

THE EFFECT ON THE KIDNEYS OF TEMPORARY ANEMIA, ALONE AND ACCOMPANIED BY PERFUSION *

C. C. GUTHRIE, M.D.

PITTSBURGH, PA.

INTRODUCTION

As set forth in a previous paper,¹ investigations in this direction were suggested by the character of the results following transplantation of kidneys. To quote: "From the results on the kidneys, although my animals lived for several weeks, I came to the conclusion that permanent success was improbable owing to the latter factor" (i. e., injurious effect of the perfusion practiced). The results there reported have since been extended and confirmed, as shown below.²

METHOD

As previously described in the papers cited^{1, 2} the method consists in temporarily shutting off the circulation in a segment of the aorta, including the origin of the renal arteries and then perfusing the kidneys by injecting the solution to be tested into this segment by means of a small trochar (or needle) thrust through the wall of the aorta (Fig. 1), the instrument being connected by means of a rubber tube with a reservoir containing the solution.

On withdrawing the needle the puncture in the wall of the aorta is closed by several simple stitches which penetrate all the coats of the vessel. The clamps (serrefines) are then removed from the aorta and the abdominal wound closed. The animal is then bandaged and placed in the hospital.

It should be remarked that, in addition to the aorta, all arteries other than the renal arising from the segment are clamped, as well as all other vessels that may give a collateral circulation—e. g., the ureters with their surrounding tissues are compressed *en masse* by means of encircling coarse ligatures,³ which are temporarily fastened by means of ordinary

* From the Physiological Laboratories of Washington and Pittsburg Universities.

1. Guthrie, C. C.: Some Physiologic Aspects of Blood-vessel Surgery. Jour. Am. Med. Assn., 1908, li, 1658.

2. Guthrie, C. C.: Washington Univ. Bull., 1908, vii, 40.

3. Narrow strips of cloth are very good for this purpose.

hemostatic forceps or serrefines (Fig. 1). Even with such precautions more or less patent arterial connections are maintained, as shown by the fact that on withdrawing the perfusion needle, as a rule, arterial-hued blood soon begins to escape from the opening.

In some of the experiments the adrenals were shut off by the anterior serrefine and sometimes they were not, so that we have some data on the

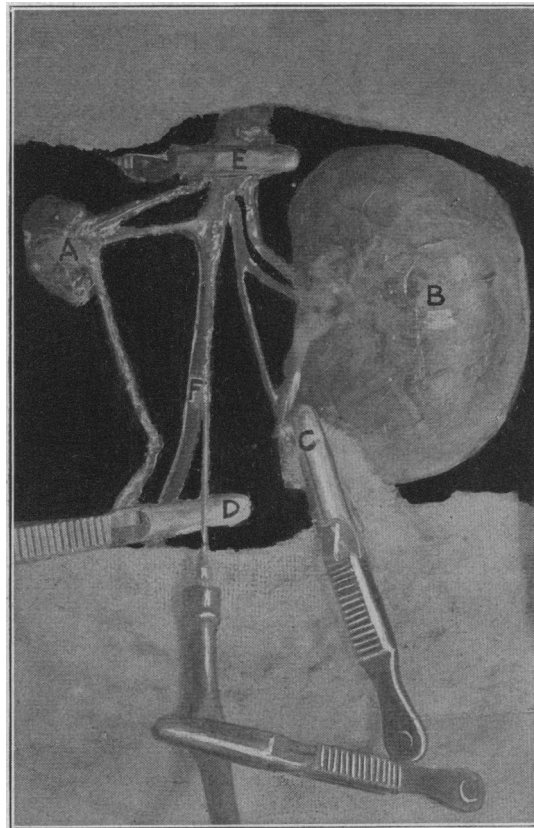


Fig. 1.—Kidneys and aorta of Cat 22; operated on May 23, 1908; photographed May 22, 1909. A, right kidney, rendered anemic and perfused; B, left kidney, anemic only; E and D, temporary clamps on aorta; C, showing method of clamping ureteral vessels; F, perfusion needle, thrust into aorta and connected by tube with reservoir holding solution.

