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Baboon renal and chimpanzee liver heterotransplantation

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In late 1963, we were led and stimulated in a new direction by Dr Keith Reemtsma. I am here mainly to pay tribute to Reemtsma's remarkable feat of 25 years ago. It was an experience and an accomplishment which as the years have gone by has assumed an even greater significance than at that time. I have confidently predicted, as has Keith in an indirect way, that the magnitude of the accomplishment will become even greater in the years ahead because like Keith, I am convinced that animal to human transplantation is close to the horizon. The main reason to believe this is how close efforts at both chimpanzee-to-human and baboon-to-human transplantation came to succeeding in that now distant time. However, failure to complete the task over a span of 25 years introduces a note of very real caution because the inability to exploit this kind of breakthrough generally means that the problem is deeper and more unfathomable than was actually appreciated.

Baboon renal heterotransplantation

Our studies with baboon-to-human renal heterotransplantation were done at the University of Colorado in 1963. Some of the people who are here today came to Denver in 1963 and have now resurfaced as handsome as ever, Dr Moor-Jankowski being included. The participants in these studies brought to the University of Colorado an international group. Of that group, Professor K.A. Porter of St. Mary's Hospital and Medical School may have been the single most important contributor because of what he did, and was able to do, thanks in part to the generosity of Dr Reemtsma.

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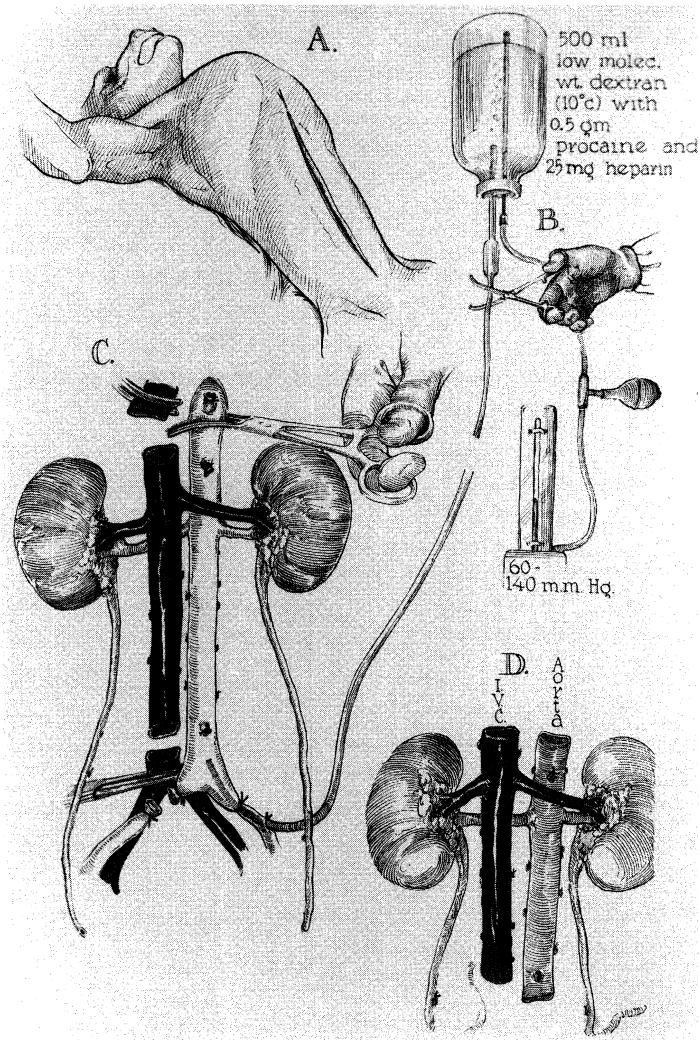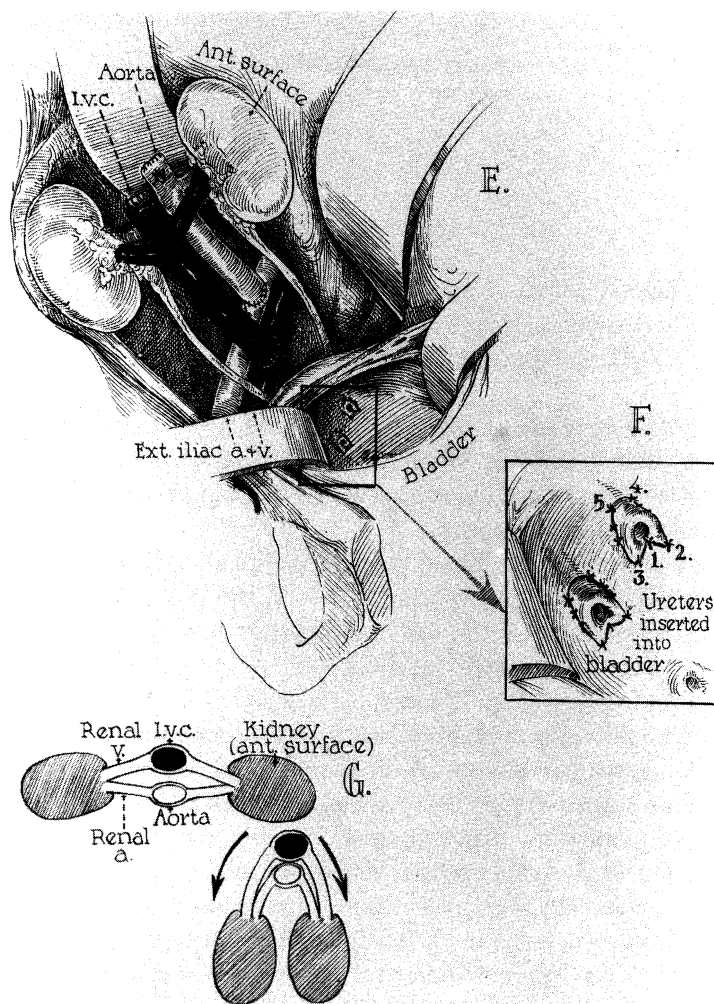
Fig. 1. (Above) Preparation of heterografts. A, complete midline incision; B, method used to cold perfuse the heterograft complex; C, in situ perfusion; D, the heterograft after removal. (Opposite) Insertion of

