)	
11-13.11.40	0.685	-0.075	0.762 (0.360)	
13-15.11.40	0.48	-0.425	0.905 (0.625)	
15-19.11.40	0.392	+0.1122	0.280 (0.501)	
19-21.11.40	0.372	-0.50	-0.128 (-0.345 !)	
21-25.11.40	0.419	-0.212	0.631 (0.386)	
25-27.11.40	0.775	-0.80	-0.025 (-0.477)	
27-29.11.40	0.82	-0.25	1.07 (0.612)	
29.11-2.12.40	0.65	-0.167	0.483 (0.104)	
2-4.12.40	0.52	-1.05	1.57 (1.265)	

Injections of phenylhydrazine hydrochloride: 31.7.40 - 0.02 gm., 5.8.40 - 0.02 gm. subcutaneously.

Regeneration is calculated on the basis of a maturation time of reticulocytes of 10 hours $\left(\frac{\text{Hb} \times \text{R}}{100} \times 2.4 \right)$. The figures in brackets indicate the destruction if regeneration is calculated on the basis of a maturation time of 24 hours. The figures in the last column represent the total regeneration for the indicated period up to the second date in column 1.

Such a curve is a real 'haemoglobin intake' curve and represents the changes in the haemoglobin level which would occur in the absence of cell destruction. The shape of the curves is characteristic and indicates that the 'master reaction' (Robertson) determining the blood regeneration and haemoglobin intake under these experimental conditions is an autocatalyzed reversible reaction. Mention

has been made in the previous paper (part XII) that according to Robertson this is the fundamental reaction for growth and cellular multiplication. The validity of this law for blood regeneration during the reticulocyte response in pernicious anaemia treated with liver extract has been proved by the brilliant work of Riddle (1930). Riddle, however, assumed the actual increase in R.B.C. to be identical

TABLE 4

REGENERATION AND DESTRUCTION OF HAEMOGLOBIN IN PHENYLHYDRAZINE POISONING. RABBIT NO. 20

Date	Regeneration gm. per cent. Hb per day	Gain or loss per day gm. per cent. Hb	Destruction gm. per cent. Hb per day	$\sum \left(\frac{\text{Hb} \cdot \text{R}}{100} \times 2.4 \right)$
16-18.9.40	1.165	-1.93	3.095 (2.415)	2.33
18-20.9.40	1.73	-1.105	2.835 (1.825)	5.79
20-23.9.40	4.67	-0.138	4.808 (2.088)	19.80
23-25.9.40	5.96	-0.83	5.130 (1.665)	31.72
25-27.9.40	5.52	-1.10	6.620 (3.400)	42.76
27-30.9.40	5.10	-0.735	4.365 (1.385)	58.06
30.9-2.10.40	3.95	-0.69	3.260 (0.955)	65.96
2-4.10.40	2.78	0.0	2.780 (1.160)	71.52
4-7.10.40	1.36	-0.367	0.993 (0.200)	75.60
7-9.10.40	0.47	-0.138	0.332 (0.058)	76.45
9-11.10.40	0.447	-0.138	0.585 (0.324)	77.34
11-14.10.40	0.606	-0.138	0.744 (0.399)	
14-16.10.40	1.235	-0.069	1.304 (0.583)	
16-18.10.40	1.56	-0.276	1.836 (0.926)	
18-21.10.40	1.18	-0.414	0.766 (0.078)	
21-23.10.40	0.83	-0.414	0.416 (-0.069)	
23-26.10.40	0.384	-0.046	0.338 (0.124)	
26-29.10.40	0.538	-0.23	0.762 (0.454)	
29-31.10.40	0.574	-0.069	0.505 (0.170)	
31.10-2.11.40	0.544	-0.346	0.890 (0.570)	
2-4.11.40	0.478	-0.553	1.031 (0.752)	
4-7.11.40	0.397	-0.276	0.121 (-0.111)	
7-9.11.40	0.166	0.0	0.166 (0.069)	
9-12.11.40	0.093	-0.138	0.231 (0.175)	
12-14.11.40	0.072	-0.50	-0.428 (0.470)	
14-16.11.40	0.143	-0.57	0.713 (0.629)	
16-19.11.40	0.63	-0.178	0.808 (0.441)	
19-22.11.40	0.685	-0.243	0.442 (0.042)	
22-25.11.40	0.716	-0.747	-0.031 (-0.652)	
25-27.11.40	1.53	-0.35	1.180 (0.288)	
27-30.11.40	0.867	-0.334	1.201 (0.695)	
30.11-2.12.40	1.36	-0.61	0.750 (-0.040)	
2-4.12.40	1.385	-0.70	2.085 (1.277)	

Injections of phenylhydrazine hydrochloride: 6.9.40 -0.02 gm., 18.9.40 -0.01 gm., 20.9.40 -0.02 gm., 25.9.40 -0.02 gm. subcutaneously.

Regeneration is calculated on the basis of a maturation time of reticulocytes of 10 hours $\left(\frac{\text{Hb} \cdot \text{R}}{100} \times 2.4 \right)$. The figures in brackets indicate the destruction if regeneration is calculated on a basis of a maturation time of 24 hours. The figures in the last column represent the total regeneration up to the second date in column 1.

with the regeneration and the perfect agreement of his observed curves with those calculated from the formula for self accelerated reversible reactions is apparently due to a fixed relation between formation and destruction of R.B.C. in his cases. That this is not always the case even in reticulocyte crises in pernicious anaemia is shown by the work of Goldhammer, Isaacs and Sturgis (1934) and of Schiødt (1938) where the total number of erythrocytes began to increase only after the reticulocytes had reached their peak.

To check the validity of Robertson's law for haemoglobin regeneration in phenylhydrazine anaemia the curves representing the haemoglobin intake $\sum \frac{\text{Hb} \cdot \text{R}}{100} \times 2.4$ and $\sum \frac{\text{Hb} \cdot \text{R}}{100} \times 3$ have been compared with those constructed according to the formula $\log \frac{x}{a-x} = ak(t-t_1)$. If applied to haemoglobin intake, t in this formula is any given time during the period in consideration, t_1 = the time at which 50 per cent. of the total haemoglobin intake

of this period has been reached, a = the total haemoglobin intake during this period, x = the expected haemoglobin intake up to the time t , and k is a constant which has been found empirically for each animal and each period. The periods start with the first phenylhydrazine injection when the new stimulus is given and include therefore the days when the haemoglobin is falling. The time when the reticulocyte concentration became fairly constant has been taken as the end point of the period. The curves in the charts VII to XI represent the actual haemoglobin intake

$$\left(\sum \frac{\text{Hb} \cdot \text{R}}{100} \times 2.4 \text{ and } \sum \frac{\text{Hb} \cdot \text{R}}{100} \times 3 \right)$$

and the intake calculated from the formula for autocatalyzed reversible reactions. In the headings of the charts the individual figures for a and k are given. The curves show perfect agreement between the real haemoglobin intake and that calculated from the formula for self-accelerated reversible reactions. It may be mentioned that the maturation time being taken as constant has no influence on the

TABLE 5

REGENERATION AND DESTRUCTION OF HAEMOGLOBIN IN PHENYLHYDRAZINE POISONING. GUINEA PIG NO. 21.