results of perfusing them also. In some cases the renal artery (or arteries) to one kidney was clamped during the perfusion so that it is possible to compare on one animal the result of anemia alone, with ane-

mia accompanied by perfusion. The temperature of the perfusion liquid varied in different cases, as noted in the table. The pressure employed was, for the most part, practically constant for the non-colloid solutions (plain sodium chlorid, Ringer's and Locke's solutions), but with colloid solutions (e. g., starch) more pressure was required to force the liquid

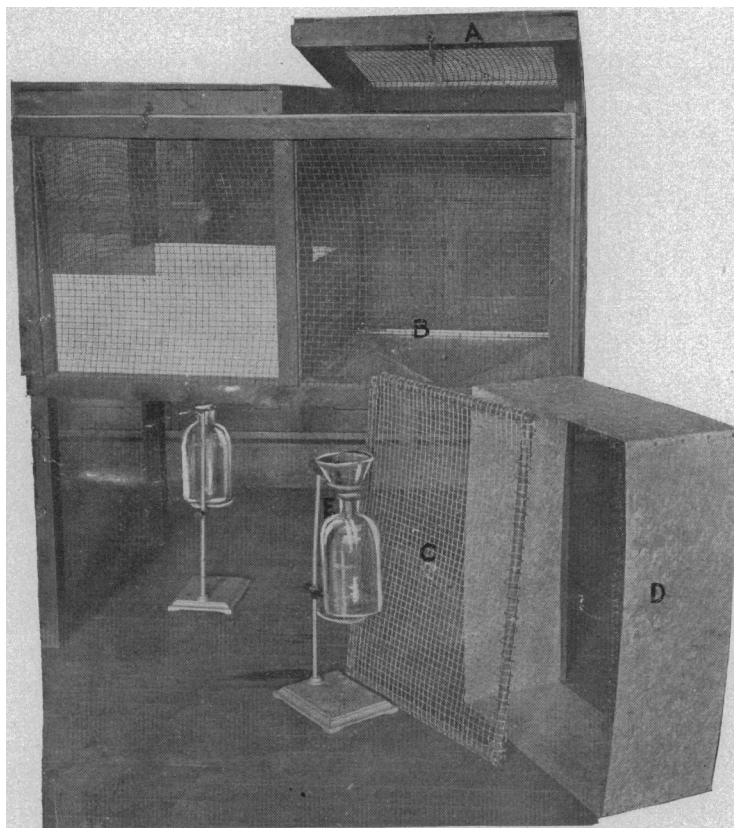


Fig. 2.—Practical form of metabolism cage, containing two compartments: Right compartment assembled ready for animal; left dismantled as for cleaning, showing parts, as follows: (A) hinged lid; (B) funnel-shaped metal bottom; (C) removable metal screen bottom on which the animal rests; (D) metal guard which fits inside upturned flange on (B); (E) funnel and bottle for receiving urine. Frame finished with waterproof paint. All metal parts galvanized or tinned.

into the capillaries. In those cases in which the urine was collected the animals were kept in metabolism cages along with control cats (Fig. 2). The diet, etc., was the same in all cases.

IMMEDIATE RESULTS

On beginning the perfusion, the kidneys could be observed through their capsules to become paler, the degree of paleness being taken as an index of the degree of perfusion. In addition, puncture of the kidneys was made (also through the capsules) with a very fine needle (No. 14

TABLE OF EXPERIMENTS, ANEMIA AND PERFUSION OF KIDNEYS

(Continued from table in Journal of the American Medical Association, 1908, li, 1658)

