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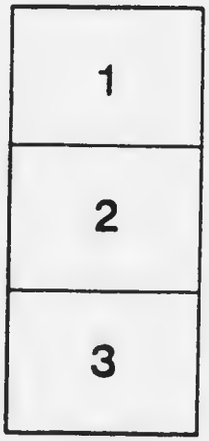

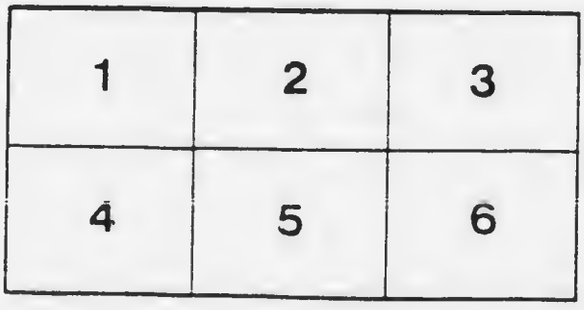

PLASMA REMOVAL WITH RETURN OF CORPUSCLES
(PLASMAPHAERESIS)

FIRST PAPER

BY

JOHN J. ABEL, L. G. ROWNTREE AND B. B. TURNER

From the Pharmacological Laboratory of the Johns Hopkins University

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I. In connection with our experiments on vividiffusion[1] with a view to the ultimate use of the method for the relief of toxaemia the idea suggested itself to try the effects of the repeated removal of considerable quantities of blood, replacing the plasma by Locke's solution and reinjecting this together with the sedimented corpuscles.

While this work was in progress our attention was called to an article in a recent number of Russki Vrateh (No. 14, pp. 637–639, St. Petersburg, May 16, 1914); by V. A. Yureviteh and N. K. Rosenberg, entitled: Washing the Blood Outside the Organism and the Survival of the Red Corpuscles, in which experiments similar in general outline to our own are reported. The authors worked on rabbits, using sodium citrate to obviate clotting. Only about 50 per cent of the blood volume was withdrawn (carotid) and the washed corpuscles reinjected. In two experiments a second amount of blood, about half as great as the first was withdrawn to show by the survival of the animal that the corpuscles reinjected were physiologically active.

The fact that washed corpuscles obtained from one animal can be introduced into another animal of the same species (dogs) and function naturally for a number of days at least, also follows from the experiments made by P. Morawitz in the course of his studies on the restoration of the proteids of the blood, although no blood counts are given (Beitr. z. ehem. Physiol u. Pathol., vii, 150, 1906).

Boruttau Nagel s, Hd'b. der Physiol. des Mensch. Erganzungsband, p. 32, 1910) states that the centrifuged corpuscles of defibrinated

[1] This Journal, v, p. 275, 1914, and the preceding paper.

mammalian blood may be reinjected and function normally after having been kept on ice for four or five days.

The question whether the red blood corpuscles of one animal can exist permanently in the blood of another animal of the same species appears to require further consideration. According to Todd and White (Proc. Roy. Soc., Ser. B, vol. 84, p. 255, 1912) in this case, the injected corpuscles are treated by their host as foreign, and in fact act as antigens.

If this method can be employed without harmful consequences it is probable that it could be applied in a bolder manner and in a greater variety of morbid states than the time honored but often debatable venesection of medical practice. An empirical method so universally practiced since prehippocratic days almost certainly contains a basis of truth. The decline in its use was brought about more by the ill effects of the anaemia consequent on its abuse, and by its natural limitations, than by any disproof of its intrinsic merits. As revived again in our own time and used with discretion, "bloodletting not only relieves symptoms but may save the patient's life as in engorged conditions of the right side of the heart, whether due to mitral incompetence or pulmonary affections."[2]

In view of the well known and appalling excess to which blood letting was at one time carried, as may be read (to give but one example) in Marshall Hall's "Observations" we would have it distinctly understood that the suggestions here made are given with all reserve subject to further experiments.

The experience of the past has shown that it is possible only in the case of vigorous adults to remove at one time more than one third of the blood without subsequent danger, while children, the aged, the obese and convalescents do not tolerate the loss of this quantity.[3] Venesection practiced at intervals of two or more days permits of the withdrawal of a considerable amount of blood though this method also is not without its dangers.[4]

[2] Sir Lauder Brunton: Therapeutics of the Circulation. London, 1908, p. 172.

[3] Hermann's Handb. der Physiol 1880, vol. iv, p. 139.