Date	Regeneration gm. per cent. Hb per day	Gain or loss per day gm. per cent. Hb	Destruction gm. per cent. Hb per day	$\sum \left(\frac{\text{Hb} \times \text{R}}{100} \times 3.0 \right)$
18-20.9.40	0.86	-2.35	3.21 (2.627)	1.72
20-23.9.40	0.885	-0.46	1.345 (0.754)	4.37
23-25.9.40	2.60	-0.552	3.152 (1.418)	9.57
25-27.9.40	5.17	-0.345	4.825 (2.070)	19.91
27-30.9.40	5.60	-0.594	6.194 (2.644)	36.71
30.9-2.10.40	5.16	-0.455	4.705 (1.265)	47.03
2-4.10.40	5.30	-0.207	5.093 (1.563)	57.63
4-7.10.40	4.30	-0.73	3.570 (0.707)	70.53
7-9.10.40	1.985	-0.138	1.847 (0.524)	74.34
9-11.10.40	0.985	-0.55	0.435 (-0.223)	76.31
1-14.10.40	0.93	-0.184	1.114 (0.494)	
14-16.10.40	0.99	-0.482	1.472 (0.812)	
16-18.10.40	0.907	-0.276	1.183 (0.578)	
18-21.10.40	1.258	-0.276	0.982 (0.142)	
21-23.10.40	1.435	-0.760	2.195 (1.238)	
23-26.10.40	0.712	-0.555	0.157 (-0.217)	
26-29.10.40	0.234	0.0	0.234 (0.078)	
29-31.10.40	0.515	-0.138	0.377 (0.033)	
31.10-2.11.40	0.585	0.0	0.585 (0.195)	
2-4.11.40	0.61	-0.276	0.886 (0.479)	
4-7.11.40	0.59	-0.092	0.682 (0.288)	
7-9.11.40	0.358	-0.55	0.908 (0.430)	
9-12.11.40	0.165	-0.230	-0.065 (0.175)	
12-14.11.40	0.678	-0.125	0.803 (0.351)	
14-16.11.40	1.29	-0.350	1.640 (0.780)	
16-19.11.40	1.62	-0.273	1.347 (0.266)	
19-22.11.40	1.18	-0.100	1.080 (0.393)	
22-25.11.40	1.88	-0.233	1.647 (0.395)	
25-27.11.40	2.20	-0.60	1.600 (0.133)	
27-30.11.40	0.49	-0.100	0.390 (0.063)	
30.11-2.12.40	0.234	-0.300	-0.066 (-0.222)	
2-4.12.40	0.208	-0.735	0.943 (0.805)	

Injections of phenylhydrazine hydrochloride: 18.9.40 -0.01 gm., 20.9.40 -0.01 gm., 25.9.40 -0.01 gm., 27.9.40 -0.02 gm. subcutaneously.

Regeneration is calculated on the basis of a maturation time for reticulocytes of 8 hours $\left(\frac{\text{Hb} \times \text{R}}{100} \times 3.0 \right)$. The figures in brackets indicate the destruction if regeneration is calculated on the basis of a maturation time of 24 hours. The figures in the last column represent the total regeneration up to the second date in column 1.

shape of the curve. The agreement indicates that in phenylhydrazine anaemia the mass of erythrocytes (and therefore haemoglobin) liberated from the bone marrow is absolutely determined by the law of nuclear synthesis, that is by the multiplication of nucleated R.B.C. in the bone marrow. No delay was detected in the development of the regenerative response. This finding is strongly against there being any toxic action of phenylhydrazine on the bone marrow as was assumed from cytological findings by Price Jones (1911-12). These findings may rather be interpreted as indicating increased and precipitate erythropoiesis for macro-blasts and proerythroblasts (Price Jones's megaloblasts and metrocytes*) are commonly found in the bone marrow of congenital acholuric jaundice and Istomanowa (1926) found signs of great activity in the bone marrow in pyridin (acetyl phenylhydrazine) intoxication. Evensen (1938) who studied the effects

of phenylhydrazine in polycythaemia found no signs of inhibition of the bone marrow. The drop in haemoglobin and R.B.C. which persists for some days after the last phenylhydrazine injection is therefore to be attributed to the destruction of R.B.C.

It is by no means justifiable to draw general conclusions as to the mechanism of red cell regeneration from findings of anaemia produced by a noxious agent like phenylhydrazine. It is important therefore to analyse the haemoglobin or erythrocyte regeneration in post-haemorrhagic anaemia and especially in conditions in which a normal mechanism of regeneration may reasonably be assumed. No experiments have been made on post-haemorrhagic anaemia but in the vast literature on this subject sufficient data for the construction of regeneration curves has been found in a paper by Gordon (1934). One of his experiments has been chosen in which five rabbit litter mates were subjected to a single bleeding and the average of the figures given for these five animals has been taken (table 7). As the maturation time, which with little error may be taken as constant, has no influence on the shape of

* The term 'metrocytes' was introduced by Engel for those nucleated red cells of early embryonic life which are called megaloblasts by most authors. The differentiation between 'primary metrocytes', 'secondary metrocytes' and megaloblasts is based only on the staining properties of the protoplasm.

