ANALYSIS OF THE MECHANISM OF DRUG INDUCED TOLERANCE TO RENAL ALLOGRAFTS IN DOGS*

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INTRODUCTION

The nature of drug induced tolerance to renal allografts remains obscure. Suggested mechanisms include chance histocompatibility (Calne et al., 1962), elution of transplantation antigens (Russel, 1964), replacement of certain elements of long term grafts by host tissues (Woodruff, 1959). Other possibilities are that the recipient becomes tolerant to renal antigens, that continuous release of antigen from the renal allograft causes dose-dependent immunological unresponsiveness, that the recipient is unable to mount an effective immunological response. This report records the results of experiments designed to analyse the mechanism of drug induced tolerance to renal allografts in dogs. Experiments reported previously (Alexandre et al., 1963; Murray et al., 1964) are included.

MATERIALS AND METHODS

Subjects were twenty-one bilaterally nephrectomized dogs with long functioning pelvic renal allografts. Survival was 61-770 days after transplantation. Drug management, function and histology of the allografts have been reported (Alexandre et al., 1963; Calne et al., 1962; Sheil et al., 1964). Most dogs were maintained on low doses of azathioprine, but in some cases all immunosuppression had been stopped. Dogs were tested in the following ways:

1. Skin grafts (a) from the specific donor of the long functioning renal allograft; (b) from indifferent donors and (c) from specific and indifferent donors in dogs off drugs. Skin grafts were full thickness, 5 cm square, and sutured in prepared beds on the right thorax. No attempt to reverse rejection of skin by increased drug therapy was made, and survival as determined macroscopically and by biopsy was the time to complete loss of viable graft.

2. Second renal allografts (a) from the same (specific) donor of the long functioning renal allograft and (b) from donors other than those of the first kidney (indifferent donors). Renal vessels of further allografts were anastomosed to iliac vessels of the pelvis, or to the jugular vein and common carotid artery of the neck. In the pelvis the ureter was implanted in the bladder; in the neck a cutaneous ureterostomy was fashioned. As detailed in the results, some dogs which received second renal allografts had already rejected skin grafts.

3. Re-implantation of long functioning renal allografts. In four experiments, to test the effects of trauma and ischaemia, renal vessels of long functioning renal allografts were dissected and the renal vein occluded while the renal artery was divided and reanastomosed.

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315
RESULTS

1. Skin grafts in dogs with long functioning renal allografts

   a. Specific donor skin grafts in four dogs maintained on drug therapy (Table I).

   Skin survival was 15-32 days (average 23). Dog 46 rejected the renal allograft and died 15 days after receiving the skin graft. At this time the skin was intact. Dogs 44, G. A. 113, G. A. 61 rejected skin grafts on days 26, 27, 16. In two of these (44, G. A. 113) there was considerable elevation of the BUN at the time of skin graft rejection. Dog 44 subsequently received two further skin grafts while on drug therapy. These were rejected by days 32 and 24 without significant elevation of the BUN.

   b. Indifferent skin grafts in six dogs on maintenance drug therapy (Table I).

   Skin survival was 14-20 days (average 17). In all cases minor elevation of the BUN occurred at the time of skin graft rejection.

   c. Skin grafts in three dogs off drug therapy (Table I).

   G.A. 154 rejected indifferent skin transplanted 310 days after cessation of drug therapy, in 10 days. Histology showed typical first set rejection. This dog subsequently rejected a

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**TABLE I**

*Skin grafts from the specific kidney donor and from indifferent donors in dogs with long functioning renal allografts*

