KIDNEY PRESERVATION AND AUTOTRANSPLANTATION

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The increasing use of cadaver kidneys for transplantation led us to study the preservation of the dog kidney.

Cadaver transplants raise important problems which do not apply to living donors. The greatest problem, besides that of the selection of a suitable cadaver, is the ischaemic damage that occurs to the kidney during agony, collection and transplantation. Current medical ethics do not allow us to protect failing renal tissue during agony. After the death of the donor the time interval between collection and transplantation is likely to exceed many hours, hours during which the damage to the kidney will become irreversible [(Hume, Merrill, Misler & Thorn (1955), Joekes, Porter & Dempster (1957) and Calne (1965)], if no protective measures are taken.

Many authors have attempted to prevent or lessen ischaemic functional impairment by cooling. By now hypothermia is almost universally accepted as the best method for short term kidney preservation. The inconsistent results described and the difference in details of methods used still raise many questions, among others those of immersion versus perfusion, the right temperature level for storage, the optimal speed for cooling and maintenance of normal distribution of renal vascular flow during perfusion.

REQUIREMENTS FOR OPTIMAL PRESERVATION

According to the literature and own experiments requirements for optimal extracorporeal hypothermic kidney preservation are

1. Constant renal vascular volume

Defalco, Mundth, Brettschneider, Jacobson and McClenathan (1965) stressed the necessity for an adequate venous pressure during perfusion for normal flow in the renal cortex.

2. Optimal hypothermia

This will be reached in two stages. The most rapid cooling of the kidney can be attained with renal artery perfusion, using a fluid of 0°C. at 100–120 mm Hg combined with external cooling by sterile ice packs (Figure 1). In this manner cortical and medullary temperatures are dropped evenly within three minutes to 9-10°C. Mazur (1961) reasoned that once this level is reached, further lowering of the temperature should be gradual in order to avoid intracellular ice-crystal formation. Temperatures above 15°C. must be avoided. Re-heating during transplantation should be kept at a minimum (Markland and Parsons, 1963) before the vascular anastomoses are ready.

3. Composition of the perfusion fluid

Several authors, Markland and Parsons (1963), Hitchcock, Kiser, Telander, Peterson (1964)
and Manax, Bloch, Longerbeam, Lillehei (1964) have experimented with different compositions of the hypothermic perfusate. Their results and previous experiments of our own made us use a perfusate composed of Ringer's lactate, glucose, Difco amino-acids, low molecular weight dextran, procaine, heparin and penicillin (Table 1). During perfusion oxygen bubbled through the perfusate (pO₂ 15 mm Hg-0 °C).

<table>
<thead>
<tr>
<th>Composition of the perfusate</th>
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<tbody>
<tr>
<td>L.M.W.-dextran</td>
</tr>
<tr>
<td>Ringer's lactate</td>
</tr>
<tr>
<td>'DIFCO'-Aminoacids</td>
</tr>
<tr>
<td>Procaine 1%</td>
</tr>
<tr>
<td>Heparin 50 mg</td>
</tr>
<tr>
<td>Penicillin</td>
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<tr>
<td>pH 7.4</td>
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4. Simple technique and organization

During the last minutes of life, unavoidable anoxia and hypotension will occur. Experiments should be conducted, imitating hypotension and the ischaemic period of agony. The total preservation time needs hardly ever exceed 4-5 hours, the time required for preparing and implanting the kidney in the recipient. Technique and organization must be simple to meet the challenge of the dramatic moment.

