OBSERVATIONS BY THE CARBON MONOXIDE METHOD ON A CHLOROTIC ANÆMIA IN A RABBIT, AND ON TRANSFUSION.¹

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VIII. A CASE OF CHLOROTIC ANÆMIA IN A RABBIT.

WHILE we were trying without much success to produce anæmia in rabbits, one of the animals in hand (*Mary*, Table XIII. Fig. 12) developed spontaneously a very definite anæmia, partly real and due to a deficiency in total hæmoglobin, but for the most part brought about by a large increase in the volume of the plasma. In this respect, therefore, it was of the type of anæmia which Lorrain Smith showed to obtain in human chlorosis;² his observations have more recently been confirmed by Plesch and Oerum.

The early history of this animal, comprising four observations scattered over rather more than a month, showed nothing abnormal. The percentage oxygen capacity and total hæmoglobin were then observed to be falling, though the animal did not appear to be definitely ill till the percentage oxygen capacity had fallen to one-half, and the total oxygen capacity to three-quarters, of the normal. Thereafter the general symptoms became progressively more severe, and a marked dilution of the blood developed till, shortly before death, the blood volume was more than doubled. Blood films showed great variation in size of red cells,³—a few being too big and many too small,—a good deal of poikilocytosis and polychromatophilia with many nucleated reds (normoblasts) and some granular myelocytes. Post-mortem, thick pus was found infiltrating the muscles of the abdominal wall and upper part of the thigh; there was no reaction by the tissues, the abscess “wall” being purely necrotic. The heart was much distended, and the great veins blown up with very watery blood. The organs showed nothing noteworthy on histological examination. The organism found in pure culture in the pus was a non-motile, Gram-negative cocco-bacillus, growing freely on artificial media at 37° C. and at room temperature, liquefying gelatin, producing a small

¹ Received February 26, 1909.

² Some of Smith's cases of chlorosis show, besides a great increase in the volume of the blood, a deficiency in total oxygen capacity of about 25 per cent.

³ Allowance, is, of course, made for the fact that all these histological signs of blood regeneration may be present to a greater or less degree in “normal” rabbits.

amount of indol (para-dimethyl-amido-benzaldehyde test), fermenting dextrose with gas production, no effect on lactose, no growth in MacConkey's bile-salt

TABLE XIII.—RABBIT, *Mary*, ♀.—*Spontaneous Anæmia*.

Date.	Weight.	Percentage O ₂ Capacity.	Total O ₂ Capacity.	Blood Volume.	Per 100 Grms. Body Weight.		Relative Values.			Remarks.
					C.c. Blood.	C.c. O ₂ Capacity.	Per Cent. O ₂ Capacity.	Total O ₂ Capacity.	Blood Volume.	
..	3140	12·5	23·1	185	5·9	0·735	100	100	100	Average of May 14 and 22.
April 15	2960	12·4	20·7	167	5·6	0·70	99	90	90	
May 1	3450	11·5	22·2	193	5·6	0·64	92	96	104	
„ 14	3200	12·0	23·0	192	6·0	0·72	96	100	104	May 12 : 4 fetus = 215 grms.
„ 22	3080	12·95	23·2	179	5·8	0·75	104	100	97	
June 2	3070	9·25	19·5	210	6·85	0·64	74	84	114	
„ 4	3000	9·6	18·8	196	6·5	0·63	77	81	106	
„ 11	2880	7·6	18·9	248	8·6	0·655	61	82	134	
„ 12	2850	6·5	17·3	266	9·35	0·61	62	75	144	First obviously ill.
„ 13	2740	6·7	15·3	228	8·35	0·56	54	66	123	
„ 15	2820	4·9	13·6	277	9·8	0·48	39	59	150	
„ 18	2620	3·05	13·5	443	16·9	0·515	24	58	239	Died June 18-19.

broth, no change in litmus milk. A culture was inoculated into a second animal (*Marky*, Table XIV.) subcutaneously in the belly. This gave rise to a

TABLE XIV.—RABBIT, *Marky*, ♀.

