LONG-TERM PRESERVATION OF THE KIDNEY

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At the EDTA meeting in Lyons last year, Kemp and his colleagues from Leeds described attempts to obtain long-term preservation of rat kidneys by cooling to —79°C, without ice crystal formation. This work was based on the report by Farrant in 1965, who cooled strips of smooth muscle to this temperature in solutions containing varying concentrations of dimethyl sulphoxide (D.M.S.O.). The muscle strips retained their contractile properties after they had been warmed and the D.M.S.O. removed from them.

Kemp showed that the process of cooling, and rewarming resulted in a large increase in the total weight of the kidney and a considerable loss of protein, potassium and enzymes.

This present paper reports further experiments from Leeds, in which the technique of perfusion employed by Kemp was modified. Twelve rat kidneys were perfused and the resultant show that weight gain was eliminated and protein loss reduced to a minimum.

Table I illustrates the principal modifications adopted:

1. The composition of the perfusing fluid was altered. High Molecular Weight Dextran (molecular weight 110,000) was found to contribute greatly to reducing weight increase and was therefore substituted for the Low Molecular Weight Dextran previously used.

2. As before the concentration of D.M.S.O. in the perfusing fluid was increased during cooling in 5% steps, but by indirect measurements using a refractometer, it was found that only 2 ml of solution were required to produce this increase. Previously 8-10 ml had been used.

3. Perfusion pressures were kept below 70 mm Hg. Kemp had used pressures of up to 300 mm Hg.

4. All solutions were filtered through a 1.2 µ millipore filter immediately before perfusion, thus removing solid particles which might otherwise block the small vessels of the kidney.

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<td>Mannitol</td>
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Fig. 1. Protein loss. Modified technique—up to 7.5 mg. Original technique—up to 40 mg.

5. Mannitol was added to the perfusion fluid during rewarming to counter the osmotic effect of progressively lowering the D.M.S.O. concentration.

RESULTS

a. Weight gain. This was completely prevented using the modified technique. Previously the kidney had gained up to 80% of its original weight.

b. Protein loss (Figure 1). Up to 7.5 mg of protein were lost from the kidney throughout perfusion. Using the original technique protein losses of up to 40 mg were recorded. The loss was found to be greatest at the higher temperatures. At low temperature, that is with a higher concentration of D.M.S.O., the protein loss was minimal and in some cases

Fig. 2.

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reduced to zero. In kidneys in which weight gain was allowed to occur protein loss was
invariably greater.

c. Histology (Figure 2). Sections stained with supravital dye and with haematoxylin and eosin
were studied. These showed good cellular detail and clear nuclei. Brush borders were
intact in many places, but some cells contained droplets and vacuoles. There was no
interstitial oedema.

Summary

The method employed by Kemp in an attempt to obtain long-term preservation of rat
kidneys was modified in the following manner:
1. A dextran of molecular weight 110,000 was substituted for the Low Molecular Weight
Dextran as the main colloid in the perfusion fluid.
2. The volume of the perfusate used and the perfusion pressure were reduced.
3. Mannitol was added to the perfusion fluid during the rewarming phase.
4. The perfusion fluid was filtered through a millipore filter immediately prior to use.

The result of these modifications was to prevent weight gain and to reduce protein loss
from the kidney during perfusion.

REFERENCES

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DISCUSSION

Le Président (Legrain, Paris): Je voudrais demander au Dr. Vantelon si les résultats qu’il a notés à propos de la forte mortalité chez les malades ayant fait un rejet et traités ultérieurement par dialyse chronique n’est pas la meilleure preuve qu’il faut éviter dans tous les centres qui ont les deux possibilités de traitement, de vouloir à tout prix chercher à traiter les malades en crise de rejet par de hautes doses de corticoïde qui conduiront à une situation dramatique, dans laquelle l’hémodialyse ultérieure est fréquemment voué à l’échec.

Vantelon (Paris): Je pense que c’est effectivement la raison de la mortalité élevée chez ces malades. Toutefois, le nombre de malades traités, les causes assez variées de décès (infarctus du myocarde, hémorragie après ulcère cortisonique, etc.) ne permettent pas d’avoir une opinion définitive à ce sujet. Mais il y a toute évidence pour que ce soit effectivement la cause de la mortalité excessive chez ces malades.

Funck-Brentano (Paris): Le nombre de reins de cadavres susceptibles d’être prélevés dans des conditions suffisamment bonnes pour faire l’objet après cela d’une transplantation est très réduit. Les listes que nous avons proposées M. Traeger pour évaluer les chances qu’ont des malades maintenus en dialyse chronique de recevoir un rein de cadavre ne paraissent être d’un intérêt certain. Il faut apparaître sur ces listes des malades qui, du fait des circonstances, sont justement placés hors liste et ont de ce fait très peu de chances de rencontrer un rein de cadavre susceptible de leur être transplanté. Etant donné les progrès réalisés dans les méthodes de conservation des reins prélevés après la mort, je me demande si le moment n’est pas venu de suggérer que les centres s’intéressant à la fois à la dialyse et à la transplantation se communiquent dans le cadre de l’EDTA la liste de leurs malades “hors liste” avec leurs groupes sanguins érythrocytaires et leucocytaires, pour bénéficier des possibilités de transplantation de reins de cadavre éventuels appartenant à des groupes rares.