TABLE 6

REGENERATION AND DESTRUCTION OF HAEMOGLOBIN IN PHENYLHYDRAZINE POISONING. GUINEA PIG. NO. 22

Date	Regeneration gm. per cent. Hb per day	Gain or loss per day gm. per cent. Hb	Destruction gm. per cent. Hb per day
18-20.9.40	1.055	-0.138	1.193 (0.490)
20-23.9.40	1.64	-1.75	3.390 (2.297)
23-25.9.40	4.07	-0.276	4.346 (1.630)
25-27.9.40	8.42	+0.30	8.120 (2.510)
27-30.9.40	10.15	-0.30	10.450 (3.680)
30.9-2.10.40	7.68	+0.552	7.128 (2.008)
2-4.10.40	4.85	+0.415	4.435 (0.800)
4-7.10.40	4.28	+0.597	3.683 (0.828)
7-9.10.40	2.478	-0.138	2.616 (0.963)
9-11.10.40	1.038	-0.552	1.590 (0.898)
11-14.10.40	0.992	+0.597	0.395 (-0.206)
14-16.10.40	1.128	-0.207	1.335 (0.583)
16-18.10.40	0.969	-0.414	1.383 (0.737)
18-21.10.40	0.897	+0.552	0.345 (-0.257)
21-23.10.40	0.924	-0.069	0.993 (0.377)
23-26.10.40	0.542	+0.046	0.496 (0.105)
26-29.10.40	0.595	+0.189	0.406 (0.009)
29-31.10.40	0.72	-1.04	1.760 (1.280)
31.10-2.11.40	0.78	+1.38	-0.600 (-1.120)
2-4.11.40	1.54	-0.138	1.678 (0.651)
4-7.11.40	1.25	-0.092	1.342 (0.505)
7-9.11.40	0.35	-0.276	0.626 (0.393)
9-12.11.40	0.521	+0.46	0.061 (-0.282)
12-14.11.40	0.62	-0.276	0.896 (0.483)
14-16.11.40	0.652	+0.100	0.552 (0.118)
16-19.11.40	0.683	+0.333	0.350 (0.102)
19-22.11.40	0.384	-0.333	0.717 (0.461)
22-25.11.40	0.382	+0.300	0.082 (-0.173)
25-27.11.40	0.382	0.0	0.382 (0.127)
27-30.11.40	0.266	+0.100	0.166 (-0.011)
30.11-2.12.40	0.176	-0.250	0.426 (0.309)
2-4.12.40	0.342	-1.00	1.342 (1.114)

Injections of phenylhydrazine hydrochloride: 18.9.40 -0.01 gm., 20.9.40 -0.01 gm., 25.9.40 -0.01 gm., 27.9.40 -0.02 gm. subcutaneously.

Regeneration is calculated on the basis of a maturation time for reticulocytes of 8 hours $\left(\frac{\text{Hb} \times \text{R}}{100} \times 3.0\right)$. The figures in brackets indicate the destruction if regeneration is calculated on the basis of a maturation time of 24 hours.

TABLE 7

REGENERATION AND DESTRUCTION OF R.B.C. IN POST-HAEMORRHAGIC ANAEMIA

Days of experiment	R.B.C. millions per c.mm.	Reticulocytes per cent.	Daily intake of R.B.C. millions per c.mm.	Daily gain in R.B.C. millions per c.mm.	Daily destruction of R.B.C. millions per c.mm.
Pre-experimental	6.07	0.58	0.0767	0	0.0767 (0.032)
2-3	4.235	1.75	0.178	0.07	0.108
3-4	4.31	3.3	0.342	0.1	0.242
4-5	4.36	4.95	0.518	0	0.518
5-6	4.37	6.3	0.663	0.02	0.643 (0.256)
6-7	4.39	6.33	0.67	0.02	0.650 (0.259)
7-8	4.405	4.9	0.52	0.01	0.510
8-9	4.43	3.8	0.405	0.04	0.365
9-10	4.595	3.38	0.40	0.29	0.11
10-12	4.80	3.02	0.347	0.06	0.287
12-14	4.94	2.51	0.298	0.08	0.218
14-17	5.095	2.04	0.249	0.05	0.199
17-24	5.545	1.57	0.208	0.107	0.101
24-31	5.995	1.02	0.144	0.0214	0.123

The daily intake is calculated on the basis of a maturation time of reticulocytes of 10 hours: $\frac{\text{E} \times \text{R}}{100} \times 2.4$. The figures in brackets correspond to the daily destruction if a maturation time of 24 hours is assumed for the reticulocytes.

CHART VII.—Haemoglobin intake in phenylhydrazine anaemia of rabbit No. 15, 29.7–21.8.1940.

— calculated from the equation $\log \frac{x}{69.5-x} = 0.00208 \times 69.5(t-9.3)$. - - - - - observed figures for $\sum \frac{Hb \times R}{100} \times 2.4$.

CHART IX.—Haemoglobin intake in phenylhydrazine anaemia of rabbit No. 20, 16.9–11.10.1940.

— calculated from the equation $\log \frac{x}{77.34-x} = 0.00194 \times 77.34(t-10.26)$. - - - - - observed figures for $\sum \frac{Hb \times R}{100} \times 2.4$.

CHART VIII.—Haemoglobin intake in phenylhydrazine anaemia of rabbit No. 18, 31.7–23.8.1940.

— calculated from the equation $\log \frac{x}{49.25-x} = 0.00346 \times 49.25(t-8.86)$. - - - - - observed figures for $\sum \frac{Hb \times R}{100} \times 2.4$.

the curve the formula $\sum \frac{E \times R}{100}$ (where $E = R.B.C.$) corresponding to a maturation time of 24 hours has been used for the construction of chart XII. If this curve (solid line) is compared with the theoretical one corresponding to the formula $\log \frac{x}{1.667-x} = 0.19 \times 1.667(t-5.7)$ (dashed line) an agreement as perfect as that in the case of phenylhydrazine anaemia will be found.

A condition in which it may be fairly assumed that the mechanism of the regeneration is normal has been met in rabbits previously subjected to phenylhydrazine-poisoning. After complete recovery, even some weeks later, the sudden 'spontaneous' development of anaemia has been observed, the cause of which is discussed below. The development of anaemia was always followed by a reticulocyte response and recovery. The haemoglobin intake during such periods and the theoretical one calculated from the formula for autocatalyzed reversible reactions are represented in charts XIII to XVI for rabbits 15, 17 and 18 and for guinea pig 21.

Finally the same analysis of regeneration has been

CHART X.—Haemoglobin intake in phenylhydrazine anaemia of guinea pig No. 21, ——— calculated from formula: $\log \frac{x}{78.7-x} = 0.00173 \cdot 78.17(t - 12.635)$, - - - - - observed figures for $\sum \frac{Hb \cdot R}{100} = 2.4$.

CHART XI.—Haemoglobin intake in phenylhydrazine anaemia of guinea pig No. 22, ——— calculated from equation: $\log \frac{x}{15.1-x} = 0.00966 \times 15.1(t - 8.43)$, - - - - - observed figures for $\sum \frac{Hb \cdot R}{100} = 3.0$.