Reemtsma gave Porter pathology material from the chimpanzee-to-human hetero-transplants and Porter was then able to study the pathology with three species of heterograft donors. These were the Rhesus monkey which Keith did not mention, the chimpanzee which was the best donor and, in between these in biological desira-bility, the baboon. A kind of comparative pathology spectrum was laid out by a single world class pathologist.

The surgical techniques were similar in Reemtsma's chimpanzee heterotransplan-

TABLE I
Heterotransplantation of baboon kidneys[a]

Duration of function	10–60 days (mean 36)
CCr at one day	34–61 ml/min (mean 46.3)

[a]Raw data in Refs. 1 and 3.

of the time [1–3]. In some of the cases, single baboon kidneys were placed into the adult human and in others double kidneys were used. The organs functioned for 10–60 days, mean 36 (Table I), and that allowed liberation from dialysis for this period of time.

This means of course that none of these kidneys underwent hyperacute rejection. The creatinine clearance after 1 day was 34–61 ml/min or for a mean of 46 (Table I). That was about half of what one could expect with a homograft as I will show you with direct comparison in just a moment.

Moor-Jankowski had determined the blood types of these baboons [4]. In 1963, it was thought that the baboon had only blood types A, B and AB. In half of the recipients there was a confrontation of the ABO blood group barriers, 2 AB to O and 1 B to O (Table II). These three grafts functioned for 10, 49 and 25 days. The other three grafts were ABO compatible. These kidneys survived for 23, 49 and 60 days. Blood group compatibility was thought to be a favorable condition at the time because it was in 1963 that we had delineated for the first time the rules of ABO matching. The events of this discovery are summarized elsewhere [5]. We showed in human recipients of renal homografts that hyperacute rejection could be caused by ABO incompatibilities, and we provided evidence that preformed antigraft isoagglutinins were responsible. Incidentally, I should mention that the expression of ABO antigens in baboons is faint so that the animals were difficult to type. Typing was done by Moor-Jankowski with salivary collections. The poor representation of ABO antigens in tissues might have explained the non-effect of blood group incompatibility.

