groups of virus must not be overlooked. For, whereas murine Lansing, MEF and Y-SK virus can often, if not always, be maintained in an unbroken series of monkey generations, murine Col-SK, MM, High Lansing and EMC virus produce paralysis in monkeys only for a limited number of passages. The abrupt extinction of paralyzing power for primates, despite the presence of a potentially virulent agent and the manifestation of extensive lesions in the central nervous system of the infected monkey, constitutes an as yet unsolved problem. The problem is further accentuated by the fact that monkey passage fails to bring about a permanent conversion of the "viscerotropic" Col-SK strain into the "neurotropic" Y-SK strain. If one contrasts the obvious depression of virulence in monkeys—a frequent phenomenon for the Col-SK-MM group of virus—with the ease with which the same viruses can be propagated in mice, one is confronted with several possible conclusions. Either different murine strains of human poliomyelitis virus possess different innate degrees of pathogenicity for monkeys and mice; or, some of these viruses have suffered an irreversible loss of their intrinsic monkey pathogenicity, subsequent to their experimental adaptation to rodents. A third consideration would be that in the case of the Col-SK-MM-EMC-Mengo group of viruses we are dealing with a serologically homogenous group of aberrant members of the poliomyelitis family which, though capable of producing paralytic poliomyelitis in monkeys and probably also in man,(6) are genetically not of human but of rodent origin in nature. This hypothesis might receive support from the recent demonstration of the regional occurrence of virucidal antibodies against EMC and MM virus in the sera of wild rats.(7) Obviously, further study of the complex problem is necessary in order to provide the correct answer.

Conclusions. 1) Col-SK, MM, High Lansing and EMC virus, harvested from remote rodent passages, produce typical poliomyelitis in intracerebrally infected cynomolgus monkeys. 2) Two strains of highly virulent Theiler virus, GDVII and FA, are non-pathogenic for cynomolgus monkeys. 3) The Col-SK-MM group of virus can be carried in cynomolgus monkeys only for a limited number of passages. 4) Judging from serological and biological characteristics, monkey passage fails to bring about a permanent conversion of Col-SK virus into Y-SK virus.

6. Jungeblut, C. W., in preparation.

7. Warren, J., Russ, S. B., and Jeffries, H., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 376.

Received September 8, 1949. P.S.E.B.M., 1949, v72.

Artificial Kidney. II. Construction and Operation of an Improved Continuous Dialyzer.* (17491)

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The insertion of a continuous dialyzer (artificial kidney) in the circulatory system of an animal or a human in uremia has been shown to be capable of removing nitrogenous waste products(1-10) and to result in the adjustment of electrolyte equilibrium.(1,3,8,

* Supported in part by a grant from the Miles Laboratories, Elkhart, Ind.

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1. Kolff, W. L., *New Ways of Treating Uraemia*, London, J. and A. Churchill, 1947.

2. Murray, G., Delorme, E., and Thomas, N., *Arch. Surg.*, 1947, v55, 505.

3. Murray, G., Delorme, E., and Thomas, N., *J. A. M. A.*, 1948, v137, 1596.

4. Alwall, N., and Norviit, L., *Acta med. Scand.*, (Suppl. 196), 1947, v128, 250.

5. Alwall, N., Norviit, L., and Steins, A. M., *Acta med. Scand.*, 1949, v132, 477.

10,11). This continuous dialysis of blood has proved valuable(12,13) in the isolation of substances which are present in blood in low concentrations. The method may also be applied to fundamental physiological studies. (14,15) For such dialysis to be reasonably successful the apparatus used should contain a large area of dialyzing membrane so that the rate of dialysis may be rapid and the process be completed in 1-4 hours. The volume of blood which it contains should be relatively small and constant in order to avoid undue changes in the subject's blood volume. The dialyzing solution on the other side of the membrane should be in a closed system. This is necessary in order to keep a certain amount of CO_2 in solution which makes possible a physiological pH and avoids precipitation of calcium phosphate. The apparatus must be easy to clean, assemble and sterilize and should preferably be inexpensive.

