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VARIOUS CONSTITUENTS OF THE
BLOOD OF AN IMMUNISED HORSE
AFTER A LARGE BLEEDING

BY

R. A. O'BRIEN, M.D.

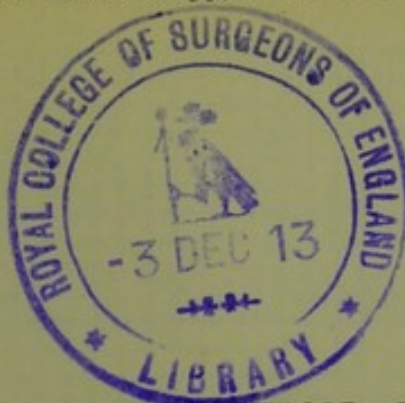
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From

THE WELLCOME PHYSIOLOGICAL RESEARCH LABORATORIES
BROCKWELL HALL
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LONDON, S.E.





THE RATE OF REPRODUCTION OF VARIOUS CONSTITUENTS OF THE BLOOD OF AN IMMUNISED HORSE AFTER A LARGE BLEEDING.¹

By R. A. O'BRIEN, M.D.

From the Wellcome Physiological Research Laboratories, London, S.E.

IN physiological literature there are many observations on the effects of bleeding on the various constituents of the blood, and in the literature of immunity there are many publications dealing with the effect of bleeding on the antibody titre in immunised animals. These observations have in large part been made upon goats, rabbits, etc., so that, having the opportunity, it seemed worth while to repeat the observations on horses, from which one can obtain much larger quantities of blood for determinations and so work with a smaller experimental error.

The results of observations on horses possess, moreover, great practical value, as the animals are utilised in the manufacture of sera for therapeutic purposes.