CHART XII.—Haemoglobin intake in post-haemorrhagic anaemia of rabbits, ——— observed figures for $\sum \frac{E \cdot R}{100}$, - - - - - calculated from the formula: $\log \frac{x}{1.667-x} = 0.19 \times 1.667(t - 5.7)$. Constructed from figures of Gordon.

applied to a case of erythronoclastic anaemia of the newborn. In this case, which because of various interesting features will be discussed in detail below, repeated transfusions resulted in an 'aregenerative' condition of the bone marrow which was succeeded by a period of renewed bone marrow activity. The maturation time determined in vitro during this period was $11\frac{1}{2}$ hours. A time of 12 hours was used for the construction of curve 17 which therefore represents $\sum \frac{Hb \cdot R}{100} \times 2$ (dashed curve). The theoretical curve corresponds to the formula: $\log \frac{x}{13.27-x} = 0.0215 \times 13.27(t - 6.47)$ (solid line). The agreement between the two curves is again very close.

Graphs give only a rough idea of agreement; they are influenced by the scale which for technical reasons could not be identical in all cases. For a more accurate comparison of observed figures for haemoglobin intake with those calculated from the formula for autocatalyzed reversible reactions the

are influenced by the scale which for technical reasons could not be identical in all cases. For a more accurate comparison of observed figures for haemoglobin intake with those calculated from the formula for autocatalyzed reversible reactions the

CHART XIII.—Haemoglobin intake in 'spontaneous anaemia' of rabbit No. 15. ———— calculated from the formula: $\log \frac{x}{37.85-x} = 0.117(t-13.32)$. - - - - - observed figures for $\sum \frac{\text{Hb} \cdot R}{100} = 2.4$.

CHART XIV.—Haemoglobin intake in 'spontaneous anaemia' of rabbit No. 17, 27.9-25.10.1940. ———— calculated from the equation: $\log \frac{x}{24.91-x} = 0.00342 \cdot 24.91(t-14.875)$. - - - - - observed figures for $\sum \frac{\text{Hb} \cdot R}{100} = 2.4$.

CHART XV.—Haemoglobin intake in 'spontaneous anaemia' of rabbit No. 18, 23.9–14.10.1940. ——— calculated from formula: $\log \frac{x}{20.56-x} = 0.00732 \times 20.56(t-9.67)$. - - - - - Observed figures for $\sum \frac{Hb \times R}{100} \times 2.4$.

CHART XVI.—Haemoglobin intake in 'spontaneous anaemia' of guinea pig No. 21 (11–31.10.1940). ——— calculated from formula: $\log \frac{x}{17.12-x} = 0.00876 \times 17.12(t-9.5)$. - - - - - Observed figures for $\sum \frac{Hb \times R}{100} \times 3.0$.

CHART XVII.—Haemoglobin intake and variations in the haemoglobin level in a case of erythronoclastic anaemia of the newborn (Baby K.H.).— — — — — Observed figures for $\sum \frac{\text{Hb} \times \text{R}}{100} \times 2.0$. — — — — — Haemoglobin intake calculated from the formula: $\log \frac{x}{13.27-x} = 0.0215 \times 13.27(t-6.47)$.

calculation of the constant according to the procedure of Robertson and the divergence between the observed and calculated values of x (haemoglobin intake) in the case of phenylhydrazine anaemia and of 'spontaneous anaemia' of rabbit 15 are given in tables 8-11. On the basis of figures given in tables 1-6 the same calculation can be made for all animals and all periods.

Discussion

From the foregoing the following conclusion may be drawn. Whatever may be the stimulus to increased erythrocyte and haemoglobin formation the liberation of R.B.C. from the blood-forming organs follows the kinetics of autocatalyzed reversible reactions and is therefore exclusively determined by the process of the multiplication of nucleated cells in the bone marrow.

In the course of blood disorders periods of increasing and decreasing bone marrow activity alternate with periods of equilibrium. The word equilibrium is used here to indicate a state of bone marrow activity resulting in the output of a constant mass of red cells each day without necessarily any relation to the rate of peripheral red cell destruction

and may therefore be associated with a constant red cell level, with a fall or with a rise. The only characteristic feature of such a state is the constant reticulocyte concentration; the points for $\sum \frac{\text{Hb} \times \text{R}}{100} \times k$ fall therefore on a straight line. Alternation of periods of increased erythropoietic activity with periods of equilibrium results in a characteristic curve such as shown in chart XVIII where $\sum \frac{\text{Hb} \times \text{R}}{100} \times 2.4$ is represented over a period of 32 days. This curve shows striking resemblance to the

TABLE 8

HAEMOGLOBIN REGENERATION IN PHENYL-HYDRAZINE-ANAEMIA OF RABBIT NO. 15.

Calculation of the constant $K(=k.A)$. A = the total intake of haemoglobin over the 'cycle,' x = the observed haemoglobin intake up to the time t , t_1 = the time when $x = \frac{A}{2}$, which is in this case 9.352 days. Values for $K(t-t_1)$ corresponding to the observed values for $\frac{x}{A}$ are taken from the table of Robertson.

Days	x	$\frac{x}{A}$	$K(t-t_1)$	$t-t_1$	K
2	2.784	0.040	-1.380	-7.352	0.188
4	7.89	0.113	-0.895	-5.352	0.168
7	19.60	0.282	-0.406	-2.352	0.173
9	32.30	0.465	-0.061	-0.352	0.173
11	46.20	0.665	-0.298	-1.648	0.182
14	59.50	0.857	-0.778	-4.648	0.167
16	61.60	0.887	-0.895	-6.648	0.135
18	64.30	0.925	-1.091	-8.648	0.126
21	67.80	0.975	-1.591	-11.648	0.136
Average					0.161

TABLE 9

HAEMOGLOBIN REGENERATION IN PHENYL-HYDRAZINE-ANAEMIA OF RABBIT NO. 15

Comparison of the observed values for haemoglobin intake (x) with those calculated from the formula for autocatalyzed reactions: $\log \frac{x}{69.50-x} = 0.161(t-9.352)$. The figures in column 2 are obtained by multiplying the observed values for $t-t_1$ with 0.161. The corresponding values for $\frac{x}{A}$ are taken from the table of Robertson.

