An alternative explanation for the smallness of the isotope shifts is obtained if one realizes that the last added neutrons should be approximately uniformly distributed over a region including a dense core of nuclear matter and a less dense region at the nuclear surface about 1.5×10^{-13} cm thick. Then only the part of the neutron wave function inside the core radius is effective in displacing protons outward and so causing isotope shifts. The isotope shift is hence about 50% of that calculated when the gradual decrease in nuclear density at the surface is neglected.

References

1. CHRISTY, R. F., and KELLER, J. M. *Phys. Rev.*, **61**, 147 (1942).
2. BROCH, E. K. *Arch. Math. Naturvidenskab*, **48**, 25 (1945).
3. ROSENTHAL, J. E., and BREIT, G. *Phys. Rev.*, **41**, 459 (1932).
4. BETHE, H. A., and LONGMIRE, C. *Ibid.*, **75**, 306 (1949).
5. BEARDEN, J. A., and WATTS, H. M. *Ibid.*, **81**, 73 (1952); DU MOND, J. W. M. National Research Council Report (Dec. 1950).
6. CRAWFORD, M. F., and SCHAWLOW, A. L. *Phys. Rev.*, **76**, 1310 (1949); BRIX, P., and KOPFERMANN, H. *Z. Physik*, **126**, 344 (1949).

Manuscript received November 19, 1951.

An Artificial Kidney— A Simplified Apparatus

John R. Guarino and Louis J. Guarino

Brusch Medical Center, Cambridge, Massachusetts
and *Pan-Engineering Company, New York*

The object of this report is to present and to describe an apparatus suitable for routine clinical use as an artificial kidney. It is of small size, is simple in design and construction, and has uncomplicated and safe operation.

The ideal indications for use of an artificial kidney may be found in acute reversible conditions causing retention or a critical increase of the crystalloid metabolins, or of other diffusible toxic products in the blood. It becomes necessary then to remove the toxic products by artificial means to sustain life for the period of repair. Such conditions may be mechanical or pathologic, of renal, extrarenal, or mixed origin. They include the nephritides of acute toxemias, of infection, pregnancy, and poisons; the nephroses such as the bichloride of mercury kidney; trauma to kidneys and ureters causing anuria; urinary obstructions; anuria following blood transfusions, severe burns; poisoning caused by diffusible chemicals, such as the barbiturates, sulfonamides; crystalloid and electrolyte imbalance.

The extrarenal routes attempted for excretion of retention products include skin, peritoneal lavage, gastrointestinal tract, kidney transplantation, crossed blood transfusion, plasmapheresis, perfusion through an isolated loop of intestine, and vividialysis or vividiffusion (artificial kidney).

The artificial kidney, a method of vividialysis, has proved the most effective and practical means for the extrarenal excretion of retention products. This work began with Abel, Rowntree, and Turner, in 1913 (*1*), and was followed by Haas (*2*) and Thalhimer (*3*). With the use of heparin in place of the more toxic hirudin, and cellophane as the dialyzer in place of collodion, celluidin, or peritoneal membrane, further progress continued with Kolff (*4*), Murray, Delorme, and Thomas (*5*), Lam and Ponka (*6*), Alwall (*7*), Skeggs and Leonard (*8*), Vanatta, Muirhead, and Grollman (*9*), and Merrill, Smith, Callahan, Thorn, and Walter (*10*).

The various apparatus differ in design, but are basically similar and have one common principle: blood of the subject is introduced through a cellophane tube which is immersed in a bath of perfusion fluid. The diameter of the tube ranges from 0.1 cm to 2.54 cm, with lengths of 10–45 m. The tubing is arranged in parallel series, wound spirally about a vertical stationary drum, or spirally about a horizontal revolving drum. The volume of the bath fluid has varied from 25 to 100 liters, and the fluid is stationary, or it is agitated by a motor-driven propeller. A blood volume of 0.5–1.0 liter is contained in the tubing. To reduce the blood volume and the size of the apparatus, Alwall (*7*) maintains compression of the cellophane tubing by a special device; Skeggs (*8*) employs a series of "kidney units," each consisting of a single layer of sheet cellophane between two corrugated rubber pads.

These systems are necessarily large or complicated and present serious operational and technical difficulties. In circulating a large volume of blood through such extensive extracorporal systems, blood clotting, sterility, hemolysis, pyrogen reactions, blood flow, blood viscosity, hemorrhage into the tubing, and requirements of large amounts of heparin become major problems and increase the operational risk, limiting the use of the artificial kidney as a routine clinical procedure. Although the therapeutic value of the artificial kidney is well established, the treatment is considered radical and heroic.

