6. Kinsell, L. W., Michaels, G. D., Partridge, J. W., Boling, L. A., Balch, H. E., and Cochrane, G. C., *J. Clin. Nutrition,* 1953, v1, 224.

7. Ahrens, E. H., Jr., Blankenhorn, D. H., and Tsaltas, T. T., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 872.

8. Bronte-Stewart, B., Antonis, A., Eales, L., and Brock, J. F., *Lancet,* 1956, v1, 521.

9. Ahrens, E. H., Jr., Tsaltas, T. T., Hirsch, J., and Insull, W., Jr., *J. Clin. Invest.,* 1955, v34, 918.

10. Beveridge, J. M. R., Connell, W. F., and Mayer, G. A., *Circulation,* 1956, v14, 484.

11. Keys, A., Anderson, J. T., and Grande, F., *Lancet,* 1957, v1, 66.

12. Keys, A., *J. Chronic Dis.,* 1956, v4, 364.

13. Malmros, H., and Wigand, G., *Lancet,* 1957, v11, 1.

14. Ahrens, E. H., Jr., Insull, W., Jr., Blomstrand, R., Hirsch, J., Tsaltas, T. T., and Peterson, M. L., *ibid.,* 1957, v1, 943.

15. Anderson, J. T., Keys, A., and Grande, F., *J. Nutrition,* 1957, v62, 421.

16. Beveridge, J. M. R., Connell, W. F., and Mayer, G. A., *Fed. Proc.,* 1957, v16, 11.

17. Kingsley, G. R., and Schaffert, R. R., *J. Biol. Chem.,* 1949, v180, 315.

18. Anderson, J. T., and Keys, A., *Clin. Chemistry,* 1956, v2, 145.

19. Abell, L. L., Levy, B. B., Brodie, B. B., and Kendall, F. E., *J. Biol. Chem.,* 1952, v195, 357.

20. Keys, A., Anderson, J., and Brozek, J., *Metabolism,* 1955, v4, 427.

Received June 21, 1957. P.S.E.B.M., 1957, v96.

Procedure for Transplantation of Dog Kidney without Interruption of Renal Blood Supply.*† (23462)

P. NATHAN, L. J. WILCHINS AND BENJAMIN F. MILLER

May Institute for Medical Research, Cincinnati Jewish Hospital Assn., and Departments of Physiology and Medicine, University of Cincinnati College of Medicine.

Kidneys transplanted from one dog into another are usually rejected within five to ten days(1). A characteristic pathology develops in these tissues and it is generally accepted that this rejection is mediated through an immunological reaction(2). The operative procedure previously employed for transplantation requires an interruption in the blood supply for at least 20 minutes. The contribution of the ischemic period to the development of the subsequent metabolic and pathologic states is unknown; however, it seems likely that it may produce non-specific damage to the renal tissues. We have devised a technic whereby the kidney can be transplanted without interruption of its blood supply, thus permitting study of the rejection process without the complicating factor of ischemia.

Methods. In principle, the new procedure takes advantage of the fact that the aorta and vena cava form a "T" connection with their respective renal vessels. This structural arrangement permits perfusion of the renal vessel from below during the time when the upper section of the aorta or vena cava must be occluded for the vascular suturing. For example, in the arterial system the blood may flow into the renal vessel either from above as it normally does or from below the point of origin of the artery on the aorta. In our procedure, the accessory circulation provides arterial blood for the kidney through the aorta via a connection through a plastic tube with the host's carotid artery. This blood enters the aorta below the point of origin of the renal vessel. The same principle is used to return the renal venous blood through the vena cava below the point of origin of the renal vein, and back through the external jugular to the donor's heart. The donor animal is prepared by dissection of the left kidney, its ureter and the renal vessels. The aorta and vena cava are also freed for a distance of 1½ inches above and 1½ inches below the renal vessels. All of the major abdominal vessels except those referred to above,

* Supported in part by the Josiah Macy, Jr. Fn.

† The authors gratefully acknowledge the assistance of Genevieve Edwards, R. N.

METHOD FOR TRANSPLANTATION OF KIDNEY WITHOUT INTERRUPTION OF ITS BLOOD SUPPLY

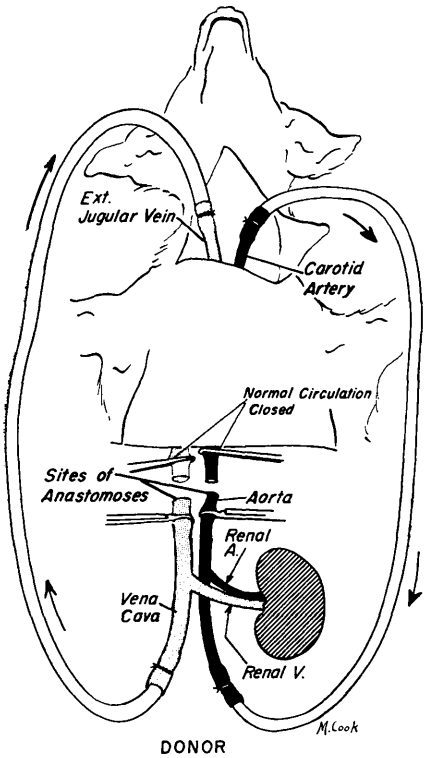

FIG. 1. This diagram illustrates state of the donor just before kidney is transferred to the host. Note sites of anastomoses which will be connected to iliac vessels of the host.