METHODS.

The specific gravity was obtained by weighing 10 c.c. of blood taken from the jugular vein immediately into a gravity bottle kept at room temperature, which varied during the experiments from 45° F. to 56° F.

The duplicate readings are given in the tables. In Figs. 1, 2, 3, 4 the last two figures are the basis on which percentages were estimated.

Red and white cells.—Quantities of 1 c.c. of blood were diluted and counted in a modified Bürker apparatus.

The volume of red cells.—After trials with the centrifuge the method adopted consisted in reading the height of the red cell column in a 50 c.c. tall graduated cylinder after standing forty-eight hours at room temperature, by which time the volume had become constant.

Hæmoglobin was estimated by the Haldane-Gowers' apparatus—human scale.

The above constituents were all estimated in duplicate or triplicate, as will be seen from the table,—the average experimental errors being, for specific gravity, 2 per cent.; red cells, 5 per cent.; white cells, 5 to 10 per cent.; hæmoglobin, 2 per cent.

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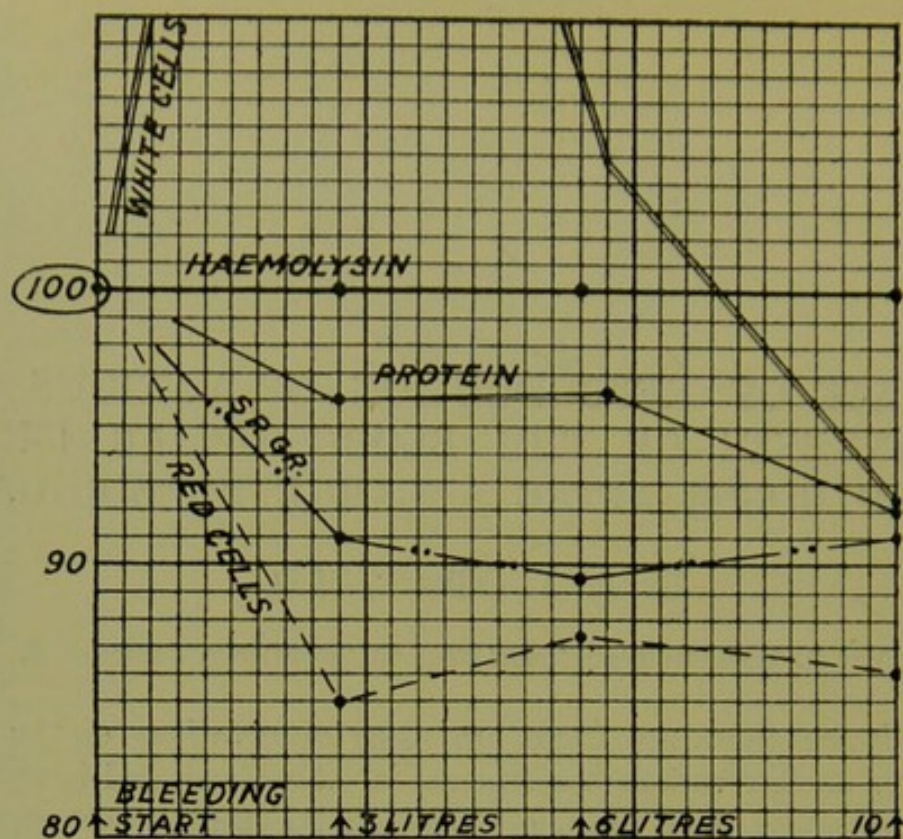