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Days after renal transplantation</th>
<th>Source of skin graft</th>
<th>Survival of skin (days)</th>
<th>Drug therapy at time of skin graft</th>
<th>BUN Before skin graft rejection</th>
<th>After skin graft rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>71</td>
<td>Specific donor</td>
<td>15*</td>
<td>Maintenance</td>
<td>17</td>
<td>*</td>
</tr>
<tr>
<td>44</td>
<td>61</td>
<td>—</td>
<td>26</td>
<td>—</td>
<td>20</td>
<td>70</td>
</tr>
<tr>
<td>G.A. 113</td>
<td>106</td>
<td>—</td>
<td>27</td>
<td>—</td>
<td>27</td>
<td>149</td>
</tr>
<tr>
<td>G.A. 61</td>
<td>222</td>
<td>—</td>
<td>16</td>
<td>—</td>
<td>15</td>
<td>32</td>
</tr>
<tr>
<td>44</td>
<td>182</td>
<td>—</td>
<td>32</td>
<td>—</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>44</td>
<td>292</td>
<td>—</td>
<td>24</td>
<td>—</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>83</td>
<td>80</td>
<td>Indifferent donor</td>
<td>17</td>
<td>—</td>
<td>20</td>
<td>36</td>
</tr>
<tr>
<td>84</td>
<td>81</td>
<td>—</td>
<td>17</td>
<td>—</td>
<td>13</td>
<td>33</td>
</tr>
<tr>
<td>R.Y.C. 110</td>
<td>98</td>
<td>—</td>
<td>20</td>
<td>—</td>
<td>20</td>
<td>48</td>
</tr>
<tr>
<td>86</td>
<td>125</td>
<td>—</td>
<td>14</td>
<td>—</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>L. 201</td>
<td>154</td>
<td>—</td>
<td>20</td>
<td>—</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>L. 273</td>
<td>135</td>
<td>—</td>
<td>20</td>
<td>—</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>G.A. 154</td>
<td>730</td>
<td>Indifferent donor</td>
<td>10</td>
<td>None</td>
<td>47</td>
<td>35</td>
</tr>
<tr>
<td>G.A. 154</td>
<td>770</td>
<td>Same indifferent donor</td>
<td>5</td>
<td>None</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>S.M. 143</td>
<td>227</td>
<td>Indifferent donor</td>
<td>11</td>
<td>None</td>
<td>30</td>
<td>**</td>
</tr>
<tr>
<td>44</td>
<td>734</td>
<td>Specific donor</td>
<td>5</td>
<td>None</td>
<td>20</td>
<td>62</td>
</tr>
</tbody>
</table>

* Died in uraemia. Skin graft intact.
** Died in uraemia after rejection of skin graft.
### Table II

*Second renal allografts from the same donor in dogs with long functioning renal allografts*

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Days after first renal transplantation</th>
<th>Disposal of first renal allograft</th>
<th>Drug therapy at time of second renal transplantation</th>
<th>Result of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.M. 257</td>
<td>75</td>
<td>Left in situ</td>
<td>Maintenance</td>
<td>The second kidney was placed in the neck. Both kidneys continue to function well 100 days later. BUN 13 mg per 100 ml.</td>
</tr>
<tr>
<td>G.A. 113</td>
<td>260</td>
<td>Left in situ</td>
<td>Increased to maximal</td>
<td>The second kidney was rejected and was removed at 23 days. The first kidney continued to function at an impaired level for a further 50 days. Death was caused by haemorrhagic pneumonia.</td>
</tr>
<tr>
<td>G.A. 61</td>
<td>550</td>
<td>Returned to original donor</td>
<td>Maintenance</td>
<td>The second kidney did not function and the dog died 2 days later in uraemia. Microscopy showed a generalized tubular lesion with preservation of the glomeruli. Infarcted kidney removed after 2 days. Kidneys from indifferent donors were then transplanted (Table III).</td>
</tr>
<tr>
<td>G.A. 32</td>
<td>295</td>
<td>Returned to original donor</td>
<td>Maintenance</td>
<td>Infarcted kidney removed after 2 days. First kidney continued to function at same level.</td>
</tr>
<tr>
<td>D.M. 12</td>
<td>154</td>
<td>Left in situ</td>
<td>Maintenance</td>
<td></td>
</tr>
</tbody>
</table>

Second skin graft from the same indifferent donor in an accelerated fashion in 5 days. S. M. 143 rejected indifferent skin, transplanted 45 days after drug therapy was stopped, in 11 days. The BUN began to rise at this time, and the dog went on to reject the kidney despite reinstallation of full drug therapy. Dog 44 had rejected three skin grafts from the kidney donor while on drug therapy. A fourth specific skin graft, transplanted 314 days after cess-