METHODS

Two groups of dogs were used. In the first one 18 dogs were anaesthetised with sodium pentobarbital and intubated. The vessels and ureter of the left kidney were carefully exposed. This kidney was now removed, and the renal artery immediately cannulated. The kidney was cooled (Figure 2) with cold perfusate at pressures not exceeding 180 mm Hg. A venous pressure of 5-10 cm H₂O was maintained. When a temperature of 9-10 °C. was reached the perfusion was discontinued and the organ was stored in the perfusate in a refrigerator at 0-4 °C, for an average period of 7 hours. Following the time of cold ischaemia the kidney was reimplanted in the contralateral iliac fossa, as described by Starzl. Ureter reimplantation was simply done straight through the bladder wall. Approximately three weeks later contralateral nephrectomy was performed.
With a second group of dogs the same procedure was followed, with the exception that prior to nephrectomy and preservation the dogs were made hypotensive, followed by a period of total renal ischaemia. Arterial pressure was maintained at 30–40 mm Hg for half an hour and total normothermic ischaemia produced by clamping the renal pedicle for 15 minutes. In this group contralateral nephrectomy was performed, directly after reimplantation of the stored kidney. Preservation time was standardized at 4 hours.

Blood and urine samples were taken, biopsies before and after transplantation were seen by the pathologist and electron microscopist; arteriography of perfused kidneys was performed.

RESULTS

The results of the first group are shown in Table II. Eighteen kidneys were preserved, 13 dogs showed a normal renal function as shown by urea and creatinine levels and the capability to concentrate urine to a specific gravity of 1045–1050. Observation is now between 3 and 12 months.

<table>
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<th>TABLE II</th>
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<tr>
<td><strong>Results Group I</strong></td>
</tr>
<tr>
<td>Preservation duration 7 ± 3 hours</td>
</tr>
<tr>
<td>Preserved kidneys</td>
</tr>
<tr>
<td>Normal renal function</td>
</tr>
<tr>
<td>Primary anuria</td>
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</tbody>
</table>
KIDNEY PRESERVATION AND AUTOTRANSPLANTATION

TABLE III
Results Group II

<table>
<thead>
<tr>
<th>Preservation duration 5 ± 1 hours</th>
<th>Hypotension (30-40 mm Hg) — 30 min.</th>
<th>15 min. normothermic ischaemia</th>
<th>Preserved kidneys</th>
<th>Functioning kidneys</th>
<th>Primary anuria (all death within 24 hours)</th>
<th>Still alive</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparing the venous flow during perfusion in group I and group II kidneys, no significant difference was obtained. Vasoconstriction by autoregulation of the dog kidney in shock did not persist, if present, during perfusion. Arteriograms of perfused kidneys after shock and normothermic ischaemia showed a normal cortical flow, which proved that the requirement of normal vascularization in this condition was possible. Here again, no vasoconstriction was observed. There was a difference in weight gain after perfusion of the normal and shocked kidney, the latter being more oedematous than the other.

This might support the experiments of Marchioro, Huntley, Waddell and Starzl (1963), who showed that lowering of perfusion pressures gave less weight gain and better survival of this type of kidney.

DISCUSSION

Only recently the first publications concerning successfully preserved and reimplanted kidneys appeared in literature. Couch, Cassie and Murray (1958) showed, using an extracorporeal heart-lung machine, that kidneys could produce some urine during perfusion. Lachinsky (1960) was the first to show that kidneys preserved at 2–4 °C. after cooling with a blood containing perfusate could function after reimplantation and contra-lateral nephrectomy. Only 8 of 52 dogs survived however. Kiser et al. (1961) using the same technique, showed improving results while 13 out of 17 dogs survived after preservation between 45 minutes and 7 hours. By adding dialysis to the heart-lung circuit Shatkin, Anthon, Anthon and MacNeill (1962) showed that after 5 hours perfusion no change in flow and pressure of the perfusate and in the production of urine had appeared.

Hypothermia alone permits viable storage of the kidney for 8 hours at the most [Birckland, Vogt, Krog and Semb (1959) and Calne, Pegg, Pryse-Davies and Brown (1963)]. Even longer periods were bridged. Humphries, Moretz and Converse Peirce (1964) reported a successful twenty-four hour storage with a canine autotransplant after total nephrectomy. Mannix, Bloch, Largiader and Lyons (1964) started to reach for our ultimate goal to preserve whole organs for even longer periods using the combination of hypothermia and hyperbaric oxygen. Although the role of oxygen under pressure is not clear they were able to preserve kidneys for 48 hours. Even though our goal is to preserve whole organs for weeks or months in organ banks, what is needed at the present time is a simple method to preserve organs in vitro for only a few hours.

