48-Hour Storage of Canine Kidneys after Brief Perfusion with Collins' Solution

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THE most successful kidney preservation in recent years has been achieved by continuous perfusion with cooled, oxygenated blood or plasma. This technic, as perfected by Belzer [3] for 3-day storage of canine kidneys and extended in one instance to 5-day storage in our laboratory [14] and to 7-day storage by J. E. Woods (personal communication), requires bulky, complex and expensive apparatus, so that it is not widely available and cannot be used easily to transport kidneys.

Collins *et al.*,[6] have concentrated recently on simple methods. With extra-careful technic they showed that simple immersion of kidneys in iced saline was effective for 12 hours' preservation. They then extended the storage time by first briefly perfusing a solution similar to *intracellular* fluid in electrolyte composition.[7, 8] With this "C_4" solution that also contained procaine hydrochloride, heparin sodium, glucose, and phenoxybenzamine hydrochloride, they were able to preserve canine kidneys for 30 hours as well as any stored by continuous perfusion systems. In December, 1969, Collins and Terasaki [33] described their slightly modified C_5 solution which included 1 Gm./100 ml. human albumin. In October, 1970, Terasaki announced in Chicago that human kidneys briefly perfused with Collins' solution were transported long distances in better condition than those briefly perfused with other solutions.

The primary purpose of this report was to try to improve the Collins C_5 solution. We chose to add to it the nutrients and other substances present in tissue culture medium 199. This storage solution did preserve kidneys for 48 hours fairly well, but no better than did C_5 alone. The post-transplant maximum serum creatinine levels were not significantly different; they averaged, respectively, 4.9 mg./100 ml. and 4.1 mg./100 ml. ($p > 0.1$).

By contrast no kidney stored after brief perfusion with a third solution, medium 199 containing Hanks' balanced salt solution which is similar to extracellular fluid, was able to maintain life. Thus we were able to confirm the efficacy of the C_5 solution, but were not able to improve it.

Materials and Methods

Young, female mongrel dogs that had been dewormed were used. They weighed 15–25 Kg.; two dogs of about the same weight were chosen as donor and recipient. They were allowed water *ad libitum* but no food the 12 hours before operation. The dogs were anesthetized and maintained with thiamylal sodium (Surital) given intravenously; they were intubated and ventilated at 14 respirations per minute by a Harvard piston respirator delivering 14 cc.

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air/stroke/Kg. dog weight. The blood pressure was not measured.

Operation on Donor

The donor dog received intravenously 500 ml. of Ringer's lactate and 1,000 ml. of half-normal saline with 12.5 Gm. of mannitol before the renal artery was clamped. The kidney was approached by "non-touch" technic through a flank or midline incision. After the artery and vein were freed and the ureter cut, the kidney was left untouched literally for 10–15 minutes.

The kidney was immersed immediately into 0° C. iced saline slush. Then 150 ml. of the storage solution at 1–3° C. was suspended at 100 cm. (74 mm. Hg) and perfused via the renal artery through the kidney in an average time of 3 minutes. The kidney was immersed in storage solution inside a polypropylene jar which was kept in iced normal saline slush inside a refrigerator at 0°C. for the 48 hours.

Operation on Recipient

Through a midline incision both kidneys of the recipient were removed intracapsularly. The left iliac vein and artery were anastomosed end-to-end to the renal vein and artery of the donor kidney within 25 to 35 minutes while the kidney was surrounded with an ice cold sponge. Surgicel® * was wrapped around the artery to decrease bleeding. The vein was released. Air bubbles were removed from the artery before it was released. Phenoxybenzamine hydrochloride ** 5.0 mg. diluted in 0.5 ml. of saline was injected into the artery slowly. The kidney was left untouched for 5–10 minutes. The ureter was anastomosed to the dome of the bladder with continuous 5-0 chromic suture, or implanted by simple puncture technic. During the operation, the dogs were given 500–800 ml. of Ringer's lactate and 1,000 ml. of half normal saline with 12.5 Gm. of mannitol. In the next 2 hours 500 ml. 10% dextrose in water was given and this repeated the next day. Beginning the day of operation azathioprine (Imuran) 1 to 5 mg./Kg./day was given. Serum creatinine measurements were made from day 1 to day 5, then day 7 and every week thereafter.