FIG. 1.—Variations in blood constituents of horse during 10-litre bleeding. Time taken, fifteen minutes. Horse 1.

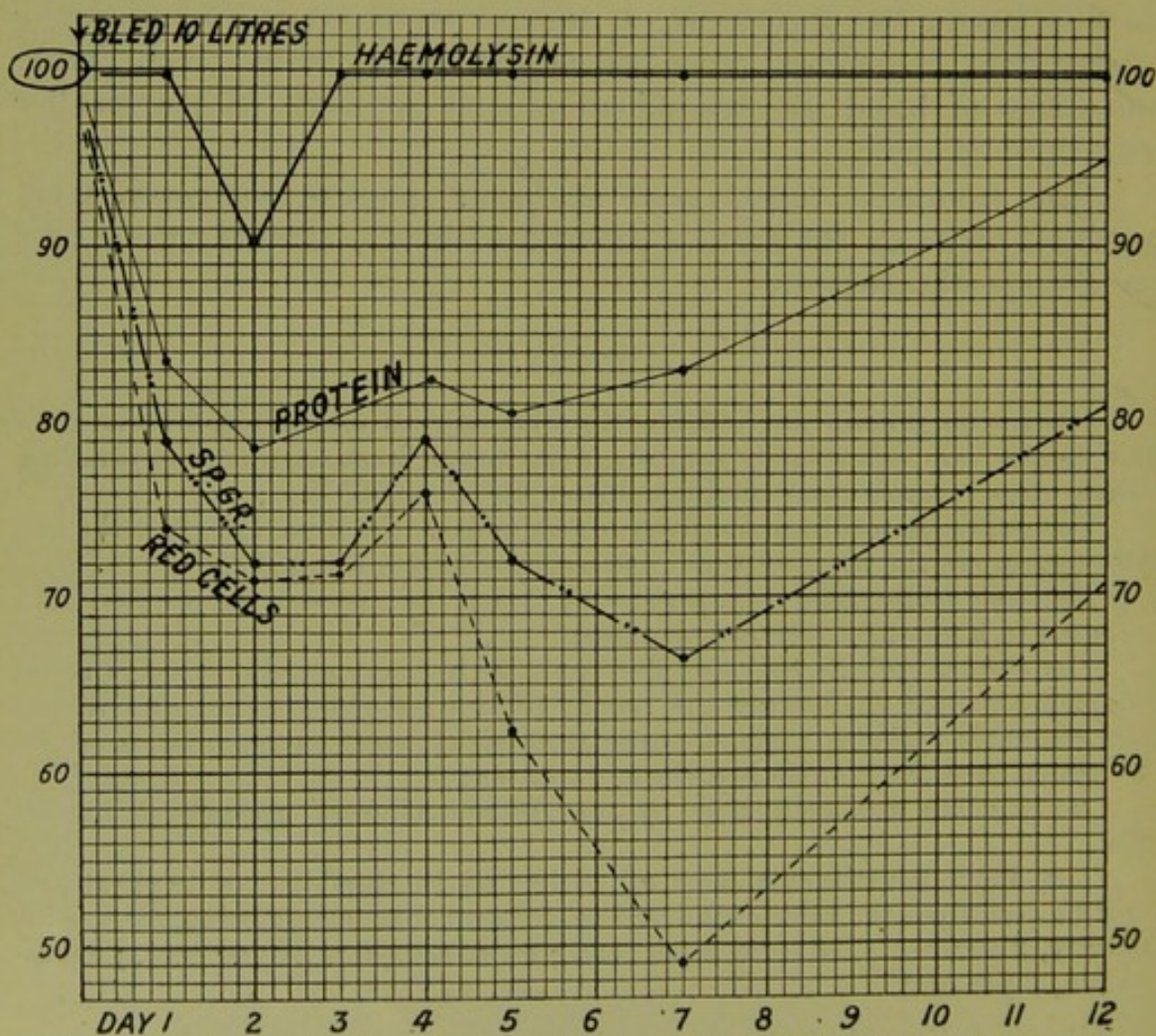


FIG. 2.—Effect of a 10-litre bleeding on blood constituents of Horse 1. Amounts of constituents expressed as percentage of amount present before bleeding.

The total proteins in serum were estimated daily by the refractometer. The refractometer readings were checked at various points by coagulating the total proteins and weighing the precipitates.

For estimating *hæmolytic titre*, 0.5 c.c. of 1 per cent. suspension of sheep's red cells containing 26,000 to 28,000 cells per c.c. was employed, 0.05 c.c. fresh guinea-pig serum as complement, and varying dilutions of the horse's serum were added, the whole being made up to 2.5 c.c. with saline solution and incubated for one hour in a water bath at 37° C.