Date.	Weight.	Percentage O ₂ Capacity.	Total O ₂ Capacity.	Blood Volume.	Per 100 Grms. Body Weight.		Relative Values.			Remarks.
					C.c. Blood.	C.c. O ₂ Capacity.	Per Cent. O ₂ Capacity.	Total O ₂ Capacity.	Blood Volume.	
..	3210	13·2	23·7	176	5·5	0·72	100	100	100	Average of July 14 and 15.
July 14	3180	13·4	24·3	174	5·5	0·73	101·5	102·5	99	
„ 15	3240	13·0	23·1	178	5·5	0·71	98·5	97·5	101	↓ Inoculated with culture from <i>Mary</i> .
„ 16	3120	11·7	19·1	163	5·2	0·61	89	81	92·5	
„ 17	3200	12·2	21·0	172	5·4	0·66	92	89	98	
„ 18	3200	11·1	20·3	183	5·7	0·63	84	86	104	
„ 21	3280	10·4	19·0	183	5·6	0·58	79	80	104	
„ 23	3250	9·5	18·0	189	5·8	0·55	72	76	107	↓ Two 40 mm. abortions.
„ 25	2870	9·6	20·3	212	7·4	0·71	73	86	120	Died July 26-27.

spreading suppuration of the same type as that found in *Mary*, and proved fatal in ten days ; but the changes in the blood, though in the same sense, were

not nearly so definite as in that animal. The organism soon lost its virulence on artificial media, and after three months produced no ill effects in rabbits by any method of administration.

The chlorotic condition of the animal *Mary* cannot be explained by the hæmoglobin outside the circulating blood (pp. 264, 271).

FIG. 12.—Rabbit, *Mary*.—The thick line represents the total oxygen capacity, the thin line the percentage oxygen capacity, the interrupted line the blood volume.

Such an explanation would involve the assumption that the absolute amount of extravascular hæmoglobin had, in about a fortnight and during the progress of the anæmia, been increased four to seven-fold. Even assuming that as much as 10 per cent. of the oxygen capacity

was not in the blood, we get a normal circulating oxygen capacity of $23.1 - \frac{23.1 \times 10}{100} = 20.8$ c.c. and a blood volume of $\frac{20.8 \times 100}{12.5} = 166$ c.c.; for the last two determinations we have—(1) $13.6 - 2.3 = 11.3$ c.c. oxygen capacity, and a volume of $\frac{11.3 \times 100}{4.9} = 230$ c.c.; and (2) $13.5 - 2.3 = 11.2$ c.c. oxygen capacity and $\frac{11.2 \times 100}{3.05} = 367$ c.c.

blood volume; or, in other words, volumes still in excess of the normal by 39 per cent. and 121 per cent. respectively. To explain the apparent volume of 277 c.c. on 15th June, the extravascular oxygen capacity would have to be $13.6 - \frac{185 \times 4.9}{100} = 4.5$ c.c., or 19

per cent. of the normal total oxygen capacity; and on 18th June, 8.0 c.c. or 35 per cent. of the normal total, and 59 per cent. of that actually present at the time. Our own observations (p. 264) would lead us to suppose that the normal extravascular oxygen capacity in *Mary* was about 1.5 c.c. The marrow area in rabbits is usually so full of red marrow that there hardly seems to be much room for any prodigious increase here; exact measurements are, however, much needed. F. Müller has recorded a remarkable case of a dog suffering from distemper in which only 38 (instead of about 80) per cent. of the hæmoglobin was in the blood, the total hæmoglobin in the body being probably reduced by about three-eighths and the absolute amount in the marrow being apparently increased. Dogs, however, evidently differ a great deal from rabbits in the distribution of the hæmoglobin in different parts of the body.

How far the extreme degree of blood dilution which existed in *Mary* shortly before death is to be regarded as being an essential feature of the anæmia seems rather doubtful. The great increase in blood volume coincided rather closely with the onset of definite illness (lassitude, failure of appetite), and may have been a secondary effect due perhaps to a low blood pressure.

In another rabbit, a female of 2600 grms. under observation prior to experiment, a real anæmia was found (Table XV.).