No.	Date Operated.	Aorta Occluded.		Period Perfused.		Solution Perfused With.	Death After, Days.	Remarks.
		Min.	Sec.	Min.	Sec.			
22	5/23/08	9	45	8	20	Locke's	364*	Anemia of both kidneys. Right only perfused.
23	5/23/08	12	30	0	0	0	40*	Anemia only of kidneys. Escaped in July in good condition. Showed no symptoms of renal insufficiency.
24	11/ 3/08	22	0	0	0	0	8*	Anemia only of kidneys. Cat in splendid condition until 8th day when abdominal wound opened owing to absorption of gut suture material, allowing the intestines to escape.
25	11/ 3/08	0	0	0	0	0	2.5	Ligated renal vessels permanently.
26	11/ 3/08	0	0	0	0	0	6	Excised kidneys.
29	12/16/08	20	35	7	0	.9 NaCl	84	Partial perfusion only of both kidneys.
30	12/16/08	28	0	16	30	Starch	1.5	Both kidneys perfused.
31	12/22/08	16	0	8	0	.9 NaCl	131	Left kidney excised. See Fig. 7.
32	1/26/09	25	0	15	30	.9 NaCl	20*	Clot in aorta. Some temporary paralysis. Chloroformed after recovery.
33	1/26/09	9	0	4	15	.9 NaCl	123*	Partial perfusion both kidneys.
34	1/26/09	16	30	12	30	.9 NaCl	105*	Both kidneys and adrenals.
35	1/26/09	14	10	10	0	Locke's	7	Both kidneys and one adrenal perfused.
36	1/27/09	10	0	2	15	.9 NaCl	122*	Partial perfusion both kidneys. Still alive and in fair condition.
37	1/27/09	12	30	5	0	.9 NaCl	101	Partial perfusion both kidneys and suprarenals.
38	1/27/09	10	30	7	0	Locke's	122*	Right kidney removed. Still alive in fair condition.
39	1/27/09	9	0	5	30	Ringer's	36	Both kidneys perfused.
40	1/27/09	11	30	5	30	Ringer's	33	Both kidneys perfused.
41	1/27/09	14	15	9	30	Locke's	32	Both kidneys and adrenals perfused.
42	2/ 4/09	18	0	12	0	NaCl	7	Both kidneys and right adrenal perfused.
44	2/ 4/09	19	30	12	0	NaCl	14	Both kidneys perfused. Young cat.

* Indicates that the animal escaped, was killed, or is still alive. This is usually indicated under "remarks." Nos. 22, 33 and 34 are exceptions. The former is explained in legend under Figure 4; the latter were chloroformed after they became too weak to stand. The amount of liquid perfused varied from about 10 to 30 c.c., in general the amount varying with the period of perfusion. In no case, with the possible exception of Cat 30, was the perfusion made with the pressure exceeding an average blood-pressure in cats. The solutions were brought to body temperature before beginning the perfusions in most cases, but when the room was very cold the temperature of the solution was considerably lowered before it entered the blood-vessels. The following temperatures were recorded: For No. 30, 15°; No. 31, 37°; No. 36, 30°; No. 38, 15°; No. 39, 20°; and No. 41, 12° C. These temperatures were taken by allowing the liquid to spurt through the needle on to the bulb of a thermometer.

cambric, which was the size usually employed for closing the puncture in the aorta), which permitted a minute quantity of liquid from the blood vessels to escape. By closely observing this liquid as it spread out on the capsule the presence of red blood corpuscles could be observed. It is doubtful whether in any instance was such liquid altogether free of red

corpuscles. Our perfusions, therefore, were relatively and not absolutely complete. The same was in general true for our anemias, though the amount of arterial blood reaching the kidneys was small—a mere dribble.

On releasing the temporarily occluded arteries the kidneys rapidly assume an appearance indicative of a very active circulation. The primary recovery of the animal is uneventful. One striking feature of the results, though not bearing directly on the major problem, may be men-

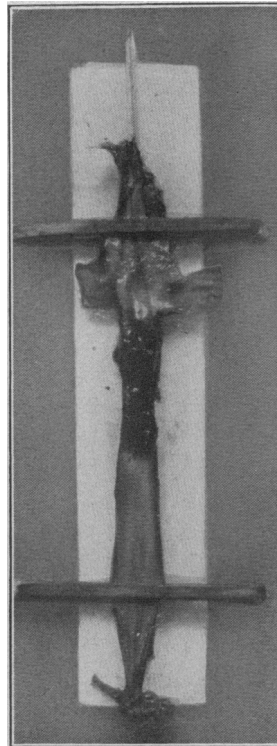


Fig. 3.—Aorta of Cat 32, showing occlusion by thrombus just posterior to renal arteries, kidneys perfused with salt solution, Jan. 26, 1909. Cat killed Feb. 15, 1909.