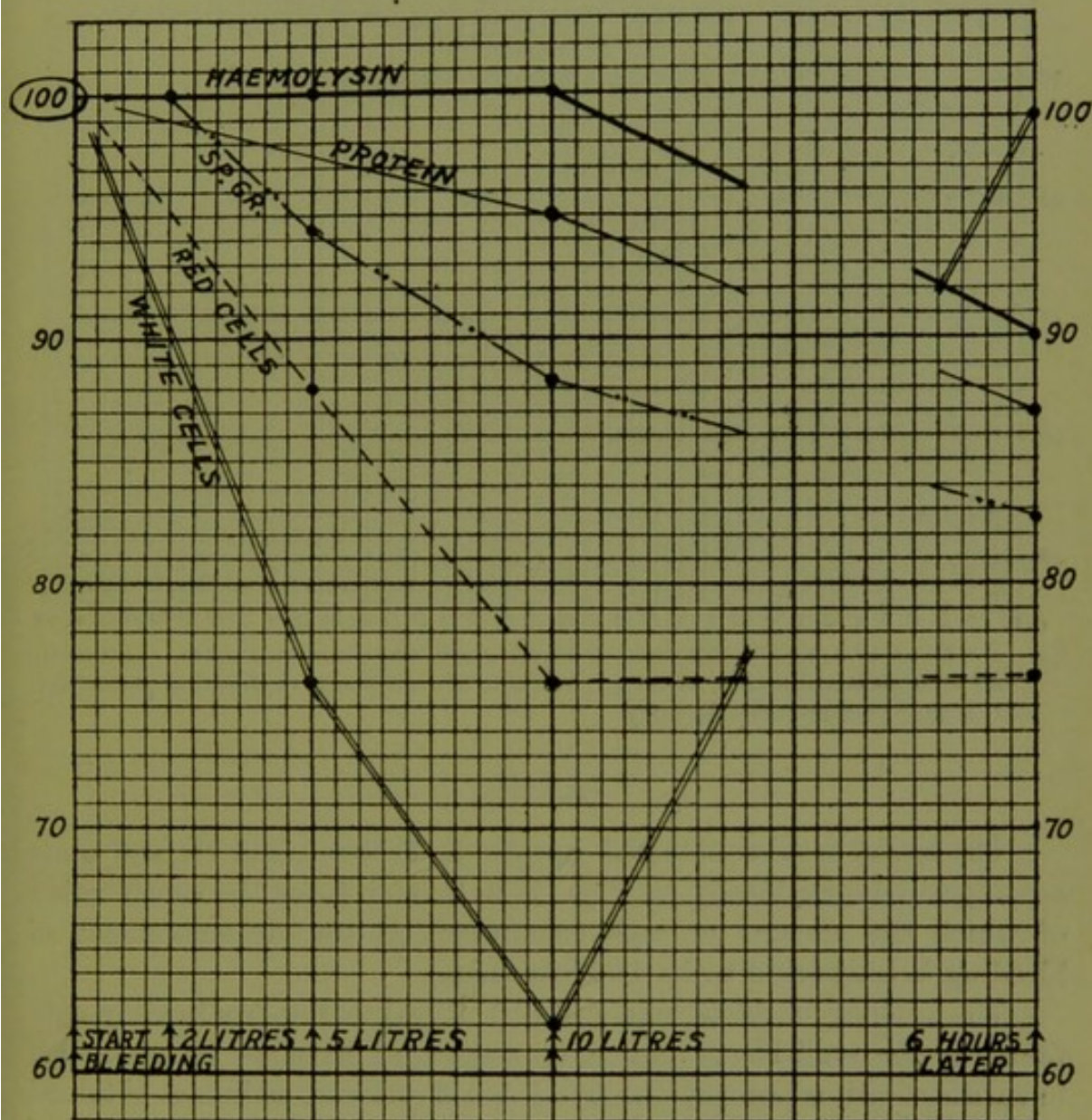


FIG. 3.—Variations of blood constituents of Horse 2 during a 10-litre bleeding, and six hours later.

The samples kept in the cold room were titrated in batches of three, and finally the whole series in one experiment, so that each sample has been estimated two, three, or more times.

Each tube in the series contained 10 per cent. less horse serum than the preceding. A "standard" old stable hæmolysin was used throughout, so that variations in complement on the various days were provided for, and each day's sample was read as a percentage of the stable hæmolysin.

No wide discrepancies in readings were noted on the various occasions. The quantities given in the tables represent the mean of two or three readings in each case.

The average error of any point on the curve is from 5 per cent. to 10 per cent. *Diphtheria antitoxin* was estimated in the usual way, the individual reading being correct to 5 per cent.