The percentage oxygen capacity of the blood was observed to fall from 12.95 to 6.1 in the course of nine days without any alteration in body weight or signs of general illness. It was then treated by Welcker's method (p. 267), and hæmoglobin equivalent to an oxygen capacity of 8.96 c.c. was recovered, giving a blood volume of 147 c.c. calculated on its body weight; this gives 0.34 c.c. oxygen capacity and 5.6 c.c. of blood per 100 grms. body weight as against normal values of 0.71 c.c. and 5.5 c.c. Assuming that at the beginning this animal was roughly "normal," its percentage oxygen capacity had fallen by 53 per cent., its total oxygen capacity by 51 per cent., while its blood volume had remained stationary. This is in marked contrast to the case of *Mary*, detailed above, in which the percentage oxygen capacity fell 76 per cent., the blood volume rose 140 per cent., while the total oxygen

TABLE XV.

Date.	Body Weight.	Hæmoglobin per Cent. on Human Scale.	Percentage Oxygen Capacity.
October 5 . . .	2500	70	12·95
„ 6 . . .	2620	65	12·0
„ 7 . . .	2650	64	11·8
„ 8 . . .	2670	59	10·9
„ 9 . . .	2580	59	10·9
„ 10 . . .	2400	58	10·7
„ 12 . . .	2390	46	8·5
„ 13 . . .	2510	39	7·2
„ 14 . . .	2650	33	6·1

capacity only diminished by 42 per cent. Post-mortem, besides a good many hydatids in the omentum and liver, nothing abnormal was found except a large spleen weighing 7·5 grms.¹ This increase in size was due to enlargement of the pulp; there was great proliferation of endothelial cells, which showed some phagocytosis of red cells, but not to an abnormal degree, and excessive phagocytosis of polynuclear leucocytes. No histological signs of excessive blood destruction were found in the spleen or elsewhere. The femur marrow was markedly less leucoblastic than normal. Blood films during life showed many microcytes, no poikilocytosis, excessive polychromatophilia, some punctate basophilia, and a few nucleated reds; leucocytes apparently normal.

From cultures from the spleen three different organisms were isolated. They need not be further particularised, since in a long series of experiments none of them showed any pathogenic effect on rabbits to which they were administered.

IX. OBSERVATIONS ON TRANSFUSION.

Experiments on transfusion of rabbit's blood into rabbits were first undertaken with the view of testing the accuracy of the carbonic oxide method, and in this respect have been already detailed (p. 266). The fate of the injected blood was afterwards followed, and various points of interest arose which are dealt with here.

The material injected was in all cases fresh citrated blood from normal rabbits, each injection containing between 0·4 and 0·5 gram. of sodium citrate. This amount of sodium citrate is enough to keep any quantity of rabbit's blood up to about 100 c.c. liquid at 37° for, at any rate, many hours, so long as the blood is that obtained from the first main gush which issues from the divided carotid. If, thereafter, one waits a short time till the animal's circulation has to some extent filled up again, the second lot of blood often coagulates with 0·5 gram. sodium citrate in less than 50 c.c. blood. We do not suppose that this citrated blood is not different from normal blood in respect of the plasma

¹ The spleen of a normal rabbit of this size weighs about 1·5 gram.

moisty. It was, however, in these experiments necessary to know accurately the amount of hæmoglobin injected; this would be difficult to determine exactly if the blood were transfused directly from artery to vein without intermediate manipulation in vitro. We had no evidence that the citrate plasma was in any respect toxic.

The details of the experiments are shown in Tables XVI.-XVIII. and Figs. 13-15.

In the case of the rabbit *Brown and White* (Table XVI. Fig. 13) the total oxygen capacity had been steadily rising for four weeks before the

TABLE XVI.—RABBIT, *Brown and White*, ♀.—*Transfusion*.