Our experimental data, as well as those of others previously cited indicate that by preserving the dog kidney in the simple way described maximal protection against renal damage is given.

This concept is undoubtedly useful clinically when procuring organs from living donors or from donors dying in an operating room. A more complex problem exists when the agony is imitated in that much of the permissible ischaemic time has been lost during a period of low organ perfusion before death. It is evident that kidneys cannot be repaired during storage, but survival after a suitable period of preservation even with severely damaged kidneys is possible.

Five dogs died in anuria after contralateral nephrectomy. Here total venous obstruction caused by a fault in the anastomosing technique occurred three times. Release of this venous congestion gave neither return of normal appearance nor function. In one animal ligaturing
one of two renal arteries of the transplanted kidney caused permanent ischaemia of the greater part of it. Only once could no reason for non-function be found.

It is evident that a flawless technique is of the utmost importance and, in particular, venous congestion should be feared. In our cases it caused irreparable changes in the kidney. In 1950 Dempster already emphasized this danger of venous congestion and underlined the importance of technical errors as a reason for transplantation failures.

Urea and creatinine concentrations in the blood showed only a minimal rise when no difficulties were met during the procedure. In primary anuria a quick rise occurred, followed by death within 4–5 days.

Light microscopy revealed no abnormalities before or after preservation, except that in some kidneys calcium or pigment was found in the tubules. Even with electron microscopy only very slight changes of the proximal tubules were observed. Disappearance of the brush-border as mentioned by Kiser, Telander and Peterson (1961) was not seen (Figure 3).

**Fig. 3a** Before preservation

In the second group of 8 dogs in which the agony was imitated we were faced with a major problem. Uncontrollable bleeding as a cause of renewed or prolonged shock made interpretation rather difficult (Table III). Four dogs died within 24 hours because of prolonged shock and bleeding.

Four dogs had an initially functioning kidney, of which 2 dogs died within 2 weeks because of impaired kidney function.

Two dogs are still alive with a normal renal function as shown by urea and creatinine levels and concentration test.

Light microscopy after reimplantation showed tubular necrosis after early death as seen in shock-kidneys. In the dogs who died within two weeks tubular necrosis and venous congestion was observed. No abnormalities were seen in the biopsy specimens of the two living dogs.
Fig. 3b After preservation

Fig. 3c After preservation. Intact brush border
REFERENCES


DISCUSSION

The Chairman: Les présentations de M. Schimmel, M. Alexandre et M. van Elk posent évidemment des problèmes d'un grand intérêt pour chacun de nous.

We could now ask Dr. Dossetor, who has a very large experience of cadaver kidney transplantation to give us his opinion to introduce the discussion.

Dr. J. B. Dossetor (Montreal): At the risk of making this little more than a comment, may I just show a couple of slides?

Fig. 1. This is the result of survival on 28 cadaver transplants, done since October 1963. The data are arranged in 3 lines and there are 26 of these that were done more than 3 months ago, of which a total of 14 survived for 3 months. There were 8 done more than 1 year ago, of which 2 have survived for 1 year.

The gross survival figure, then, is the bottom line and there is an approximate 6 months survival of 50%. However, if one removes 5 technical failures, one gets the middle line, and if one then removes 5 paravesical abscess infections, all of whom died (a complication of which we seem to have had a predominant number), then one might achieve conceivably 75% surviving at 6 months.

This is the first point that I think is relevant to the discussion this morning.

The second point that I should like to make is on the next 2 slides. This is a comparison of the ischaemic time, in minutes, on the ordinate and the time, in days, along the abscissa before the serum creatinine fell to a level of below 2 mg%.

The solid dots are the total ischaemic time and the open circles are the hypothermic period of the total ischaemic time, and there is no correlation either between the period of hypothermic ischaemia and survival or the total ischaemia.

Fig. 2. This slide shows that there is a correlation, between the normothermic ischaemic time, the time between the patient being declared dead and his kidney being cooled, and the number of days before kidney function is normal as judged by serum creatinine of less than 2 mg%.
DISCUSSION

Fig. 2. Relationship of early function in the transplant and ischaemic time.