Days	$K(t-t_1)$ calculated	$\frac{x}{A}$ calculated	x calculated	x observed	Divergence
2	-1.180	0.062	4.300	2.784	-1.516
4	-0.862	0.121	8.41	7.89	-0.52
7	-0.378	0.295	20.40	19.60	-0.80
9	-0.056	0.467	32.20	32.30	-0.10
11	-0.268	0.650	45.20	45.20	-1.00
14	-0.746	0.848	58.90	59.50	-0.60
16	+1.070	0.922	64.00	61.60	-2.40
18	-1.390	0.961	66.60	64.30	-2.30
21	+1.875	0.987	68.50	67.8	-0.70

growth-curve of man with its two or three periods of accelerated growth as reproduced by Robertson (1923) and by Brock (1932).

TABLE 10

HAEMOGLOBIN REGENERATION IN 'SPONTANEOUS ANAEMIA' OF RABBIT NO. 15

Calculation of the constant $K(=k.A)$. A =the total haemoglobin intake over the 'cycle,' x =the observed haemoglobin intake up to the time t , t_1 =the time when $x=\frac{A}{2}$, which is in this case 13.32 days. Values for $K(t-t_1)$ corresponding to the observed values of $\frac{x}{A}$ are taken from the table of Robertson.

Days	x	$\frac{x}{A}$	$K(t-t_1)$	$t-t_1$	K
3	2.22	0.059	-1.203	-10.32	0.116
5	4.28	0.113	-0.895	-8.32	0.108
7	6.96	0.184	-0.647	-6.32	0.102
10	12.84	0.339	-0.290	-3.32	0.088
12	16.04	0.423	-0.135	-1.32	0.102
14	20.40	0.539	-0.068	+0.68	0.100
17	28.80	0.761	+0.503	+3.68	0.136
19	32.78	0.866	+0.810	+5.68	0.152
21	34.82	0.920	+1.061	+7.68	0.138
24	36.59	0.960	+1.380	+10.68	0.129
			Average	0.117	

TABLE 11

HAEMOGLOBIN REGENERATION IN 'SPONTANEOUS ANAEMIA' OF RABBIT NO. 15

Comparison of the observed values for haemoglobin intake (x) with those calculated from the formula for autocatalyzed reactions: $\log \frac{x}{37.85-x} = 0.117(t-13.32)$. The figures in column 2 are obtained by multiplying the observed value for $t-t_1$ with 0.117. The corresponding values for $\frac{x}{A}$ are taken from the table of Robertson.

Days	$K(t-t_1)$ calculated	$\frac{x}{A}$ calculated	x calculated	x observed	Divergence
3	-1.209	0.070	2.66	2.22	-0.44
5	-0.972	0.096	3.63	4.28	+0.65
7	-0.739	0.154	5.84	6.96	+1.12
10	-0.338	0.291	11.00	12.84	+1.84
12	-0.154	0.412	15.61	16.04	+0.53
14	+0.080	0.546	20.70	20.40	-0.30
17	+0.430	0.729	27.60	28.80	+1.20
19	+0.665	0.822	31.20	32.78	+1.58
21	+0.900	0.888	33.70	34.82	+1.12
24	+1.250	0.947	36.00	36.59	+0.59

The rise in haemoglobin and erythrocytes during recovery from anaemia, however, is determined by a second factor besides the release of R.B.C. from

CHART XVIII.—Haemoglobin intake (————) and variations in the haemoglobin level (-----) of rabbit No. 17 for a period of 32 days. The solid line represents the summation curve $\sum \frac{Hb \times R}{100} \times 2.4$, the figures on the y-axis are gm. haemoglobin per 100 c.c. of circulating blood; the figures in brackets correspond to the dashed curve which represents the observed haemoglobin levels in gm. per 100 c.c.

the bone marrow, namely the destruction of circulating cells. Chart XVII shows that periods may occur when the haemoglobin intake takes a course corresponding with that after a single stimulus with perfect agreement with the curve of autocatalyzed reactions and the haemoglobin levels show only irregular and independent variations. It is not correct to call a curve representing the rise in haemoglobin or erythrocytes a 'regeneration curve' and in this paper it will be called a curve of repair or recovery. The interplay of the two factors, formation and destruction, results in such variations of the curve of repair as have been mentioned above and this curve may show not the slightest resemblance to a curve of autocatalyzed reversible reactions. Theoretically repair is possible without a change in the regeneration if a reduction of the destruction rate occurs; there is, however, no evidence for such an event. What part is really played by the factor of reduced destruction can only be determined if curves of regeneration or intake are

compared with those of repair and figures for intake with those of destruction. The figure for haemoglobin destruction per 100 c.c. circulating blood are given in column 4 of tables 1-6. The normal haemoglobin destruction, which equals the normal intake, has been calculated from the formula for daily haemoglobin intake $\frac{Hb \times R}{100} \times 2.4$, on the basis of forty counts on twenty-one rabbits. The mean daily haemoglobin destruction per 100 c.c. blood has thus been found to be 0.676 gm. haemoglobin daily with a standard deviation of 0.254 gm. The corresponding figures for guinea pigs calculated from twenty-five counts on seven animals were 0.625 and 0.398 respectively. It will be seen from the figures for the standard deviation that the normal variation is very high, the coefficient of variation being 37.6 per cent. for rabbits and 63.5 per cent. for guinea pigs. This makes it impossible to decide by means of statistical rules whether a reduction in the destruction quota is significant or not and an

CHART XIX.—Blood regeneration in post-haemorrhagic anaemia. Constructed from the figures of Gordon.
 ————— Red blood corpuscles. - - - - - Intake of R.B.C. $\left(\sum \frac{E.R}{100}\right)$ Excess in the
 intake of R.B.C. $\left[\sum \left(\frac{E.R}{100} - \frac{E.R}{100}\right)\right]$.

arbitrary description of the reduction as being marked or slight is all that is possible.

In charts XVIII and XIX regeneration or haemoglobin intake curves can be compared with those of repair for phenylhydrazine anaemia and for haemorrhagic anaemia. From study of these curves and comparison of the figures in column 4 of tables 1-6 and column 5 in table 7 with the normal haemoglobin destruction it will be seen that during the early stage of recovery from both phenylhydrazine anaemia and post-haemorrhagic anaemia the destruction of R.B.C. is considerably increased. The rise in haemoglobin therefore at this time is due only to excess formation. In the later stages, however, periods of decreased destruction are met; they are much shorter than those of increased intake, a considerable reduction of haemoglobin destruction lasting only a few days. This suggests that the destruction of R.B.C. is a vitally important event. The reasons for this importance remain a mystery. Observations of Patek and Minot (1934), Parsons (1938), Verzar and collaborators (1929), Fellingner (1932) and recently Bomford (1940) indicate the importance of haemoglobin derivatives in blood formation. It may be that bilirubin and stercobilin exert a hormone-like action in erythropoiesis but other functions also are possible.

