acids on cholesterol metabolism are not due to the exhaustion of amino acids used in formation of glycine and taurine conjugates.

1. Beher, W. T., Baker, G. D., *Am. J. Physiol.*, 1959, v197, 1339.
2. Beher, W. T., Anthony, W. L., Baker, G. D., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 317.
3. Siperstein, M. D., Jayko, M. E., Chaikoff, I. L., Dauben, W. G., *ibid.*, 1952, v81, 720.
4. Beher, W. T., Baker, G. D., Anthony, W. L., *Am. J. Physiol.*, 1960, v199, 736.
5. Norman, A., *Arkiv. f Kemi*, 1955, v8, 331.
6. Sjövall, J., *ibid.*, 1955, v8, 317.
7. Beher, W. T., Baker, G. D., Anthony, W. L., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 385.
8. Frantz, I. D., Davidson, A. G., Dulit, E., Mobberley, M. L., *J. Biol. Chem.*, 1959, v234, 2290.
9. Siperstein, M. D., *Am. J. Clin. Nutrition*, 1960, v8, 645.
10. Beher, W. T., Anthony, W. L., Baker, G. D., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 531.

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A Method for Transplantation of the Rabbit Kidney.* (26530)

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The study of kidneys transferred from one dog to another has provided important information on the transplantation reaction. Hume(1) has recently reviewed the work of Carrel, Williamson, Dempster, Simonson and others in this field. However, extensive employment of the kidney in transplantation research has been limited because currently available surgical technics necessitate the use of large animals such as dogs. The expense of preparation, maintenance and sterile surgical procedures required in dogs has discouraged their widespread use in such studies. Some of these difficulties can be eliminated by transplanting organs in the rabbit. This report describes a method which for the first time makes possible chronic homologous transplantation of the rabbit kidney by vascular anastomosis. This method has been employed to show the histological changes in the rabbit kidney during the rejection process.

Method. Selection and preparation of host. The recipients of kidney transplants were randomly bred albino rabbits, weighing about 4.0 kg. They were anesthetized by intravenously administered Nembutal (25 mg/kg) and placed on their backs with legs securely tied to the operating table. In this position, the retraction of the rabbit's neck often produces difficulties with respiration. To overcome this problem, 100% oxygen was administered under the surgical drape which covers the animal's head. The drape acted as a tent which partially contained the gas.

An intravenous drip containing 30 mg Nembutal and 2.5 mg heparin per 100 cc saline was connected to a peripheral ear vein. The rabbit received about 75 cc of this solution during a 2½ hour period. The heparin was given during surgery because it appeared to help prevent clotting at sites of the anastomoses. No additional anticoagulant was given to the host after operation.

A clean surgical procedure was used, but sterile precautions were not observed. The carotid artery and jugular vein on one side of the recipient rabbit's neck were isolated, clamped and divided, then their distal ends

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were tied with silk ligatures. Their proximal stumps were later anastomosed to the donor vessels.

Selection and preparation of donor. Kidney donors were young, outbred New Zealand or Dutch rabbits, weighing one-half to one kilogram. Size of the donor and recipient was selected so that the vessels to be connected would be approximately of the same diameter. The left kidney, renal vessels, and ureter were isolated through an abdominal incision. Because of the lower position of the left kidney and the length of its renal vein, it is more easily transplanted than the contralateral organ. A few centimeters of abdominal aorta and vena cava *above and below* the origin of the renal vessels were dissected free. All of the branches, except the renal vessels, were tied and divided. The donor was then given an intravenous injection of one mg of heparin.

Preparation of anastomoses. The isolated aorta and vena cava were next tied off about 2 cm above and below the origin of the renal vessels to obtain adequate T-sections. A small incision just above the distal ligatures on the major abdominal vessels permitted insertion of short lengths of plastic tubing about one-half the diameter and 5 cm longer than the isolated abdominal vessels. The plastic tubes were pushed forward until they reached the anterior ligature. Proximal ends of aorta and vena cava were then divided just below their anterior ties. The elasticity of the vessels caused them to shorten so that the plastic tubes protruded beyond the cut edges. Ligatures were then tied around aorta and vena cava below the origin of the renal vessels to prevent the plastic tubes from slipping out. The aorta and vena cava were severed between the 2 distal ties. When the vessels were thus prepared and the ureter freed and cut at the bladder, the kidney with all its attached structures was removed from the donor.

