because of the instability of the phenylalanine-oxidizing liver enzyme(3). It should be noted, however, that the controls gave positive results, although the livers were removed somewhat later than in the phenylketonuric patients. Moreover, the amount of tyrosine formed in Control 1 was comparable to that reported by Udenfriend and Cooper(3) in an experiment with rat liver slices in which the liver was immediately removed and all operations carried out in a cold room. In order to avoid further deterioration of the enzymatic activity, no attempt was made at dialyzing the liver preparation and only the crude extract was used.

Although there is a remote possibility that the observed failure to find tyrosine in the phenylketonuric preparations might be the result of a very rapid deamination of phenylalanine to phenylpyruvic acid, the most likely explanation is that the specific enzymatic system described by Udenfriend and Cooper(3) is absent in phenylketonuria. This absence is apparently the essential metabolic characteristic of the disease; in fact, as previously noted(5), the main biochemical findings may be explained on the assumption of a failure in the normally occurring conversion of phenylalanine to tyrosine. Furthermore, the demonstration of the absence of a specific enzyme clarifies the observation that phenylketonuria is determined by a single recessive gene(1), since several instances are on record of specific enzymatic reactions being controlled by single genes.

Summary. Liver extracts of two phenylketonuric patients failed to catalyze the conversion of phenylalanine to tyrosine in the presence of oxygen, phosphopyridine nucleotide and nicotinamide.

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Cross-Dialysis: Description of a Possible Method of Temporary Kidney Substitution. (20164)

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The several types of artificial kidneys and their clinical applications have been adequately described(1). Basically they consist of two compartments separated by a dialyzing membrane. Blood of the patient is circulated through one compartment and a bath solution is circulated through the other. The dialyzable substances tend to pass from the compartment in which their concentrations are higher into that compartment in which their concentrations are lower. The rate of transfer depends to a great extent on the physical characteristics of the dialyzable substances; the type, thickness and area of the dialyzing membrane; and the concentration gradient present.

The purpose of this study was to design a system of cross-dialysis whereby the blood of a uremic subject would be cross-dialyzed with that of a subject with normal kidneys. There would be no mixing of blood between the two circulations. In such a system it is anticipated that the normal subject would remove through dialysis "toxic products" from the blood of the uremic subject and then utilize its own kidneys to excrete them. This system would not be an artificial kidney in the true sense as it would use a normal pair of kidneys to regulate certain functions which the patient with diseased kidneys would be unable to do. The following results might be expected: a) Re-

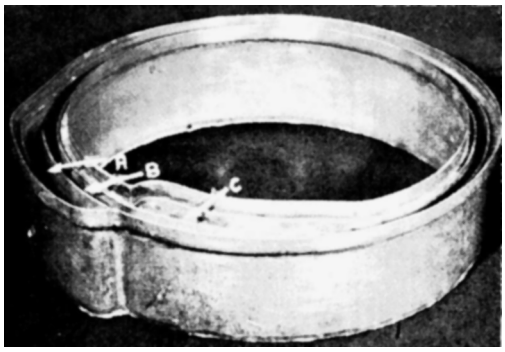

FIG. 1. Dialyzing unit with cover removed. A and C indicate sections where ends of cellophane tubing are brought out for connections. B indicates circular dialyzing compartment which measures 14" in diameter, 3⅛" in height and 5/16" in width.

moval and excretion by the normal subject of such products of metabolism as non-protein nitrogen compounds and possible unknown toxic products which might play a role in the uremic syndrome. b) Automatic control of electrolytes. Those in abnormally low concentration in the patient would be corrected by inflow from the normal subject, and those in abnormally high concentration in the patient would dialyze out and be excreted. c) Similar control as in (b) but for elements and compounds not measurable by present-day laboratory procedures or for which the clinical significance is not yet known.