We believe, therefore, that one must hasten very fast to get the kidneys cool, but once they are cooled an interval of up to 2 or even 3 hours is still compatible with good kidney function.

Furthermore, of all those kidneys that have not functioned initially, all have subsequently functioned except one.

The function of those who have had tubular necrosis extending over 2 weeks is in no way different, at an interval later, from the late function of those that functioned initially.

We do not believe that a period of tubular necrosis is incompatible with an adequate renal function, long term.

Fig. 3. Now with respect to the factors conditioning survival, the large asterisks indicate major rejections and the top group indicates those 8 patients in whom major rejection occurred.

The squares indicate minor rejections and you can see that, in the middle section, there are 10 patients who had 1 or more episodes of minor rejection. The bottom 3 patients have had no detectable rejection episode on immunosuppressive drugs.

Fig. 4. This shows a breakdown in the same 3 categories and I want to point out first the difference with respect to sex, because you can see that of the women that have been transplanted all but one have died. Of the men who have been transplanted, only 1 has died from an immunological reason. The reason for this we were unable to explain at the Washington meeting.

However, since then I have analysed the figures with respect to other factors. There is no evidence that the blood group incompatibility plays any role even for group O into Group A. The Rhesus factor was incompatible on a number of occasions, but this factor does not appear to have been important. The only point of difference is in the frequency of haemodialysis prior to transplant—those with major rejection were dialysed fewer times—data similar to that of Dr. Michielsen from Leuven.

Difference between the female survival and the male survival is, statistically, significant.
DISCUSSION

The difference between the number of dialyses of the major rejection group and the number of dialyses of the minor rejection group is not quite significant at the 5% level.

![Diagram of rejection episodes](image)

**Fig. 3.** The horizontal lines represent periods of survival after transplantation, each complete line representing 6 months survival. Group 'A' had major episodes of rejection indicated by asterisks, as well as some minor episodes, indicated by solid squares. Group 'B' had minor episodes of rejection only. Group 'B2' have had no detectable rejection episodes.

The Chairman: Thank you, Dr. Dossetor. It is interesting that we had exactly the same experience, at one time, and believed that women were definitely not as good as men, although the difference was perhaps not statistically significant. Then, the results in women were better than in men and now there is no more difference between women and men. Don’t you think, Dr. Dossetor, that it is difficult to get firm statistical conclusions from series in which the total number of cases is not very large and the factors involved in the survival time are numerous.
**DISCUSSION**

**POSSIBLE RELATIONSHIP BETWEEN AGE, SEX, BLOOD GROUPS AND NUMBER OF HEMODIALYSES ETC., AND SEVERITY OF SUBSEQUENT EPISODES OF REJECTION**

<table>
<thead>
<tr>
<th>A ) GROUP WITH MAJOR REJECTION:</th>
<th>R, S.</th>
<th>28</th>
<th>F</th>
<th>(♀)</th>
<th>♀</th>
<th>0</th>
<th>UV</th>
<th>0</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.P.</td>
<td>46</td>
<td>F</td>
<td>(♀)</td>
<td>♀</td>
<td>0</td>
<td>UV</td>
<td>♀</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>L.T.</td>
<td>16</td>
<td>F</td>
<td>(♀)</td>
<td>♀</td>
<td>7</td>
<td>UP</td>
<td>UP</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>A.B.</td>
<td>27</td>
<td>F</td>
<td>(♀)</td>
<td>♀</td>
<td>14</td>
<td>Mean No. of hemodialyses</td>
<td>UV</td>
<td>♀</td>
<td>No</td>
</tr>
<tr>
<td>V.T.</td>
<td>55</td>
<td>M</td>
<td>♀</td>
<td>♀</td>
<td>0</td>
<td>UV</td>
<td>♀</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>C.B.</td>
<td>21</td>
<td>F</td>
<td>(♀)</td>
<td>♀</td>
<td>4</td>
<td>UV</td>
<td>♀</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>E.L.</td>
<td>28</td>
<td>M</td>
<td>♀</td>
<td>♀</td>
<td>6</td>
<td>UP</td>
<td>♀</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>D.N.</td>
<td>28</td>
<td>F</td>
<td>♀</td>
<td>X</td>
<td>2</td>
<td>UV</td>
<td>♀</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>N.O.</td>
<td>25</td>
<td>M</td>
<td>♀</td>
<td>X</td>
<td>22</td>
<td>UP</td>
<td>♀</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