Our apparatus is shown in Fig. 1. It consists of a silicone-coated glass chamber, with a detachable upper portion. There are conduits attached for the entrance and exit of blood, and for the entrance and exit of a prepared rinsing or perfusion fluid. Twenty-three ft of ¾-in. cellophane tubing are incorporated in the chamber in concentric layers. The cellophane tubing requires no support and is simply placed in the chamber with its free ends connected to a reservoir containing the rinsing fluid. A continuous circulation of the rinsing fluid is first established through the cellophane tubing in the chamber. The fluid leaves the reservoir through conduit *"A"*, and is pumped through conduit *"B"* into the cellophane tubing in the chamber, and leaves the chamber through conduit *"C"* to return to the reservoir, providing a continuous flow of the rinsing fluid through the cellophane tubing. This circulation of the fluid can be accomplished by gravity; however, since this would require two reservoirs and manual attention, it has been found preferable to use

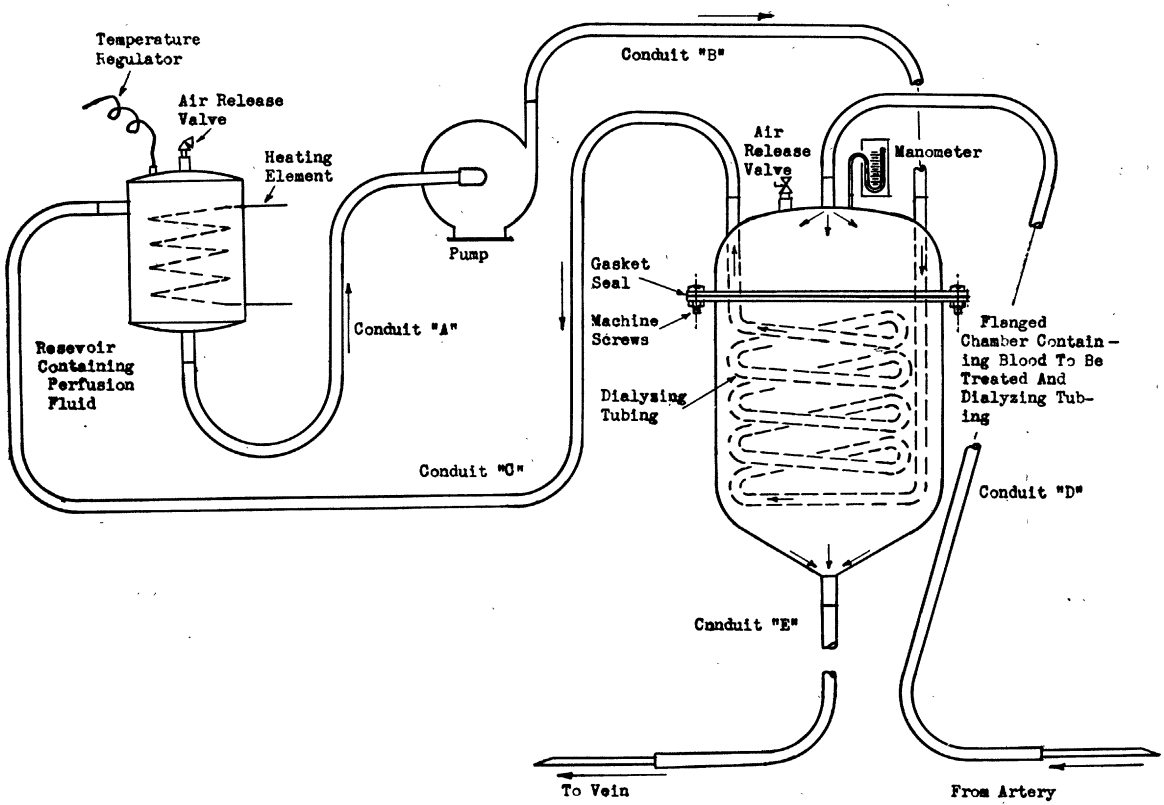

FIG. 1. Schematic diagram of artificial kidney. Diameter of chamber, 4"; length of chamber 7"; length of dialyzing tubing, 23'; diameter of dialyzing tubing, ¾"; dialyzing surface area, 4115 sq cm; volume of blood in chamber, 50 ml.

a pump to circulate the rinsing fluid. Circulation through the cellophane tubing, arranged as described, is controllable and has been maintained without difficulty at rates up to 300 ml/min. A mixture of oxygen and carbon dioxide may enter the reservoir to control the pH of the fluid.