The donor kidney placed in the neck of the host is shown in Fig. 1. In the schematic drawing, the distal ties (not labelled) which hold the plastic tubing are located on the abdominal vessels above the renal artery and vein. Two holding sutures using 6-0 silk were

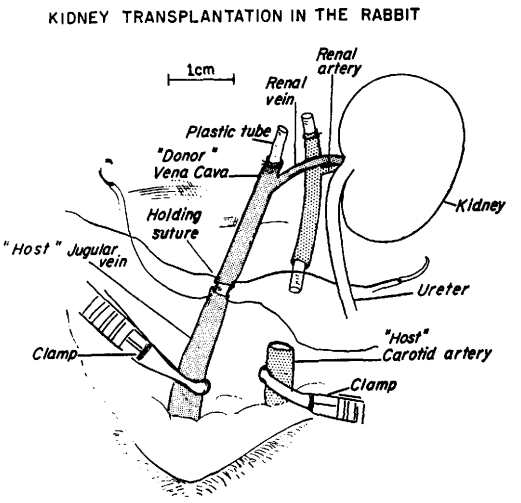

FIG. 1. Schematic drawing of part of procedure for transplantation of rabbit kidney demonstrates the homotransplant placed in neck of recipient just prior to suturing the vessels. Later, the ureter will be exteriorized through a stab wound in the skin near top of kidney.

placed so that the cut ends of the vena cava and jugular vein could be approximated. Next, the protruding plastic tube in the vena cava was inserted into the cut end of the host's jugular vein. Note that the tubing prevents the collapse of both donor and recipient vessels. The veins were then connected by a continuous silk suture and arteries were similarly intubated and anastomosed.

When the anastomosis had been completed, the distal ties holding the plastic tubes were cut and the tubes removed through the open ends of the vessels. The aorta and vena cava were next tied just above the renal vessels (Fig. 1). When the vascular clamps were removed, the blood of the recipient flowed from its carotid artery into the aorta and renal artery of the donor. The return flow is through the donor's renal vein and abdominal vena cava to the jugular vein of the host.

The recipient's skin was closed over the kidney, and the ureter of the transplant was brought out through a separate stab wound. A small plastic tube was inserted into the ureter and tied, and thus served as a catheter. An antibiotic salve was applied to keep the surface of the exposed ureter in good condition.

Preparation of autotransplants. As a con-

TABLE I. Homologous Rabbit Kidney Transplants.

Exp. No.	Biopsy on POD	Urine flow observed on POD	Transplant removed POD	Ischemia (min.)
1	10, 14, 17	16	17	99
2	7, 14, 21, 28	19	28	54
3	4, 15, 22	19	22	60
4	5, 7	5	7	32
5	9	—	9	39
6	2	1	2	175
7	4	3	4 (host died)	50
8	—	3	4 (" ")	110
9	—	3	7	153
10	—	3	4 (" ")	64
11	5	3	5	60
12	7, 13	3	13	55

trol for the homotransplants, 2 autotransplants were prepared in which the renal artery and vein of the host's kidney were connected to vessels in his neck. For this procedure it is not possible to use the method described above. However, plastic tubes were employed during part of the operation and were removed through the anastomotic site after the major portion of the connecting suture line was completed(2). The small size of the renal artery and vein make this suturing difficult.

To obtain biopsies, several days or weeks after surgery, a small incision was made in the skin over the kidney to expose the transplant. Specimens of the tissue were removed and prepared for histologic study. Tissue sections were all stained with hematoxylin and eosin.

Results and observations. Table I summarizes results of 12 consecutive kidney transplantations in rabbits. Most of the transplants were removed 2 to 9 days after operation in order to compare the time-course of rejection with well documented observations on dogs(1) in which the major histologic features of kidney rejection occur within a week. Three of the transferred kidneys were allowed to remain in place for 17 to 28 days. Urine flow was observed from these organs for more than 16 days after surgical procedures.

Table I shows that the period of renal ischemia required for operation is about one hour. In some instances fewer than 40 minutes was sufficient to complete the operation. Three of the 12 animals referred to in Table I died for unknown reasons 4 days after the transplantation.