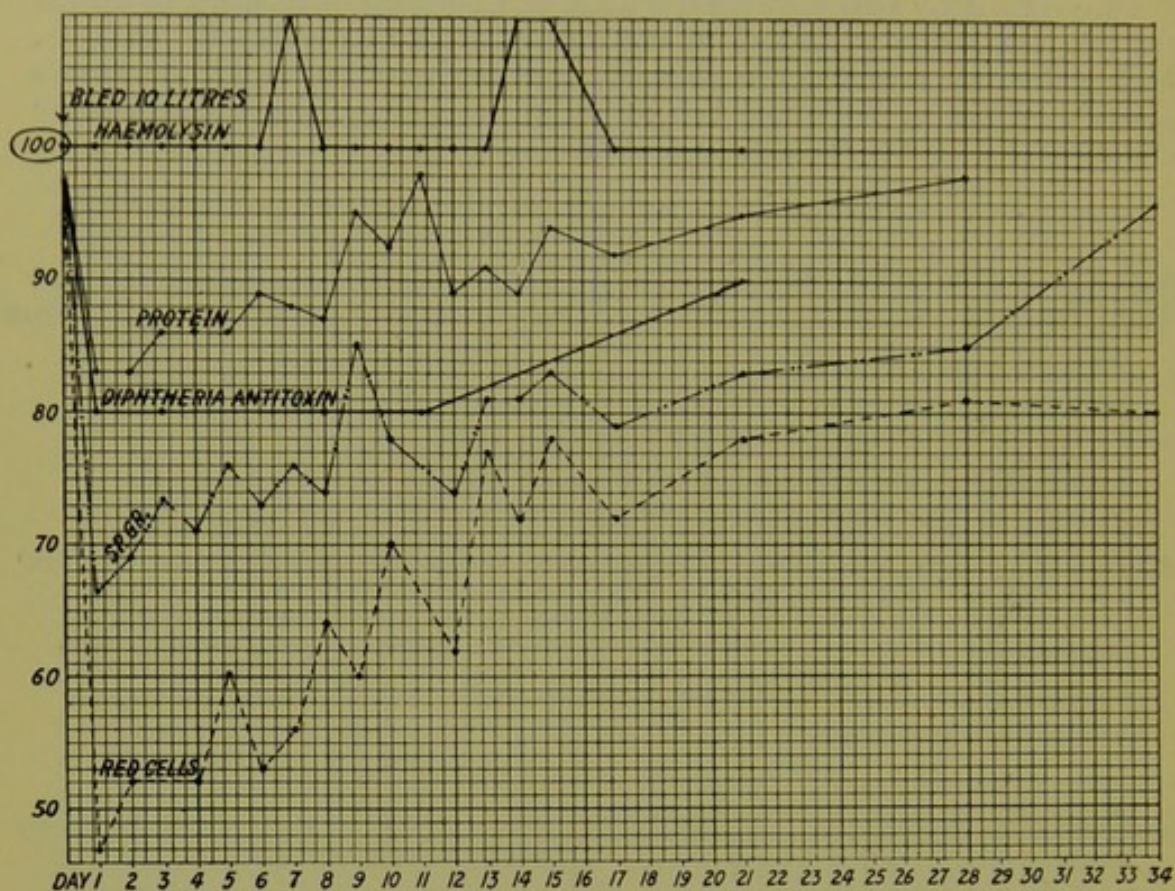


FIG. 4.—Effect of a 10-litre bleeding on blood constituents and antibodies,—haemolysin and diphtheria antitoxin. Horse 2. Amounts of constituents expressed as percentages of amount present before bleeding.

RESULTS.

The actual results obtained appear in Tables I. and II., and some are expressed graphically in Figs. 1, 2, 3, and 4, for the construction of which the amount of each constituent at the commencement of bleeding is considered to be 100 per cent.

The amount of blood removed (10 litres) was approximately one-fourth of the total blood volume, for although I have not yet succeeded in satisfactorily measuring the total blood volume, estimates obtained by bleeding out and by intravenous injection of known volumes of saline solution justify the assumption that the blood volume of horses of the same weight as those used is from 36 to 40 litres. The weight of Horse 1 was 680 kilos.

It is stated by Athanasiu (1909¹) that the blood volume is the first of the blood constituents to regain the normal figure. If this be so, one would expect, after the removal of 10 litres, *i.e.*, 25 per cent. of the total blood volume, that fluid would pour into the blood vessels until the volume was re-established, at which stage the blood constituents should be about 75 per cent. of the initial figure. Yet in the case of Horse 1 the hæmoglobin and red cells drop on the third day

to about 70 per cent. of the prehæmorrhagic level, and in Horse 2 to below 50 per cent. on the second day, the figure for the total proteins being respectively 73 per cent. and 83 per cent. Assuming that the blood volume returns to the initial figure in two days, the 50 per cent. fall in red cells due to the loss of 10 litres of blood in the case of Horse 2 would give by calculation 20 litres for the total blood volume.

TABLE I.—*Results of 10-litre Bleeding. One Year after Immunisation.*

Horse 1.