Group 1 (Four Kidneys—Storage Solution: Hanks' Solution Plus Medium 199 Plus C_5 Additives)

For four control kidneys the storage solution was the complete Medium 199 made with Hank's base * to 500 ml. of which was added the amounts of glucose, heparin, procaine hydrochloride, phenoxybenzamine hydrochloride and albumin present in 500 ml. of C_5. The Hanks' base in milliequivalents per liter consisted of: Na^+ 137.6, K^+ 5.8, Ca^{++} 2.5, Mg^{++} 1.6, Cl^- 144.8, SO_4^{--} 1.6, and phosphate 1.1. Sodium bicarbonate was not added. The extra ingredients in Medium 199 included amino acids, vitamins, coenzymes, nucleic acid derivatives, and glutamine.[18] The measured osmolality was 332 mOsm./Kg. H_2O.

Group 2 (Four Kidneys—Storage Solution: C_5)

The C_5 solution (Tables 1 and 2) including the non-electrolytes was made in glass-distilled, Cutter Laboratories distilled water exactly as recommended by Collins except that Parts A and B were filtered separately through a 0.22 micron millipore filter (Millipore Corporation) rather than autoclaved. The average pH measured at 10° C. was 7.2. The average osmolality measured by freezing point depression was 349 mOsm./Kg. H_2O.

Group 3 (Ten Kidneys—Storage Solution: C_5 Plus Medium 199 without Its Hanks' Base)

To modify the basic C_5 solution, the amino acids, vitamins and coenzymes, lipid

* Oxidized Regenerated Cellulose, Johnson & Johnson, New Brunswick, New Jersey.

** Dibenzyline®. Kindly supplied by Miss L. B. Luonga of Smith, Kline, and French, Philadelphia, Pennsylvania.

* Medium 199, Hanks' base, Catalog No. 52061, BioQuest, Cockeysville, Maryland.

TABLE 1. *Method of Making C_5*

Components	Gm./Liter	Volume
Part A		
KH_2PO_4	4.1	
Glucose	50.0	
$MgSO_47H_2O$	14.8	500 ml.
Procaine HCl	0.2	
Part B		
$K_2HPO_43H_2O$	19.4	
KCl	2.24	500 ml.
$NaHCO_3$	1.64	

Parts A and B sterilized by millipore filter and kept refrigerated before use.

Immediately before use:

1). Add phenoxybenzamine 25 mg., and Human Serum Albumin 10 Gm. to Part A.
2). Add Heparin (without preservative) 5,000 u. to Part B.
3). Add B to A and perfuse kidney from 100 cm.

sources, nucleic acid derivatives, and glutamine but not the Hanks' salts * normally present in 500 ml. of tissue culture Medium 199, were added along with the phenoxybenzamine hydrochloride 12.5 mg. and albumin ** 5 Gm. to 250 ml. of Part B of the C_5 solution. Immediately before use, this modified Part B was mixed with 250 ml. of Part A to which had been added the heparin sodium *** 2,500 units. The final mixture had an average pH measured at 10° C. of 7.16 and an osmolality measured by freezing point depression of 363 mOsm./Kg. H_2O.

* Cultur STAT Medium 199, without serum. Catalog No. 52024, BioQuest, Cockeysville, Maryland.

** Human serum albumin, Salt-poor. Cutter Laboratories, Berkeley, California.

*** Heparin sodium without preservative. Fellows-Testagor, Detroit, Michigan.

TABLE 2. *Concentration of Ions in C_5 Solution and Other Solutions for Comparison*

	C_5	Keeler	Martin	Abouna	Human[37] Kidney
K^+	115	150	115	30	57
Na^+	10	10	10	120	82
Mg^{++}	60	50	30	16	8.6
Ca^{++}	0	2	0		7.0
HPO_4^-	85		70	22	57
$H_2PO_4^-$	15		30		(mMol.)
SO_4^-	60		30		
HCO_3^-	10		10		
Cl^-	15		15		68

Results

When the kidneys were removed from the storage container, the perinephric fat was firm and encased in a layer of ice. The results are summarized in Table 3.

Group 1. All four kidneys assumed a fairly normal color when transplanted, but none excreted urine. Two excreted urine the next day but all dogs were moribund by the second or third day and were sacrificed. One of the four had a venous thrombosis and so may have failed partly from technical problems. The other three by microscopic examination showed severe tubular necrosis particularly in the medulla compatible with acute ischemic injury.

Group 2. All four kidneys assumed a fairly normal color and all excreted urine during implantation of the ureter. All maintained life for at least 1 week before they were rejected. The maximum rise in serum creatinine was on day 2 or 3 and averaged 4.1 mg./100 ml.