The radial artery of a subject is exposed and interrupted, and a cannula is inserted into the proximal stump. Blood will flow from the subject through conduit *"D"* into the chamber, and leave the chamber through conduit *"E"* to return to the subject. As the blood enters the chamber, an air cushion is formed above the surface of the blood. The air cushion and air vent may be used to control the volume or level of blood in the chamber (*11*). The hydrostatic pressure of the perfusion or rinsing fluid is maintained equal to, or above, the hydrostatic pressure of the blood. The blood is allowed to flow over the cellophane tubing in a nondirectional flow, forming, in effect, a blood shower. The treated blood leaves the chamber through conduit *"E"* and is returned to the subject through a cannula inserted into the distal stump of the radial artery or to a suitable vein. An artery-to-artery flow in place of artery-to-vein may eliminate the adverse effect of an arteriovenous shunt that is present in the latter method at high flow rates. Fig. 2 shows a convenient method by which an artery-to-artery flow is accomplished with the use of a double-passage cannula. The cannula is of sufficient length to extend outside the operative field. It avoids clutter at the site of dissection and requires only one cut-down.

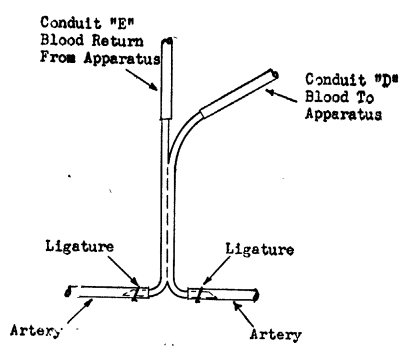

FIG. 2. Schematic diagram of artery-to-apparatus-to-artery blood flow with use of single double-passage cannula.

In the one chamber, then, there is formed at the same time, in a closed system, a continuous circulation of blood enveloping a continuous cross-circulation of the perfusion fluid. The dialyzing tubing with its circulating rinsing fluid may occupy part or nearly the whole volume of the chamber. Since the volume of the blood in the chamber is controlled, a very large dialyzing surface area can be exposed, utilizing only a minimum amount of blood, eliminating dead space and excess or waste blood. The efficiency of the ap-

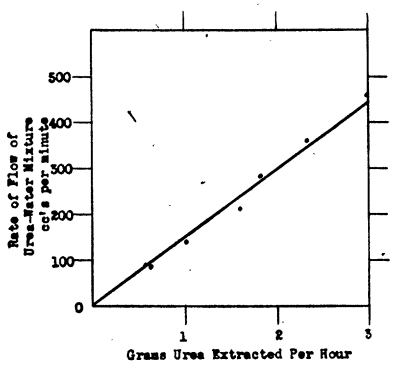

FIG. 3. The effect on rate of urea extraction with increasing flow rate of urea-water mixture. Dialyzing surface area, 1370 sq cm; rate of flow of perfusion fluid, 100 cc/min; urea nitrogen in urea-water mixture, 226 mg%; temperature of urea-water mixture and perfusion fluid, 65° F; volume of urea-water mixture in chamber, 50 cc; volume of perfusion fluid in reservoir, 4 liters; length of cellophane tubing, 7½'.

paratus is greatly increased thereby and permits a small-sized chamber.

There is a rapid change of blood in cross-circulation with a rapidly changing rinsing fluid. In this way the concentration of the diffusible substances in the blood will be constantly maximal, and the concentration of the diffusate in the perfusion fluid can be maintained near zero. This allows highest diffusion gradients to exist at all times between the blood and the perfusion fluid. These conditions are conducive to a maximal rate of diffusion.

In a tubular blood flow of other systems, dialysis occurs only with the layer of blood at the periphery of the tube in contact with the dialyzing membrane; the blood away from the periphery is surplus. Furthermore, the blood flow at the periphery of the tube is slowest, and this factor becomes increasingly important as the viscosity of the blood increases, and when the rate of blood flow decreases.

Since the amount of the blood used in this apparatus is small (10–50 ml), and since the blood traverses a very short extracorporal path, the heart of the subject functions very efficiently as the sole propagating force. This has been demonstrated with the application of the apparatus on dogs, where a pulsating blood flow can be maintained with negligible loss of blood pressure gradients.

The efficiency and capacity of the apparatus have been studied, using urea-water mixtures and water. The actual experimental values are plotted in graphs (Figs. 3, 4). Similar results were obtained with human blood. In consideration of possible errors in measuring flow rates and in computing the actual dialyzing surface area, since the tubing was never fully inflated, an average trace was made in each case.