TABLE II
ABO blood types

			n	Days of function
AB	→	O	2	10, 49
B	→	O	1	25
A	→	A	1	23
B	→	B	1	49
B	→	AB	1	60

In Fig. 2 is shown the course of one of the baboon heterograft recipients. This young man was discharged from the hospital 30 days after receiving a baboon hetero-graft. Eventually, the baboon kidneys were removed because the recipient was sick even though the heterograft still had life-supporting renal function with urine urea concentration of about 500 mg%. A homograft now was found and was transplanted on the same day as heterograft removal. The homograft doubled the urine urea con-centration. The creatinine clearances were in the 40–50 ml/min range throughout most of the residence of the heterograft and were still about 20 ml/min on the day the heterograft was removed. The creatinine clearance of the homograft was 100 ml/min immediately. Eventually the patient died of sepsis after 40 more days with a total survival of more than 100 days. The important observation was the astonishing per-formance of an animal heterograft for 2 months in a human.

The baboon kidneys were sent to Porter who demonstrated cell mediated rejection.
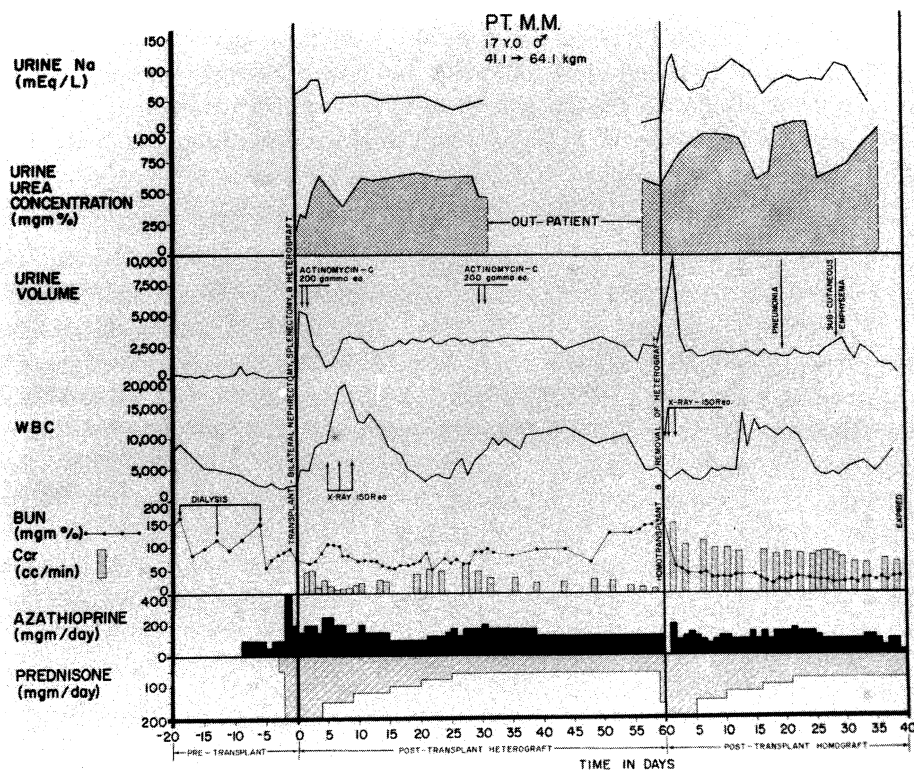

Fig. 2. Patient SD 3. Recipient was AB+ blood group, and donor was B. The patient was anuric preopera-tively. The difference in quality of function of the heterografts, compared to the secondarily placed homo-grafts, is evident. Heterotransplant rejection crises occurred after five and 50 days. Urine function contin-ued until heterografts were removed. With permission. From Ref. 3.

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The cell composition of the infiltrate was no different than in homograft rejection, and Porter told us that he could not really say from histopathological examination that this was a heterograft after some 2 months in residence. However, these kidneys had the infarcts, cortical and subcortical (Fig. 3), which have come to be associated with vascular lesions of humoral antibody rejection. With such infarcts comes sepsis.

With special stains, Porter showed widespread vascular lesions in the baboon heterografts. There is nothing specific about these lesions. They are also found in homografts. In fact, the homograft that was eventually retrieved at autopsy from the patient whose course is shown in Fig. 2 had the same vascular lesions as were in the preceding heterografts.