	Specific Gravity of Blood.	Red Cells, Millions per c.mm.	Hæmo-globin.	Volume of Red Cells per cent. of Total Blood.	Leuco-cytes per c.mm.	Total Protein per cent.	Hæmolytic Titre.
At start of bleeding	1057.7	8960 9200 8320 100	104 100	49 100	6050 6600 100	7.2 100	0.0022 100
After 3 litres	1052.6	7600 7500 85	96 92	43.5 88	9000 8900 142	6.92 96	0.0022 0.002 100
After 6 litres	1051.2	8160 7440 87.5	93 89	44 89.5	7000 6250 105	6.95 96.5	0.0022 100
After 10 litres	1052	7520 8080 86	90 86.5	44 89.5	5250 6400 92	6.65 92.5	0.002 0.0022 100
DAY 1 . .	1045.8 1045.5 79	6880 6560 74	73 76 71	76 67	7600 8800 124	5.9 83.3	0.0022 0.002 100
DAY 2 . .	1041.2 1042.1 72	6420 6080 71	64 66 62.5	30 61	8800 8100 133	5.65 78	0.0024 90
DAY 3 . .	1043.8 1041.6 72	6400 6240 71.5	76 80 75	35.5 72.5	6500 6500 100	5.8 90.5	0.0024 0.0022 100
DAY 4 . .	1046 1045 79	6960 6600 76	68 70 70 67.5	33 67	8700 7800 130	5.94 82.4	..
DAY 5 . .	1041.6 1041.6 72	5680 5540 62.5	72 71 68.2	31.8 31.3 64.8	7600 6200 110	5.8 80.5	0.0022 0.002 100
HORSE SUFFERING FROM MAMMITIS.							
DAY 7 . .	1038.4 1038.4 66.5	4240 4400 49	54 52 51	27 55	9300 8800 144	5.98 83	0.0022 0.002 100
DAY 12 . .	1047.9 1045.8 80.5	6320 6160 70.5	74 77 72	34.5 71	8600 8500 74	6.85 95	0.0022 100
DAY 15 . .	1055 1053 96.5	8160 8080 92	93 95 90	44 89.5	6000 6200 100	6.85 95	0.0022 100
DAY 47 . .	1050 1048 86	8360 8240 95	85 87 87 83.5	..	3750 4000 61	..	0.0022 100

The figures in heavy black represent percentages of the quantity of the particular constituent present at the commencement of the experiment.

TABLE II.—*Effects of 10-litre Bleeding. One Year after Immunisation.*
Horse 2.

	Specific Gravity of Blood.	Red Cells, Millions per c.mm.	Hæmoglobin.	Volume of Red Cells per cent. of Total Blood.	Leucocytes per c.mm.	Total Protein in Serum.	Diphtheria Anti-toxin Units per c.c. of Serum.	Leucocytes.					Hemolytic Titre.
								Total Counted.	Polymorpho-nuclear Cells.	Mono-nuclear Cells.	Eosino-phil Cells.	Mast Cells.	
At start of bleeding .	..	9520 9320 100	..	41.6 100	9500 9600 100	6.82 100	50 100	367	81.5	12.7	5.4	1	0.0081 0.0078 0.006 0.008 0.0097 100 0.0087 0.0097 100 ..
After 2 litres .	1055 100	..	104 100	..	..	..	..	..	..	..	..	..	..
After 5 litres .	1052 94.2	8320 8240 88	96 92	..	7300 7300 76	..	..	..	..	..	..	..	..
After 10 litres .	1049 1048 88.2	7280 7200 7320 7040 76	83 84 80	42 101	5600 5800 62	6.52 95	..	346	85	7	5.8	1	0.0078 0.0088 0.006 100
Six hours later .	45 46 82.8	7200 7200 6960 76	72 74 71	37.2 89	9200 9600 99	5.94 87	..	353	85	10	4.5	1	0.0078 100
Day 1 (24 hours later)	36 37 66.3	4720 4400 47	53 50 50	24 58	7200 6600 72	5.65 83	40 80	378	79	19	2	..	0.0081 100
Day 2 .	38 38 69	4720 5220 52	54 56 56	27 65	6600 7600 74	5.7 83	..	395	76	22	2	..	0.0081 0.013 (?) 100
Day 3 .	40 41 73.6	4800 4772 5400 52	60 60 58	27 65	9300 9560 99	5.94 86	40 80	372	80.5	18	1.5	1	0.0097 0.01 0.009 100 0.0081 0.013 (?) 100
Day 4 .	39 38 70.7	4840 4960 52	50 52 49	25 60	7600 7600 80	5.9 86	..	275	76	21.4	5	..	0.0081 0.013 (?) 100