Date.	Weight.	Percentage Oxygen Capacity.	Total Oxygen Capacity, c.c.	Blood Volume, c.c.	Per 100 Grms. Body Weight.		Relative Values.			Remarks.
					C.c. Blood.	C.c. O ₂ Capacity.	Per Cent. O ₂ Capacity.	Total O ₂ Capacity.	Blood Volume.	
April 27	3420	11.0	18.1	165	4.8	0.53	97	80.5	83	
May 6	3670	11.6	19.9	171	4.7	0.54	102	88	86	
" 13	3650	10.4	20.8	200	5.6	0.57	92	92	100.5	
" 20	3500	10.5	21.3	202	5.8	0.61	93	84.5	101.5	
" 26	3890	11.3	23.5	199	5.1	0.58	100	100	100	
" 27	3750	13.7	28.5	208	5.5	0.76	121	127	104	↑ Transfused 52 c.c.
" 28	3780	14.4	28.3	196	5.2	0.75	128	126	98	= 6.5 c.c. O ₂
" 29	3760	14.4	29.0	201	5.4	0.77	128	129	101	capacity.*
" 30	3700	12.8	30.8	240	6.5	0.83	113	137	121	
June 1	3570	13.3	27.7	209	5.85	0.78	118	123	105	
" 2	3520	12.95	27.2	210	6.0	0.77	114	121	105	
" 4	3750	11.8	23.3	198	5.3	0.62	104	104	99.5	
" 5	3740	11.1	24.1	217	5.8	0.645	98	107	109	
" 10	3810	9.8	22.1	228	5.9	0.58	87	98.5	114	
" 11	3680	9.8	21.25	217	5.9	0.58	87	94.5	109	
" 13	3520	10.4	23.7	228	6.5	0.67	92	103	115	
" 15	3680	11.3	22.8	202	5.5	0.62	100	101	101.5	↓ Transfused 61 c.c.
" 16	3680	13.6	27.8	202	5.5	0.75	120	122	101.5	= 8.0 c.c. O ₂
" 17	3650	13.5	23.8	174	4.8	0.64	119	104	87	capacity.
" 18	3650	13.2	26.0	197	5.4	0.71	117	116	99	
" 19	3630	12.4	23.1	188	5.2	0.65	110	108	85	
" 20	3560	11.7	23.1	188	5.55	0.65	104	103	99.5	
" 25	3500	10.2	23.3	228	6.4	0.65	90	104	114	↓ Transfused 60 c.c.
" 26	3570	12.8	26.7	208	5.8	0.75	113	119	107.5	= 6.4 c.c. O ₂
" 27	3630	11.7	24.2	207	5.7	0.67	104	108	104	capacity.
" 29	3700	10.8	25.0	232	6.3	0.68	96	111	117	
July 1	3640	11.7	25.1	215	5.9	0.69	103.5	112	108	
" 3	3530	11.1	26.7	240	6.7	0.745	98	119	121	
" 6	3600	11.45	23.6	223	6.2	0.71	101	114	112	
" 8	3700	11.7	25.4	218	5.9	0.685	103.5	112	110	
" 10	3660	10.6	26.0	246	6.7	0.71	94	116	124	
" 13	3650	11.1	24.4	220	6.0	0.67	98	108	111	
" 15	3660	11.3	24.2	214	5.8	0.68	100	107	107	
" 17	3560	11.2	23.3	208	5.8	0.65	99	104	105	
" 20	3500	12.0	26.1	218	6.2	0.75	106	116	110	
" 21	3500	12.6	21.4	170	4.9	0.61	112	95	85	Killed July 21.

* The sign † indicates that the transfusion preceded the determination entered for the same date; ‡ that it was done afterwards.

first transfusion. It was at first an animal with a low percentage oxygen capacity (about 60 per cent. on the hæmoglobinometer scale), and only 0.5 c.c. oxygen capacity per kilo body weight instead of about 0.7 c.c. During this period its body weight also rose 12 per cent. Having then a total oxygen capacity of 22.5 c.c., a hæmoglobinometer reading of 61 per cent. and a blood volume of 199 c.c., it received intravenously at 11.10 a.m. 52 c.c. of blood¹ from another rabbit which gave on the hæmoglobinometer a reading of 67.5, and therefore contained hæmoglobin having an oxygen capacity of 12.5 c.c. per cent. or 6.5 c.c. for the amount injected; this may be assumed

¹ These volumes include about 4 c.c. of citrate solution.

to be 4.85 grms. of hæmoglobin, more or less. No symptoms were observed. The carbon monoxide determination of the total oxygen capacity two hours after transfusion gave a value of 28.5 c.c. as against a calculated figure of 29.0 c.c., and the same blood volume as before the injection. For the next two