Microscopic examination of these four kidneys at 1 week to 10 days after transplant showed severe rejection which obscured observations about ischemic injury.

Group 3. Each of the ten kidneys when transplanted excreted some urine within minutes after transplantation. Six kidneys appeared of normal pink color and consistency and excreted urine in spurts from the ureter.

Six kidneys maintained a serum creatinine level * sufficient to sustain health (Fig. 1). The maximum serum creatinine came on the second postoperative day with the average being 4.9 mg./100 ml. In one dog the maximum serum creatinine was only 2.6 mg./100 ml. Four dogs lived 21 days or

* Measured by automated chemistry system, Technicon Corporation. Chromogens in serum were not first removed.

TABLE 3. *Post-transplant Serum Creatinine Levels in Dogs of Group 2 and Group 3*

Dog No.	Storage Solution	Serum Creatinine (mg./100 ml.) Day 1	2	3	4	5	6	7	
4463		3.8	4.2	3.3	2.8	2.6		3.4	
4482	C_5	2.3	4.4	4.9	4.4	5.2		6.9	Rejection
4488	(Group 2)	3.6	3.9	3.7	3.5			3.0	
4529		3.3	3.5	3.4	2.7			14.0	Rejection
3812		4.4	7.9	6.6+					+Died, artery thrombosis
3695		3.4	5.1	4.7		1	2.9		
3913		4.0	6.3	6.6		6.1	+		+Died, intussusception
3807	C_5 + Med. 199	5.0	8.3	10.0	+				+Died, intussusception
3853	(Group 3)	3.0	4.5			3.0		2.8	
3921		4.7	7.8			6.5		6.0	
3849		3.5	4.3			3.7		7.2	Rejection
3895*		5.4	8.9	11.9	13.3			13.5	Rejection
4074		3.9	5.4	5.5	4.8	5.1		4.9	
4128		2.4	2.6	2.3		1.8		3.0	

The range of serum creatinine levels in normal dogs with 2 kidneys in our laboratory is *0.8 to 1.8* mg./100 ml.
* We judged this kidney to be preserved satisfactorily but injured very early by rejection, as confirmed by histological examination.

longer and one dog had a serum creatinine of 1.4 mg./100 ml. on day 39.

We judged the other four kidneys to have been preserved equally well but they suffered early complications. One dog excreted urine but died on the third day after transplant with arterial thrombosis. Another two died because of intussusception within 1 week. Both had had good urine output.

Intussusception was found in another when it was sacrificed on the 14th day; the serum creatinine on the tenth day had been 1.8 mg./100 ml.

Discussion

Canine kidneys briefly perfused with Collins' C_5 solution as a storage solution and then kept hypothermic for 48 hours were preserved well. Collins' solution plus medium 199 was equally effective but no more so. By contrast when the storage solution was Hanks' solution plus medium 199 no kidneys were able to maintain life.

This work confirms that reported by Collins. These kidneys were stored longer but not preserved so well as those reported by Collins which kept the post-transplant serum creatinine levels at 2.6 mg./100 ml. or less.

Most other investigators have had good results with Collins solution.[21, 28, 35] Collste and associates[9] briefly perfused C_4 but without phenoxybenzamine hydrochloride.* Of four kidneys stored for 48 hours, three survived and maintained serum creatinine levels similar to those we report herein. However, Smellie and associates[29] reported briefly that they could not reproduce Collins' results. Miller and associates[23] successfully preserved kidneys for 24 hours after briefly perfusing with Belzer's cryoprecipitated plasma but not with Collins' solution.

Collins was not the first to try a solution similar in electrolyte composition to intracellular fluid. Keeler and associates[16, 17]

* Terasaki[33] reported by Newsletter that phenoxybenzamine hydrochloride deteriorates rapidly when added to the Collins' solution and that "Solution C_3" without phenoxybenzamine hydrochloride further tested after the Lancet article[7] has given good results.

(Table 2) tested the concept in 1965, and Martin *et al.*,[22] in 1969 preserved kidneys by perfusing them continuously for 8 hours with solutions similar to intracellular fluid.