*Fig. 1. G.A. 113. Second renal allograft from the same donor. The clinical course of this dog is shown in Fig. 2. Histology of the kidney 23 days after transplantation is shown. There is widespread mononuclear cell infiltration and extensive interstitial oedema. The changes are those of drug modified acute rejection.*

317
Fig. 2. Second kidney from the same donor. Clinical course of dog G.A. 113. The second kidney, transplanted 260 days after the first, was rejected and removed after 23 days. The first kidney continued to function at an impaired level for a further 50 days. A kidney from a different donor was transplanted shortly before death which was caused by haemorrhagic pneumonia.

ation of drug therapy, was rejected in a second set fashion in 5 days. At this time the BUN rose from 20 mg to 62 mg per 100 ml.

2a. Second renal allografts from the same donor (5 dogs, Table II)

The second donor kidney in one experiment (S.M. 257) was placed in the neck 75 days after the first operation. At the same time the first kidney was re-implanted in the pelvis. Drug therapy was continued at maintenance levels only. Both kidneys continue to function well 100 days later.

Experiment G.A. 113 has been reported previously (Murray et al., 1964). The dog received the second kidney 260 days after the first, had been maintained on drug therapy throughout, and had previously rejected a donor skin graft. The second kidney was rejected after 23 days despite maximal drug therapy (Fig. 1). Biopsies of the first kidney performed during the 23 days showed increased cellular infiltrate, and this was reflected in rise in the BUN. The first kidney continued to function at an impaired level for a further 50 days (Fig. 2). Death, which occurred after further operation, was caused by haemorrhagic pneumonia. Post mortem histology of the first kidney shows only progression of changes characteristic of long functioning renal allografts (Fig. 3).

A third different result in this series occurred in experiment G.A. 61. This dog received the second donor kidney 550 days after the first. Ischaemia time for the second kidney was short.
MECHANISM OF DRUG INDUCED TOLERANCE TO RENAL ALLOGRAFTS IN DOGS

Fig. 3. G.A. 113. First renal allograft day 333 (post mortem). The changes are those characteristic of long functioning renal allografts. There are bands of tubular atrophy and interstitial fibrosis; other tubules are dilated with flattened epithelium.

(20 minutes). Drug therapy was at maintenance levels at the time of the second operation, but had been stopped between days 453 and 518; deterioration of function of the first kidney had required reinstitution of therapy. The dog had previously rejected a donor skin graft. The kidney did not function and was removed. Histology shows a pattern of sensitized rejection. There is a widespread tubular lesion with preservation of the glomeruli (Fig. 4), and no renal vessel occlusion.

Two further experiments of this type failed for technical reasons.

2b. Further renal allografts from donors other than those of the first kidney (5 dogs, Table III)

D. M. 31 received the indifferent kidney 69 days after the first. There had been no skin graft, and the dog had been maintained on drug therapy throughout. Drug therapy was

Fig. 4. G.A. 61. Second renal allograft from the same donor. The second kidney was transplanted 550 days after the first. Drug therapy was at maintenance levels but had been stopped between days 453 and 518. The dog had previously rejected a donor skin graft. The kidney did not function. Histology shows preservation of the glomerulus and some tubular epithelium but there is widespread tubular destruction.
### TABLE III

Second renal allografts from dogs other than the first kidney donor in dogs with long functioning renal allografts