[4] For instances of the withdrawal of large amounts of blood at varying intervals see Wagner's Handwörterbuch der Physiologie, vol. i, p. 207, 1842. The statement here made that Piorry abstracted an amount of blood from a young dog almost

The drawbacks of venesection are dependent on the immediate (syncope) or subsequent effects of the anaemia. The removal of so large a number of cells (40 per cent of the blood volume) entails a prolonged effort on the part of the haemapoietic organs for their regeneration. This is naturally a slow process, but the organism is generally in a position to furnish rapidly the liquid medium of the blood either from stores of reserve material or by synthesis from substances absorbed from the digestive tract.

If the immediate, or more especially, the later effects of the anemia following venesection can be obviated by the speedy return of the corpuscular elements of the blood, the value of this therapeutic procedure would be greatly enhanced and its field of application extended. The possibility of increasing or decreasing (if only temporarily), the volume of fluid in the vascular apparatus, or of removing an excess of deleterious substances with the plasma opens the way for the use of a method of this kind in cases where bleeding has hitherto been contraindicated because of the danger of reducing the oxygen carrying capacity of the blood, as for example, in aneurysma, in cardiac decompensation with a low blood count and in various toxaemias. But especially would a method of this kind appear to be advantageous for the patient in most of those instances in which venesection is now being done admittedly with good results.

Other applications of such a procedure also suggest themselves. In view of the fact[5] that mammalian corpuscles retain their stability for three or four days when kept on ice a supply of human corpuscles might possibly be kept in this manner in operating rooms for rapid injection in emergencies that would otherwise prove fatal.

In the preparation of antitoxic sera, great economy might be effected in the use of horses if the method were found to be applic-

equal to half its weight in five days is manifestly a misquotation. Piorry himself states that he withdrew 2 pounds of blood in fifteen days from a young dog weighing 10 pounds. See Arch. gén de Méd., vol 10, p. 139, 1826. See also Marshall Hall: Observations on Blood-Letting. London, 1836, and Piorry, Procédé Opératoire, etc., et Collection de Mémoires. Paris et Londres, 1831, pp. 208–291.

[5] Nagel's Hdb. der Physiol. des Menschen, Ergänzungsband, p. 32.

able, since, instead of bleeding about five to eight litres every two weeks, probably fifteen or twenty litres might be taken in the same or even at shorter intervals if the animal did not have to regenerate any morphological elements. Other applications, which will not be discussed at present suggest themselves.

It is shown by the following experiments that it is possible to withdraw by repeated bleedings, in a single day, a volume of blood more than twice that ordinarily contained in the body with no apparent injury to the animal if only the corpuscles suspended in Locke's solution be returned after each bleeding. On the day following the animal's condition is quite normal and we have not hesitated to continue the process on the second and the third day. Owing to technical difficulties, which we hope to conquer, the limits of the method have not yet been reached.

II. METHODS

Two different methods were used in bleeding, one by inserting a hollow needle into a vein, the other by cutting down on a vein and tying in cannulae.

In the former method the bleeding apparatus consists of a large test tube or flask of at least 200 cc. capacity fitted with a rubber stopper carrying two short bent glass tubes. One of these serves for suction while the other is attached by a short rubber tube to a No. 14 needle, 2 inches long and 0.064 inch diameter. The inner surface of the needle and rubber tube is coated with a thin layer of soft vaseline while the glass inlet and collecting tubes are coated with paraffin. Sufficient hirudin is placed in each tube at the start, to prevent for two or three hours the coagulation of the amount of blood to be drawn.

After shaving the skin, and washing with alcohol or tincture of iodine, the vein, preferably the external jugular, or one of the prominent leg veins, is dilated by applying pressure with the finger between the site of operation and the heart, to facilitate the introduction of the needle. This procedure is also maintained throughout the operation to increase the flow of blood. The needle is inserted and suction applied. The bleeding at a rate not exceeding about 20 cc. a minute, is continued till the color of the mucous membranes, and the state of the heart or respiration indicate the approach of the limit of safety.

The repeated introduction of the needle inevitably results in injury to

the walls of the vessel and in haemorrhage into or infiltration of the perivascular tissues as well as in setting free thromboplastic substances, all of which make the procedure more difficult each time.

The use of cannulae necessitates one or two small incisions over a superficial vein and isolation of the vessel over a length of at least 2 cm. The cannulae are introduced in the usual way, one directed peripherally for extraction of blood and the other centrally for reinjection. Both are guarded by small bull dog clamps which do not exert sufficient pressure to injure the intima.