Hypothermia *per se* is the most important factor in preservation by non-perfusion methods. Hypothermia may exert its beneficial effect by reducing oxygen requirements of that part of the cell's machinery that works for the animal as a whole such as net tubular transport of sodium by renal tubules. Hypothermia may, however, become deleterious when it depresses that part of the metabolic machinery that operates to maintain cell structure and intracellular electrolyte composition which is radically different from the surrounding interstitial fluid. Perhaps it is this latter component of cellular activity that accounts for the inexorable metabolism that can be measured in an organ even when it is detached from the body. This machinery includes those enzymes involved in pumping out sodium that diffuses in through the cell membrane along concentration gradients. These enzymes are less active when cold, particularly in non-hibernators.[4] It is well established that kidney slices gain water and sodium and lose potassium when made cold and/or when deprived of oxygen or nutrients.[36]

Therefore, it seems reasonable to postulate that any cell that is unable to pump out sodium, will be better preserved if the normal extracellular fluid is replaced with fluid containing electrolytes similar to those in intracellular fluid. Despite the appeal of this concept, it is still rather odd that the fluid Collins chose works for kidneys as well as it does. The true composition of renal parenchymal and vascular cells is difficult to ascertain because of the large and variable amount of extracellular fluid in kidneys. Swann[32] reported that the volume of a kidney under normal arterial pressure *in situ* will decrease to 68% of its normal value after the artery is clamped and blood and interstitial fluid are allowed to drain from the vein. Furthermore, the main work of kidney cells is to reabsorb sodium from the glomerular filtrate, so that their sodium content may vary more than does the sodium content of cells of other organs of the body. Then, too, the medulla is rather high in sodium concentration particularly if it is excreting hypertonic urine.

Nevertheless, it is of interest that in milliequivalents per kilogram of fresh whole human kidney one analysis showed: sodium 82, potassium 57, chloride 68, magnesium 8.6, calcium 7.0, and phosphorus (in millimoles) 57.5.[37] And another dry weight analysis of canine kidneys showed only a slightly greater potassium concentration than sodium.[30]

It seems paradoxical that Collins' solution with a potassium content of 115 mEq./l. has preserved kidneys well, whereas, it has not preserved canine livers well. Abouna (personal communication) was not able after briefly perfusing C_4 to preserve *canine* livers for 6 hours, whereas, he was able after briefly perfusing a solution containing only 28 mEq./l. of potassium (Table 2) to preserve *porcine* livers for 6 hours.[1] Abouna first reported upon such work in 1968 and cited similar conclusions from experiments reported in 1950.[11]

If the primary benefit of the Collins' solution is to prevent accumulation of sodium in the cell, then a corollary may be that Collins' solution protects the cell by not requiring it to pump out sodium during storage and thereby exhaust its storehouse of energy substrates. Therefore the cell is better able to cope with the demands put upon it during the first few minutes after transplantation.

It was with this second consideration in mind—the need for energy substrates in the cell before it has time to extract substrates from the host's blood—that we chose to add the amino acids and vitamins (and coenzymes) of medium 199 to the Collins' C_5 solution in our attempt to improve the solution further.

We chose medium 199 because it served well when continuously perfused for 3

days,[13] and because it contains amino acids and glutamine which the renal cortex metabolizes more readily than it does glucose. Normally, at least when supplied with oxygen, which was not the case, the renal cortex derives most of its energy from free fatty acids.[24] Medium 199 contains a free fatty acid, but it has never been ascertained if the kidney can make use of this fatty acid because it is oleic acid bound to sorbital as Tween 80. Actually the kidney cortex can metabolize glucose when other substrates are not available and the medulla does so by preference.[24] Thus the glucose in C_5 solution may have sufficed as a source of energy; and the extremely high concentration, 2,500 mg./100 ml., may have "forced" the glucose across the cell membrane.

It should be pointed out that despite our emphasis in this discussion so far upon protection of the tubular cell by the similarity of C_5 to intracellular fluid, no studies of tubular function such as clearance of para-amino hippurate were done after transplantation. Instead the criterion for success was serum creatinine levels which depend upon renal plasma flow and glomerular filtration rate. It may be that the efficacy of C_5 has more to do with its ability to preserve the vascular endothelium and smooth muscle. When the vascular lumens are full of C_5, the lack of the usual sodium-potassium gradient will cause the membrane potential of the vascular smooth muscle to be low. Indeed, the muscle may be depolarized and unable to contract.[18]

A major concern with C_5 solution has been that a precipitate will frequently form after a few hours even though C_5 contains no Ca^{++}. A perfusion fluid made up to the same ionic concentration as *plasma,* but without its proteins to bind and stabilize the ions, will form a precipitate at pH 7.4 which increases at higher pH's.[26] The precipitate in C_5, magnesium phosphate by our analysis may form even though C_5 does include albumin of 1 Gm./100 ml. In an attempt to minimize the precipitate we

