A streptococci *in vivo* had a synergistic relationship with the latter in utilizing hyaluronate as a substrate for energy. On the basis of these findings, a mechanism of pathogenesis by hemolytic streptococci alone and with associated agents of infection was proposed. Certain clinical findings, interpreted as evidence for the existence of such a process, were cited.

It is a pleasure to acknowledge the assistance of Miss Clara Boyd.

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A New Dialyzer for Use as an Artificial Kidney. (18527)

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In the wake of pioneer experimental studies of Abel, Rowntree and Turner(1), the process of dialysis is receiving steadily increasing attention in the treatments of patients with acute anuria(2-8), and in the study of hypertension and renal disease(9,10). Our special interest has been in the application of continuous extracorporeal dialysis of blood for the correction of disturbed ionic equilibrium and the removal of accumulated nitrogenous constituents of the body water as occurs in uremic states. The instrument that was designed incorporates the following desirable features: (1) sterilization by autoclaving, (2) complete visibility of blood and wash channels during operation, (3) provision for rapid exclusion and short-circuiting of defective tubing, (4) true countercurrent flow, (5) surface area adaptable to needs, (6) relative constancy of blood volume, (7) optional and controlled pressure differential between blood and wash electrolyte solution compartments, and (8) reasonable compactness.

A complete dialyzer* is made up of a variable number of units according to the surface area desired, an assembled unit being shown in Fig. 1. Each unit has a cellophane surface area of approximately 4000 cm^2. Fig. 2 and 3 are diagrammatic representations of the top and cross sectional views of such a unit. Top and bottom are formed from a heavy, thermal stress-resistant sheet of plate glass (A), the steel frame (B) is in the middle, and clear plastic gasket sheets (I) are placed between glass and steel. As seen in Fig. 1 the steel frame of each unit forms two double channels, each about one meter in length. After preparation of the cellophane tubing (Visking Corp., 36/32 in. inflated diameter) by repeated boiling in water, and testing with air pressure for leaks, a length of tubing (D) is connected to the nylon fittings (E), sandwiched between the flexible steel, woven, chain-linked screens (C) and placed into a channel. The nylon fittings (cross section Fig. 2) are constructed to provide smooth flow without dead space, folding or leakage. Attachment is made by drawing the end of the cellophane tubing over the inner nylon piece and slipping the steel collar on top. The nylon fittings are then inserted into the openings at both ends of the channel. Turn-

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2. Kolff, W. J., *J. Mt. Sinai Hospital*, 1947, v14, 71.

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

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

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10. Grollman, A., Muirhead, E. E., and Vanatta, J., *Am. J. Physiol.*, 1949, v157, 21.

* Made by Brosites Machine Co., New York City.

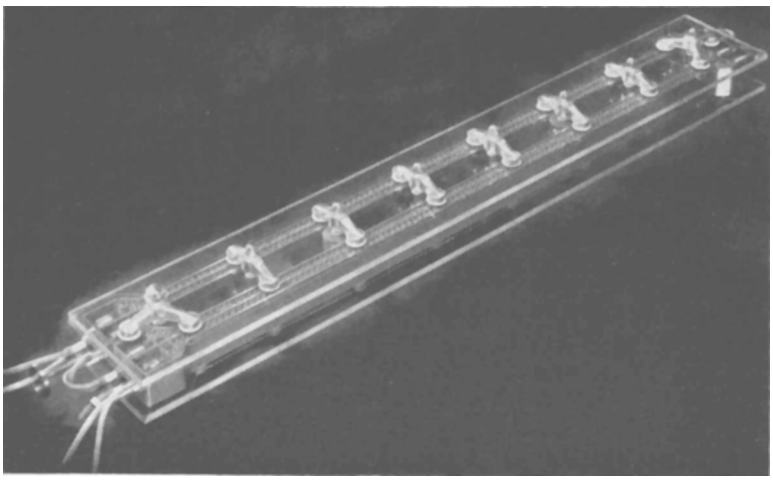

FIG. 1.
Assembled dialyzer unit showing 4 channels.