| B ) GROUP WITH MINOR OR MINIMAL REJECTION EPISODES: | A.B. | 30 | M | ♀ | X | 11 | UP | Yes |
| J.D. | 24 | M | ♀ | X | 6 | UV | Yes |
| F.A. | 34 | M | ♀ | X | 28 | Mean No. of hemodialyses | UV | Yes |
| R.V. | 55 | M | ♀ | ♀ | 0 | UV | ♀ | Yes |
| R.D. | 27 | F | (♀) | ♀ | 60 | UV | ♀ | Yes |
| N.K. | 16 | F | ♀ | ♀ | 17 | UV | ♀ | Yes |
| J.B. | 18 | M | ♀ | ♀ | 18 | UP | ♀ | Yes |
| D.B. | 48 | M | ♀ | ♀ | 0 | UV | ♀ | Yes |
| D.V. | 13 | F | ♀ | ♀ | 0 | UP | ♀ | Yes |
| E.P. | 22 | M | ♀ | X | 7 | UP | ♀ | Yes |
| B.T. | 16 | M | (♀) | ♀ | 16 | UP | ♀ | Yes |
| A.S. | 37 | M | ♀ | ♀ | 3 | UV | ♀ | Yes |
| H.G. | 32 | M | ♀ | ♀ | 25 | UP | ♀ | Yes |

| C ) SIX IMMEDIATE OR TECHNICAL FAILURES |

**Fig. 4.** Groupings identical to Figure 3. There is a difference between the numbers of haemodialyses in groups 'A' and 'B'. It is not significant at the 5% level.

Dr. P. R. Uldall (Newcastle): I wonder if I could just have some clarification of the paper by Dr. Schimmel, the contribution from Paris? Because of the language difficulty, I do not know whether I fully understood him.

I gathered that the kidneys which they merely cooled, and which had never undergone ice-crystal formation, recovered well and functioned normally, but finally underwent pyelonephritis, whereas the ones that he definitely froze underwent severe necrosis and did not function. Am I right about that?

The Chairman: Je aimerais moi-même demander à M. Schimmel quel avantage il trouve au refroidissement par un gaz par rapport aux méthodes de refroidissement classiques?

Dr. Schimmel (Evreux). Cela dépend en effet du refroidissement que l'on veut obtenir par circulation interne. Au dessous de -14° les liquides ne sont plus utilisables, même avec du DMSO. Seul un gaz permet de maintenir une circulation au dessous de cette température.

The Chairman: In other words, you used gas because you could much more easily obtain freezing temperatures than with liquid media. But what is the use if the frozen kidneys were severely damaged in all cases?
DISCUSSION

Dr. P. R. Uldall (Newcastle): I should just like to say in experiments that we did on similar lines to Dr. Schimmel and his colleagues using rat kidneys, we did not use intravascular nitrogen gas; we used surface cooling, but we achieved cooling and thawing times very comparable to his. The kidneys also behaved in a very comparable manner to Dr. Schimmel’s kidneys. The ones which were merely supercooled to round about −15°, without ice-crystal formation ever taking place, these recovered extremely well and became normal functioning kidneys and were able to maintain the life of the animal.

As soon as you froze the kidney and got ice-crystal formation, there was severe necrosis, similar to that which he described.

I should like to ask him if he has become aware of the work by Dr. Farrant at Mill Hill in which he suggests that solid organs should be frozen in a step-wise fashion, gradually increasing the concentration of dimethylsulphoxide as the temperature is lowered. We feel that this offers considerable promise and the first experiments we have done, adapting this method to kidneys, are much more promising than the results we had using the old method.