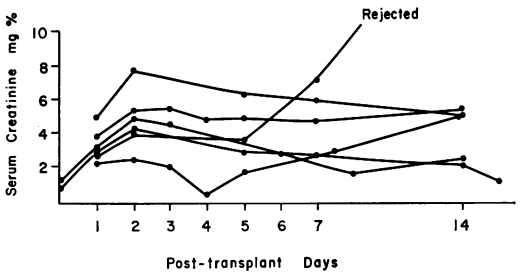

FIG. 1. Serum creatinine levels in the six dogs of Group 3 with no complications.

sealed the kidney and test solution in a plastic jar without air bubbles since the loss of CO_2 would increase the precipitation of magnesium phosphate; the P_{CO_2} once measured at 37° C. at the end of the 48 hours was 40 mm. Hg. Despite these precautions crystals have sometimes been present after 48 hours. They must not have formed in the kidneys themselves or at least not enough to impair blood flow. Some other investigators have had problems with crystals. Rudolf and associates[28] cleared them away from C_4 by filtration, added 3 Gm. albumin to each 100 ml. and then perfused kidneys continuously for 24 hours. The results were good but not better than those reported by Collins.

Watkins and associates[35] have used the Collins C_5 solution with good results. They also obtained equally good results with a simplified hyperosmolar, hyperkalemic, phosphate-buffered solution that was basically C_5 from which was omitted phenoxybenzamine, heparin, procaine, and magnesium (Table 1). Magnesium may be unnecessary since Conway and Cruess-Callaghan[10] reported that magnesium inside muscle is quite indiffusible over short periods and loses only about 10% of its value after soaking for 24 hours in magnesium-free Ringer solution at 2–3° C.

The potential benefits of the magnesium ion should be listed. Magnesium ion infusions of 0.8 to 4 mEq./min. into the renal artery increases blood flow and even blocks the vascular constriction caused by intravenous norepinephrine, pitressin, or angiotensin.[19] When C_5 solution is perfused at

50 ml./min. the magnesium ion delivered is at the rate of 2 mEq./min. Furthermore, magnesium ion activates intracellular processes for the generation and transfer of energy-rich phosphates, governs transmembrane sodium-potassium flux, and may stabilize the cell under conditions of marginal flow.[34] Furthermore Webb has reported that magnesium ion in high concentration is a metabolic inhibitor. Recently he and associates[15] used a Tyrode's solution containing 2% $MgSO_4$ and 5% dextran 40 for preserving rat hearts for 48 hours at 4° C.

To preserve canine hearts Bayliss and Maloney[2] chose to include 2 mEq./l. of magnesium and to omit instead all phosphates. They briefly perfused the coronary system with this modified Collins' solution before storage in ice for 24 hours. When tested by parabiotic perfusion, these hearts performed far better than those cooled by other solutions.

The low pH of 7.0 to 7.2 of the Collins' solution may be an important feature since the pH governs the charge on the protein molecules which in turn effects the intracellular ionic concentration.[27] For cells that are cold with inactive sodium pump enzymes, the ionic concentration within the cell depends even more upon the Donnan Equilibrium forces. The normal intracellular pH cannot be agreed upon some studies suggesting it is as low as 6.0.[5] Interestingly for the kidney, although one study listed it as 6.93[38] another listed it as 7.32, a value significantly higher than for other tissues.[30]

The pre- and intra-operative management outlined by Collins is probably important. We did not use nitrous oxide for anesthesia or measure blood pressures, but did follow carefully his fluid administration regimen. Mannitol, as well as chronic diuresis and ethacrynic acid, decrease renal medullary osmolality and inhibit experimentally induced acute renal failure.[12]

Mannitol is used widely now for live human kidney donors. Even in doses large enough to cause extensive vacuoles in tubules, it does not seem deleterious.[31] However, one recent study showed that mannitol in high enough concentrations causes renal vasoconstriction.[20]

The results with C_5 were not significantly different from those with C_5 plus medium 199. We conclude that for 48-hour preservation by brief perfusion followed by storage in ice that Collins C_5 solution is satisfactory. The addition of the nutrients in Medium 199 to the C_5 storage solution did not improve the results ($p > 0.1$).

For human kidneys we have used C_5 on two occasions with great satisfaction. The first was a live donor kidney not stored at all; the second was a cadaver kidney stored for 10 hours while transported 1,000 miles. We share the feeling of many others that further clinical use is indicated.