ing a nut (H) on the outside of the apparatus seals the cellophane-nylon junctions by compressing the collar over the inner piece. The sturdy screens (C), placed on both sides of the cellophane tubing, compress it to about 1 mm thickness. The volume of the blood inside the tubing thereby depends only on the elasticity of the cellophane with changes in pressure and is constant at constant pressure (the volume at a differential pressure of 50 mm Hg is about 300 cc per unit). The connections to the nylon fittings of adjacent channels are made externally with short segments of Tygon tubing. The wash solution flows along the cellophane tubing, though opposite in direction to the blood, to the end of a channel, passes through an opening in the steel frame and enters the adjacent channel. The wash is drained off from the top through a metal tube drain (F). The highest efficiency of transfer is established by a true counter-current flow without channeling. A concentration gradient that is constant, with an optimal rate of exchange, is thereby established. This principle has been extensively used in various commercial dialyzers for laboratory and technological purposes and was first incorporated by our group for clinical use(18). The unit becomes completely sealed upon the tightening of bolts (J) extending through fibre washers in top and bottom plates. This instrument has been reassembled

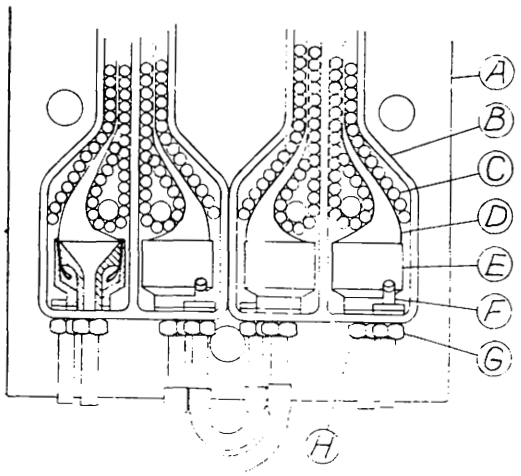

FIG. 2.
Top view of the front end of the dialyzer.

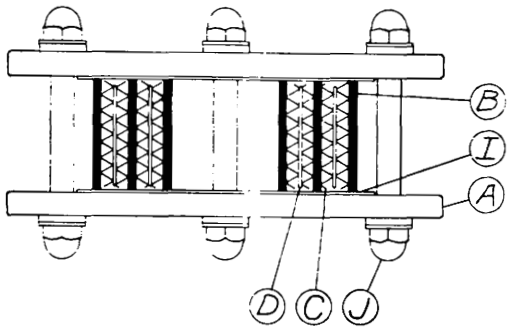

FIG. 3.
Cross-sectional view at the middle of the dialyzer.

18. Rosenak, S. S., Oppenheimer, G. D., Saltzman, A., Exhibit at the annual meeting of the Amer. Urol. Soc., Boston, 1948.

many times, with and without autoclaving, and we found it sufficient to achieve a snug fit by tightening the bolts with an ordinary wrench without danger of breakage of the glass plates. After assembly, and testing for leaks by placing water in the wash compartment and air in the cellophane under pressure of 120 mm Hg, the unit is drained off, nuts are loosened to permit expansion and the unit autoclaved as a whole. Immediately thereafter, while the membrane is still damp, all fittings are tightened, the connecting tubing applied to corresponding fittings and the inner compartment filled with sterile saline. Should the instrument be sterilized for possible use within a time period of about three weeks, instead of filling the units with saline, all outside blood channel connections are joined with each other by means of autoclaved Tygon tubings, while the wash channel connections are similarly joined with autoclaved rubber tubings. The dialyzer thus sealed will maintain sterility for at least 30 days as tested by us repeatedly. The sealed-in moisture prevents the dialyzing membrane from shrinking and getting brittle.

All connections to the dialyzer can be handled from the outside without breaking sterility. Complete visibility of operation is assured by the clear top and bottom plates, clear gaskets and solutions. Any subsequent leakage of blood, which may occur, is immediately detected, located and by-passed. Constant temperature is assured by slow dissipation of heat from the instrument, by warming the wash solution prior to entry into the dialyzer, and is checked by thermometers inserted into channels (Fig. 1). For the sterile propulsion of materials to the dialyzer at appropriate rates, a suitable pump of new design, previously described(15), has been used. In operation our pump permits conclusions (by pre-calibration) as to the speed of blood flow through the dialyzer, this being the resultant of 2 components: the setting of the amount of compression of the tubing and the operation pressure as recorded by a manometer at the blood inlet. The latter is attached to a bubble-catcher which also acts as a sampler.