tioned, viz.: the result of injury to the aortic intima. In two cases only have we observed occluding thrombi in the aorta, and in both these cases the result was due to gross fault in technical procedure connected with the production of temporary hemostasis, by which not only the intima, but the outer walls of the aorta, were crushed and otherwise maltreated. In the first case (Cat 18, reported in a previous paper¹) the thrombus

extended for some distance both above and below the origin of the renal arteries and the cat died during the first day. The second cat (No. 32) showed partial paralysis of the hind limbs for a few days, but this soon entirely disappeared. On the twentieth day the animal was killed with chloroform, as its kidneys were desired for examination. Much to my surprise, on examining the aorta, which is part of the routine of such a post-mortem, the lumen was found to be completely occluded by an old thrombus, beginning 2 to 4 mm. posterior to the origin of the renal arteries and extending backward for 11 to 14 mm. (Fig. 3). A few days later I received a reprint from Dr. Halsted,⁴ in which he gives the results of gradual occlusion of the aorta in dogs, the occlusion being produced by means of an aluminum band clasped around the vessel by an ingenious

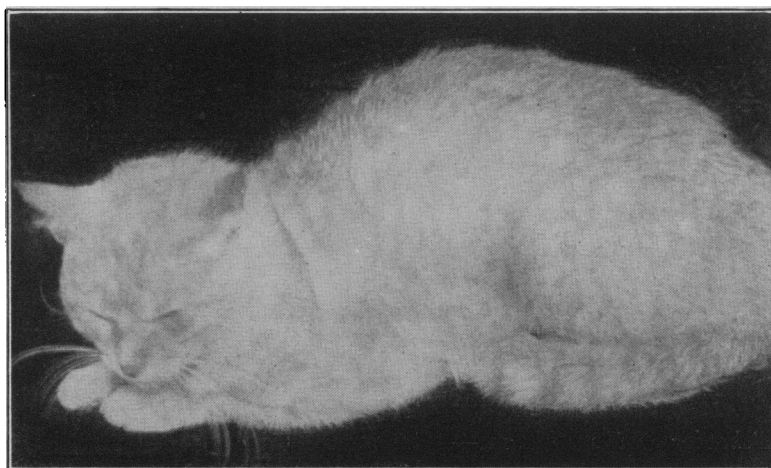


Fig. 4.—Cat 22; May 23, 1908, arterial circulation to both kidneys restrained for 9 minutes 45 seconds. During part of this period the right kidney was perfused with Locke's solution. May 22, 1909, animal photographed and chloroformed (See Fig. 1).

instrument devised by him for the purpose. Essentially the above observations on the cat agree with his observations on dogs. He has observed "in a number of instances, about three months after the operation, a deposit of extradural fat about the cord below the site of the aortic band" (p. 377). He promises a full report of the work soon, as well as a paper giving the literature and history of the subject.

4. Halsted, W. S.: Partial, Progressive and Complete Occlusion of the Aorta and Other Large Arteries in the Dog by Means of the Metal Band, *Jour. Exper. Med.*, 1909, xi, 373.

LATER RESULTS: ANEMIA ALONE

Behavior of the Animal.—In the case of anemia alone of the kidneys, or of the kidneys and suprarenals together, no abnormal symptoms were observed and recovery was uneventful. When one kidney was subjected to anemia alone and the other to anemia with perfusion (Cat 22) the result was the same. Anatomically the changes thus far observed have been less marked than when perfusion was practiced.

Anatomical Changes.—In the case of Cat 22, in which one kidney was rendered anemic, while the other was in addition perfused with Locke's solution, a marked increase in size of the former (compensatory hypertrophy?) with practically complete disappearance of the latter (perfused) kidney was observed 155 days after the operation. Twelve months later the animal was photographed (Fig. 4) and then killed with chloroform and the kidneys photographed (Fig. 1).

Histological Changes.—No marked alterations have been observed, but the data on this point are as yet incomplete.

Chemical Changes.—No studies have been made when anemia alone has been practiced. The metabolism of such animals has suffered no marked alteration, as judged by the condition, behavior, etc. It may be remarked that Cat 22, although having all the appearances of a vigorous male, since the operation seemed to have absolutely no sexual desire, according to the attendant. This seems rather unusual from our experience with cats.