FIG. 13.—RABBIT, *Brown and White*.—The thick line represents the total oxygen capacity, the thin line the percentage oxygen capacity, the interrupted line the blood volume. At A the animal was transfused with 52 c.c. of blood having 6.5 c.c. oxygen capacity, at B with 61 c.c. = 8.0 c.c. oxygen capacity, and at C with 60 c.c. = 5.4 c.c. oxygen capacity. In the curve of total oxygen capacity the ringed points represent actual determinations, the others calculated values.

days the total oxygen capacity remained the same. It then rose suddenly by about a tenth, and subsequently declined. It nearly reached its pretransfusion level eight days after transfusion, though the first determination which gave a value at or below that point was made on the fourteenth day. Nineteen days after the first transfusion the rabbit received 61 c.c. of blood and hæmoglobin having an oxygen capacity of 8.0 c.c. This should have raised the total oxygen capacity from 22.8 c.c. to 30.8 c.c.; on making a determination twenty-two hours after transfusion we found, however, only 27.5 c.c., and twenty-four hours later only 23.5. The hæmoglobin injected was evidently being got rid of much more quickly on the second transfusion than after the first. This was confirmed at a third transfusion, when 60 c.c. of injected blood should have raised the total oxygen capacity from 23.3 c.c.

TABLE XVII.—RABBIT, *Rachel*, ♀—Transfusion.

Date.	Weight.	Percentage O ₂ Capacity.	Total O ₂ Capacity.	Blood Volume.	Per 100 Grms. Body Weight.		Relative Values.			Remarks.
					C.c. Blood.	C.c. O ₂ Capacity.	Per Cent. O ₂ Capacity.	Total O ₂ Capacity.	Blood Volume.	
June 26	3600	12.2	25.9	212	5.9	0.72	100	100	100	Average of June 26, 27, 29.
" 27	3570	11.8	25.3	216	6.0	0.71	97	98.5	102	
" 29	3580	12.8	26.2	204	5.7	0.73	105	101	96	
" 30	3650	12.1	26.0	215	5.9	0.71	99	100	102	
" 30	3520	13.2	33.5	246	7.0	0.82	108	123	116	↑ Transfused 56 c.c. = 7.055 c.c. oxygen capacity.
July 1	3500	12.75	33.7	264	7.5	0.96	104	130	125	↓ Transfused 80 c.c. = 9.2 c.c. O ₂ capacity.
" 2	3550	15.2	30.3	200	5.6	0.85	125	122	94	
" 3	3400	15.3	31.3	206	6.1	0.92	125	126	97	
" 4	3550	16.2	30.8	188	5.3	0.86	133	118	89	
" 6	3500	16.3	33.0	202	5.8	0.94	134	128	95	
" 7	3500	16.8	34.1	203	5.8	0.975	138	132	96	
" 8	3550	14.1	33.5	238	6.7	0.94	116	130	112	
" 10	3540	13.7	29.3	214	6.05	0.83	112	113	101	
" 12	3400	14.2	31.4	221	6.5	0.92	116	121	105	
" 13	3420	14.2	30.2	213	6.2	0.88	116	117	100	
" 15	3580	13.0	27.5	212	5.9	0.77	107	106	100	
" 17	3500	13.0	27.6	212	6.1	0.79	107	107	100	
" 20	3600	13.7	26.7	195	5.4	0.74	112	103	92	
" 21	3500	14.1	...	...	...	...	116	...	...	
" 22	3400	16.4	34.2	209	6.1	1.01	134	132	98.5	
" 23	3500	15.7	31.3	199	5.7	0.895	129	121	94	
" 24	3500	14.8	31.2	211	6.0	0.89	121	121	100	
" 25	3350	14.4	25.9	190	5.35	0.77	118	100	85	
" 27	3590	15.2	25.1	165	4.6	0.70	125	97	78	
" 28	3530	13.9	26.0	187	5.3	0.74	114	100	88	
" 29	3500	15.0	32.4	216	6.1	0.92	123	125	102	↑ Transfused 70 c.c. = 9.3 c.c. O ₂ capacity.
" 30	3420	16.8	30.3	179	5.25	0.88	137	117	84.5	
" 31	3440	14.4	31.0	216	6.3	0.90	118	120	102	
Aug. 1	3400	14.5	31.9	220	6.5	0.94	119	123	104	Killed August 5.
" 3	3380	15.5	29.3	189	5.7	0.88	127	113	89	
" 4	3310	16.1	26.3	163	4.9	0.79	132	102	77	
" 5	3200	15.9	25.8	162	5.1	0.81	131	100	76	