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Days after first renal transplantation</th>
<th>Disposal of first renal allograft</th>
<th>Drug therapy at time of second renal transplantation</th>
<th>Result of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.M. 31</td>
<td>69</td>
<td>Left in situ</td>
<td>Increased to maximal levels</td>
<td>Both kidneys continued to function well until death from pneumonia at 210 days.</td>
</tr>
<tr>
<td>R.Y.C. 110</td>
<td>290</td>
<td>Left in situ</td>
<td>Increased to maximal levels</td>
<td>Death occurred in uraemia 35 days later. The second kidney was acutely rejected. The first merely showed progression of the chronic changes found in long functioning allografts.</td>
</tr>
<tr>
<td>G.A. 52</td>
<td>295</td>
<td>Returned to original donor</td>
<td>Increased to maximal levels</td>
<td>A second donor kidney (Table II) and a first indifferent kidney failed to function and were removed. A second indifferent kidney functioned and was acutely rejected after 13 days.</td>
</tr>
<tr>
<td>S.M. 142</td>
<td>343</td>
<td>Re-implanted in pelvis at time of transplantation of second kidney</td>
<td>All drug therapy had been stopped on day 265</td>
<td>The second kidney was rejected in an accelerated fashion in 3 days. Four days after removal of the second kidney the first underwent a mild rejection process, easily reversed by reinstition of drug therapy. Renal function is good past day 450.</td>
</tr>
<tr>
<td>B.N. 28</td>
<td>210</td>
<td>Left in situ</td>
<td>Increased to maximal levels</td>
<td>The second kidney did not function and was removed after 2 days. Histology showed infarction. The first kidney continued to function at the same level.</td>
</tr>
</tbody>
</table>

increased to that used for the first kidney. Both kidneys functioned well until death from pneumonia 141 days after the second operation. Microscopy of both kidneys shows typical changes of long functioning renal allografts.

R. Y. C. 110 received the second kidney 290 days after the first, which at this time showed deterioration of function. Drug therapy had been maintained throughout, and the dog had previously rejected an indifferent skin graft. Maximal drug therapy was reinstated, but the dog died in uraemia 35 days later. Microscopy shows a violent rejection pattern in the second kidney with extensive cellular infiltration, and widespread tubular destruction. A striking feature is acute arteritis involving the main renal artery and its branches (Fig. 5), as well as arterioles in the kidney and in the wall of the ureter. No similar lesions were found in other organs. Histology of the first kidney shows only progression of changes found in long functioning allografts (Fig. 6).

Experiment G. A. 52 had a somewhat similar result. The dog had been maintained on drug therapy throughout, and there had been no skin graft. The first kidney was removed and a second donor kidney transplanted at day 295. The second donor kidney and a first indifferent kidney both failed to function because of vascular thrombosis. A second indifferent kidney then functioned for 13 days until it was rejected despite high levels of drug therapy.
Microscopy again reveals a violent rejection pattern (Fig. 7). Moreover, as reported previously (Murray et al., 1964), post mortem histology reveals arteriolar necrosis with fibrinoid change affecting not only the kidney but also the stomach, intestine and pancreas. Associated with these lesions are many polymorphs and mononuclear cells (Fig. 8).

S. M. 142. Here the indifferent kidney was transplanted to the neck 343 days after the first. At the same time the first kidney was reimplanted in the pelvis. All drug therapy had been stopped 78 days previously. The second kidney secreted copiously for 2 days, and lowered the BUN from 30 mg per 100 ml to 13 mg per 100 ml. On day 3 urine formation stopped, and the kidney was removed. Microscopy reveals a pattern of accelerated rejection with interstitial oedema and haemorrhage (Fig. 9). The first kidney functioned at the same level for 4 days after removal of the second, and then underwent a rejection crisis (BUN 144 mg per cent). Reinstitution of drug therapy caused reversal of rejection, and the dog is alive and well with good renal function past day 450.

One experiment in this series failed for technical reasons.

Fig. 6. R.Y.C. 110. First kidney day 325 (post mortem). The changes are those of long functioning renal allografts. A band of atrophy with fibrous tissue replacement is shown. Mononuclear infiltration remains focal.
3. Long functioning renal allografts which were re-implanted (4 dogs)

Design and results of these experiments are shown in Table IV. One dog (S. M. 144) died in uraemia 15 days after re-implantation of the long functioning allograft. Histology does not reveal typical renal rejection, but a pattern similar to an acute Shwartzman reaction. A second dog (S. M. 142) underwent a rejection crisis following re-implantation but this experiment was complicated by transplantation of a kidney from an indifferent donor at the time of re-operation. The function of two other allografts was unaffected by re-implantation.