LATER RESULTS: ANEMIA WITH PERFUSION

Behavior of the Animals.—After anemia with perfusion of the kidneys, cats as a rule show no unusual symptoms for twenty-four hours or more. During this time they appear the same as any cat on which a major surgical operation has been performed. In the acuter types of cases the usual symptoms of renal insufficiency rapidly develop, terminating in the death of the animal within a week or ten days, while in the slower types such pronounced symptoms do not appear for weeks or months. In all cases where such symptoms have been pronounced, death has invariably occurred within a few days. As a rule death follows the appearance of such symptoms more quickly in the acute than in the slow types of cases. In the latter especially, the animals usually showed great emaciation before death (Fig. 5) or even before the onset of convulsive symptoms. With the onset of convulsions, in all cases death occurred within a few days. Particularly in the acuter cases the character and train of symptoms were indistinguishable from the symptoms following

simple ligation of the renal blood-vessels or double nephrectomy. For a day or two preceding death there appeared to be a more or less complete suppression of urine. With the lighter convulsions and during the onset of the stronger ones the pupils usually showed marked constriction, but during the height of the stronger convulsions the pupils dilated. It may be remarked that such changes in the pupils are probably due to deficient respiration, it being known that partial asphyxiation produces a constriction (Guthrie and Ryan), followed by a dilatation if the asphyxia be sufficiently complete.⁵

Anatomical Changes.—As a rule when examined up to a few weeks after the operation the grosser changes consist in what we have termed a “subnormal resiliency,” i. e., the kidneys feel more or less flabby, in appearance they are pale, on section the cortex is pale, the medulla congested, while in the boundary zone a marked stripe of congestion is seen. The tissue has more or less of the “cooked” appearance that pathologists

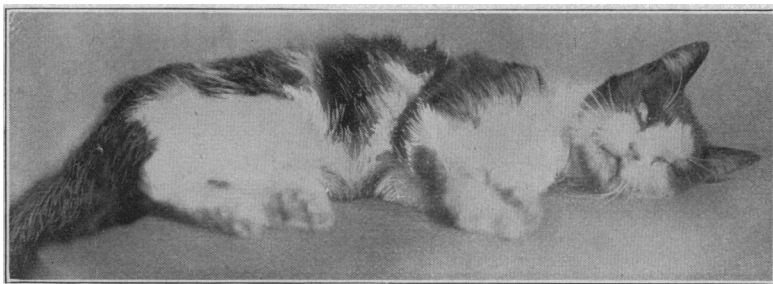


Fig. 5.—Cat 41, photographed shortly before death, showing emaciated, weak and spasmodic condition. Note the constricted pupils. Jan. 27, 1909, renal and adrenal arterial circulation restrained for 14 minutes 15 seconds. During part of this period both pairs of glands were perfused with Locke's solution. The animal lived thirty-two days.

have associated with parenchymatous degeneration (Fig. 6). Ultimately the kidney becomes harder, and on section less medullary congestion is seen. The cortex is paler than normal.

Histological Changes.—The most prominent feature in early examinations is the congestion. This is greatest in the boundary zone and medulla. Later, interstitial hemorrhages occur throughout the entire organ. At this stage cloudy swelling, of the tubular cells especially, is observed. This and the succeeding degeneration is also very marked in the cortex. Cellular infiltration also occurs, particularly in the boundary

5. Stewart, Guthrie and Pike: Jour. Exper. Med., 1906, viii, 289.

region. The degenerative processes may proceed until cell structure is lost. As a rule, such processes are more marked in certain areas than in others, so that such areas are surrounded by tissue showing more nearly normal structure. Such degeneration may result in the disappearance of Malpighian corpuscles as well as tubules proper.

Chemical Changes.—The observations so far have been confined to the urine. At first there seems to be a decrease in the amount of urine. Such urine shows a high specific gravity and a high percentage of normal solids, e. g., urea and chlorids. In the more protracted cases the amount