to 28.7 c.c., while only 26.7 c.c. and 24.2 c.c. were found after nineteen and forty-three hours respectively. After the third transfusion the animal was kept under observation for more than a fortnight, but the total oxygen capacity and blood volume did not return to their former level. That this was a result of repeated transfusion seems very doubtful; the figure supports the idea that the rise above the arbitrary "normal" level towards the end of the experiment is a continuation of the increase in total oxygen capacity which was going on before the experiment began. The last two determinations are very irregular, and can hardly be correct. No disease was found post-mortem.

During the whole series of three transfusions the hæmoglobinometer readings roughly followed the curve of total oxygen capacity, and, taken by themselves, present a regular and reasonable curve. In detail, however, they give an inaccurate picture of what really happened.

FIG. 14.—RABBIT, *Rachel*.—The thick line represents the total oxygen capacity, the thin line the percentage oxygen capacity, the interrupted line the blood volume. At A the animal received 56 c.c. blood (7.05 c.c. oxygen capacity), at B 80 c.c. (9.2 c.c.), and at C 70 c.c. (9.3 c.c.).

TABLE XVIII.—RABBIT, *Dr. Nicholson*, ♂—Transfusion.

Date.	Weight.	Percentage O ₂ Capacity.	Total O ₂ Capacity.	Blood Volume.	Per 100 Grms. Body Weight.		Relative Values.			Remarks.
					C.c. Blood.	C.c. O ₂ Capacity.	Per Cent. O ₂ Capacity.	Total O ₂ Capacity.	Blood Volume.	
..	2625	14.8	21.8	147	5.6	0.83	100	100	100	Average of July 14 and 15.
July 14	2570	15.0	22.3	149	5.8	0.87	101	102	101	↓ Transfused 51 c.c. = 6.1 c.c. oxygen capacity.
" 15	2680	14.6	21.3	146	5.4	0.79	99	97.5	99	
" 16	2640	18.0	27.8	155	5.9	1.06	122	127	105	
" 17	2590	19.1	31.1	163	6.3	1.20	129	143	111	
" 18	2570	19.2	30.8	160	6.2	1.20	130	141	109	
" 20	2670	18.3	30.3	166	6.4	1.18	124	139	113	
" 22	2720	14.6	26.4	181	6.7	0.97	99	121	123	
" 23	2650	14.8	26.3	178	6.7	0.99	100	120	121	
" 24	2600	13.9	27.1	195	7.5	1.04	94	124	133	
" 25	2800	13.9	23.7	170	6.1	0.85	94	109	116	
" 27	2610	14.2	23.3	164	6.3	0.89	96	107	111	
" 28	2690	15.0	20.7	138	5.1	0.77	101	95	94	
" 30	2550	13.2	23.2	176	6.9	0.91	89	106	120	
" 31	2520	17.5	2.7	156	6.2	1.09	118	125	106	
Aug. 1	2540	17.2	28.5	165	6.5	1.12	116	131	112	↓ Transfused 63 c.c. = 6.6 c.c. oxygen capacity.
" 3	2640	15.7	24.9	159	6.0	0.94	106	114	108	
" 4	2690	15.4	23.3	151	5.6	0.87	104	107	103	
Killed August 4.										

In the animal *Rachel* a similar series of three, and in *Dr. Nicholson* of two, transfusions were done with very similar results, the details of which are shown in Figs. 14 and 15. In both cases the animals were found to be healthy when they were killed.

As is well known, the plasma part of transfused blood is usually quickly thrown out of the circulation. The following table summarises our observations on this point, the blood volume being corrected on the assumption that 95 per cent. of the total original hæmoglobin was in the circulation (see above, p. 264).

TABLE XIX.