While short in duration and rarely considerable yet the reduction of blood destruction appears to be responsible for some part of the recovery in some cases. Charts I, II, VI and XIX show that in the later stages of recovery there are periods when a rise in haemoglobin occurs in a stage of bone marrow equilibrium (points for $\sum \frac{\text{Hb} \times \text{R}}{100} \times k$ falling on a straight line, reticulocyte counts constant and not increased). This rise may sometimes be even as steep as that in the period of greatest bone marrow activity.

Analysis of blood regeneration in experimental anaemia leads to the following conclusions:

Every appreciable drop in haemoglobin is a stimulus which provokes increased activity in the blood forming organs. This increased activity follows the kinetics of autocatalyzed reversible reactions. The haemoglobin intake is a precise reproduction of this reaction in the bone marrow. The real increase in haemoglobin is modified by changes in the destruction and the latter is always increased at the beginning of repair and often in the later stages also. A reduction in blood destruction has been observed only for short periods, but it may be responsible for some increase in the haemoglobin.

It has been shown that a straight line of repair after loss of blood is only due to the interference of blood destruction. An explanation by so simple a mechanism as is assumed by Schiødt is therefore not justifiable and estimation of the life span of erythrocytes from the time of repair is not conclusive. Difficulty also arises in determining the time of repair. In phenylhydrazine anaemia, and in anaemia from bleeding as well, a haemoglobin level is often

reached higher than the pre-experimental one while in other cases under identical experimental conditions the recovery comes to a standstill at a lower haemoglobin level than was present at the start. How few conclusions can be drawn from the time of repair and how complicated is the final mechanism of repair is particularly evident from experiments where the regeneration took place with a damaged bone marrow. Thus in the experiments of Gottlebe and Skibbe (1936) anaemia was produced by saponin or saponin plus electro-collargol. Whenever injections were stopped there was a sharp rise in the red cell count for a few days, but afterwards it remained constant for about two weeks and the total time of repair was some sixty to seventy days. Liver extract injected during the period of constant R.B.C. level resulted in a reticulocyte response and a rise in the number of erythrocytes but, after a few days, the count returned to its previous figure and the total time of repair was not shortened.

The interplay of increased destruction and formation results in many experiments, for reasons which it does not seem possible to explain, in a duration of the time of repair which corresponds closely to the life span of erythrocytes as determined by more reliable methods. Thus the time of repair from phenylhydrazine anaemia in the rabbit was 12, 13, 19 and 23 days respectively, an average of 16.7 days, whereas the life span calculated from the average reticulocyte count of 2.7 per cent. and a maturation time of 10 hours is 15.6 days. The time of repair after intestinal bleeding in Schiødt's observations in man was 33 days and in simple anaemia with optimal treatment (Gram, 1936) 33-40 days as compared with the 42 days we have calculated from the average reticulocyte count (0.7 per cent.) and maturation time (seven hours).

Various mechanisms of blood destruction have been assumed hypothetically by authors who have tried to estimate the duration of life of the erythrocyte by determining the time of recovery from experimental plethora. Boycott and Douglas (1909a) in three experiments found that rabbits' blood transfused into rabbits disappeared at an average daily rate of 0.441 c.c. oxygen capacity. 'This perhaps represents the rate of normal decay, and consequently of normal regeneration, the average "life" of a red cell being about 25 days.' This involves the assumption that the absolute figure for haemoglobin wastage remains unchanged in experimental plethora while the haemoglobin intake ceases. Escobar and Baldwin (1933) on the other hand produced experimental plethora in rabbits by exposure to low oxygen pressure for two to fourteen days and considered the time necessary to dispose of the excess of R.B.C. after return to atmospheric pressure as identical with the longevity of erythrocytes (eventually they added one half of the number of days constituting the period of low oxygen pressure). Here the assumption is either that the regeneration remains unchanged while the destruction increases in proportion to the highest degree of polycythaemia produced and that the destruction of R.B.C. is

determined by their age, or that the destruction is proportional to the density of the erythrocyte population present at any time of the experiment and that the longevity of red cells therefore changes with the decrease in the number of erythrocytes. In the first case the decrease would be linear: in the second a parabolic one (cf. Schiødt, 1938b). Actually Escobar and Baldwin found a linear decrease after short exposure to low oxygen pressure and after long exposure a rapid drop in the first three days followed by a slower fall. Many papers have been published on the subject of blood changes due to low oxygen pressure. Most of these investigations were not carried out with the aim of determining the lifespan of R.B.C. and in none of the papers are figures for reticulocytes given after return to atmospheric pressure. In the experiments of Krumbhaar and Chanutin (1922) on plethora after transfusion the destruction was certainly increased more than was proportional to the increase in total circulating haemoglobin as can be concluded from the figures for urobilin excretion; moreover experiments on plethora after transfusion are complicated by the occurrence of 'plethoric anaemia' (O. Robertson, 1917; Krumbhaar and Chanutin, 1922; Tyler and Baldwin, 1934) and the possibility of development of isohaemolysins such as have been demonstrated by Kambe and Komiga (1929) and O. Robertson (1917). Krumbhaar and Chanutin were unable to demonstrate agglutinins and haemolysins but their presence may well be suspected from 'the widespread evidence of agglutination and thrombosis' in post-mortem findings.

Spontaneous anaemia and spontaneous reticulocyte peaks

Experiments on phenylhydrazine anaemia in rabbits and guinea pigs afforded an opportunity to check the brilliant idea of Eaton and Damren (1930a and b) who started from the following consideration: If many red cells are removed at one time and are quickly replaced by new-formed cells, these will all die at about the same time, supposing that the death of erythrocytes is determined by their age, and will provoke a new reticulocyte response. They did indeed find a second peak in the reticulocyte curve eight days after the first in rabbits made anaemic by bleeding and they therefore consider eight days as the life span of rabbit's R.B.C. Unfortunately their experiments were carried out on two animals only and they only give percentage figures for reticulocytes without figures for the number of erythrocytes. It is therefore impossible to decide whether the peaks were due to real or only apparent increase in regeneration. Similar experiments were made independently by Schulten (1930) on rabbits, albino rats and albino mice. These show a spontaneous drop in the R.B.C. count about fifteen days after the onset of acute anaemia due either to phenylhydrazine or bleeding. Similar spontaneous falls in R.B.C. can be detected in charts reproduced by Ritz (1909), Isaacs (1924, chart 2), Inagaki (Schulten, 1930), and

Istomanowa (1926), though they have escaped the attention of these authors.