As I went back over these papers [1–3] in preparation for this talk, I wondered why there were not better humoral antibody studies in these patients. I soon remembered that cytotoxic antibodies as a cause for humoral rejection were not recognized by Terasaki until more than a year later [6]. In 1963, we were working with antibody systems that may or may not have been really relevant to the problem of humoral rejection. Figure 4 summarizes observations in one of those patients who received an ABO incompatible kidney showing anti-A and anti-B isoagglutinins, as well as heterospecific hemagglutinins. After placement of the heterograft, both ABO isoagglutinins and the heterospecific hemagglutinins declined, indicating that there was antibody binding to the transplant. Electronmicrographic studies were confirmatory. In contrast, with an A-to-A transplant the isoagglutinins were not altered during the

Fig. 3. Heterografts removed from patient whose course is shown in Fig. 2.
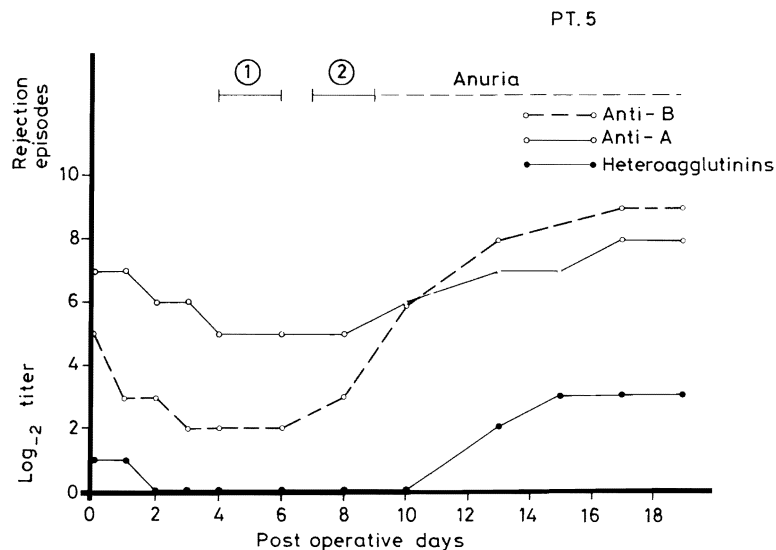
Fig. 4. Serial measurements of the activities of anti-A and anti-B hemagglutinins and heteroagglutinins of Patient SD 5, type O, following transplantation of the kidneys from a type AB baboon. Note that the antibody titers rose after the patient became anuric during the second rejection episode. With permission. From Ref. 3.

postoperative course, but the heterohemagglutinins which were present in every one of these human recipients fell as if they were being screened out by the grafts (Fig. 5).

So much for the baboon studies. The death of six patients was a devastating loss. We never tried again. Porter's conclusions were as follows after comparing the baboon kidneys to transplants of Rhesus monkeys and chimpanzees [3].

"On the basis of present information, which is admittedly rather scant, it would appear that a treated chimpanzee renal heterotransplant fares no worse in the early stages than a treated human renal homotransplant from an unrelated donor. It is clear, however, that baboon heterotransplants, and particularly Rhesus monkey transplants, invoke a fierce response on the part of the host despite any treatment that is available at present. In the resulting rejection process, cellular infiltration and peritubular capillary destruction are prominent early features, but by nine days the vasculonecrotic element is marked. There is some circumstantial evidence to suggest that, whereas the peritubular capillary damage is mediated by cell-bound antibody, the fibrinoid necrotic vascular lesions are caused by circulating antibody."

Porter's comment about antibodies was prophetic. The humoral component of rejection has been the central topic for any discussion of heterotransplantation between divergent species since that time. In fact, xenograft models have been used to evaluate treatment of hyperacute rejection with the assumption that the mechanisms of de-
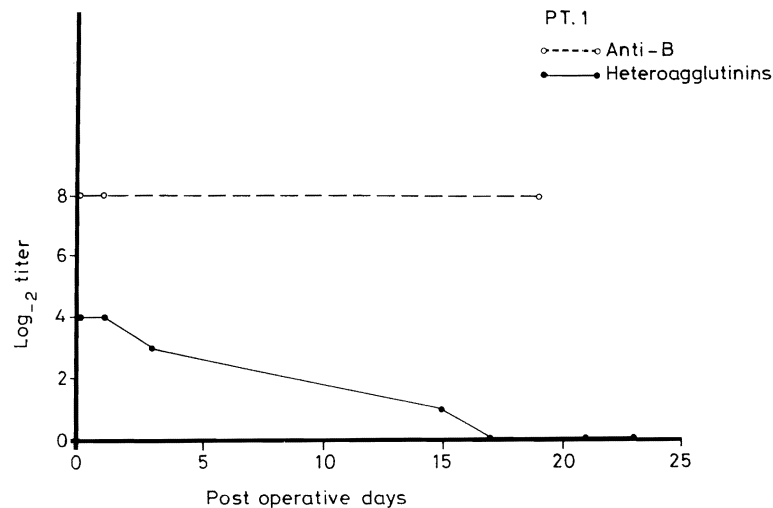
Fig. 5. Serial measurements of the anti-B hemagglutinin and heteroagglutinin activities in Patient SD 1, blood group A, following transplantation of kidneys from a type A baboon. With permission. From Ref. 3.