In Fig. 3 a straight-line relationship is evident between the rate of flow of urea-water mixture and amount of urea extracted, with the dialyzing surface area and rate of flow of perfusion fluid remaining constant. As the rate of flow of urea-water increases, the amount of urea extracted increases directly.

In Fig. 4 a straight-line relationship is also evident between the dialyzing surface area and the amount of urea extracted, with the rate of flow of urea-water and the rate of flow of perfusion fluid remaining constant. As the dialyzing surface area increases, the amount of urea extracted increases directly.

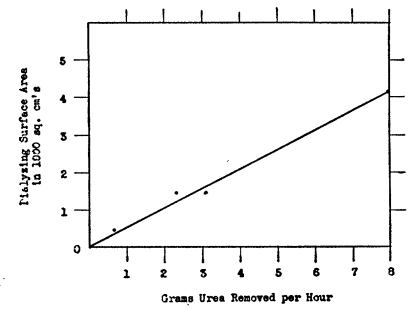

FIG. 4. The effect on rate of urea extraction with increasing dialyzing surface area. Rate of flow of urea-water mixture, 240 ml/min; urea nitrogen level, 213 mg%; rate of flow of perfusion fluid, 75 cc/min; volume of urea-water mixture in chamber, 50 ml; volume of perfusion fluid in reservoir, 4 liters; temperature of urea-water mixture and perfusion fluid, 65° F.

In the apparatus, which is 4 in. in diameter and 7 in. long, and which contains 23 ft of tubing with a dialyzing surface area of approximately 4115 sq cm, 8 g urea/hr were removed. The rate of flow of the rinsing fluid through the tubing was approximately 75 ml/min, and the rate of flow of urea-water mixture was 240 ml/min.

This apparatus in its present capacity is a highly efficient artificial kidney. The chief advantages are:

1) Small size of apparatus, with simplicity of construction and operation;

2) Requirement of small amount of blood in the chamber for a large dialyzing surface area;

3) Elimination of blood pumps, machinery, and air traps;

4) A rapid change of blood in a nondirectional flow;

5) A constant change and circulation of perfusion fluid, with no evaporation of fluid;

6) No opportunity for reabsorption of the diffusate by the blood;

7) The requirement of a minimum amount of cellophane tubing and perfusion fluid;

8) The need for only a small amount of heparin;

9) Elimination of problems of blood circulation, blood clotting, hemorrhage, blood viscosity, sterility, hemolysis, pyrogen reactions, and heparin toxicity.

This artificial kidney is small, safe, and efficient, and can be used with little difficulty in small hospitals and clinics as a routine treatment.

References

1. ABEL, J. J., ROWNTREE, L. G., and TURNER, B. B. *J. Pharmacol. Exptl. Therap.*, **5**, 275 (1914).
2. HAAS, G. *Abderhaldens Handbuch biol. Arbeitsmethoden*, **8**, 717 (1935).
3. THALHIMER, W. *Proc. Soc. Exptl. Biol. Med.*, **37**, 641 (1938).
4. KOLFF, W. J. *New Ways of Treating Uremia; The Artificial Kidney, Peritoneal Lavage, Intestinal Lavage.* London: Churchill (1947).
5. MURRAY, G., DELORME, E., and THOMAS, N. *Brit. Med. J.*, **2**, 888 (1949).
6. LAM, C. R., and PONKA, J. L. *J. Lab. Clin. Med.*, **32**, 1434 (1942).

7. ALWALL, N. *Acta Med. Scand.*, **128**, 317 (1947).
8. SKEGGS, L. T., JR., and LEONARD, J. R. *Science*, **108**, 212 (1948).
9. VANATTA, J., MUIRHEAD, E. E., and GROLLMAN, A. *Am. J. Physiol.*, **156**, 433 (1949).
10. MERRILL, J. P., *et al. J. Clin. Invest.*, **29**, 412 (1950).
11. GUARINO, J. R. *A Study of Gas Emboli Formation with a New Method of Supplying Oxygen and other Gases to the Blood.* New York: Pan-Engineering Company (1946).

Manuscript received November 14, 1951.