Several artificial kidneys have been described.(1,2,7,12,14,16) Although differing in constructional details they are all characterized by the use of long lengths of cellophane tubing (sometimes greater than 100 feet) and the design most widely used at the present(1) is of an undesirably large size. In a previous communication(17) we described a new design of a continuous dialyzer which met the above requirements more closely than any previously described. Although it has been used in many successful experiments it had one main disadvantage in that the blood being dialyzed was exposed to a large surface of rubber which accelerated clotting and necessitated coating the rubber with a non-wetting silicone resin. This involved a considerable amount of work. The present report describes an improved model in which the above objection is overcome and in which the dialyzing efficiency has been greatly increased.

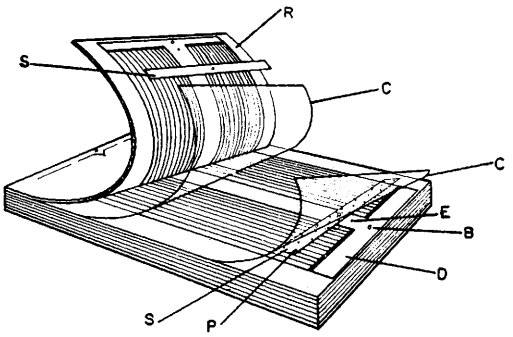

FIG. 1.

Diagram of the artificial kidney. The top rubber pad of an assembly of 4 units has been bent back to show the relafive position of the inner parts.

R—Grooved rubber pads.

C—Cellophane sheets.

S—Stainless steel plates between the two sheets of cellophane. These plates are separated one millimeter by projections (P) in one of the plates.

D—Depression in rubber pads to accommodate the steel plates.

B—Interconnecting tubes for blood. The holes in the steel plates fit exactly over B.

E—Interconnecting tubes for dialyzing solution.

The dialyzer is composed of a variable number of units, each unit consisting of two identical longitudinally-grooved rubber pads‡ (12 inches by 18 inches by 3/16 inch), between which are placed 2 sheets of cellophane (Fig. 1). The two pieces of cellophane are separated at either end by 2 identical pairs of stainless steel plates which at one end provide

6. Alwall, N., Norviit, L., and Steins, A. M., *Acta med. Scand.*, 1949, v132, 587.

7. Alwall, N., *Acta med. Scand.* (Suppl. 229), 1949, v133, 3, 16, 22.

8. Bywaters, E. G., and Joekes, A. M., *Proc. Roy. Soc. Med.*, 1948, v41, 420.

9. Maclean, J. T., Ripstein, C. B., deLeeuw, N. K. M., and Miller, G. G., *Canad. Med. A. J.*, 1948, v58, 433.

10. Vanatta, J., Muirhead, E. E., and Grollman, A., *Am. J. Physiol.*, 1949, v156, 443.

11. Wener, J., and deLeeuw, N. K. M., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 18.

12. Abel, J. J., Rowntree, J. G., and Turner, B. B., *J. Pharm. and Exp. Therap.*, 1914, v5, 275.

13. Abel, J. J., Rowntree, J. G., and Turner, B. B., *J. Pharm. and Exp. Therap.*, 1914, v5, 611.

14. Reineke, R. M., Holland, C. R., and Stutzman, F. L., *Am. J. Physiol.*, 1949, v156, 290.

15. Lim, R. K. S., and Necheles, H., PROC. SOC. EXP. BIOL. AND MED., 1926, v24, 197.

16. Alwall, N., *Acta med. Scand.*, 1947, v128, 317.

17. Skeggs, L. T., Jr., and Leonards, J. R., *Science*, 1948, v108, 212.

‡ The authors wish to acknowledge the invaluable assistance of the Houck Machine Co., Barberton, Ohio, for machining the specially designed steel mold and of A. E. Sidnell and the personnel of the Seiberling Latex Products Co., of Akron, Ohio, for molding the rubber pads.