In this method we injected into the animal intravenously sufficient hirudin to decrease materially the coagulability of the blood, but with due care to avoid injury to the vessel walls it is probable that this might be dispensed with. Blood is collected in the suction apparatus already described, containing at the commencement an appropriate amount of hirudin. After each bleeding the vein is clamped and both cannula and vein are washed free of blood and filled with hirudin solution. By plugging the end of the cannula temporarily and freeing the clamp, a little hirudin may with advantage be forced back into the vein.

For the separation of plasma, the blood collected is diluted with an equal volume of Locke's solution containing 0.85 % NaCl and the mixture centrifugalized for 15 minutes at 3000 revolutions per minute. A suction pipette, on the familiar wash bottle plan, is used to draw off the supernatant plasma. With practice and care a very close separation can be effected removing from 80% to 85% of the volume of the diluted blood without removing more than a small proportion of the white corpuscles which form a fairly coherent skin-like layer on top of the erythrocytes. The blood platelets being smaller and slower settling may well have been removed to a great extent in our experiments. The process of dilution and centrifugalizing may of course be repeated if desired, but it was not considered desirable, in view of the time consumed and consequent reduction in amount of blood extracted. For reinjection the corpuscles are mixed with a Locke's solution containing only 0.6% NaCl, using sufficient to restore approximately the original volume of the blood and pouring back and forth from tube to tube to produce a well mixed emulsion of the corpuscles. The smaller proportion of salt was used to avoid introducing excessive amounts of this substance; where this precaution was not observed the animals showed great thirst.

This procedure, bleeding, centrifugalizing and reinjection of corpuscles was repeated as often as practicable (4 to 8 times) during the day, the individual bleedings varying from 2 to 3.5 per cent of the animal's body

weight. Bleeding and injection were always done slowly about fifteen minutes being required for each manipulation.

Bleeding through a needle is satisfactory only for the removal of moderate quantities of blood. The process is likely to be interrupted by the formation of clots. Nevertheless we have drawn from the veins in this way a volume of blood equal to three times the normal blood volume. The needle method involves the use of several of the larger veins where much blood is drawn and on the third day it will be found difficult to introduce the needle or to secure a free flow of blood from veins that have been repeatedly pricked. In several cases we were obliged to discontinue work on dogs on the third day on account of the difficulty of inserting the hollow needle.

Cannulae tied into a vein allow of more rapid and effective bleeding and one vein may usually serve for a whole day, the cannula being left in and the wound properly bandaged in the intervals between bleedings and injections.

III. PROTOCOLS

The following two protocols are good examples of the experiments carried out.

Experiment 8. Young excitable dog, age about 6 months. Weight 9.6 kg.

June 29, 1914. 1.30 p.m. Drew by needle method 200 cc. blood, diluted to 400 cc. with Locke's solution, centrifugalized 15 mins., separated plasma and reinjected corpuscles made up to 175 cc. at 2.15 p.m.

2.30. Drew 237 cc.

2.35. Dog drank ½ pt. milk eagerly.

3.20. Reinjected corpuscles made up to 250 cc.

3.35. Drew 230cc. Dog rather fatigued, but still walks about voluntarily. Passes stools.

4.35. Reinjected corpuscles made up to 280 cc.

Dog somewhat depressed immediately after operation, vomits milk. Pulse at 5 p.m., 180. Recovers after a short time and eats meat and drinks milk.

Total amount drawn in afternoon 667 cc. Returned 705 cc.

Volume of blood in dog's body estimated at 7.5 per cent of body weight = 720 cc.

Plasma separated 1200 cc. from 1460 cc. diluted blood.

June 30. Dog is very lively and excitable again.

Has eaten meat this morning.

11 a.m. Drew 250 cc. blood, diluted to 500 cc. Separated 430 cc. after centrifugalizing. Plasma very milky.

11.55. Reinjected corpuscles made up to 220 cc.

12.15. Drew 260 cc. blood. Plasma still milky.

12.35. Pulse 136. Dog is quiet, lies down.

Reinjected 240 cc. about 12.45.

2 p.m. Drew 245 cc. blood. Plasma clearer.

2.25. Dog subdued. Pulse 165 regular, low tension.

3 p.m. Reinjected corpuscles made up to 280 cc.

3.50. Pulse 160.

4.10. Drew off 220 cc. Plasma nearly clear. Dog is in good condition, not depressed.

4.30. Reinjected corpuscles made up to 260 cc.

Dog in good condition: eats heartily.

Total for day: blood drawn 975 cc., corpuscle mixture returned 1000 cc.

July 1. Dog is in fine condition, has eaten well.