DISCUSSION

Unmodified renal allografts sensitize dogs to subsequent donor skin allografts (Dempster, 1953). However, in dogs with long functioning renal allografts, on low dosage drug therapy, skin grafts are not rejected in accelerated fashion. Instead, skin survival is prolonged 2 to 3 times that usual for unmodified canine skin rejection. Donor skin survives longer than

Fig. 8. G.A. 52. An arteriole in the submucosa of the stomach is shown. There is fibrinoid degeneration of the arteriolar walls with accumulation of immature mononuclear cells and some polymorphonuclear neutrophils.
Fig. 9. S.M. 142. Second kidney from a different donor. The second kidney functioned for 2 days but then urine formation ceased. This section shows the presence of mononuclear cells; however, the striking feature is interstitial haemorrhage.

indifferent skin which suggests that part of the increased survival may result from absorption of immune effectors by the functioning renal allograft (Sheil et al., 1964; Moseley et al., 1966); however, non-specific inhibition of the immune response by drug therapy plays a major role because, after stopping drugs, prolongation of skin survival is slight. The conclusion that dogs on maintenance drug therapy have significantly impaired immune capabilities is further supported by the observation that one dog did not react to specific donor skin in a sensitized fashion, even after repeated grafting.

Rejection of skin causes deterioration of function of the resident kidney. Specific skin causes greater impairment of function than non-specific skin. It seems likely that skin and kidney from the same donor share antigens, and that the heightened level of immunity caused by the skin graft expresses itself as more effective reaction against the kidney (Sheil et al., 1964; Moseley et al., 1966). However, it is important to note that rejection of indifferent skin also causes some impairment of renal function. Perhaps the antigens of indifferent skin sufficiently resemble those of the kidney to evoke a heightened reaction against the kidney. It is also possible that skin stimulates the immune response in a non-specific way, such as by increased production of complement, and this allows the dog to react more effectively against the kidney. If the latter suggestion proves correct, it will be important to evaluate the effect of other immunological stimuli, such as blood transfusion, pregnancy, viral and other infections, on function of renal allografts.

After withdrawal of drugs there is a dramatic change in response of the dogs; indifferent skin is rejected in usual first set fashion, and a second graft from the same indifferent donor in second set fashion; specific donor skin is rejected in accelerated fashion. Yet, despite these evidences of normal, or near normal, immunological capacity, these dogs tolerate the long functioning kidney, one for more than 3 years since drug therapy was stopped. This demonstrates specific decreased response to the renal allograft. However, it is possible that after stopping drug therapy the immunological balance with the resident kidney is less stable; the dog grafted 3 times while on drug therapy showed no significant elevation of the BUN with the second and third grafts; with the fourth specific skin graft while off drugs, significant elevation of the BUN occurred; in a second dog off drugs an indifferent skin graft precipitated a rejection process which could not be reversed by reinstition of drug therapy.

The experiments in which second donor kidneys were transplanted gave a spectrum of
<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Days after transplantation</th>
<th>Ischaemia time mins.</th>
<th>Drug therapy</th>
<th>Design of experiment</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.M. 144</td>
<td>203</td>
<td>37</td>
<td>Maintenance</td>
<td>Re-implantation only.</td>
<td>The dog died in uraemia on day 218. Renal histology showed a picture similar to an acute Shwartzman reaction.</td>
</tr>
<tr>
<td>S.M. 142</td>
<td>343</td>
<td>32</td>
<td>None</td>
<td></td>
<td>The second kidney was acutely rejected by 3 days and was removed (Table III). Function of the first kidney remained good for a further 4 days but then a rejection crisis caused the BUN to rise to 144 mg per 100 ml. This was reversed by reinitiation of drug therapy. Renal function remains good past day 450.</td>
</tr>
<tr>
<td>S.M. 176</td>
<td>148</td>
<td>30</td>
<td>Maintenance</td>
<td>Both donor kidneys were transplanted to the pelvis at the initial operation. On day 148 the right pelvic kidney was removed and placed in the neck.</td>
<td>Both kidneys functioned and the BUN remained constant at the pre-operative level for 30 days. Over the next 5 days the BUN rose to 320 mg per 100 ml despite reinitiation of full drug therapy and the dog died on day 195. Histology of both kidneys showed only extensive atrophy of the type characteristic of long functioning renal allografts. Both kidneys continue to function well 100 days later. BUN 13 mg per 100 ml.</td>
</tr>
<tr>
<td>S.M. 257</td>
<td>75</td>
<td>35</td>
<td>Maintenance</td>
<td>The first kidney was re-implanted in the pelvis at the time of transplantation of the second donor kidney to the neck.</td>
<td></td>
</tr>
</tbody>
</table>
results; one kidney was accepted, and two rejected in different fashions. On the occasion when the second kidney was accepted the second operation was relatively soon after the first, the dog has received no skin graft and had been maintained on drug therapy throughout; a similar result was achieved in an experiment involving transplantation of a second kidney from a donor other than the original donor. In both cases it appears that degree of sensitization was slight, immune capacity low, and histocompatibility differences relatively minor.