an opening through which blood may enter the space between the pieces of cellophane and flow in a uniform film to the opposite end where it may leave the apparatus through the second pair of steel plates. Dialyzing solution flows in a countercurrent direction through the grooved spaces in the rubber pads on both sides of the thin layer of blood contained between the two pieces of cellophane. As many such units as desired are easily assembled one above another and clamped with a simple holder composed of 2 flat steel plates. When so assembled all units are automatically connected in parallel through suitable interconnecting holes at both ends of the rubber pads. By appropriate placement of small slips of cellophane between the individual units, they may be connected in series or in series parallel, several combinations being possible. This makes it possible to alter the resistance of the "kidney" to the available flow of blood. For dogs weighing between 15 and 20 kg in which blood is drawn from the femoral artery and returned to the femoral vein the best assembly has been found to be a total of 8 units, 2 parallel sets of 4 units being connected in series.

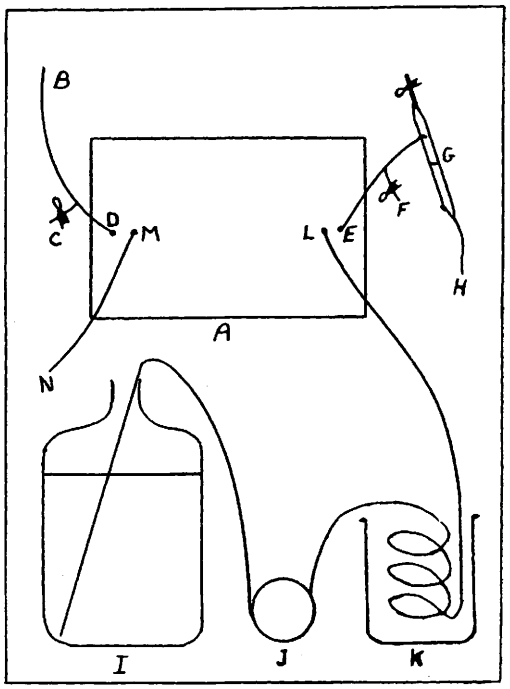

FIG. 2.
Diagram of connections of the artificial kidney to the animal and to the source of dialyzing solution. See text for explanation of letters.

If the "kidney" is properly assembled, there will be no leakage of blood into the dialyzing solution, or between the rubber pads. To detect assembly errors, air is introduced into the spaces to be occupied by the blood at 150-200 mm of mercury above atmospheric pressure. If no decrease in air pressure occurs, the apparatus is considered satisfactory. No holes have yet been found in the cellophane sheets and leaks have never developed during the dialysis. DuPont No. 300 non-moisture-proof cellophane sheets may be purchased cut to size.

The connections between "kidney" and animal are shown in Fig. 2. A rubber tube (B) sufficiently long to lead to the arterial cannula is connected to the blood spaces at one end of the "kidney." A similar tube (H) leads from the other end to the venous cannula. In this line is placed a simple glass air trap (G). It is also convenient to place glass T connections (C and F) in both arterial and venous lines. These are used to fill the "kidney" with blood and to administer intravenous solutions. During sterilization these 2 T tubes may be connected to the dialyzing solution inlet (L) and outlet (M). Steam at 5-15 lb. pressure is run through the arterial tube (B), the "kidney," glass trap (G), and the venous lines (H) for 30 minutes. Following sterilization the "kidney" is cooled and washed by running cold water for several hours through the dialyzing solution spaces only. Just before use the assembled "kidney" is rinsed by passing 6-10 liters of sterile saline through the blood spaces. We have found it convenient to place the kidney on an ordinary hospital bedside table and to reserve the lower part of the table for an apparatus designed to deliver dialyzing solution warmed to 37.5°C at any desired rate. This apparatus consists of a small centrifugal pump§ (J) controlled by a rheostat which propels dialyzing solution from a 5 gallon bottle (I) through a 20 foot length of ¼ inch diameter copper tube coiled in a thermostatically controlled

§ Eastern Engineering Co., New Haven, Conn., Model B-1.