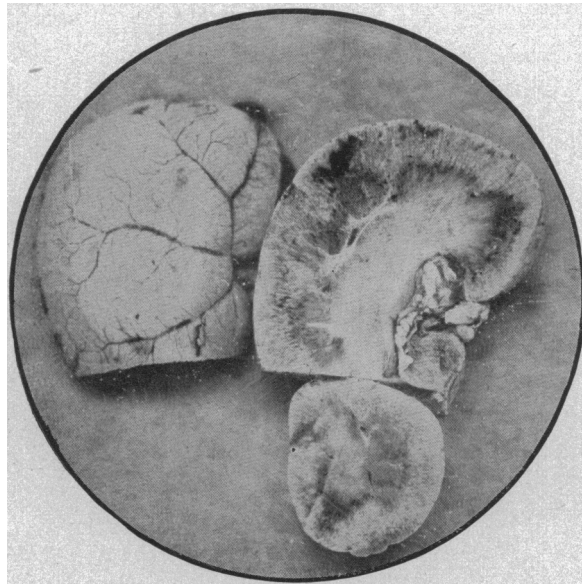


Fig. 6.—Kidneys of Cat 32, which were perfused with salt solution Jan. 26, 1909. The animal died Feb. 15, 1909, twenty days after perfusion.

and composition may approach the normal for a time, but prior to death another change occurs so that the specific gravity and content of normal solids may sink below the normal.⁶ Neither albumin nor sugar have been

6. It is interesting to note the composition of normal cat urine. The daily averages for one of our control cats for one week was as follows: Amount 91.4 c.c.; Sp. G. 1.059; chlorids abundant; urea 17.5 per cent. (by the hypobromite method). This figure for urea is unquestionably too high, and I wish to call attention to the fact that the figure given in the table accompanying the paper in the *Journal of the American Medical Association* (1908, li, 1658) should not be taken as indicating the actual amount of urea present. For some reason as yet unknown to me, the hypobromite method has indicated far too much urea in all cats' urines to which it has been applied. I may say that when time permits, we intend making more thorough analytical studies of the urines, samples of which we have carefully preserved for this purpose.

observed, at least in significant quantities. Since the interpretation of such results is a matter requiring great care owing to the great complexity of factors, e. g., amount and composition of the food eaten, liquids drunk, body weight, physiological state, etc., a more complete discussion is withheld for a later paper. The metabolism appears to suffer great alterations as judged not only by changes in the urine, but by the loss of appetite and of weight and change in general behavior.

SUMMARY OF RESULTS

1. Anemia alone is apparently much less harmful than when accompanied by perfusion of any of the commoner salt solutions.
2. The commoner salt solutions do not seem to differ greatly in toxicity.

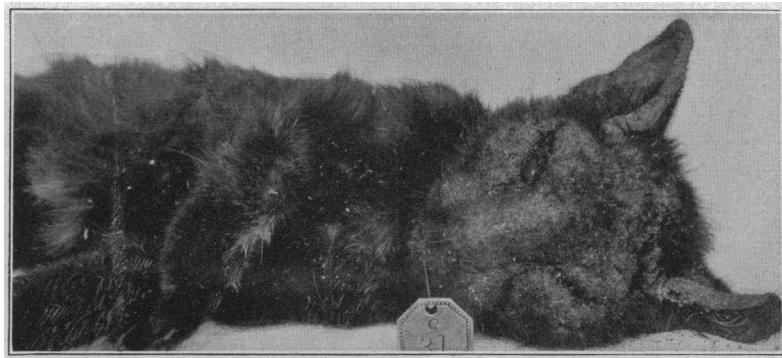


Fig. 7.—Cat 31, of which the kidneys were perfused for eight minutes with 0.9 per cent. sodium chlorid solution, Dec. 22, 1908. Left kidney excised at end of operation. At the time of death, 131 days later, this animal presented a very remarkable thickening of the skin of the head, such as we have never observed before in a cat. There was no indication of it at the time of the operation. We do not necessarily conclude, however, that the condition was due to temporary anemia and perfusion of the kidneys or that the condition itself was solely responsible for the death of the animal.

3. Attempts thus far to devise a non-toxic solution for perfusing, e. g., salt-starch solution, have received no encouragement from the results.
4. Anemia with perfusion of the kidneys as a rule is followed by death of the animals within a few months—the majority dying in a few weeks.
5. The cause of the differences in time of death of the animals are unknown. Individual peculiarities may be (and probably are) an important factor.

6. Metabolic disturbances, seemingly in the direction of increased protein metabolism especially, occur.

7. Decrease in urinary secretion preceding death is probably an important factor to consider in interpreting the final symptoms, which are those of uremic poisoning.