The structural changes observed in the biopsy material can be summarized as follows: Within 4 days after grafting there is a definite perivascular accumulation of white cells (Fig. 2). These continue to infiltrate the tissue until the major portion of the parenchyma is no longer visible. By 7 days after transplantation, the outer layers of the cortex contain

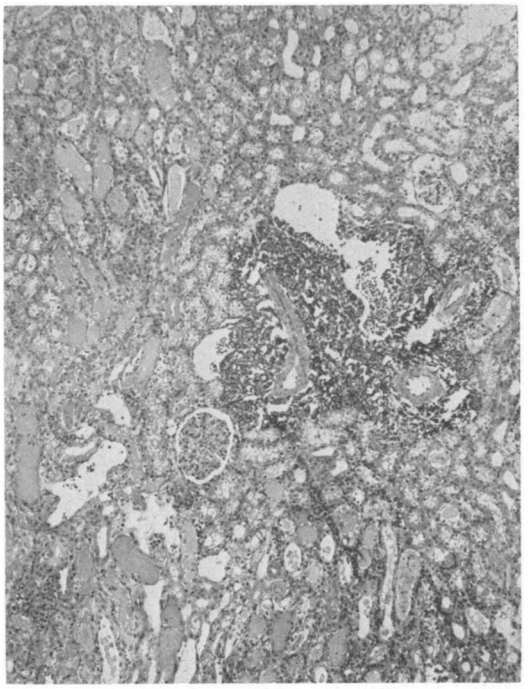

FIG. 2. Perivascular infiltrate in rabbit kidney can be observed 4 days after transplantation. Casts are present in many of the tubules. (75×)

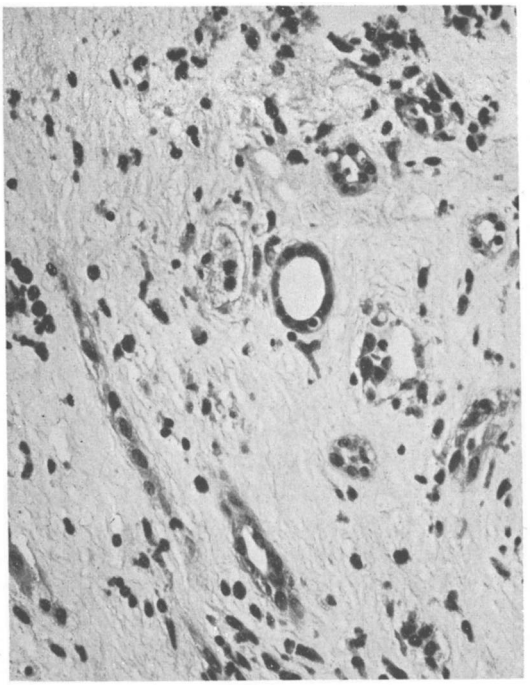

FIG. 3. Section from cortex of a kidney 28 days after homologous transplantation shows surviving tubules. (250×)

only a few viable cells.

Part of the blood supply of the transplanted kidney remains intact as long as 3 weeks after operation. In one animal the graft still bled when cut to obtain biopsy material 28 days after transfer of the kidney. Fig. 3 shows some surviving tubules in the cortex of this transplant on the 28th postoperative day. In some unpublished experiments we have shown that the total function of these relatively long-term transplants is not sufficient to keep the host alive when its own kidneys are removed.

In contrast, the autotransplanted kidneys showed a normal tissue pattern. Twenty days after the operation, the second kidney was removed from one of the animals. The host survived in good condition until 10 days later, when the autotransplant was recovered for histologic examination. Totally nephrectomized rabbits die of uremia within 3 days. Also, in the second autotransplant, this single kidney alone was sufficient to maintain the host for more than 2 weeks.

In addition to the 2 autotransplants mentioned above, the kidneys in 2 other rabbits were subjected to one hour periods of ischemia during which the renal pedicle was clamped. Upon removal of the clamps, the kidneys were allowed to remain *in situ*. Four days later tissue from these organs was examined for damage due to ischemia. Some dilated tubules and casts were seen in these sections; however, no cellular infiltrate was evident. The histological appearance in the homologous transplants described above appears to be due to the specific rejection process.

Discussion. The biopsy sections show that rejection in the rabbit is similar to that in the dog and suggests that the rabbit can be substituted for the dog in many transplant studies. We observed the following differences between kidney transplants in the dog and rabbit: Urine secretion may continue in the rabbit for almost 3 weeks after transplantation; the dog kidney almost invariably stops in less than 10 days. Some blood may flow through the rabbit kidney as long as 4 weeks after operation; in the dog, the major blood vessels tend to thrombose in less than 10 days. It is conceivable that the special renal vasculature in the rabbit may permit blood to flow after the major portion of these vessels has been destroyed. This suggestion is supported by the observation that homologous skin grafts in our rabbits are uniformly rejected in less than 12 days.