A second experiment involving transplantation of a second donor kidney resulted in its rejection after 23 days. The first kidney, in situ, sustained increased immunological damage as documented histologically and as reflected in increased levels of BUN, but continued to function. Here it is clear that the dog had not become tolerant of renal antigens after bearing the first renal allograft for 260 days. It also appears that unresponsiveness to the first kidney is not due to dose-dependent antigen overloading, as transplantation of the second donor kidney merely doubles the antigenic load. Indeed, the second kidney was rejected despite levels of drug therapy which allowed acceptance of the first. The factors which should be considered here are:—the presence of the functioning first kidney at the time of the second operation may have allowed better excretion of the drug load, with decreased effectiveness of the drug regimen; the dog may be less responsive to drugs after prolonged therapy; prolonged contact with renal antigens and previous rejection of a donor skin graft may have produced sensitization rather than tolerance, so that a more effective response was mounted against the second kidney than was produced against the first.

Whichever of these possibilities proves correct, it remains that the first kidney functioned for 520 days after rejection of the second, and is therefore less susceptible to effects of a rejection process than is the second. This observation has also been made by others (Pierce and Vareo, 1963). It is possible that the second kidney is more vulnerable because of trauma and ischaemia associated with transplantation, and that the first kidney might have been rejected had it been subjected to the same operation. Our re-implantation studies were designed specifically to test this point, and the result of one experiment shows that it is important; here a dog died in uraemia 15 days after re-implantation of a long functioning allograft. The possibility that the first kidney is less vulnerable because of modification during long residence in the host should also be examined. Previous experiments (Murray

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Fig. 10. This kidney functioned as a renal allograft for 550 days. It was then returned to the original donor and functioned as the only kidney until the experiment was completed after 100 days. Histology at this time shows normal tubular epithelium but presence of focal mononuclear infiltration.
et al., 1964) show that the long functioning renal allograft has not lost all antigenicity because it is rejected after transplantation to a third party, and there is no major change in antigenicity because it is accepted when retransplanted to the original donor. In the latter experiments, however, histology reveals reaction of an immunological type by the original donor against the retransplanted kidney (Fig. 10) which implies some form of host modification during residence as an allograft. There is abundant evidence that antigen-antibody reactions continue in long functioning renal allografts, and decreased susceptibility may simply be an expression of diminished number of antigenic receptor sites available because of previous reactions with antibodies.

A third different result in this series occurred when a second donor kidney was acutely rejected. The degree of sensitization and immunological capacity of this dog were high at the time of the second operation because of a previous skin graft from the kidney donor and because drug therapy was stopped for a period of 65 days shortly before the second kidney was transplanted. Histological changes are those of sensitized rejection. As shown in a skin graft experiment it appears that the dog can maintain the long functioning renal allograft even though sensitized against transplantation antigens. A further point apparent in both experiments where second donor kidneys were rejected is that exposure to renal antigens of the second kidney evoked a heightened immunological response or, to express this in another way, that, before the second procedure, there was decreased reaction against the long functioning first kidney. This indicates lessened antigenic effectiveness due to qualitative or quantitative change or decrease in accessibility to the circulation.