are tied and divided. The carotid artery and jugular vein are also isolated and, as mentioned above, these vessels are employed to provide the accessory circulation. A polyethylene tube about 2½ feet long is filled with saline and inserted into the carotid artery. The opposite end of this tube is put into the aorta below the renal artery. Clamps are applied until a flow of blood through the tubes is desired. A similar procedure is used for the venous system to connect the vena cava distal to the renal vein with the donor's external jugular vein. Clotting in the tube is controlled by intravenous administration of heparin. During the manipulations described above, the normal channels for renal blood flow are intact. The clamp on the tubing is now removed so that blood flows from the carotid artery into the aorta and from there through the renal artery to supply the kidney. At the same time that the clamp is removed from the tubing, another clamp is placed on the aorta above the renal artery so that the normal route of blood flow is blocked. The venous system is treated in a similar manner. The venous blood now flows from the renal vein into the vena cava and then via the plastic tubing into the external jugular vein of the donor. The aorta and vena cava are tied and divided rostral to the renal vessels. The kidney can be lifted from its bed and moved to an adjoining operating table without interruption in its blood supply. Fig. 1 illustrates the conditions prevailing just prior to the time the donor kidney is removed from its normal position.

The host has already been prepared on the adjoining operating table. Its iliac vessels on the side nearest the donor are isolated, clamped and divided. The aorta and vena cava are anastomosed to the iliac artery and vein, respectively. The most difficult part of the surgery results from the need to adjust the diameter of the cut end of the vessels so that there will be no subsequent constriction at the suture line. The circumference of the smaller iliac vessels can usually be enlarged by cutting it at an angle. In this way it is possible to match it to the larger aorta or vena cava.

When the vascular anastomoses are completed, the clamps on the host's vessels are removed to allow the host's blood to pass through the kidney. At the very moment that these clamps are released, another set is applied to the tubing, blocking the donor blood flow to the kidney. Thus, at no time is there an interruption in the blood supply to the kidney nor an interchange of donor and host blood.

The transplanted kidney is placed in the abdominal cavity of the host and its ureter is exteriorized through a stab wound. Urine can be collected from the isolated ureter.

The kidney has a continuous blood supply throughout the procedure. Pulsations from the renal artery can be directly observed during the operation. The capsule retains its normal pink color, and some of its vessels are noted to bleed freely. The ureter remains so well vascularized that the blood loss from its cut end must be controlled. Equally im-

portant, urine is observed to flow throughout the operation, and continues without interruption when the host's blood is started through the kidney.

Twenty-four operations have been performed. One of the animals failed to produce urine and was discarded within 4 hours following surgery. Twenty-three of the operations were acutely successful. However, 10 of these kidneys failed to function for more than one day due to the formation of a thrombus in the renal vein or artery. Thirteen of the transplants continued to secrete urine for periods of one to 8 days, at which times the kidneys were removed.

The lack of ischemia during the transplantation operation does not confer a marked prolongation of function to the kidney. However, there appear to be some benefits from transplantation without interruption in the blood supply. The tissue in Fig. 2 illustrates the extent of the rejection process six days after transplantation. The cellular infiltration is not prominent. On the other hand, a kidney which has been subjected to a period of ischemia during the operation (Fig. 3) shows a marked infiltration of white cells as well as a considerable amount of necrosis when it has been present in the host for 6 days (see also 2).

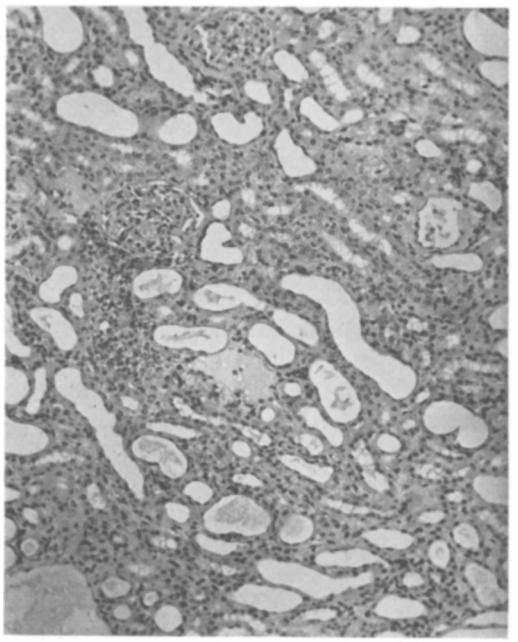

FIG. 2. Section from kidney transplant 6 days after introduction into host (H & E, 100×). This kidney was transplanted without an interruption in its blood supply. Note lack of white cell infiltration.