struction of xenografts are the same as hyperacute rejection of homografts in sensitized recipients. Such techniques to prevent humoral rejection have been summarized elsewhere [5]. Those include plasmapheresis; antibody removal with a Staph A column; transplantation of serial grafts to reduce the antibody titer; infusion of the chelating agent and anticoagulant, citrate, which also is a very effective way of preventing complement activation; and as described elsewhere in these proceedings by Makowka the use of prostanoids and inhibitors of the inflammatory response. All of these treatment protocols were tried in dogs which were given pig kidney grafts. There was prolongation of survival from a few minutes in untreated dogs to several hours in animals treated with the various methods [5]. However, getting beyond the several hours of prolongation before the supervention of hyperacute rejection has really confounded investigators to this day.

Chimpanzee liver heterografts

Before closing, I want to mention three chimpanzee-to-human orthotopic liver grafts. These were important cases, also done in the dark ages when we had primitive immunosuppression by today's standards. Yet here also there was near success. The first patient was a child with biliary atresia. After operation, the bilirubin fell to normal and stayed there until death from sepsis (Fig. 6). In commenting in 1969 about this chimpanzee-to-human heterograft Porter said that he could not distinguish the changes from those in homografts. His exact words were [8]:
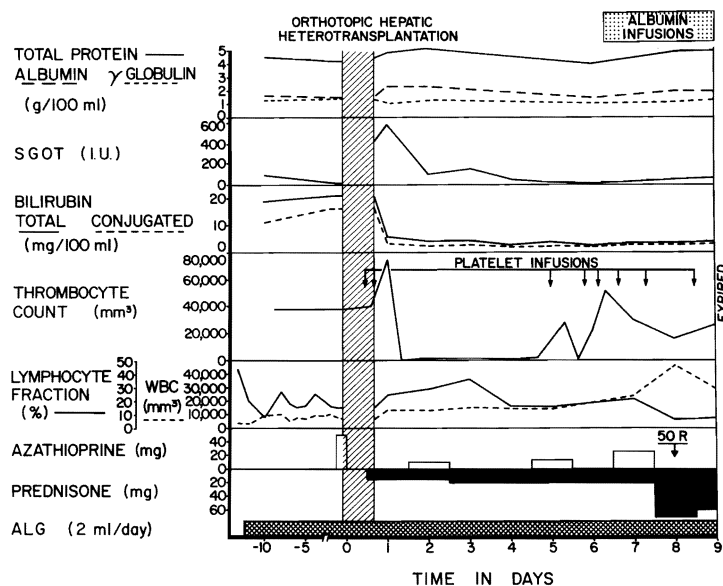
ORTHOTOPIC HEPATIC
HETEROTRANSPLANTATION
ALBUMIN
INFUSIONS

TOTAL PROTEIN ——
ALBUMIN γ GLOBULIN
(g/100 ml)

SGOT (I.U.)

BILIRUBIN
TOTAL CONJUGATED
(mg/100 ml)

THROMBOCYTE
COUNT (mm³)

PLATELET INFUSIONS

EXPIRED

LYMPHOCYTE
FRACTION WBC
(%) —— (mm³)

AZATHIOPRINE (mg)

50 R

PREDNISONE (mg)

ALG (2 ml/day)

TIME IN DAYS

Fig. 6. The course of a child with intrahepatic biliary atresia who received an orthotopic chimpanzee heterograft on July 15, 1966. ALG was started two weeks in advance of operation. The 50 R indicates local homograft irradiation. With permission. From Ref. 7.

"The histologic changes in this liver heterograft were very like those that occur in hepatic homografts. The lymphoid cell infiltration in the portal tracts was dense, but no more so than in the grafts from patients OT7-to-10 and in many treated canine hepatic homografts. There were no lesions of large blood vessels. Fibrinoid necrosis of arterial walls was conspicuously absent. It was difficult to believe that this child's death had been the direct result of hepatic failure produced by rejection. The analysis of the postoperative clinical events given in Chapter Nineteen tended to support the conclusion that considerable liver function was maintained until almost the end of life."