Rae (Seattle): I should like to ask Dr. Vantelon, who gave the first paper, what was the evidence of tertiary hyperparathyroidism in the patients he mentioned, what indication was there for parathyroidectomy following transplantation and at what stage after transplantation was this parathyroidectomy performed? I should also like to know what the calcium in the dialysate was in these patients who developed so-called tertiary hyperparathyroidism.

Secondly, Dr. Vantelon mentioned the high incidence of gastroduodenal ulceration. At the Swedish artificial kidney centre in Seattle, where patients dialyse twice a week in the centre, there is a high incidence of calcium and bone problems and also a high incidence of gastrointestinal ulceration and X-ray negative dyspepsia. I should like to know if he thinks there is any connection between these two circumstances.

Vantelon (Paris): Ceci est une très longue question. Chez les malades en dialyse chronique, on pense qu’il existe une hyperparathyroïdie autonome lorsque la calcémie est normale ou un peu élevée, alors que le bain de dialyse ne contient que 3 meq par litre de calcium. Lorsqu’il existe des lésions osseuses qui sont évacutrices des lésions d’hyperparathyroïdie, en particulier au niveau des mains et des dents, nous avons rarement la preuve qu’il existe bien une hyperparathyroïdie. En effet, les signes biologiques de l’hyperparathyroïdie sont totalement masqués chez ces malades par les dialyses d’une part, et par l’anurie d’autre part.

Au contraire, chez les malades transplantés qui ont une fonction rénale pratiquement normale, les signes classiques de l’hyperparathyroïdie peuvent réapparaître; chez deux malades, quelques semaines après la transplantation, la calcémie s’est spontanément élevée jusqu’à des taux de 140 milligrammes par litre; chez l’un d’entre eux, il a été nécessaire de
faire une parathyroïdectomie partielle pour ramener le taux de réabsorption tubulaire du phosphore à la normale; chez l’autre, on a pu observer une lithiase rénale apparaissant dans les semaines suivant la transplantation et ce malade a dû subir d’une part l’ablation de ces pierres urétérales, d’autre part l’ablation des parathyroïdes. Par contre, trois autres malades, qui étaient très suspects d’avoir une hyperparathyroïdie autonome sur la base des données cliniques avant la transplantation, n’ont eu aucun trouble particulier après la transplantation et il est possible—sans pouvoir l’affirmer—que ces hyperparathyroïdies autonomes guérissent par la corticothérapie qui suit la transplantation.

En ce qui concerne les ulcères gastro-duodénaux, nous n’avons observé qu’un cas chez un malade qui n’avait pas d’hypercalcémie.

MIGNONE (Parme): Je voudrais demander quelle est l’interprétation la plus probable pour expliquer l’effet favorable que l’hémodialyse exerce sur la préparation à la transplantation rénale; s’agit-il d’un mécanisme immunologique, ou d’autre chose?

VANTELON (Paris): Je pense que le Docteur Michielsen répondrait beaucoup mieux que moi à cette question. L’hypothèse est que d’une part l’insuffisance rénale très prolongée diminue les réactions immunologiques et que, d’autre part, les transfusions multiples pourraient éventuellement avoir le même effet.

MIRKOVITCH (Lausanne): I should like to make two comments about the two papers concerning preservation of the kidney. The first paper is an excellent demonstration that the kidney cell can be stored and preserved, but, according to our work in kidney preservation, there is also a transport across the capillary membrane that is even more difficult to preserve than the function of kidney cells.

Regarding the second paper, as long as we were using solutions with artificial colloids, we could not preserve the transport across the capillary membrane after reimplantation. As soon as we started using blood or plasma, the picture changed to 50% of successes after 24 hours preservation.

KEMP (Copenhagen): I should like to congratulate Dr. Carruthers and his colleagues. I have just one question: did you experience any freezing of the tissue when you went down in temperature, because, in our experience, if we did not use 5 ml/g tissue, we very often had freezing?

CARRUTHERS (Leeds): We did indeed get freezing on occasions and we modified our technique then to take our temperatures down a lot slower than you had done previously. We then managed to do it without freezing.

HOME (London): I should like to ask a question concerning the preservation of canine kidneys. Were any measurements made of the concentration of adenosine triphosphate or the ability of the renal tissue to esterify inorganic phosphate before and after storage? This I think might be a more fundamental pointer towards the anabolic capacity of the kidney.

LANNON (Montreal): No, we did not do these studies, but what we did do was to break down oxidative phosphorylation with dinitrophenol and I produced two curves, one for plain succinate and another curve for succinate that was stimulated with DNP. At a stage you saw on those slides—somewhere around 36 hours—there was no longer any stimulation of oxygen utilisation with dinitrophenol and this, in an indirect way, indicates that at that stage there was a breakdown in oxidative phosphorylation. We did not measure it directly: if we had done so it might be a better parameter.