Summary

Eighteen canine kidneys were removed, immersed in iced saline slush, briefly perfused with a cold storage solution, and then stored for 48 hours at 0° C. Each was then transplanted into a bilaterally nephrectomized host treated with azathioprine.

Group 1. The storage solution included Hanks' solution, medium 199, and the non-electrolytes in Collins' C_5 solution. Hanks' solution is similar in electrolyte composition to *extracellular* fluid. Medium 199 contains amino acids, vitamins, coenzymes, and other nutrients. The non-electrolytes in C_5 were albumin, heparin sodium, procaine hydrochloride, glucose, and phenoxybenzamine hydrochloride. None of the four kidneys excreted urine when transplanted or maintained life for more than 3 days.

Group 2. The storage solution was the C_5 solution described by Collins which includes electrolytes similar to those in *intracellular* fluid plus the "non-electrolytes" listed above. All four kidneys excreted urine immediately. The average maximum serum creatinine level was 4.1 mg./100 ml.

Group 3. The storage solution was C_5 plus medium 199. All ten kidneys were judged successful, but four were difficult to evaluate because of complications. The

other six functioned well but no better than those of Group 2 for which the storage solution was Collins' solution alone.

The rationale for using a storage solution similar in electrolyte composition to intracellular fluid is discussed.

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Discussion

Dr. Leslie E. Rudolf (Charlottesville): At the present time there are two basic methods of preserving kidneys. One is to perfuse the organ on some type of pulsatile apparatus and, more generally, pump it with some type of cryoprecipitated plasma. The other method is simpler, less complex, not as expensive, and does not require individual monitoring. This method, as Dr. Humphries has done, is to flush out the kidney with some type of a solution and place it under hypothermic storage.

The result of Dr. Humphries' work and that of others has enabled the latter methods to approach some of the results that we achieved with pulsatile perfusion. We realize that with pulsatile perfusion we encounter many difficulties. We are dealing in this country with five or six regional organ donor programs that are interlinked with a computerized system, and when we consider transplanting or transporting organs between and among institutions, we should provide for the simplest, easiest, and least expensive technics. Some of the work that Dr. Humphries has been doing has made a significant contribution to the area.

Preservation experiments include many variables: storage temperatures, perfusate composition, perfusate flows and rates, pressure, and the element of storage duration. Dr. Humphries' study has been well controlled, and I believe the most important aspect of this work has been to measure preserved function by the ultimate test, and the ability of the kidney to sustain the dog's life.

I would like to ask Dr. Humphries one question: Would it not have been better to reimplant the 48-hour preserved kidneys as autografts rather than allografts, in an attempt to obviate some of the subsequent impairment of function that may have resulted from graft rejection, rather than changes that occurred as a result of organ preservation?

In closing, I would like to be somewhat philosophic and say that I think all of us interested in organ perfusion and preservation should be looking more closely at the effects of our technics on the vascular endothelium in the preserved organ. After all, it is the endothelium and its surrounding smooth muscle that is responsible for vasoconstriction, vasodilation, the transcapillary movement of water, nutrients and electrolytes, and hence ultimately responsible for organ edema, metabolism and ion exchange, all of which determine an organ's basic function.

Dr. John McDonald (New Orleans): Dr. Campbell's paper nicely defines the various syndromes that rejection may produce. His manuscript outlines the many diagnostic tools that can be used in establishing the diagnosis of rejection. The multiplicity of these methods makes it obvious that it is sometimes difficult for the clinician to determine whether or not he's dealing primarily with rejection or some other intercurrent problem, and even if all of these methods were ideal, it is evident that all of the signals of rejection that are now in use occur too late; that is, they are all a reflection of injury to the grafted organ. Of more value would be a means of establishing when rejection is about to occur, before organ damage is apparent. In this regard I would like to relate some experiments currently proceeding in our laboratory.

All people have a circulating antibody to rat erythrocytes which is apparently a naturally occurring immunity. Milgrom and colleagues noted a few years ago that the titer of this antibody was substantially higher in patients bearing renal allografts that were doing poorly than in patients bearing allografts that were functioning normally. We began to study this heterophile antibody for other reasons, but have encountered an interesting set of observations.

(Slide) This slide is not current. It shows the changes in the heterophile titer which occurred in 14 patients. Our data now include 25 patients, and 31 rejection episodes. The titer rose more than four times the control in only two of twelve patients studied who never had any rejection episode. However, it rose sixteenfold in seven of eight patients who had rejection crises which were not controlled. The dotted portions of these lines show the temporal relationship with the rise in titer and the change in clinical function.

Small changes in titers occurred in four of five