10.45. Drew off 180 cc. blood. Diluted to 380 cc. Separated 320 cc. Plasma moderately milky.

11.30. Reinjected 180 cc. corpuscle mixture.

12.15. Drew off 215 cc. blood.

1.30. Reinjected corpuscles diluted to 180 cc.

Stopped on account of difficulty with veins. No signs of haemolysis.

Total for day: blood drawn 395 cc: corpuscle mixture returned 360 cc.

Total for whole experiment (parts of 3 days). Blood drawn 2037 cc. Returned 2065 cc.

Experiment II. Small full grown female dog: weight 8 kg.

July 6th. Prepared vein in leg and inserted Bernheim cannulae under ether anesthesia.

12.15. Drew off 135 cc. blood. Took 20 cc. for analysis. Diluted remainder to 260 cc. Separated 215 cc. plasma.

1.55. Reinjected corpuscles diluted to 150 cc.

2.10. Drew off 183 cc. blood.

3 p.m. Reinjected corpuscles made up to 190 cc.

3.25. Drew off 165 cc. blood.

4.45. Reinjected corpuscles diluted to 175 cc.

5 p.m. Drew off 40 cc.

5.35. Reinjected 70 cc. Wound sewed up with benzoylperoxide. Dog is very well and lively, runs about, eats meat.

Total for day. Blood drawn 523 cc. Reinjected 585 cc.

July 7th. 9.30 a.m. Cannulae inserted under ether into external jugular vein.

10.40 a.m. Drew off 227 cc. blood.
11.15. Reinjected corpuscles made up to 255 cc.
11.40. Drew off 220 cc. blood.
12.40. Reinjected corpuscles diluted to 245 cc.
1 p.m. Drew off 205 cc.
2 p.m. Reinjected corpuscles diluted to 230 cc.
2.25. Drew off 205 cc.
3.15. Reinjected corpuscles diluted to 245 cc.

Stopped work as dog was weak with very rapid feeble pulse owing to accidental introduction of considerable amount of thymol with the hirudin used. Wound sewn up as before.

Dog off table at 3.40, seems better, stands up, drinks.

About 5.30 dog is in fine condition again, anxious to fight dog in adjoining cage.

Total for day. Blood drawn 857 cc. Returned 975 cc.

July 8. Dog in fine condition. Weighs 8.05 kg.

1st bleeding using needle method. Drew off 245 cc., after separation made corpuscles up to 260 cc.

12.40. Reinjected 240 cc. of above mixture.
2 p.m. Drew off 210 cc. blood.
3 p.m. Reinjected corpuscles made up to 220 cc. and 50 cc. left from last injection.

Stopped on account of difficulty in drawing blood. Dog is very well and cheerful.

Total for day. Blood drawn, 455 cc. Returned 480 cc.

Total for experiment (parts of 3 days). Blood drawn 1835 cc. Returned 2040 cc.

Blood volume (estimated) 600 cc.

The following is a more condensed account of our other experiments.

Experiment 1. May 21, 1914. Normal dog weight 11 kg.
3 to 3.30 p.m. Blood drawn 270 cc. Returned 160 cc.
3.45 to 4.20 p.m. Blood drawn 200 cc. Returned 160 cc.
4.45 to 5.05 p.m. Blood drawn 100 cc. Returned 270 cc.
Total for day: Blood drawn 570 cc. Returned 590 cc.
May 22. 2.50 to 3.05 p.m. Blooddrawn 262 cc. Returned 190 cc.
Total for experiment: Blood drawn 832 cc. Returned 780 cc.

Note. This dog was in good condition a month later and was used for the nephrectomy experiment described on page 636.

Experiment 9. July 1, 1914. Brown dog about 1 year old. Weight 7.75 kg.

2.15 to 3.50. Blood drawn 232 cc. Returned 250 cc.

4 p.m. to 5 p.m. Blood drawn 270 cc. Returned 310 cc.

Totals. Blood drawn 502 cc. Returned 560 cc.

Note. Discontinued experiment on account of smallness of dog's veins and difficulty in bleeding.

Experiment 10. July 2, 1914. Mature dog about 2 years old. Weight 12.8 kg. 10.50 to 12 noon. Blood drawn 345 cc. Returned 360 cc.

Clots due to weak hirudin caused loss of 50 to 60 cc. blood. 12.45 to 1.40. Blood drawn 392 cc. Returned 395 cc.

1.45 to 3.15. Blood drawn 370 cc. Returned 370 cc.

About 25 cc. more blood lost in clots.

3.30 to 4.20. Blood drawn 170 cc. Returned 265 cc.