We have had experience with two other liver heterotransplantations. Although I reported them, I may have tried subconsciously to hide the experience by stashing the reports in obscure corners and funny places. Hugh Auchincloss smoked me out by some marvellous detective work in his review of heterotransplantation which was published recently [9]. Much to my amazement (possibly even chagrin), Auchincloss had discovered all of these cases with one exception. Table III presents a summary of these orthotopic liver heterotransplantations. The dates of the operations were in 1966, 1969 and 1973. The first heterograft was the one which I have already mentioned [7, 8]. The second heterograft may have been hyperacutely rejected [10]. There were many dumb things that were done in this case, and some are mentioned in Table III. One thing which I wanted to point out is that Fritz Bach, who is a participant at these Proceedings, studied many chimpanzees with MLC and found two potential

TABLE III
Chimpanzee-to-human liver transplantation

Recipient age	Date	Days graft function	Pathology	Refs.
28 months	15 July 1966	9	Like homograft	7, 8
7 months	3 December 1969[a,b,c]	0	Normal	10
23 months	9 June 1973	14	No rejection; centrilobular cholestasis, was a retransplant	11

[a]Positive cytotoxic crossmatch.
[b]MLC donor selection (F. Bach).
[c]Kidneys placed first to deplete antibodies.

donors out of a collection of many at the Holloman Air Force Base, Alamogordo, New Mexico, that provoked relatively little reaction by the lymphocytes of the recipient. Unfortunately, this child was studied so exhaustively that transfusions were required to replace blood drawn and by the time the child was transplanted, there was a positive cytotoxic crossmatch with the chimp donor. We made another foolish mistake at operation when we actually heparinized this child after the liver was put in. The child bled to death. The liver one day later was normal [10].

I operated on the third patient on 9 June 1973 and had a description of the case in a paper honoring the late Dave Hume in 1974 at his memorial service in Richmond (addressed to Dr Hugh Auchincloss for his records). Very complete data about this last case was given, but this is not mentioned in the title of my article [11]. The recipient was 23 months old. The heterotransplantation was performed under desperate circumstances. Ten days previously, this child had received a homograft which we thought might have been hyperacutely rejected because there was a powerful cytotoxic crossmatch with the original human donor. The chimpanzee liver was placed into the dying child in replacement of the homograft, and it functioned for 14 days. This child died of sepsis in the same way as had occurred in the 1966 patient. There was no rejection, although there was some centrilobular cholestasis. This liver was in amazingly good shape and, in retrospect, one has to really wonder, because of the troubles we were having with biliary tract reconstruction until a year or so after that, if there may not have been a technical component.

Future prospects

Over the years, we have had a lively interest in the humoral component of what we perceive to be a double-edged problem, that is control of cellular immunity and more

importantly of humoral immunity. The prospect of a genuine breakthrough in controlling preformed heterospecific antibodies has been bleak, and I do not see a solution to this problem in the near future. Perhaps the work that Makowka and the group in Belgium will present at this meeting holds some promise.

I will end my discussion hoping to help some of those who will deal in their presentation with ethical and social issues of heterotransplantation. About 5 years ago, I approached the NIH a month or 6 weeks before the Baby Fae case about the possibility of using chimpanzee livers for some of our very tiny biliary atresia patients for whom we could not find organs at that time. The rather extensive dialogue with people at the NIH escalated to the Director and eventually it came to the Ethics Committee of that agency. The proposal was shelved by mutual agreement. By this time the Baby Fae case had come up quite unexpectedly. We realized what a firestorm of publicity and of condemnation further heterograft trials were apt to bring down on us. I was stunned when I saw the reaction to the Baby Fae case. In the earlier trials (mine and all of Reemtsma's) which are described in this volume, there was no particular sense of outrage. These earlier trails were not secret. Perhaps, the climate was different.

If we could have helped our patients, the prospect of receiving abuse would not have been a deterrent. There was another factor and that was a White Paper, issued by the NIH, at the end of a 5-year study. The conclusion published in Science was that only between 25 and 50 chimpanzees per year would be available in the United States for all of biomedical research, including that in the important fields of hepatitis and AIDS. The use of chimpanzees would further jeopardize an already endangered species, but without having an impact on the organ shortfall. We dropped the matter and have done nothing with it since.

Acknowledgement

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