DAY 5	41	5900	60	31	8300	5.87	..	..	..	..	0.0097 0.01 0.0081 100
	42	5440	58	76	7900	86	..	..	..	..	0.0097 0.0097 100
	76	5640	57		86		..	..	2.7	..	0.0081 100
DAY 6	40	5250	54	28	9400	6.12	..	80	17.3	..	0.0097 0.0097 100
	40	4800	56	68	98	89	..	..	..	..	0.0081 100
DAY 7	73	53	53	32	7800	6.09	..	75.5	22.3	2	0.0081 100
	42	5400	64	77	8500	88	..	..	..	..	0.0081 110
	43	5320	66		88		..	..	..	..	0.0097 100
DAY 8	76	56	62	28	8300	6	40	77	20.3	2.3	0.0097 100
	41	6400	62	68	7500	87	80	..	..	..	0.0097 100
	74	6160	63		83		..	..	..	..	0.0097 100
DAY 9	47	5680	70	31	7800	6.52	..	76.3	20.1	3.3	0.0097 100
	46	5720	72	74	9500	95	..	..	..	..	0.0097 100
DAY 10	85	60	68	29	82	6.3	..	75.5	20.3	4	0.0097 100
	43	6400	69	69	8500	92.5	..	..	..	..	0.0097 100
	43	6950	68		89		..	..	..	..	0.0097 100
	45	70	65				..	..	..	..	0.0097 100
DAY 11	78	..	80	36	8000	6.72	40	74.2	22.7	3	0.0097 100
	42		82	86	8100	98	80	..	..	..	0.0097 100
	43		79		88		..	..	..	..	0.0097 100
DAY 12	76	4960	62	28.5	5800	6.08	..	76.5	21.2	2.3	0.0097 100
	41	5250	59	27.5	6200	89	..	..	..	..	0.0081 100
	74	62		67	63	6.25	..	75	21.8	3.2	0.0097 100
DAY 13	46	6000	71	33	9300	91	..	..	..	..	0.0081 110
	44	6640	72	32	97		..	..	..	..	0.0081 110
	81	77	69	78		6.12	..	79.5	18	2.4	0.0073 100
DAY 14	44	5840	76	29	8300	89	..	..	..	..	0.0073 100
	45	6000	72	71	8700		..	..	..	..	0.0081 110
DAY 15	81	72	70	38.5	89	6.45	..	..	..	..	0.0081 110
	46	6400	76	92	87	94	..	..	..	..	0.0081 100
	46	78	74				..	..	..	..	0.009 0.0073 100
DAY 17	83	5840	65	30.5	9800	6.32	..	..	..	..	0.009 0.0073 100
	44	6000	67	73	9300	92	..	..	..	..	0.009 0.0073 100
DAY 21	79	72	64	31.5	100	6.5	45	71.5	23	..	0.009 0.0073 100
	45	6400	74	34	1040	95	90	..	..	..	0.009 0.0073 100
	47	6480	76		1030		..	..	..	..	0.009 0.0073 100
DAY 28	83	78	72	80	109	6.69	..	..	..	5	0.009 0.0073 100
	48	6480	81	36.8	7800	98	..	..	..	..	0.009 0.0073 100
	46	6950	85	37	7700		..	..	..	..	0.009 0.0073 100
DAY 34	85	81	80	89	81	..	..	78.5	16	5.5	0.009 0.0073 100
	54	7360	92	37	1050		..	..	..	..	0.009 0.0073 100
	52	7840	94	36.8	9800		..	..	..	..	0.009 0.0073 100
	96	80	89	89	106		..	..	..	..	0.009 0.0073 100

The figures in heavy black represent percentages of the quantity of the particular constituent present at the commencement of the experiment.

We know from bleeding out experiments on many horses that the total blood volume is much more than 20 litres, therefore there must be either a hyperhydræmia or a destruction of red cells in the body.

I did not observe hæmoglobinæmia at any time, nor can I find any records by other workers of hæmoglobinæmia or hæmoglobinuria following bleeding, so that we are justified in discarding the hypothesis of destruction of red cells. An actual increase in total blood volume similar to that found by Boycott (1909²) for a week after a large bleeding in a rabbit seems to occur.