4.30 to 6 p.m. Blood drawn 400 cc. Returned 460 cc.

Total for day. Blood drawn 1677 cc. Returned 1850 cc.

Dog in good condition, eats meat, drinks.

July 3, 10 a.m. to 11.25. Blood drawn 264 cc. Returned 270 cc.

1.15 to 2.45. Blood drawn 105 cc. Returned 150 cc.

Total for day. Blood drawn, 369 cc. Returned 420 cc.

Total for experiment. Blood drawn, 2046 cc. Returned 2270 cc.

Experiment was discontinued on account of difficulty in getting into veins. Some haemolysis was observed due to a mistake in using hirudin solution without salt, but this disappeared rapidly. Dog in good condition at end of experiment, but less lively for several days. Lost 1 kg. by July 8 but regained this by July 10.

IV. Plasmaphaeresis after bilateral nephrectomy.

Several experiments were carried out to ascertain the effects of plasmaphaeresis on nephrectomized animals. It is stated by Ascoli, Vitzou, Strubell and others[6] that after this operation dogs usually live from about 48 to 72 hours which is supported by our control experiments (see experiments Nos. 3 and 5 below). A longer life was observed in some cases by Jackson and Saiki.[7] Possibly the difference is due to the low nitrogen diet given in the latter case some days before the operation. Our results except in one

[6] Ascoli: Vorlesungen über Urämie. Jena, 1903. Vitzou: Journ. de Physiol et Pathol. générale, iii, 901, 926, 1901. Strubell: Wien. klin. Wochenschr., vol. 14, p. 689. 1901. Elmendorf: Biochem. Ztschr. vol. 60, p. 438, 1914.

[7] Archives of Int. Med., vol. 9, p. 79, 1912.

case (experiment 6) show a prolongation of life, compared to the controls, of 1 to 1½ days and we have every reason to believe that it would have been greater had it not been for various accidents which were immediately followed by death in each case. Thus, two dogs were overbled and died before the corpuscles could be reinjected, a third died from a profuse haemorrhage into the peritoneal cavity (500 cc.) at a time when his clinical condition was apparently satisfactory, while a fourth reacted in an abnormal manner to an injection of pituitrin, dying immediately upon the injection intramuscularly of 2 cc. of the commercial solution.

The clinical improvement in the animals after the treatment was always very marked. While therefore we do not claim that these results, which we regard as merely preliminary, are as conclusive as might be desired, they give encouraging indications of the advisability of continuing such experiments with a view to the possible use of plasmaphaeresis in the relief of toxaemic conditions. Doubly nephrectomized animals of course constitute a very severe test for a therapeutic procedure, intended only for the relief of a temporary impairment or suppression of urinary function. The fact that the animals do not eat is itself a handicap, as starvation decreases the rate of blood regeneration (Piorry).

Protocols of experiments on nephrectomized dogs.

Experiment 2. Bilateral nephrectomy. Dog. Weight 13½ kg.

June 2, 1914. Operation performed about 3 p.m.

June 5, 4 p.m. to 5 p.m. Blood drawn 415 cc. Returned 250 cc.

5.15 to 6.30. Blood drawn 150 cc. Returned 325 cc.

Total for day. Blood drawn, 575 cc. Returned 575 cc.

Dog showed tremors. Received 0.25 gram calcium lactate.

June 6, 12 noon. Blood drawn 260 cc. Injected 180 cc. fresh blood from normal animal + 150 cc. saline solution.

2 p.m. Returned dog's own blood made up to 260 cc. and immediately bled 210 cc. Dog died suddenly from overbleeding.

Total for day. Blood drawn 470 cc. Returned 590 cc.

Total for experiment. Blood drawn 945 cc. Returned 1165 cc.

Postoperative life 95 hours.

It is to be noticed that in these experiments, which were performed earlier than most of those on normal animals, variations of procedure were

tried the effects of which often proved harmful. This dog was undoubtedly bled too fast and the treatment was begun too late. The blood injected from another animal was designed partly to cover accidental losses and partly to replenish serum proteins. The dog's condition on the morning of the fourth day after operation, while serious, did not appear worse than that of the control dog twenty-four hours before death, i.e. on the second day after operation.

Experiment 3. June 3, 1914. 3 p.m. Control. Bilateral nephrectomy.

Dog, 11½ kg. Died 11 a.m. June 6. Postoperative life 68 hours.

Experiment 4. June 15, 2.30 p.m. Bilateral nephrectomy. Dog weight 11 kg.