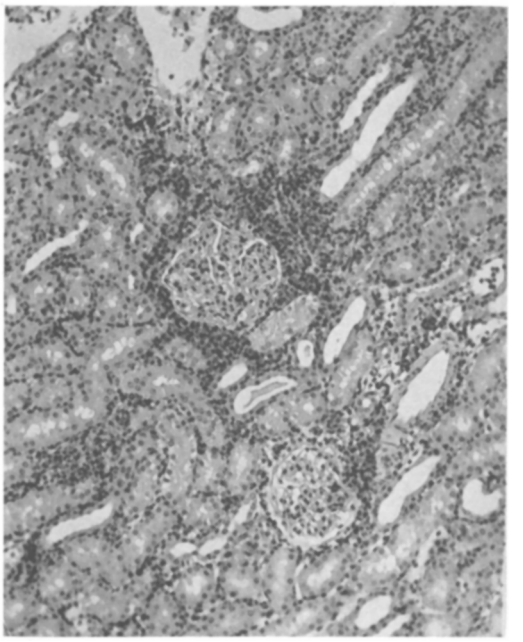

FIG. 3. Section from kidney transplant showing a marked infiltration of white cells, 6 days after introduction into the host (H & E, 160×). This kidney was subjected to a period of ischemia during the operation.

Summary. A technic is described for transplantation of the dog kidney from one animal to another without any interruption of the organ's blood supply. An accessory circulation from the neck vessels of the donor animal is provided for the kidney during the time required to connect the renal vessels to the host's vascular system. This procedure permits the study of the transplant rejection process without the ischemia which is a complicating factor in the technics usually employed. Transfer of the kidneys without ischemia does not markedly prolong the period of function, however the pathological changes which occur within the first few days following transplantation may be delayed.

After this work was completed and the manuscript submitted for publication, our attention was drawn to the papers of Paul Govaerts who used similar but not identical procedures to those described

in our article to carry out acute experiments on the effect of diuretics on kidney function (P. Govaerts, *C. R. de la Soc. Biol.*, 1928, v99, 647; *Arch. Internat. Pharmacodyn. et de Thérapie*, 1929, v36, 99). The technic was also employed by Brull (*C. R. de la Soc. de Biol.*, 1931, v107, 248, 249) for short-term experiments.

1. Williamson, C. S., *J. Urol.*, 1923, v10, 275.

2. Simonsen, M., *Biological Incompatibility in Kidney Transplantation in Dogs*, Ejnar Munksgaard, Copenhagen, 1953.

Received June 27, 1957. P.S.E.B.M., 1957, v96.

Colorimetric Method for Estimating Number of Cells in Monolayer Cultures without Physiological Damage.* (23463)

CATHERINE RAPPAPORT (Introduced by J. L. Melnick)

Section of Epidemiology and Preventive Medicine, Yale University School of Medicine, New Haven, Conn.

A colorimetric method for determining the number of cells in monolayer cultures of monkey kidney cells has been developed. The method depends on production of hydrogen ions by the cells and consists in measuring change in absorption of a standard physiological salt solution of phenol red after a 15-second exposure to the cultures. The procedure avoids the physiological damage usually resulting when cells are removed from the glass surface for counting and makes it possible to estimate reliably the number of cells in the particular culture which is being studied.

Studies on many thousands of cultures during the last 6 months have shown that the amount of phenol red acidified under these conditions is correlated with the number of cells in the culture over a wide range of cultivation conditions and of age. The method proved useful particularly as a method of selecting from among a large number of cultures those having the same cell number within ± 10%. The mechanism of the reaction is not completely understood, and it is therefore not possible to evaluate all of the factors which might give anomalous results. In this paper the details of the procedure and factors controlling the sensitivity of the method will be given. Some of the possible sources of error, as determined from these limited studies, will be considered.

* Aided by grant from The National Foundation for Infantile Paralysis.

Material and methods. Monolayer cultures of monkey kidney cells were grown out from freshly trypsinized rhesus monkey kidneys in either medium M + H (0.5% lactalbumin-hydrolysate, 2% calf serum, Hanks' salt solution) (1) or the completely synthetic, protein-free medium SM-2(2) in 2-3 oz Brockway prescription bottles. *Phenol red* was the commercial LaMotte soluble product used either as obtained or after recrystallization from dilute alcohol. *Cell counts* were done by a modification of the Parker(3) method for counting nuclei. The modifications consist in using small volumes of citric acid to obviate the need to concentrate the cells by centrifugation and in adding the crystal violet within at least 5 minutes after the citric acid in order to stop the continued swelling and bursting of the nuclei. The exact procedure is as follows: 1) The supernatant fluids from the cultures are decanted, the bottles drained, and the cell layer covered with 1% tannic acid for 1½-2 minutes. 2) The tannic acid is decanted, the bottles drained on adsorbent paper, and exactly 2 ml of 10% citric acid is added to the cell layer. 3) After about a minute in contact with the citric acid, the bottles are shaken vigorously for 10-15 seconds. The time required to remove the cells from the glass and the amount of shaking depends on the age of the cells and whether they have been grown in a serum-containing or protein-free medium. With both cell types the older cultures (more than 5 days) are removed easily in 15-30 seconds, whereas younger cul-