Method. To accomplish these aims the following apparatus has been devised and constructed. The dialyzing unit, made of stainless steel (Fig. 1) is circular in shape, measuring 14" in diameter and 3-5/8" in height. The width of the dialyzing section is 5/16". Two lengths of cellophane* tubing, measuring 2-7/8" diameter flat, are placed against each other and are then wound as one for the required number of turns into the hollow section of the unit. When this procedure is carried out, it will be found that every other turn of cellophane represents the same circuit, thereby bringing the dialyzing surface of one circuit directly in contact with the other. The two ends of each length of cellophane tubing are brought out in sections A and C respectively (Fig. 1) where connections are made to 3/8" diameter polyethylene tubing which is used to bring the subjects' blood to and from the unit. The stainless steel cover, which is not water tight, is applied. Its primary purpose is to help keep the cellophane tubing in place. The present units were used with 8 turns of cellophane tubing representing approximately 5,600 cm^2 of opposing dialyzing surfaces for each circuit. Turns 1, 3, 5 and 7 represent one circuit and turns 2, 4, 6 and 8 represent the other circuit. After assembling, the unit is washed and autoclaved. Immediately prior to operation, it is rinsed with sterile saline and primed with approximately 175 cc of citrated blood per circuit. In the experimental animals, blood from an abnormal subject (uremic) is taken from the femoral artery via a plastic catheter. Its movement is facilitated by the use of a roller-type pump which controls the flow of blood through both circuits. The blood then passes to the dialyzing chamber which is suspended in a water bath kept at 39° C. It flows through the cellophane tubing which is kept compressed, almost flat, by the surrounding tubing and the walls of the unit to give maximum surface area with minimum volume. No shifting of tubing during operation has been noted with the present units. When the blood of the uremic subject is brought into close contact (separated by permeable membranes) with the blood of a normal subject, dialysis takes place. After leaving the dialyzing chamber the blood then flows through a bubble trap and flow indicator and back into the femoral vein of the uremic subject. The blood circuit from the normal subject follows a similar course. Nembutal is used for anesthesia, and clotting is prevented by the use of heparin.

Results. In vitro studies were done with the above apparatus, using 12 liters of a solution containing urea, sodium chloride and potassium chloride in concentrations indicated in Fig. 2. This solution was dialyzed against tap water. There were approximately 6700 cm^2 of opposing dialyzable surface per circuit with a rate flow of approximately 90 cc per min per circuit. Specimens for analysis were taken at intervals from the solution being dialyzed. Results are shown in Fig. 2. As expected, the rate of dialysis decreased as the concentration gradient became smaller. This

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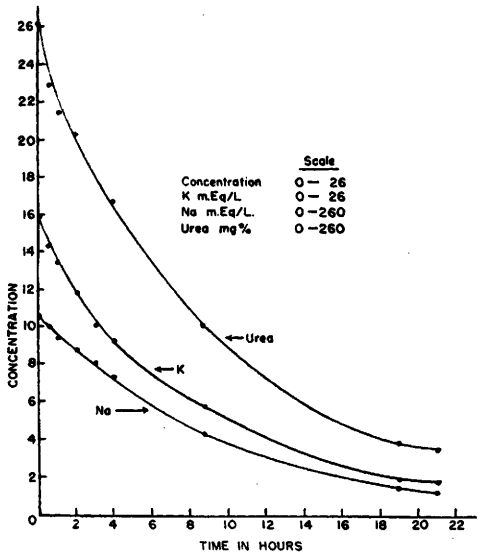

FIG. 2. Rates of dialysis. Above concentrations contained in 12 liters of solution.

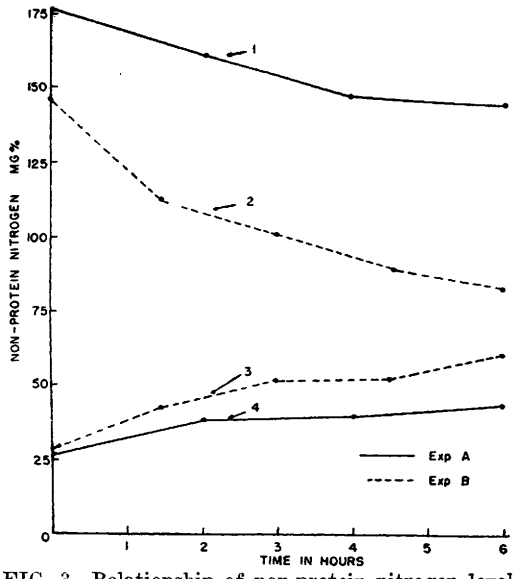

FIG. 3. Relationship of non-protein nitrogen level to hours of dialysis.

experiment was repeated three times with similar results.

In vivo studies were done using dogs as the experimental animals. At first, 2 normal dogs were dialyzed against each other for approximately 8 hours. The apparatus had no ill effects on the animals. Then dogs were made uremic by ligation and division of both ureters. Approximately 48 hours after ligation, the uremic dogs were dialyzed against normal dogs for 6 hours. Fig. 3 shows non-protein nitrogen levels plotted against hours of dialysis. Curves 1 and 2 represent animals with ureters ligated and divided approximately 48 hours before dialysis was begun. Curves 3 and 4 represent normal dogs. In Exp. A one unit was used with 4 turns of cellophane tubing per dog representing approximately 5600 cm^2 of opposing dialyzable surface per dog. The rate of flow was approximately 80 cc per min per dog. Exp. B represents 2 units similar to the above connected in parallel giving approximately 11200 cm^2 of opposing dialyzable surface per dog. The rate of flow for this run was approximately 180 cc per min per dog.