On the other hand, the protein content in both horses fell much less than the red cell or hæmoglobin percentage, and less than one would expect it to fall after a 10-litre bleeding if the total blood volume returned to normal in two days. The inference follows that the fluid first sucked from the tissues into the blood stream after a bleeding contains considerably more than the normal amount of protein. In considering these points, it must be remembered that both horses had a history of large bleedings, and may have developed a mechanism differing somewhat from that of normal animals.

HÆMOGLOBIN.—*Number and size of red cells.*—The percentages representing number of red cells and of hæmoglobin almost coincide throughout, whence it appears that the newly produced red cells contain on the average the normal amount of hæmoglobin. The figures representing volume of red cells do not always agree closely with those of red cells and hæmoglobin. It seems that the average volume of the newly produced red cells may vary somewhat from the prehæmorrhagic level.

The curve of *specific gravity* runs parallel to that of the red cells. This is to be expected, for, of the other two constituents affecting it, the *salts* do not vary much, and the total protein weighs much less than the red cells.

The *salts* in the case of Horse 1 were estimated cryoscopically, by conductivity, and by direct titration of free chlorides. The amount of total salts does not decrease, but is actually above the prehæmorrhagic figure (as was found by Loeper, 1903³), so that the fluid taken up by the blood after the bleeding contains at least the same amount of total salts as does ordinary serum.

The *total protein* figures are considered under "Total blood volume."

The *white cells* content showed considerable irregularities,—one horse manifesting great leucocytosis during bleeding, the other considerable leucopenia, the curves of both horses showing the widest irregularities during the recovery period. The samples could not be taken at the same hour every day, the variations being between 11 a.m. and 5 p.m., but the wide fluctuations on the curves do not show any definite relationship to the times at which the samples were taken.

The *differential counts* show remarkably little variation, the discrepancies being very little outside the experimental error.

ANTIBODIES.—A comparison of the curves of total protein, hæmolysin, and diphtheria antitoxin furnishes the most interesting of the results. Of the various titrations of hæmolysin there is scarcely a reading more than 10 per cent. (*i.e.*, *one test tube* in the series) on either side of the figure representing the hæmolytic titre before the bleeding. After making all allowances for experimental error, it is certain that the curve of hæmolytic titre does not run parallel to that of total protein, and that the titre remains almost constant throughout, notwithstanding fluctuations in total blood volume. This result holds good in both horses, and is therefore probably more than a coincidence.

The curve of diphtheria antitoxin titre, on the contrary, runs nearer to that of the total protein curve throughout in Horse 2. The obvious inference is that the protein first reproduced after the bleeding contains more hæmolysin than existed in the serum protein before the bleeding, and more than in that produced later, but a proportion of diphtheria antitoxin not widely different from that in the serum protein present before the bleeding, and that the various "protein fractions" containing different amounts of various antibodies are produced at different rates. The bearing of this latter suggestion is to be the subject of a further research.

SUMMARY OF RESULTS.

After taking 10 litres of blood from an immunised horse the following results occur:—

1. The number of white cells varies widely and irregularly. Very small differences are found in the differential count.
2. The hæmolytic titre remains practically constant, showing variations of at most 10 per cent. from the initial figure.
3. The total amount of salts present does not decrease, but may be increased 10 per cent.
4. The content of all other blood constituents falls, the hæmoglobin and number of red cells falling together to 50 per cent. or 60 per cent. of the initial figure. The colour index of the new red cells remains at the normal figure. The curve of volume of the red cells does not correspond very closely with that of the number of red cells.
5. The curves of total proteins and of diphtheria antitoxin show a fairly close relationship.

CONCLUSIONS.

After the withdrawal of 10 litres of blood from an immunised horse, the fluid first appearing in the blood vascular system contains an amount of protein far above the normal, hæmolysin and diphtheria

antitoxin are reproduced at different rates, and the rate of reproduction of the various proteins in the blood is probably associated therewith.

I have to thank Dr. Walpole for the whole of the cryoscopic, conductivity and protein determinations.

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2. BOYCOTT AND DOUGLAS . . *Journ. Path. and Bacteriol.*, Cambridge, 1909, vol. xiii. p. 271.
3. LOEPER Cited by Athanasiu, p. 490 (*vide supra*).