The Chairman: Thank you. Perhaps Dr. Dormont would comment on this. He made and published several experiments on this.

Dr. J. Dormont (Paris): Je n’ai pas d’expérience de la congélation du rein chez le chien mais seulement celle du refroidissement de l’organe par voie externe ou par perfusion d’un, solution saline contenant de la sérumalbumine, la viabilité du rein étant contrôlée par auto-transplantation à l’animal. Dans ces conditions, la récupération fonctionnelle de l’organe a été obtenue jusqu’à 9–10 heures de refroidissement. Au-delà, les échecs ont été fréquents, bien que quelques animaux aient survécu avec un rein réfrigéré durant 24 heures.

Dr. Cohen (London): I should like to comment on the ischaemia times and their relation to subsequent renal function when using cadaver donors. In a series of 24 patients who have received cadaver kidneys, we have found that when the normothermic period of ischaemia exceeds 85 min, we always have tubular necrosis and have to dialyse the patients for a couple of days afterwards. We very rarely see tubular necrosis when the ischaemia period is less than this. Almost inevitably, if it is longer than 85 min, of normo-thermic ischaemia we have also found, like Dr. Dossetor, that the subsequent function of these patients who have had a severe tubular necrosis in the first few days after transplantation is every bit as good as in patients who had good early function.

The big danger in this period, we find, is if they are given too large doses of immuno-suppressive drugs in the presence of poor renal function.

The Chairman: Many of us would agree entirely with your conclusions. Is there any other comment?

Mr. A. E. Kulatilake: At Hammersmith we have cooled kidneys to 4 °C for 20 hours and transplanted them quite successfully.

The technique consists of flushing out the kidney with a solution containing plasma and Rheomacrodex in the proportion of 1 : 3. One ml of Novocaine is added to the mixture, and the pH is corrected to 7.4. The kidney vasculature is held distended by placing bull-dog clamps on the vessels. At the time of transplanting a similar solution is used to rewarm the kidney but with papaverine and persantin added. Till the blood circulation is re-established 9 ml of Rheomacrodex with 1 ml of Novocaine at 40 °C is injected and held in the renal vasculature using bull-dog clamps.

The Chairman: It is time to conclude now. L’une des conclusions pratiques les plus impor-
DISCUSSION

tantes est qu’il est devenu, me semble-t-il, impossible de traiter en une matinée tout la pro-
blième de la transplantation rénale. I think it could be a good suggestion that future meetings
of our Association must no more be concerned with the whole problem of renal transplantation
but rather with more limited subjects, so that they may be discussed more extensively than
it was possible this morning.

Je pense que c’est simplement la preuve que c’est un sujet en plein expansion, dont
echaque aspect, chirurgical, immunologique, clinique, moral, etc. mérite de longs débats.

The fact that we had some difficulty to discuss the whole subject only means that the subject
has been rapidly expanding, and tends to open a number of unexpected new fields to our
medical knowledge. This is why it is such a fascinating subject. (Applause)

The President: We have now reached the end of the scientific meeting and we thank all
those who have taken part, especially Professor Hamburger for chairing this session so ably
this morning.

Before I close the Congress I should like to express my personal gratitude, especially to
Dr. Drukker, Dr. Kerr and Dr. Elliott who have worked so hard to make this meeting a
success. (Applause)

In conclusion, it only remains for me to hand over my duties as President to the in-
coming President, Professor Traeger and we look forward to seeing him in Lyon next year.
(Applause)

Professor J. Traeger (Lyon): I thank you, Mr. Swinney. It is a great honour for me to be
the President of our Association. At the request of the Committee I have accepted the risk
of organizing the next meeting in Lyon where we have good facilities, particularly for
simultaneous translation.

I said that I accepted the risk for, as you know, the next meeting will take place in only
9 months’ time from now, so I ask all members of the Society to make an effort in order that
papers should be presented, and that the life of our Association should not be interrupted.

Let me remind you that Lyon is far in the South, that the sun is always shining there
(Laughter, Applause), and that you will be on the way to the Mediterranean, so I can say to
all of you: Au revoir, à bientôt, à Lyon. (Applause)