TABLE I.
Composition of Dialyzing Solution for the Dog.

	Milliequivalents per l		
Na^+	148	Cl^-	126
K^+	3	$HCO_3^=$	24
Ca^{++}	5	$HPO_4^=$	2
Mg^{++}	3	Lactate$^-$	7
Total	159	Total	159

Glucose: 100 mg/100 ml
CO_2 gas to pH 7.35-7.45

Preparation of Concentrated Stock Solutions

Sol. A G per l		Sol. B G per l	
KCl	9.0	$NaHCO_3$	84.0
$CaCl_2$	11.1	Na_2HPO_4	5.7
$MgCl_2 \cdot 6H_2O$	12.2	NaOH	9.6
NaCl	269.0		
Glucose	40.0		
Lactic acid	27.1 ml (assay 85-90%)		

500 ml each of solutions A and B are added to 19 liters of distilled water for use. The exact amount of lactic acid required to bring the pH of the diluted dialyzing solution to pH 7.3-7.5 is determined for each lot.

water bath (K), and then to the "kidney" at inlet tube (L). After passing through the "kidney," the dialyzing solution may be collected via tube (N).

The dialyzing solution is prepared to contain the electrolyte composition of the extracellular fluid of the normal dog with the addition of 100 mg of glucose per 100 ml. This composition is calculated from actual analyses of dog plasma obtained in this laboratory over the past 10 years and reported in part by Muntwyler *et al.*(18,19) Solution A (Table I) contains a small excess of lactic acid which upon mixing with the bicarbonate of solution B yields the required amount of dissolved CO_2 to maintain the pH at 7.35-7.45. This makes possible the presence of calcium, phosphate and bicarbonate in physiological concentrations without precipitation.

Twenty dogs have been dialyzed using the above apparatus. Morphine (0.5 mg/kg) or Merperidine Hydrochloride (Demerol) (50-150 mg) was injected subcutaneously and the femoral artery and vein exposed under local anesthesia (0.5% Pontocaine Hydrochloride). A plastic catheter 4 mm in diameter and 4-6 inches long is inserted through the femoral vein into the vena cava. Three mg of heparin per kg of body weight was injected intravenously through this catheter and the femoral artery cannulated. The "kidney" was then filled with heparinized donor blood, connected to the arterial and venous cannulae and blood allowed to flow through the dialyzer. At the end of the dialysis 15 ml of 1% protamine sulfate is slowly injected intravenously.‖ This is usually adequate to restore the blood clotting time to a value of less than 15 minutes. In some animals an additional 50-100 mg of protamine sulfate were found necessary. The cannulae may then be removed. It is imperative that the clotting time be carefully watched if serious hemorrhages are to be avoided.

An arterial pressure of 100 mm Hg resulted in a blood flow of 150-250 ml per minute. Dialyzing solution flows at 400 cc per minute and is under a negligible pressure. The area of cellophane is 12,000 cm^2 which together with a total of 400 cc of blood in the "kidney" gives an average thickness of the film of blood of 0.66 mm. Under these conditions the clearance of urea from the dog's blood is usually about 50 ml of blood per minute (when calculated in a manner exactly analogous to the usual clinical method) and in occasional experiments has been as high as 60-80 ml per minute. It is of interest that creatinine dialyzes through cellophane at a rate only half as great as urea.

Most of the animals tolerated the dialysis without observable effects. Hemolysis did not occur. However, in a few cases the blood pressure decreased to as low as 30-50 mm but returned to normal within 1 to 3 hours. The cause of this effect has not been determined and is now under investigation.

In order to demonstrate the effects of treatment with the artificial kidney, dogs were given 5 mg per kg of anhydrous uranyl nitrate subcutaneously, a dose which is usually le-

18. Muntwyler, E., Mellors, R. C., and Mautz, F. R., *J. Biol. Chem.*, 1940, v134, 345.

19. Mellors, R. C., Muntwyler, E., and Mautz, F. R., *J. Biol. Chem.*, 1942, v144, 773.

‖ Kindly supplied by Paul Lewis Laboratories, Milwaukee, Wis.