June 16: 2.45 to 4.55. Blood drawn 200 cc. Returned 320 cc. containing 1 g. sodium acetate.

4.20 to 5.20. Blood drawn 50 cc. Returned 140 cc.

June 17, 11 am. to 1 p.m. Blood drawn 305 cc. Injected 250 cc saline solution containing 1½ grams Na_2CO_3. Returned corpuscle mixture 330 cc., distilled water 75cc., saline solution 125 cc., half strength saline 100cc.

2.15 to 4 p.m. Blood drawn 310 cc. Returned 355 cc. containing ⅓ gram Na_2CO_3.

4.20 to 5.40. Blood drawn 345 cc. Injected 75 cc. saline solution with 1 gram Na_2CO_3. Returned corpuscle mixture with half strength saline 315 cc.

June 18, 11 a.m. to 12.45 p.m. Blood drawn 350 cc. Injected saline 200 cc. with 1 g. Na_2CO_3. Returned corpuscles diluted with half strength saline to 350 cc.

Note. Dog in good condition, takes intelligent interest in surroundings.

2.15 to 3.35. Blood drawn 385 cc. Injected 70 cc. of fresh blood from normal dog and 75 cc. half strength saline. Returned corpuscle mixture 350 cc. containing 2 grams Na_2CO_3. Dog seems bright.

June 19, 10 a.m. to 1.20. Blood drawn 290 cc. Injected 300 cc. fresh blood from normal dog. Immediately afterwards blood drawn 210 cc. Returned corpuscles in 450 cc., together with 4 grams $NaHCO_3$.

Dog appears in fair condition: weighs 11.18 kg, though he has taken practically no food since operation.

2.30. Blood drawn 115 cc. Signs of collapse. Immediately injected 200 cc. saline solution containing 2 g. $NaHCO_3$.

Blood drawn 90 cc. Dog dies suddenly at 3.10 p.m. Post mortem examination showed great internal haemorrhage, about 500 cc. being

found in the body cavity. No obvious source of bleeding found but liver very friable. Dog evidently killed by overdistention of vascular system by excessive injections. Otherwise appeared as though it might have lived 24 to 48 hours more. Post operative life 96 hours.

Totals, June 16.	Blood drawn 250 cc.	Injected 460	
17.	Blood drawn 960 cc.	Injected 1125	
18.	Blood drawn 735 cc.	Injected 1145	
19.	Blood drawn 705 cc.	Injected 950	
Totals for experiment.	2650		3980

Blood volume (estimated) $7\frac{1}{2}\%$ of body weight = 825 cc.

Experiment 5. Control. June 15, 4 p.m. Bilateral nephrectomy. Dog, weight 9.95 kg. Died in night 17 to 18. Postoperative life less than 63 hours.

Experiment 6. June 22 3 p.m. Bilateral nephrectomy. Dog, weight 11.15 kg.

June 23. 12.15 to 2.05. Blood drawn 245 cc. Returned 320 cc.

Note. This dog becomes anaemic very quickly: air hunger shown at first bleeding. Some clots, causing loss of corpuscles, due to weak hirudin.

2.15 to 3.30. Blood drawn 185 cc. Returned 255 cc.

Dog still very anaemic.

3.45 to 4.50. Blood drawn 175 cc. Returned 255 cc. Injected 115 cc. fresh blood from normal dog and 35 cc. saline containing 2 g. $NaHCO_3$. Heart improved, pulse nearly normal.

Totals for day: Blood drawn 605 cc. Injected 980 cc.

June 24. 9.30. Blood drawn 277 cc. Dog died of heart failure about 10.20 before corpuscles could be returned. Evidently the animal, whose anaemic condition was noticed at the start, was overbled. Post-operative life $42\frac{1}{2}$ hours.

Experiment 7. June 22, 1914. 3.30 p.m. Bilateral nephrectomy. Same dog that was used for plasmaphaeresis experiment 1. Since that time has been kept in yard with larger dogs. Is now in fine condition, weighs 9.6 kg.

June 25. 9.30 a.m. to 2 p.m. Blood drawn 310 cc. Injected 100 cc. Locke's solution, and 260 cc. corpuscle mixture from dog that died the previous day (see experiment 6).

Returned 260 cc. of animal's own corpuscles in saline solution containing 2 grams $NaHCO_3$.

Blood drawn 165 cc. Returned 175 cc. with 2 grams $NaHCO_3$

Blood drawn 250 cc. Returned 165 cc.

Blood drawn 295 cc. Returned 385 cc. with $\frac{1}{2}$ gram $NaHCO_3$.