8. Structural changes of a hemorrhagic and degenerative character occur in the perfused kidneys.

DISCUSSION

The results show conclusively that renal and adrenal anemia, coupled with perfusion with all the solutions tried, is much more harmful than anemia alone. Anemia alone is certainly not to be looked on as being without effect, but it seems that for cats, under the conditions of these experiments, short periods of occlusion are not incompatible with permanent recovery of the animal. The observations have not yet been carried sufficiently far to enable us to conclude that the life of the animal is not shortened by the anemia. Neither may we conclude that anemia with perfusion as performed invariably shortens the period of life remaining to the animal; for in a few cases, when death did not occur for some months, we cannot be absolutely certain—though the evidence, on the whole, is strongly in this direction—that death was due to the operation. Carrel, in repeating Chirie and Mayer's work⁷ on the effect of temporary occlusion of the renal veins on dogs, observed death in one case in a few months after the operation, the period of occlusion being twelve minutes. He attributed death to *ostéo-périostite* of the atlas.⁸ But it seems simpler to assume that the operation had a share in the causation of death, since Chirie and Mayer observed epileptiform manifestations and rapid death of dogs in which the renal veins had been occluded for ten minutes. As before indicated, the completeness of the anemia during the period of occlusion is no doubt an important factor, and better control of this may tend to render the results of different investigators more uniform. In addition to this, however, differences in resistance to anemia as well as to anemia with perfusion will probably be demonstrated not only in animals of different species, but in individuals of the same species.

For the present, therefore, it would be unprofitable to discuss the apparent small differences in the toxicity of the solutions used on the cats. Since all solutions seemed to have a toxic action, we may see if a plausible explanation of this action can, with our present knowledge, be

7. Chirie and Mayer: *Compt. rend. hebd. d. séances Soc. de biol.*, 1907, lxii, 598.

8. Carrel: *Comp. rend. hebd. Soc. de biol.*, 1909, lxvi, 527.

given, since it is by such theoretical considerations that at least the practical aspects of an experimental investigation are advanced and a conception of the processes at least partially outlined.

Let us consider simple anemia first. In this case not only do the blood-vessels contain a fluid having normal physical properties for the cells of the kidney, but it also holds a certain amount of the pabulum for the kidney, including oxygen, in an available form. Also it is suited to receive a certain amount of the metabolites thrown out by the kidney cells into the blood, e. g., carbon dioxid, retention of which in the organ is detrimental. Also, as before stated, absolute hemostasis is difficult to accomplish under the condition of the experiment, so that renal products in the nature of "hormones" may still reach the general circulation, though, of course, in decreased amounts. However this may be, we must conclude that the factor was in favor both of the simple anemias and of the anemias accompanied by perfusion, for it is well known that (1) a tissue receiving a subminimal amount of blood⁹ is easier to resuscitate than if the circulation be entirely stopped or if the perfusion be carried out with a blood-free liquid; and (2) that after resuscitation the normality of its subsequent activities will vary directly with the degree and period of anemia.⁵

Indeed, it is well known that in simple transplantations of tissues (i. e., without anastomosis of the blood-vessels) results are less perfect when the tissues are treated with salt solution than when subjected to anemia alone (Christiani).

It is interesting at this point to note the work of Policard,¹⁰ who recently has reported in detail the results of an investigation undertaken with the view of determining what structural changes occur outside the body in the epithelial cells of urinary tubules under the influence of sodium chlorid solutions of different concentrations. He decapitated white rats, quickly removed the kidneys and cut them in very small bits, the largest being under 1 mm. in thickness. Such fragments were then immersed for fifteen minutes in a salt solution of known strength at a temperature of 15 degrees C. The tissues were then fixed in formaldehyd solution, after which they were prepared for microscopical examination. He concludes that solutions of sodium chlorid of all strengths (hypotonic, isotonic and hypertonic) change more or less the cells of the convoluted tubules.

9. Subminimal being used as indicating in the first instance an amount of blood too small to preserve the ordinary manifestations of activity, e g., in cerebral anemia, etc.; and in the second instance that the blood is too dilute to preserve such manifestations.