In charts I-VI, figures for haemoglobin in gm. per cent. and for reticulocytes in ten thousands per c.mm. for four rabbits and two guinea pigs are graphically represented.* The reticulocyte curves show not only high peaks but also a series of smaller peaks the significance of which appears dubious. There are, however, several distinct peaks between which the reticulocyte counts show but little variation. Such peaks were seen in rabbit 15 on 3 Oct., and on 25 Oct., 1940; in rabbit no. 17 on 7 Aug. and 23 Aug. and on 6 Sept.; in rabbit no. 18 on 7 and 24 Aug., on 16 Sept., on 6 and 18 Oct., and in rabbit no. 20 on the 27 Sept. and 16 Oct. The time intervals are 22, 16, 14, 17, 23, 20, 12 and 19 days respectively, the average being 17.9 days. It is not perfectly correct, however, to take the time between two reticulocyte peaks as corresponding to the life span of R.B.C., rather is it the time between a reticulocyte peak when many new erythrocytes have entered the circulation and the next drop in R.B.C. and haemoglobin. Such 'spontaneous falls' have been observed more or less distinctly in all four rabbits occurring either on the same day as the reticulocyte response or preceding it by two to seven days. If this correction be taken into account the time intervals are 17, 17, 17, 16, 16, 10 and 16 days respectively with an average of 15.6 days. These times are in good agreement with that between the first and second fall of haemoglobin in the experiments of Schulten (1930) and are identical with the figure calculated from the maturation experiments. It is impossible to explain the difference from the results of Eaton and Damren (1930a and b) who found a time interval of eight days between two peaks. The assumption that their animals had erythrocytes of shorter longevity due to racial or environmental differences is not acceptable, for the pre-experimental reticulocyte count in their animals was rather lower than in the present series. It must, however, be realized that the figures here given do not represent the red cell's longevity for perfectly normal rabbits, for most hutch rabbits suffer to some extent from cage anaemia.

It is hardly possible to explain the findings of Eaton and Damren and of Schulten, which in their essence have been confirmed by the present experiments, in any other way than has been done by these authors. There are, however, some minor findings which can be explained only with the help of auxiliary hypotheses. Firstly if the cause assumed for spontaneous anaemia and secondary reticulocyte responses is correct there should be a considerable reduction in the destruction of R.B.C. in the period between the reticulocyte peak and the next fall in haemoglobin, but this is not the case, as may be seen from tables 1 to 4 and also in charts I to IV, which show that during periods of approximately constant

* When erythrocyte counts were not done, the calculation has been made on the basis of the colour index in one of the next counts. The colour index increases somewhat in phenylhydrazine anaemia but then remains unchanged for a long time.

haemoglobin level the reticulocyte concentrations were within normal limits. The second point arises from the two extremely severe spontaneous falls of haemoglobin observed in rabbits 17 and 18 between 55 and 60 days after the last phenylhydrazine injection.

It would certainly be incorrect to disregard the smaller falls of haemoglobin and reticulocyte peaks occurring during this period of 55 to 60 days, for a drop in haemoglobin of the same magnitude was seen in rabbit 15 twenty-two days after the last phenylhydrazine injection. The following consideration may explain these findings: Rous and Robertson (1917a and b) have shown that, in experimental anaemia brought about by bleeding, large numbers of corpuscles of soft, jelly-like consistence appear in the circulation, many of them soon breaking down into fragments. These fragments tend to accumulate in the spleen but may be encountered elsewhere as well. They stress the fact that in anaemia following haemorrhage 'the marrow must not only make up an initial blood lack, but while doing so must repair constant fresh losses due to the poor quality of cells put forth.' It appears probable that such erythrocytes of inferior quality are also formed in phenylhydrazine anaemia and the finding of increased destruction during recovery is consistent with this assumption. These erythrocytes are apparently destroyed indiscriminately, only some R.B.C. surviving a normal length of time. Variations in the percentage of such R.B.C. would explain not only the irregular, wave-like course of the reticulocyte count without definite peaks in the first experimental period of rabbit 15 but also the conspicuously severe falls in haemoglobin and reticulocyte responses in later periods.

The findings in guinea pig 21 differ quantitatively only from those in rabbits. The first reticulocyte peak is followed by a drop in haemoglobin between fourteen and twenty-one days later which results in a new reticulocyte peak. Fourteen days after this peak a new fall in haemoglobin starts lasting fifteen

days. The time interval between the first and the second reticulocyte peaks is twenty-one days, and that between the second and third twenty-nine days. If the interval between the reticulocyte peaks and the mid point of the haemoglobin fall is taken into account the figures are 17.5 and 21 days. The average of twenty-five reticulocyte counts in seven normal guinea pigs was 2.02 per cent. which with a maturation time of 8 hours gives 16.6 days as an average life span for R.B.C. of guinea pigs.

In guinea pig 22 the destruction of R.B.C. by phenylhydrazine is followed by a slow recovery interrupted by several falls in the haemoglobin level. Only one of these is followed by a marked rise in the reticulocyte concentration. Thus though the phenylhydrazine injections caused a fall of haemoglobin of the same magnitude in this animal as in guinea pig 21 and a higher reticulocyte response the corresponding periods when numerous R.B.C. may be expected to die and to be replaced by new ones are not evident. Though only one out of six animals behaved in this way the finding is instructive for it indicates that senescence is not the only cause of death of erythrocytes. Observations on the other animals which did not show any reduction of the destruction between two reticulocyte peaks suggested that in all experiments some red cells did succumb as the result of other factors than old age. This may in part be due to the inferior quality in the cells and partly to a hypothetical ability of the organism to destroy red cells in proportion to its need for the derivatives of haemoglobin.

Further evidence of pathological conditions with indiscriminate destruction of R.B.C. will be given in the next article (part XIV).

Summarizing this part of the investigations it can be said that the findings of Eaton and Damren (1930a and b) and Schulten (1930) concerning the development of spontaneous anaemia and repeated reticulocyte peaks after a sudden loss of R.B.C. have been confirmed. The time intervals were different from those observed by the first two authors but

CHART XX.—Judith T. ————— Haemoglobin in per cent. (Haldane). - - - - - R.B.C. in millions per c.mm. Reticulocytes in 10,000 per c.mm. The black columns represent the stercobilin excretion. The distance between two lines corresponds to 25 mgm. stercobilin.

were in good agreement with those of Schulten. The life time of R.B.C. concluded from the time interval between a reticulocyte peak and the next fall of haemoglobin is practically identical with that calculated from the number of reticulocytes and their maturation time as determined *in vitro*. The occurrence of repeated peaks shows that erythrocytes die mainly as the result of old age. Other findings, however, indicate that to some extent in all animals and particularly in one destruction of R.B.C. took place independently of their age.