The resistance of the dialyzer itself allows fast rates of blood flow without undue elevation of intraluminal pressure as can be seen from the following. In recirculation *in vitro* arrangement with the blood passing through a circuit consisting of a reservoir (open glass receptacle), the pump, an inflow bubble catcher, a 12 channel dialyzer hooked up in series, outflow bubble catcher, and return to the reservoir, a blood flow of 500 cc/min was obtained with a pressure of 45 mm Hg inside the cellophane tubing. In *in vivo* operation we similarly find that the intraluminal pressure is more dependent on the resistance created by the catheters leading to and from the venous compartments of the patient, than on the dialyzer. If ultrafiltration is desired a small adjustable clamp is placed on the return side of the blood flow to elevate the resistance of the system to suitable levels. Such controlled hydrostatic pressure is of considerable importance as it permits lowering of the glucose concentration in the electrolyte rinsing solution to more physiological levels, while preventing the exchange of water molecules between the electrolyte rinsing and blood compartments(16). With our apparatus the calculated rate of ultrafiltration at 37°C, an average pressure differential of 105 mm Hg and a cellophane thickness of 0.0008 in., was 0.02 cc/cm^2 of surface area/hour.

A rather important feature of the described apparatus is that it can be sterilized by autoclaving after being fully assembled. Following the latter step the entire unit can be stored in the sterile state, thereby being available for instant use. The autoclaving process adds a margin of safety over instrument sterilized by other means rendering the dialyzer safe for use even after a run on a patient with infectious hepatitis.

Resistance to blood flow is low in this instrument in spite of a good surface to volume ratio obtained by restraining the membrane to 1 mm thickness. It is thus possible to connect the blood compartments in series

15. Saltzman, A., and Rosenak, S. S., *J. Lab. and Clin. Med.*, 1949, v34, 1561.

16. Alwall, N., Eglitis, P., Norviit, E., and Tornberg, A., *Acta med. Scand.*, 1950, v87, 233.

rather than parallel up to a surface area of about 10,000 cm^2 with complete avoidance of channeling which is a necessary drawback to any parallel arrangement without adequate flow control. The dialyzers of MacNeill(11) and of Skeggs and Leonards(12) which are characterized by parallel arrangement of numerous dialyzing compartments show various degrees of channeling. The blood flow in the individual compartments is necessarily uneven (13) and cannot be remedied by stepping up the rate of blood flow. Thus, the blood remaining for longer periods in the "slow" compartments may develop a clotting tendency which in turn leads to the admixture of microscopic blood clots or fibrin strands(14) to the circulating blood. The difference in the resistance of the above dialyzers and the one to be described can further be seen from data as to intraluminal pressures during blood flows of 200 to 300 cc/min. With our design the pressure is 40 to 60 mm Hg, the Skeggs and Leonards instrument runs at 170 to 180 mm Hg, at which a considerable amount of ultrafiltration is produced requiring the addition of parenteral fluids to the patient. With our instrument the pressure inside the cellophane, and thereby the degree of ultrafiltration, can be optionally regulated by the application of an external resistance.

A further advantage of our design is in the use of a chain-linked screen, made of stainless steel, for compression of the membrane. This accomplishes a minimal reduction in surface area of the membrane while providing rigidity in prevention of membrane expansion, along with ease in preparation and removal. Finally, the complete visibility of the blood channels during operation permits location and remedying of defective channels (by short-circuiting) without breaking sterility nor interruption of operations for more than one minute. This is impossible in the multilayer design in which a mishap calls for complete disassemblage. Dialysis of an aqueous solution of creatinine in various concentrations was performed with our instrument against water at 37°C with 3500 cm^2 of cellophane in the dialyzer. The creatinine solution flowed at a rate of 130 cc/min, and tap water in counter current at 100 cc/min. The rate of dialysis of creatinine under these conditions is given in Table I.

TABLE I. Dialysis of Creatinine at Various Concentrations.