Blood Factors in the Nutrition of *Trypanosoma cruzi*[1]

W. L. McRary, E. R. Noble, and E. L. Tondevold
Santa Barbara College of the University of California

Although successful media for the *in vitro* culture of the blood parasite *Trypanosoma cruzi* invariably contain blood or blood derivatives, the nature of the essential blood factors remains ill defined. It appears likely from the work of numerous investigators that hemoglobin is one source of these factors (reviewed by M. Lwoff [*1*] and von Brand [*2*]). Little and his associates (*3–5*) have described media containing as their sole blood component a coagulum made from rabbit erythrocytes, or chick red cells applied on filter paper and autoclaved. Other writers (*6*) have stated that hemoglobin is not necessary. We have obtained satisfactory results using human red cell coagulum in conjunction with a basal medium of glucose, NaCl, and peptone in the concentrations recommended by Little and SubbaRow (*3*). Attempts to extract, concentrate, and identify the essential nutrient or nutrients from the solid coagulum have not been successful. The following experiments, however, have led us to the conclusion that the active principle for our strain of the parasite is a derivative of hemoglobin.

Thrice recrystallized hemoglobin was prepared from 6 times washed human erythrocytes by the method of Drabkin (*7*). The protein was then dissolved in water, dialyzed until essentially free of salts, and stored under sterile conditions after Seitz filtration. Cultures were carried out in a diphasic medium consisting of a 3-ml agar slant and a 2-ml liquid overlay. The basal medium containing 0.2% glucose, 0.5% NaCl, and 2% peptone at pH 7.4 was used in both the agar and liquid phase. Three to 4 mg of hemoglobin was added to the agar phase prior to autoclaving, the agar being used primarily as a solidifying agent. Each subculture was carried out in sextuplicate, using 0.1 ml inocula from the preceding culture, serial transfers being made at 18–22-day intervals, at which time the parasite count was about 12,000,000/ml.

Starting from a stock culture originally obtained from Costa Rica through the courtesy of Herbert Johnstone, of the University of California Medical Center, and grown by us on human red cell coagulum, agar, glucose, NaCl, and peptone, we have carried the organism through 13 serial transfers on the hemoglobin medium, with no diminution in rate of reproduction. Concurrent with the eighth serial subculture, a series of media containing graded amounts of hemoglobin from none to approximately 3 mg was inoculated. Growth responses in this series were essentially proportional to the amount of hemoglobin present. Substitution of hemin, of acid or peptic hydrolysates of hemoglobin, and of a heme-globin mixture for the heat-treated protein have yielded negative results. We were unable to maintain growth beyond the second subculture when the hemoglobin was not heated; however, the addition of ascorbic acid or of serum to unheated hemoglobin has resulted in positive responses to date through 5 and 8 subcultures, respectively.

On the basis of these observations it appears that a moderately complex derivative of hemoglobin is the only additional essential growth factor for our strain of *T. cruzi* when peptone, glucose, and NaCl are present in the medium. Work is in progress in an attempt to determine the nature of the active substances arising from the heat treatment of protein. As part of this investigation other heme-protein combinations are also being studied.

[1] These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the University of California.

References

1. LWOFF, A., Ed. *Biochemistry and Physiology of Protozoa*, Vol. 1. New York: Academic Press (1951).
2. MOST, H., Ed. *Parasitic Infections in Man.* New York: Columbia Univ. Press (1951).
3. LITTLE, P. A., and SUBBAROW, Y. *J. Bact.*, **50**, 57 (1945).
4. SAMPATH, A., and LITTLE, P. A. *Ibid.*, **57**, 265 (1949).
5. LITTLE, P. A., and OLESON, J. J. *Ibid.*, **61**, 709 (1951).
6. SENEKJIE, H. A., and LEWIS, R. A. *Am. J. Trop. Med.*, **25**, 345 (1945).
7. DRABKIN, D. L. *Arch. Biochem.*, **21**, 224 (1949).

Manuscript received August 20, 1951.

The Archaeological and Paleontological Salvage Program at the Medicine Creek Reservoir, Frontier County, Nebraska

E. Mott Davis and C. Bertrand Schultz
Divisions of Anthropology and Vertebrate Paleontology, University of Nebraska State Museum, Lincoln

This report summarizes the results of scientific salvage operations during the past six years by the University of Nebraska State Museum at the Medicine Creek Reservoir. Medicine Creek is a major northern tributary of the Republican River in the dissected loess plains of southwestern Nebraska.

The Bureau of Reclamation completed work on the Medicine Creek Dam in 1949 as part of the Missouri Basin Development Program. Several archaeological and paleontological sites were destroyed in the course of construction work, and many more have been inundated by the Medicine Creek Reservoir, which reached normal pool level in 1951. Following the pattern set for reservoir projects throughout the Missouri Basin, a number of institutions participated in a salvage program aimed at the recovery of as much information as possible from these sites before they were destroyed.[1]

[1] There had been field work in the Medicine Creek Valley previous to the salvage investigations described here (*1–3*).