20. Donnelly, G. L., and Holman, R. L., *J. Pharm. and Exp. Therap.*, 1942, v75, 11.

21. Gustafson, G. E., Koletsky, S., and Free, A. H., *Arch. Int. Med.*, 1944, v74, 416.

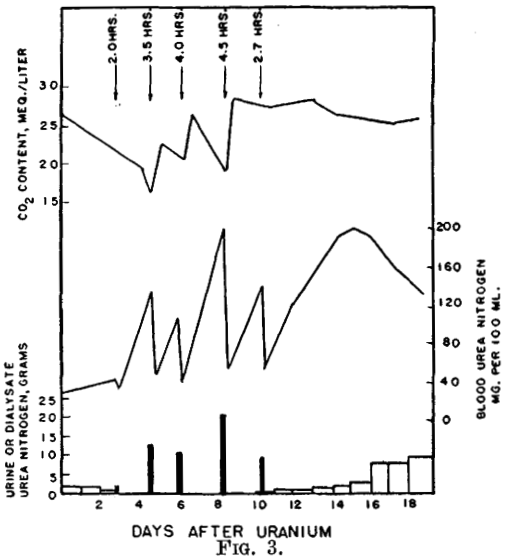

FIG. 3.

The effect of treatment with the artificial kidney on the blood urea nitrogen and the CO_2 content of plasma of a dog given a dose of uranium nitrate (5 mg per kg) which is usually lethal. The arrows represent dialyses of the durations indicated. Note the restoration of the blood urea nitrogen to almost normal levels and the elevation of the CO_2 content towards normal. The solid black bars at the bottom of the chart represent the urea nitrogen removed from the animal by the artificial kidney. This animal recovered.

thal.(20,21) The data on one of these animals are presented in Fig. 3. Three days after the injection of the uranium, the animal became anuric. At this time treatment was instituted and a total of 5 dialyses at 1-2 day intervals were successfully accomplished. The blood urea nitrogen was restored to nearly normal levels by each dialysis. A total of 57 g of urea nitrogen were recovered in the dialyzing solution. The ability of the artificial kidney to regulate acid-base balance is illustrated by the fact that the CO_2 content of the plasma which was decreased as a result of the inadequate renal function was repeatedly elevated toward normal levels. Chloride, sodium, and potassium remained within normal limits. Following the fifth dialysis, 10 days after the dose of uranium, the dog began to excrete urine and survived without further treatment.

Summary. An improved type of continuous dialyzer specifically designed for use as an artificial kidney has been described. A thin film of blood is made to flow between 2 sheets of cellophane supported between rubber pads which are grooved to allow passage of a dialyzing solution. Details of its operation are given and its application in the treatment of acute renal insufficiency in dogs is described.

Received Sept. 13, 1949. P.S.E.B.M., 1949, v72.

Intravenous Infusions of Concentrated Combined Fat Emulsions into Human Subjects.* (17492)

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The successful development of a method for the preparation of a 10% combined fat emulsion and the results after clinical investigation of this emulsion as reported by us warranted further studies in the preparation and administration of more concentrated fat emulsions.(1) The advantages to be derived from the more concentrated intravenous fat preparations, increased caloric potential in reduced volume, would constitute a further advance in the field of parenteral fat nutrition. The present report is devoted to the method of preparation of two combined fat emulsions with fat in 15 and 20% concentrations and a study of the effect of their intravenous infusion on human subjects.

Preparation of Concentrated Fat Emulsions.

* Aided by a grant from the Ben Lewis Fund in Experimental Surgery.

1. Shafiroff, B. G. P., Mulholland, J. H., Roth, E., and Baron, H. C., PROC. SOC. EXP. BIOL. AND MED., 1949, **v70, 343.**