Animal.	Blood Volume before Transfusion.	Volume Transfused.	Blood Volume after Transfusion.	Hours after Transfusion.
<i>Brown and White</i>	187 c.c.	52 c.c.	{ 199 c.c. 187 "	2 23
"	192 "	61 "	194 "	22
"	217 "	60 "	199 "	19
<i>Dr. Nicholson</i>	138 "	51 "	148 "	21
"	167 "	63 "	150 "	19
<i>Rachel</i>	204 "	56 "	{ 237 " 253 " 191 "	1 20 44
"	180 "	80 "	200 "	22
"	178 "	70 "	{ 207 " 172 "	1½ 24½

FIG. 15.—RABBIT, *Dr. Nicholson*.—The thick line represents the total oxygen capacity, the thin line the percentage oxygen capacity, the interrupted line the blood volume. At A the animal received 51 c.c. blood (6.1 c.c. oxygen capacity), and at B 63 c.c. (6.6 c.c.).

With the exception of the first experiment on *Rachel*, when the original blood volume was not restored till the third day, the injected fluid and a volume of the original plasma approximately equal to the volume of the red cells injected were turned out of the circulation very soon. In two animals (*Brown and White* and *Dr. Nicholson*) the blood volume subsequently rose in a suggestive manner, and afterwards fell roughly in correspondence with the fall in total oxygen capacity. This may have been due to the inconvenience caused by the viscosity of the concentrated blood, as happens in splenomegalic polycythæmia (Boycott and Douglas), though the highest absolute concentrations reached were only 14·4 per cent. oxygen capacity in *Brown and White* and 19·2 per cent. in *Dr. Nicholson*, corresponding to 78 and 104 on the hæmoglobinometer scale. It should be noted that the normal percentage oxygen capacities of the blood in these two animals were about at the two extremes found among ordinary rabbits.

As in the examples of regeneration of blood after hæmorrhage already dealt with (p. 274 ff.), this method allows one to trace the real history of the total hæmoglobin in the animal without the confusion introduced by variations in the blood volume. The diminution of blood volume, for example, in the case of *Rachel* (Fig. 14) after the second transfusion kept up the hæmoglobinometer readings, and entirely obscured the fact that the animal had in the course of about a fortnight made away with an amount of hæmoglobin equal to about two-thirds of that which it originally possessed. This method allows one to obtain a more or less accurate¹ quantitative expression of the rate at which transfused red cells are disposed of by the recipient animal.

In each case the first determination after the first transfusion gave practically the correct answer as calculated from the last determination before transfusion and the amount of hæmoglobin transfused :—

TABLE XX.

Animal.	Total O ₂ Capacity before Transfusion, c.c.	Hæmoglobin transfused in C.c. O ₂ Capacity.	Total O ₂ Capacity after Transfusion, c.c.	
			Calculated.	Found.
<i>Brown and White</i> .	22·5	6·5	29·0	28·5
<i>Rachel</i> . . .	26·0	7·05	33·05	32·5
<i>Dr. Nicholson</i> . .	21·3	6·1	27·4	27·8

In the next few days following a curious effect appeared in the rabbit *Dr. Nicholson* (Fig. 15). The total hæmoglobin rose above the level to which it had been put by the transfusion by some 3 c.c. oxygen capacity. The rise is attested by three concordant determinations. We cannot imagine the cause of it. It can hardly be due to a quantity of hæmoglobin normally in the tissues being brought into the circulation, for it amounts to about 15 per cent. of the total original hæmoglobin, and we have already (p. 264) shown that not more than half this proportion of the total hæmoglobin is normally

¹ It must not be supposed that the method gives entirely accurate results. The irregularities of the curves are no doubt in part due to bad experiments. We imagine that most of the results are right within 10 per cent., and many of them a good deal nearer the truth.

outside the circulating blood in rabbits. The clearness of this secondary rise in *Dr. Nicholson* suggests that the slighter increases on the fourth day in *Brown and White* and on the second and eighth days in *Rachel* are not to be accounted for by errors of experiment,—a supposition not discordant with their magnitude.

If we consider the three first transfusions as a whole we obtain the following approximate rates of disappearance of the transfused hæmoglobin.

TABLE XXI.