The results of transplantation of second kidneys from donors other than those of the first are more uniform. One kidney (discussed above) was accepted, and three were violently rejected. In two of three experiments in which second kidneys were rejected, the first kidney remained in situ, and did not share a similar fate (Table III). In one experiment the first kidney was differentially maintained despite the trauma and ischaemia of re-implantation. The implications involved in the rejection of a second kidney while the first continues to function have already been discussed in relation to second donor kidneys. These apply also to experiments in which the second kidney is from a different donor, but here there is the further consideration that the second kidney has different antigens, and for this reason may be differentially handled. This consideration is shown to be significant in experiments where two kidneys from different donors were transplanted at the same operation (Alexandre et al., 1963). In one experiment one kidney was rejected and removed after 90 days; the other still maintains the dog past 740 days. Here it is also apparent that variation in absorption and metabolism of drugs did not determine dissimilar fates of the two allografts.

It seems clear that the three dogs which violently rejected second kidneys from different donors were sensitized in some non-specific fashion at the time of the second operation; one dog developed generalized arteriolar lesions of an auto-immune type; a second had similar lesions throughout the indifferent transplant; a third dog rejected the indifferent kidney in accelerated fashion in 3 days. These results suggest that long residence of a functioning renal allograft may make it more difficult to achieve acceptance of a second kidney. This may be of importance because it is now established that in some cases in dogs (Sheil et al., 1967) and humans (Murray et al., 1964), failure of renal allografts occurs after many months of satisfactory function. However, many second kidney experiments reported here were complicated by prior skin grafts, or by periods off drug therapy. The former is known to be a powerful immunological stimulus, and the latter appears in these studies to allow release from significant non-specific inhibition of the immune response. Neither factor is usual in clinical transplantation, and these experiments suggest that they should be avoided. A further consideration is that the majority of our dogs had good function of the first kidney at the time of transplantation of the second kidney. Human patients only receive a second kidney after failure of the first and uraemia is known to depress immune capacity.
MECHANISM OF DRUG INDUCED TOLERANCE TO RENAL ALLOGRAFTS IN DOGS

SUMMARY AND CONCLUSIONS

When maintained on immunosuppressive drug therapy bilaterally nephrectomized dogs with long functioning pelvic renal allografts have significantly impaired immune capabilities, but are able to reject specific donor and indifferent skin while maintaining the renal allograft. However, skin survival is prolonged, and rejection is accompanied by impaired renal allograft function. Kidney donor skin survives longer, and causes greater impairment of renal function, than indifferent skin. Specific and non-specific factors are involved.

In some cases drug therapy may be stopped. After withdrawal of drugs indifferent skin is rejected in first set fashion and specific donor skin in accelerated fashion. During skin rejection there is increased reaction against the long functioning allograft, but this can continue to function. That is, dogs have normal, or near normal, immunological capabilities and exhibit specific, although partial, tolerance to resident renal allografts even when sensitized to transplant antigens.

Second renal allografts from the same or different donors are accepted if transplanted relatively soon after the first when drug therapy has been maintained and there has been no other immunological stimulus. In other circumstances second donor and indifferent kidneys were violently rejected while the first kidney, in situ, showed no such acute change, even when subjected to equivalent ischaemia and trauma of re-implantation.

Nature of the immunity enjoyed by long functioning renal allografts is not clear. Dogs are not wholly tolerant of renal antigens as second donor kidneys are rejected. This also excludes dose-dependent antigen overloading as the mechanism. Chance histocompatibility is important, but not the determining factor. Variation in absorption and metabolism of drugs does not appear of major significance. Long functioning allografts do not lose antigenicity as they are rejected by third parties. Despite significant host modification of renal allografts there is no major change in antigenicity as they are accepted when retransplanted to the original donor. However, histology shows the original donor to react against the retransplanted kidney. Evidence presented here supports chance histocompatibility, significant impairment of the immune response, decreased reactivity against the resident kidney, and decreased susceptibility of the long term graft, as mechanisms involved in tolerance to long functioning renal allografts.

REFERENCES