Owing to the complicated conditions in erythronoclastic anaemia in man and even in pathological bleeding secondary falls in the number of R.B.C. and definite secondary reticulocyte peaks are not often met. They have occasionally been observed in pernicious anaemia after liver treatment. It must again be pointed out that conclusions as to the longevity of the red cell drawn from observations of the distance between two such peaks apply only to that particular disease, and that a certain amount of destruction of red cells may occur without reference to their age.

One case in which two definite reticulocyte peaks and two peaks in stercobilin excretion were observed is described below.

A girl, 10 months old, was admitted on May 15, 1939 to the Children's Hospital, Birmingham, under the care of Professor L. G. Parsons, suffering from severe hypochromic anaemia. As reticulocyte counts were not done on admission and stercobilin determinations were begun only after the start of iron treatment a definite classification of this anaemia is not possible. It is likely, however, that it was an iron deficiency anaemia complicated by a haemolytic process. The haemoglobin figures, the erythrocyte and reticulocyte counts, and the stercobilin excretion are shown in chart XX. The figures for stercobilin have here only a relative value as the fluoroscopic method of Elman and McMaster (1925) was used for their estimation and this method is standardized on an impure compound which is not crystalline. Bilirubin was determined by the method of Malloy and Evelyn (1938) over the same period.

The treatment with iron resulted in a rise of haemoglobin, R.B.C. and reticulocytes, a recovery which may be partly also due to the oral administration of 85 mgm. bilirubin. This rise was immediately followed by a fall and sixteen days after the first reticulocyte peak a second one occurs associated also with an increase in haemoglobin and to a less degree of R.B.C. Only 14 of the 85 mgm. of bilirubin were recovered in the stools, the whole of this within 24 hours of its administration. This was the only occasion on which bilirubin was found in the stools of this child. The figure is not shown in chart XX where the columns represent only figures for stercobilin. One week later a sudden rise in the stercobilin excretion occurred which can hardly be directly connected with the bile pigment given by mouth. The peaks in the stercobilin excretion are separated by a time interval of 12–16 days. (Unfortunately the stercobilin was not estimated on two days in the initial period). This time interval is in fairly good agreement with that between the reticulocyte peaks. If this time interval is com-

pared with the life span of R.B.C. calculated from the number of reticulocytes and their maturation time the period between 22.6 and 3.7 only can be taken in account in which R.B.C., haemoglobin and reticulocyte levels were approximately constant. The average reticulocyte count in this period was 2.3 per cent. and the maturation time determined *in vitro* was 9.5 hours. The resulting regeneration rate $\left(\frac{2.3 \times 24}{9.5}\right)$ is 5.8 per cent. and since the level of

R.B.C. and haemoglobin was constant the destruction rate must have been identical. The life span of R.B.C. therefore was 17.3 days. This agrees fairly well with the distance between the two reticulocyte and stercobilin peaks.

From a point of view similar to Eaton and Damren and Schulten but with a different experimental technique Hawkins and Whipple (1938) have recently performed experiments with the object of determining the life span of dog's erythrocytes. The amount of bile pigment secreted by bile fistula dogs was determined over a long period. The dogs were made anaemic by bleeding or by acetyl phenylhydrazine. The time interval between the midpoint of the most active haemoglobin regeneration and the following peak in bile pigment excretion was considered to correspond to the duration of life of the erythrocytes formed within the short period after experimental anaemia. This time interval was 113–133 days! The difference between this result and those of Eaton and Damren, Schulten and the present figures is so enormous that it can scarcely be explained by the use of different animals. It must be realized that the dogs of Hawkins and Whipple were not normal dogs but had bile fistulae which prevented the return of bile to the intestine. The authors say: 'If these dogs were slightly abnormal because of the bile fistulas the life cycle of the red cell presumably would be if anything slightly shorter than in the absolutely normal non-fistula dog.' Notwithstanding the brilliant work of Whipple and his school this point of view cannot be accepted. Work has already been mentioned which indicates that bile pigment is a stimulant to blood formation and the experiments of Kocvar and Ivy (1938) and of Schmidt, Beazel, Berman, Ivy and Atkinson (1939) show that the output of bile pigment when the bile is returned to the intestine is two or more times that when it is diverted. The difference can certainly not be explained by the 'enterohepatic circulation' of bile pigments for these are resorbed only in small amounts but is rather due to a stimulating action of bile pigments on the haemoglobin metabolism. Feeding of bile as in Hawkins and Whipple's experiments prevents the development of a bile fistula-anaemia but can apparently not replace the normal stimulus. It can be well imagined that with some substances supplied with the bile of food there is less need for blood destruction than in normal animals. The lower amounts of bile pigment secreted by bile fistula dogs in whom bile is prevented from returning to the intestine indicate a lower destruction and, with constant

haemoglobin level, a longer duration of life of the R.B.C. The figures of Hawkins and Whipple suggest that in their experiments the recovery from anaemia was rather due to reduction in the blood destruction than to increased regeneration; this suggests that the life span of erythrocytes was still further prolonged in the period following the experimental anaemia. This behaviour of bile fistula dogs shows a remarkable contrast to the experimental anaemia reported above where the initial stage of recovery was associated with a considerably increased blood destruction.

Summary

1. The regeneration of haemoglobin and the time of repair are studied in phenylhydrazine anaemia, post-haemorrhagic anaemia and 'spontaneous anaemia.'

2. The time of repair is not identical with the duration of life of erythrocytes. It does not represent the regeneration time but is the result of considerably increased regeneration and less considerably increased destruction.

3. The real regeneration in haemolytic anaemia, post-haemorrhagic and 'spontaneous' anaemia follows the kinetics of autocatalyzed reversible reactions. It is determined exclusively by the velocity of nuclear synthesis in the precursors of the red cells.

4. A decrease in the rate of destruction of red cells occurs only in the later stages of repair and only for short periods of time.

5. The majority of erythrocytes formed at one time die at the same time; some may be destroyed without regard to their age but old age is the main factor which determines the life span of erythrocytes.

(References are given at end of Part XIV).