	Hæmoglobin transfused in c.c. O ₂ Capacity.	Days till total O ₂ Capacity reached normal or thereby.	Average Disappearance of Oxygen Capacity in c.c. per Day.
<i>Brown and White</i> . .	6·5	14	0·465
<i>Rachel</i>	7·05	20	0·353
<i>Dr. Nicholson</i> . .	6·1	13	0·469

The irregularities of the curves do not allow of any exact analysis. It looks, however, as if the disappearance of the blood first transfused is not a regular process, but slow (or negative) at first and quicker towards the end. A period may in this way be roughly delimited during which the first transfusion is being got rid of quickly. Subsequent transfusions disappear much more quickly; of this the figures leave no doubt, though one can here, as in other parts of the curves, calculate various absolute rates. The next table gives a series of rates of disappearance of transfused hæmoglobin; figures bracketed together are alternative values.

TABLE XXII.—Rate of Disappearance of Transfused Hæmoglobin in C.c. Oxygen Capacity per Day.

	First Transfusion.		Second Transfusion.	Third Transfusion.
	Whole period.	Rapid part.		
<i>Brown and White</i> .	{ 0·65 0·45 }	{ 1·50 0·79 }	{ 1·52 3·65 }	2·15
<i>Rachel</i>	{ 0·33 0·29 }	{ 0·825 0·57 }	2·50	1·36
<i>Dr. Nicholson</i> . .	0·55	0·95	1·30	...

We have no very clear idea of what the expression “natural senescence” of red cells may mean. If, however, the disappearance of the injected red cells were due to this process alone one would expect that the amount disappearing per day would be approximately uniform from the time of injection till the normal level was reached again. Such does not, however, appear to be the case. We have no clear evidence that any red cells disappear during the days immediately following a first transfusion. The total oxygen capacity

remains at least at the level to which the transfused hæmoglobin has raised it, and it is only after a period of delay that the body, so to speak, sets to work to get rid of the excess. The difference in the rate of disappearance of transfusions subsequent to the first makes it clear that the fall in total oxygen capacity is not due to a simple stoppage of the formation of new red cells.¹ Whether the hæmoglobin which is then destroyed is that which has been injected or an equivalent quantity of the total mixed hæmoglobin in the body is not certain. And whether the destruction is due to the fact that the animal finds itself with too much hæmoglobin, or discovers that a batch of relatively foreign red cells is obnoxious to its economy, can only be investigated by indirect methods which are now engaging our attention.

Once the animal has had practice at getting rid of transfused red cells it can dispose of them much more quickly than at first. Our figures indicate that *the hæmoglobin injected at a second transfusion disappears two to eight times as fast as on the first occasion*. The practical application seems obvious, that transfusion, which seems to have fallen into unmerited disrepute as a therapeutic procedure, cannot be done more than once on the same subject with the object of increasing the circulating hæmoglobin. Subsequent transfusions disappear so quickly that they could not be of much value in helping to tide over a period of severe oxygen deficiency.

The surprisingly rapid disposal of transfused hæmoglobin by a trained animal raises several points of interest with regard to the mechanism involved, which will be dealt with subsequently. We may, however, state here that no auto- or iso-hæmolysins could be found in the blood sera of these animals or in those of several others prepared in a similar fashion.

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REFERENCES.

1. BOYCOTT AND DOUGLAS . . . *Guy's Hosp. Reps.*, London, 1908, vol. lxii. p. 162.
2. ITAMI *Folia hæmatologica*, Leipzig, 1908, vol. vi. p. 425.

¹ Itami has shown that repeated frequent transfusion for several weeks produces histological evidence that the activity of the bone-marrow in forming red cells is much diminished. Dr. Price Jones was good enough to examine by his method the bone-marrow of a rabbit which had received during fifty-one days 542 c.c. of blood containing 44 grms. hæmoglobin, of which 354 c.c. and 28·8 grms. hæmoglobin had been transfused during the nineteen days preceding death. He found that if anything the erythroblastic apparatus in the marrow was more active than usual. By coarser methods of examination we have failed to find anything clearly abnormal in the marrow of a number of rabbits subjected to repeated, though less extensive, transfusion.

3. MÜLLER *Arch. f. (Anat. u.) Physiol.*, Leipzig, 1901,
S. 459.
4. OERUM *Deutsches Arch. f. klin. Med.*, Leipzig, 1908,
Bd. xciii. S. 357.
5. PLESCH *Centralbl f. inner. Med.*, Leipzig, 1907, S.
533.
6. SMITH *Trans. Path. Soc. London*, 1900, vol. li. p.
311.