Creatinine level, mg %	Creatinine dialyzed/hr, mg
5.6	75
7.8	101
10	166
20	335

Under the above conditions of temperature, flow rates, and surface area, with 200 mg % urea solution, a dialysis of 5 g urea per hour was effected (urea clearance of 42 cc/min). This compares favorably with the clearance of the "Artificial Kidney" of Skeggs *et al.*(12) which is 50-80 cc/min/12,000 cc surface area. Such surface area can be obtained by using 3 of our units in parallel flow. Such a battery could yield easily a urea clearance of 125 cc/min. Similarly a 5% ammonium sulfate solution was dialyzed against water over a surface area of 7500 cm^2. The sulfate solution flowed at a rate of 15 cc/min while the speed of the tap water was 400 cc/min at a temperature of 41°C. Under these conditions the concentration of the sulfate solution dropped during the run to 0.02%. Surface areas of 4000 to 12,000 cm^2 have been applied to the dialysis of uremic patients(17). To illustrate the effectiveness of this instrument we quote an experimental case of terminal uremia in a patient with chronic glomerulonephritis. In a 3 hour run on this patient with an initial blood urea nitrogen of 230 mg %, 50 g of urea nitrogen was cleared over a cellophane area of 9500 cm^2, at an average blood speed of 300 cc/min and an average electrolyte "wash" flow rate of 200 cc/min. 8 hours after conclusion of dialysis

11. MacNeill, A., Exhibit at A.N.A. convention, 1949. Atlantic City.

12. Skeggs, L. T., Leonards, J. R., and Heisler, C. R., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 539.

13. Leonards, J. R., personal communication.

14. Miller, G. E., personal communication.

17. Saltzman, A., Rosenak, S. S., Oppenheimer, G. D., Sirota, J., and Bortin, M., unpublished data.

the blood urea nitrogen dropped to 150 mg %.

The electrolyte solution was prepared from sterile concentrates† and had the following standard composition as expressed in g %. Dextrose 1, NaCl .610, $NaHCO_3$ .220, KCl .0360, $CaCl_2$. $6H_2O$ .0450, $MgCl_2$. $6H_2O$ .0107, $NaH_2PO_4H_2O$ .0058.

Summary. An efficient and safe dialyzer‡ suitable for continuous operation under sterile conditions is described. It is made up of a variable number of component channels according to surface area desired. Sterility is accomplished by autoclaving as a whole and optional controlled ultrafiltration is possible.

† Obtained by courtesy of Eli Lilly Co., Indianapolis.

‡ The authors gratefully acknowledge the cooperation of Messrs. J. L. Hutchings, G. H. Weber, and R. Burger, who gave generously of their time, materials and practical experience to the development of this instrument.

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Chemotherapeutic Effectiveness of Alloxan in Murine Bartonellosis.* (18528)

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Alloxan, an oxidation product of uric acid, is present in combined form in several compounds which are of importance in mammalian physiology, and there is some evidence for its occurrence in the free state in blood and other animal tissues(1). It has been extensively studied in recent years because of its specific toxic action on the beta cells of the pancreatic islands, resulting, when suitable dosages are used, in the experimental production of diabetes(2,3). There is evidence that its diabetogenic effect is caused by reaction with SH groups in enzyme systems(4). Alloxan, as far as we can learn, has not previously been tested for chemotherapeutic activity in any infectious disease.

Material and methods. Latently infected (carrier) rats of the St. Louis University strain were used. Previous work with several hundred rats of this strain has shown that splenectomy is invariably followed by severe bartonellosis. The organisms appear in the erythrocytes on the 2nd or 3rd day after removal of the spleen (occasionally on the 1st day), reach a maximum on the 5th or 6th day, and disappear, in surviving animals, by the 9th or 10th day. The highest daily bartonella counts are rarely below 100 per 100 erythrocytes, and the mortality is usually about 50%.

All rats in the experiments to be reported here were maintained throughout the observation periods on a low sulfur diet (LSD) with the following composition: casein 6%, salt mixture 4%, sucrose 15%, corn starch 48%, lard 22%, cod liver oil 3%, and debitterized brewers yeast powder 2%.

In Exp. 1, 20 rats, previously maintained for 10 days on LSD, were splenectomized. Ten of these served as controls. The remaining 10 were given daily intraperitoneal injections (40 mg per kilo) of freshly prepared solutions of alloxan in distilled water for 13 days, starting 4 days before splenectomy.

In Exp. 2, 14 rats, previously maintained for 3 days on LSD, were splenectomized. Four rats served as controls. The remaining 10 were given daily intraperitoneal injections

* This investigation was supported, in part, by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

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2. Dunn, J. S., Sheehan, H. L., and McLethchie, N. G. B., *Lancet*, 1943, v1, 484.

3. Lukens, F. D. W., *Physiol. Rev.*, 1948, v28, 304.

4. Lazarow, A., PROC. SOC. EXP. BIOL. AND MED., 1946, v61, 441.