The series is small, consisting of 5 experiments with uremic animals dialyzed against normal animals. In every case, the normal animals survived without apparent deleterious effects. There was no apparent hemolysis. The uremic animals subjected to the above procedure survived approximately 3 days after time of dialysis, a total of 5 days from the time the ureters were ligated. It is believed that the results in Exp. B, using the area and flow noted, were satisfactory. At 6 hours, the concentration gradient between the two animals was small, there being only 20 mg % of non-protein nitrogen difference between the two circulations. The retention of non-protein nitrogen compounds by the normal dogs as shown by curves 3 and 4 is probably to some extent the result of decreased renal function due to prolonged anesthesia(2,3) during dialysis.

Discussion. The method as described demonstrates that it might be conceivable to dialyze a subject in renal failure against a volunteer with normal kidneys. Moreover, one might consider the possibility of dialyzing a patient against a normal animal. It is questionable whether transmission of antigens would occur. The cellophane membrane used will not pass bacteria, viruses, or large molecules such as proteins. If the tubing in one circuit should rupture, it would be unable to penetrate into the other system because rupture of a membrane is immediately detected by the appearance of blood on the surface of the unit. Also, two simultaneous ruptures would be necessary,

one in each circuit, for blood to pass from one system to the other.

Summary. Apparatus and technic for cross-dialysis is described. It is believed that the procedure is relatively uncomplicated, safe and requires few laboratory checks during operation. Only further extensive studies will determine whether or not it has any value as a clinical tool.

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Conjugation of N-Allylnormorphine by Liver Slices.* (20165)

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The respiratory depression caused by an overdosage of morphine can be abolished by small amounts of N-allylnormorphine† (1,2,3). N-Allylnormorphine and morphine differ only in the alkyl group attached to the nitrogen; morphine has a methyl group at this position, whereas N-allylnormorphine has an allyl group attached to the nitrogen. Previous reports(4) have stated that the phenolic hydroxyl group in morphine is the site primarily responsible for the effect on respiration, but now it is evident that changes on the nitrogen atom are important in this respect.

In man(5) and dogs(6) morphine has been reported excreted in urine in both a free and combined state. A study of the action of liver slices on N-allylnormorphine was undertaken to determine whether it was conjugated in the liver like morphine(7) and whether there might be a significant difference in the rate of conjugation to account for the blocking ratio of one molecule of N-allylnormorphine to sixty-seven molecules of morphine (8).

Methods and results. The procedure of Snell and Snell(9) as used by Bernheim(7) was used for the estimation of morphine. This method probably involves the reactivity of the phenolic and alcoholic hydroxyl groups of morphine, for codeine and dihydromorphinone, in which these groups are altered, do not give the same intensity of color as morphine. N-allylnormorphine is identical to morphine except for the replacement of the methyl group on the nitrogen by an allyl group and would be expected to give the same color intensity with the Snell and Snell reagent. That this is so is shown in Fig. 1. The color developed with the silicomolybdic acid reagent is the same for both morphine and N-allylnormorphine in the concentrations used.

In order to study the rate of conjugation of morphine and N-allylnormorphine, livers from dogs anesthetized with sodium pentobarbital were sliced in the usual way. About 300 mg of tissue (wet weight) was placed in 4 ml of Krebs-Ringer bicarbonate solution with either 1.0 mg of morphine or N-allylnormorphine. The slices were incubated at 38°C with a gas phase of 5% carbon dioxide and 95% oxygen in a Dubnoff shaker. Suitable

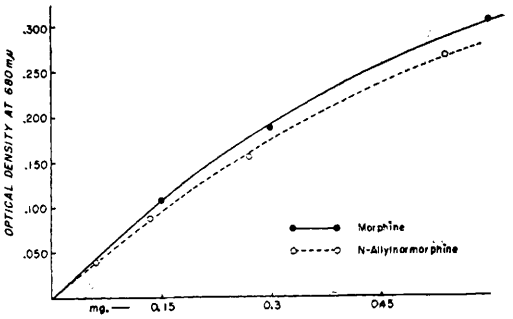

FIG. 1. A comparison of the optical density with the silicomolybdic acid reagent between morphine and N-allylnormorphine.

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† N-Allylnormorphine was obtained through the courtesy of Merck & Co., Rahway, N. J.