Although the cellular mechanism of rejection in most species appears to be similar, important differences in *rate* of kidney transplant rejection have become apparent from comparative studies. In contrast to the sudden and complete destruction of renal homotransplants in the dog, the present work with the rabbit demonstrates that some nucleated cells can survive in an immunized animal for more than 3 weeks, even within an organ which has been largely rejected. Homotransplants of human kidneys also have been known to survive for several months(3,4). Preliminary studies in our laboratory of renal transplants between outbred rats of the Sprague-Dawley strain show some viable cells more than 50 days after operation. The abrupt destruction of the dog kidney trans-

plants is probably caused by the loss of their main channels of renal blood supply. On the other hand, in the rabbit, rat and man, certain parenchymal cells which have somehow retained their vascularity, may be rejected very slowly. The importance of the blood supply for rejection of skin grafts has been stressed by Conway and Stark(5) and Rolle, Taylor and Charipper(6).

The surgical procedures developed for transplantation of the rabbit kidney can be adapted to transfer other organs such as segments of intestine or adrenal glands. Wherever a T-section of vessels is available, a plastic tube can be used in the operation to maintain their patency. This simplifies suturing and also insures against the possibility of accidental closing of the lumen.

Summary. Homologous transplants of the rabbit kidney have been carried out by a procedure in which vascular anastomoses of the donor aorta and vena cava were made to the carotid artery and jugular vein of the host. During the operation, plastic tubes were employed to maintain the patency of the vessels. These tubes were removed after completion of the anastomoses. Some nucleated cells can survive 4 weeks in homologous kidney transplants. The destructive process in the rabbit kidney is considerably slower than in the dog. It is suggested that the breakdown in the vascular supply to the transplant may be important in the rejection process.

1. Hume, D., in: *Transplantation of Tissue,* 1959, Volume II, ed. by Lyndon A. Peer. The Williams and Wilkins Co., Baltimore, pp. 484-536.

2. Snyder, C. C., Knowles, R., Mayer, P. W., Hobbs, J. C., *J. Plastic & Reconstruct. Surg.,* 1960, v26, 251.

3. Miller, B. F., *Fifth Macy Conference on Renal Function,* 1953, ed. by Stanley E. Bradley, Madison Printing Co., N. J., pp. 70-113.

4. Hume, D. M., Merrill, J. P., Miller, B. F., Thorn, G. W., *J. Clin. Invest.,* 1955, v34, 327.

5. Conway, H., Joslin, D., Rees, T. D., Stark, R. B., *J. Plastic & Reconstruct. Surg.,* 1953, v12, 74.

6. Rolle, G. K., Taylor, C., Charipper, H. A., *J. Cell. & Comp. Physiol.,* 1959, v53, 215.

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Inhibition of Cytopathic Action of Salisbury Virus by Antiviral Agent 1758.* (26531)

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In recent tissue culture studies in work related to the common cold, we have tested the activity of an antiviral agent against the cytopathic action of a Salisbury virus derived from a human cold. The reports of the work of Dr. Andrewes' group in Mill Hill and Salisbury, England(1,2,3), have renewed our interest in cold viruses. Coincidentally, we have experimented with antiviral substances from penicillium molds which demonstrated a considerable spectrum of activity in mice when virus and antiviral agent were administered by separate routes of injection(4,5,6). We are reporting here the results of tests of successful inhibition of the cytopathic action of a Salisbury virus by a penicillium antiviral agent called "1758".

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Materials. Salisbury virus H.G.P. was supplied to us as an ampoule of dried material by Dr. C. H. Andrewes. Properties of this virus have been described(1,2,3). H.G.P. virus was typed during this work with rabbit antiserum of a titer of 1-16 made with an early pool of virus. Antiviral agent 1758 was supplied to us as partially concentrated broth by Lilly Research Laboratories. This agent was derived from tank cultures of Penicillium stoloniferum (group Brevi-Compactum), and was first detected in the Lilly Labs (Powell, Culbertson, Hoehn, and McGuire, unpublished) using the technic found useful in detecting antiviral agent 8450(4). Antiviral