2 p.m. Dog in fairly good condition, drank 20 cc. milk and retained it.

3 p.m.	Blood drawn 165 cc.	Returned 200 cc.
to	Blood drawn 240 cc.	Returned 345 cc.
about	Blood drawn 150 cc.	Returned 90 cc.
6 p.m.	Blood drawn 130 cc.	Returned 230 cc.

Note. 50 cc. corpuscle mixture kept over till next day. Totals for day: Blood drawn 1705 cc. Returned 2210 cc. including corpuscles from 277 cc. blood of other animal.

Notes. Pulse was very irregular (bigeminal) at 9 a.m. At 6 p.m. pulse good 140. Drank 40 cc. milk. 10 p.m., pulse 122.

June 26, 9.30 a.m. Dog in fair condition. Pulse about 120.

10.40 to 11.05. Blood drawn 168 cc. Injected 50 cc corpuscle mixture from previous day and 126 cc. blood from normal dog.

11.15 to 11.45. Blood drawn 200 cc. Returned 215 cc.

11.50 to 12.40. Blood drawn 120 cc. Returned 150 cc. with 1 g. $NaHCO_3$.

12.50 to 1.05. Blood drawn 120 cc.

2 p.m. Injected 2 cc. pituitrin intramuscularily. Almost immediately dog died with expiratory scream. Post operative life 94½ hours. This experiment also was cut short by a mistake in procedure. The amount of pituitrin given was evidently an overdose under the circumstances. It is probable that the heart was unable to respond to the sudden increase[8] in vascular resistance. The clinical improvement effected by plasmaphaeresis gave hope of a considerably longer life even if all treatment had been discontinued at this point.

V. An examination of the above protocols bears out the statement made in an earlier part of this paper that it is possible to remove in a single day a volume of blood more than twice that ordinarily contained in the body at one time, with no apparent injury to the animal. Even in an animal whose kidneys have been removed two days previously this large amount of blood can be withdrawn not only without disadvantage but with actual benefit. In experiment 7, for example, 1705 cc. of blood were withdrawn in one day from an animal weighing 11 kg. which is more than twice the normal blood volume for an animal of this weight (7.5 per

[8] A similar phenomenon has been frequently observed in this laboratory in the anomalous and quickly fatal action of very minute doses of adrenalin on dogs under the influence of ether or chloroform, and Elliott has also called attention to this fact. Jour. of Physiol., vol. 32, p. 465, 1905.

cent = 825 cc.) How far this exceeds the quantity of blood that may be safely removed from a dog without return of corpuscles is apparent if we recall that the loss at one time of 60 to 70 per cent of the animal's blood is quickly fatal.[9]

More extended studies must determine the shortcomings and possible limitations of the method, but thus far we have no evidence that the removal of these considerable quantities of blood plasma have a harmful action which is other than temporary. In all cases in which the procedure remained uncomplicated by other operations, as nephrectomy, our animals have increased in weight, have eaten greedily and have given every evidence of perfect health. Their urine has given no evidence of anything abnormal, being free from sugar, albumin and bile pigments. The number of red corpuscles and the percentage of haemoglobin have fallen somewhat below the normal in the days immediately following the operation.

The following table substantiates the above facts:

EXPERIMENT NO.	8	10	11
Weight before operation	9.6 kg.	12.8 kg.	8 kg.
(Estimated) blood volume	730 cc.	960 cc.	600 cc.
Total blood drawn	2037 cc.	2016 cc.	1835 cc.
Ratio of volumes	2.79	2.13	3.06
Weights, July 8	10.5 kg.	11.8 kg.	8.5 kg.
Weights, July 10	10.7 kg.	12.5 kg.	8.0 kg.
Weights, July 15	11.2 kg.	12.8 kg.	8.2 kg.
Blood count (millions), July 8	1.5	5.3	
Blood count (millions), July 9	4.6	5.9	6.5
Blood count (millions), July 13	1.2	5.5	5.1
Blood count (millions), July 16	5.2	6.2	5.8
Haemoglobin, July 8	52 per cent	74 per cent	
Haemoglobin, July 9	52 per cent	65 per cent	78 per cent
Haemoglobin, July 13	60 per cent	80 per cent	79 per cent.
Haemoglobin, July 16	65 per cent	79 per cent	80 per cent
Weights, July 22*	11.0 kg.	13.35 kg.	8.5 kg.
Blood count, July 22	4.1	5.4	5.3
Haemoglobin, July 22	72 per cent	80 per cent	75 per cent

* Additions made during reading proof.