10. Policard: Jour. de physiol. et de path. gén., 1908, x, 2.

Now, if we examine the salt solutions, we find that they all contain one or more of the inorganic salts in approximately the proportion found in the blood. Yet they are all similarly toxic. The same is true of the one containing, in addition to more abundant blood salts (*viz.*, sodium, potassium and calcium), grape-sugar, which is considered another constituent of normal blood. In certain physical characters these solutions differ greatly from blood, or even serum, *e. g.*, they are non-colloidal. Such being the case, we might attribute at least a part of their harmful influence to this factor. To test this point a colloidal solution was prepared by adding boiled starch to a salt solution in such proportion that the freezing point, electrical conductivity and viscosity were nearly identical with normal cat's blood (defibrinated). Yet it was no improvement over the plain salt solution, as judged by the result (Cat 30). But it would be a mistake to draw conclusions from this experiment, as starch is an abnormal colloid for blood. Besides, owing to the cooling of the kidneys during their exposure made for observing the course of the injection (room temperature was 15° C.), it is not improbable that the temperature of the solution was lowered to such an extent that its viscosity was increased. A considerably higher injection pressure was required than for the other solutions, so this, too, must be taken into account. Also, I regret to say, no control experiment was performed to determine the effect of merely introducing it into a cat's circulation. Such experiments are outlined for later performance, as well as the employment of other colloidal solutions, *e. g.*, gum arabic, etc., as well as colloids of animal origin, *e. g.*, serum and milk products. I may add that a salt-starch solution prepared as above, in comparison with plain sodium chlorid solution, Ringer's solution and Locke's solution and turtle's blood on strips of turtle's heart, gave results most nearly like the blood itself; *viz.*, on the ventricle it had slight or no stimulating action and the strip retained its irritability well, while on the auricle it sustained the contractions for a longer time than the other salt solutions, and there was also less evidence of stimulation.

A more complete account of the solution will be reserved for future publication, as it is hoped that results with other similar solutions may be obtained and incorporated.

Unlike blood, such solutions lack not only normal physical properties, but they cannot be considered to contain an adequate pabulum—there being no evidence that grape-sugar, which is a constituent of Locke's solution, is adequate in this direction, although it is destroyed by active

tissues.¹¹ Still since the period of anemia is relatively short, and since organs or even cells, like animals, undoubtedly contain a certain amount of material that can take the place of that supplied by the blood for a time, too much stress should not be laid on this point. Even a certain store of oxygen is laid up in the tissues themselves that can be drawn on in such emergency conditions, but there is no evidence that the kidney has a sufficient store to last any great length of time, as is the case in muscle.

Although the solutions employed were well aerated, they contained but a fraction of the amount of oxygen found in arterial blood—indeed, the amount even under pure oxygen is insignificant compared with ordinary venous blood.¹² So we may conclude that the tissues received too little oxygen. Again, the carrying capacity for carbon dioxide of the solutions is far less than that of blood. Also, the total amount of solution injected into kidneys, compared to the amount of blood passing through them normally in the same length of time, is insignificant. We may conclude, therefore, that in anemia with perfusion, as well as in anemia alone, there was a profound decrease in renal respiration, and that this was probably greater in the former than in the latter case. Although some blood probably entered the renal vessels and became mixed with the perfusion solution, the total amount thus entering, considering all other factors as being the same, would be less during perfusion owing to the pressure of the perfusion liquid. Also, such as entered the vessels and became mixed with the solutions probably had a less metabolic value per unit, owing to the dilution.¹³ Numerous other possibilities might be brought forward, but what is written above is sufficient to indicate the state and complexity of the problem.

Thirtieth Street and Brereton Avenue.

11. Locke and Rosenheim (*Jour. Physiol.*, 1907, xxxvi, 205), using surviving hearts, observed a more rapid disappearance of dextrose from the perfusion fluid during activity. McGuigan (*Am. Jour. Physiol.*, 1908, xxi, 334), working in my laboratory, obtained the same result for skeletal muscle prior to the appearance of Locke and Rosenheim's announcement. So long as the isolated skeletal muscles survive (as indicated by response to electrical stimulation) the sugar disappears. Later, the perfusion fluid filters through the walls of the blood-vessels into the tissues but the sugar contained therein does not appear to be destroyed.

12. Guthrie and Pike: *Am. Jour. Physiol.*, 1907, xviii, 14.

13. It is well known from studies on isolated tissues (Cf. Guthrie and Pike: *Loc. cit.*) that beyond a certain dilution, the addition of blood to an ordinary perfusion liquid is of small value.