[9] See the experiments on the bleeding of dogs made by A. L. Soresi, New York Medical Journal, Nov. 9, 1912, p. 936. Also Marshall Hall's Experiments on dogs in his Observations on Blood-Letting. London, 1836.

Our studies in relation to these points are only preliminary but they agree with the findings of Yurevitch and Rosenberg[10] who noted that the red corpuscle count in rabbits fell from seven or eight millions to five millions or a little above this number in the course of twenty-four hours after the withdrawal of half of the blood[11] with reinjection of the red corpuscles. We see no reason for believing that the lowered count of red corpuscles is to be interpreted as proving the rapid destruction of a large number of these cells. A temporary dilution of the blood, a temporary lodgement of red corpuscles in the haemapoietic organs or a combination of these factors may well bring about the observed result. It has been stated that the urine is free from urobilin and it may also be added that in two instances in which the faeces were tested for bile pigments, though only qualitatively, it did not appear that these were present in larger amount than in the stools of normal dogs. While at present, therefore, everything points to the conclusion that destruction of red corpuscles does not take place to an abnormal degree after plasmaphaereis in healthy animals, we admit the possibility that the proceedure may lower somewhat the resistance of these cells to haemolysing agents in vitro. Theobald Smith[12] has shown that repeated bleeding of horses as in the preparation of diphtheria antitoxin lowers the resistance of the red corpuscles, in most instances producing in them a change that corresponds to a slight rise in osmotic tension of 0.04–0.09 per cent salt solution. The normal range of resistance for the corpuscles of the horse extends from 0.42 to 0.60 per cent salt solution while in the periodically bled horses it extends from 0.46 to 0.69 per cent salt solution. Numerous other investigators have found slight changes in the fragility of these cells as the result of various pathological conditions.[13]

[10] Loc. cit., p. 638.

[11] It is to be noted however that these authors have taken the total blood of a rabbit as being only 5 per cent of its body weight, i.e., they withdrew at one operation about 2½ per cent. The relative blood volume is placed higher by certain authors. (See Nagel's Hdb. der Physiol. des Menschen, Ergänzungsband, p. 20.)

[12] Journ. of Med. Research, xii, 385, 1904.

[13] See G. G. Butler: The tragility of the red blood corpuscles. Quart. Journ. of Med., vi, 145, 1912–13.

In regard to the protein content of the blood immediately after the removal of large quantities of blood plasma and in regard to the length of time required for the blood to attain again its normal composition in every way we have at the moment no data to offer, although work on these points is in progress.[14] The excellent condition of our animals in the days and weeks immediately following the removal of plasma forces us to believe that a large factor of safety obtains here as elsewhere in the animal economy and we are confident that we have not yet reached the danger point in plasmaphaeresis. The experiments of Morawitz[15] on the regeneration of the blood proteins in starving dogs, after extensive haemorrhage (with simultaneous infusion of washed corpuscles taken from other dogs) show that the blood can nearly recover its normal protein content of 6 per cent in three days, after having fallen as low as 2 per cent. Our method gives a greater opportunity for interchange between the contents of the vascular apparatus and of the tissue spaces of the organs and it is hence probable that a dangerously low protein content of the blood will not be so quickly obtained as in the experiments of Morawitz.

In this connection it is interesting to recall how rapidly fibrinogen is regenerated when a small animal is transfused with a large quantity of defibrinated blood from another animal of the same species, as shown by Whipple and Goodpasture.[16]

SUMMARY

1. It is shown that quantities of blood plasma may be withdrawn from an animal without apparent injury that exceed several times the maximum quantity of blood that can be safely drawn by the usual method of venesection provided that the corpuscular elements of the blood suspended in Locke's solution (0.6 per cent Na(Cl) be returned to the vascular system after each bleeding.

2. This procedure, which we have ventured to call plasmaphaeresis (from ἀφαίρεσις a withdrawal) has been applied by us in

[14] Extensive and careful researches on blood regeneration after venesection have been made by C. Inagaki. Zeitschr. f. Biologie, xxxi, pp. 77 to 198 (1907).

[15] Loc. cit.

[16] Amer. Journ. of Physiol., xxxiii, pp. 50 and 70 (1914).

a number of preliminary experiments on dogs for the relief of the symptoms consequent on bilateral nephrectomy. The improvement in the clinical condition of the animals treated was marked, and in all but one case of accidental overbleeding they lived distinctly longer than the control animals. It appears probable from this preliminary work that a considerably greater prolongation of life by this method will be attained in future experiments, when the limits of the procedure and the details of the most advantageous technique have been developed.