# Effect of Thyroid Hormone on Stored Kidney Preservation for Further Transplant (35912)

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(Introduced by S. Gabay)

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In recent years, great interest has developed on the problem of delayed transplant; and several procedures for organ preservation during the interval between donor removal and receptor transplantation have been tried. The most widely used method is cooling with or without hyperbaric measures (1), but the length of time during which the organ conserves viability is still too short.

According to Barker's *in vitro* experiments (2, 3), thyroxine ($T_4$), when added to incubation media, is able to maintain the $QO_2$ of kidney cortex slices at the 56% of basal values, compared with a 13% level without thyroxine, both values being obtained at 72 hr. These results led us to investigate whether thyroid hormone could preserve the viability of the whole organ for further transplant.

We have used different parameters as criteria for preservation; namely, the weight of the organs, *in vitro* $O_2$ consumption, the N content and the lactic- and malicdehydrogenases activities before and after 24 hr of the experimental procedure. The results are reported below.

*Materials and Methods.* In these experiments, 26 adult albino rats from our colony were used. After bilateral nephrectomy, both kidneys were quickly removed and washed through the abdominal aorta with the fluids to be used for perfusion or immersion experiments. One of the kidneys was immediately processed as control, while the other was used for the 24 hr experiments, described below, and then processed like the controls.

The kidneys were weighed as quickly as possible and cortex slices were performed for $QO_2$ measurements in Krebs–Ringer phosphate–glucose medium (KRPG) in a Warburg apparatus. The remainder was homogenized in 0.15 *M* saline at 4°. The homogenates were centrifuged in cold (4°) at 3000 rpm and the supernatants were used for nitrogen (N) determinations (biuret method) and LDH (EC1.1.1.27) and MDH (EC1.1.1.37) determinations according to UV optic methods.

In five rats, both kidneys were incubated in KRPG for 24 hr in continuously oxygenated flasks, to one of which $1.10^{-7}$ *M* $T_3$ was added. In the remaining 21 rats, some experimental modifications were introduced. In five rats, one kidney was perfused through the renal artery at 80 mm Hg pressure with KRPG alone. In four other animals, KRPG plus prednisone (3 mg/100 ml) was used as the perfusion medium. And finally, KRPG plus $T_3$ was the medium used in the remaining 12 animals. In some experiments, a histologic examination of the so preserved kidneys was performed.

*Results. Kidney perfusion with KRPG alone.* Table I shows the results in this experimental group. It is apparent that in this group, all the kidneys displayed a huge edema with concomitant decrease in total N content (mg of protein wet). At the same time the $O_2$ consumption in 24 hr by these kidneys was about 73% of that in the control group. LDH activity decreased in five cases and MDH in three, but differences with the control group were not significant.

*Kidney perfusion with KRPG added with $T_3$.* The addition of $T_3$ did not avoid tissue edema, with the resulting decrease in N content, or the slight decreases in LDH and MDH activities. However, a positive effect on the *in vitro* $O_2$ consumption by the kidneys was readily apparent (Table I). In fact, the $O_2$ consumption under these conditions did significantly increase above the control levels in all the kidneys examined.

TABLE I. Values Obtained in Control Kidney and in Kidneys Perfused with Krebs–Ringer–Phosphate–Glucose Solution of (KRPG) with and without $T_3$ or Prednisone.

| | Wt (mg) | | N (mg/100 mg of wet wt) | | $QO_2$ ($\mu$l/h/100 mg of dry wt) | | LDH (Bücher units/mg of N) | | MDH (Bücher units/mg of N) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | 24 hr | Control | 24 hr | Control | 24 hr | Control | 24 hr | Control | 24 hr |
| Perfusion with KRPG | | | | | | | | | | |
| No. of expts. | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| $\overline{X}$ | 1052 | 1757 | 1.86 | 0.98 | −17.2 | −12.6 | 278 | 191 | 1200 | 842 |
| SD | ±0.13 | ±0.14 | ±0.06 | ±0.20 | ±1.62 | ±3.24 | ±29 | ±54 | ±122 | ±20 |
| % in rel. to control | | +58 | | −48 | | −27 | | −32 | | −30 |
| $p$ | <.001 | | <.001 | | <.001 | | =.01 | | <.02 | |
| Perfusion with KRPG + $T_3$ | | | | | | | | | | |
| No. of expts. | 12 | 12 | 12 | 12 | 11 | 11 | 12 | 10 | 11 | 11 |
| $\overline{X}$ | 975 | 1687 | 1.72 | 0.89 | −14.7 | −16.3 | 275 | 213 | 1272 | 901 |
| SD | ±0.05 | ±0.20 | ±0.24 | ±0.18 | ±5.60 | ±3.11 | ±43 | ±58 | ±371 | ±279 |
| % in rel. to control | | +72 | | −48 | | +11 | | −23 | | −30 |
| $p$ | <.001 | | <.001 | | <.001 | | <.01 | | =.01 | |
| Perfusion with KRPG + pred. | | | | | | | | | | |
| No. of expts. | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| $\overline{X}$ | 1014 | 1634 | 1.50 | 0.83 | −15.7 | −7.7 | 273 | 366 | 1214 | 1830 |
| SD | ±0.14 | ±0.22 | ±0.28 | ±0.15 | ±2.70 | ±2.00 | ±22 | ±33 | ±84 | ±131 |
| % in rel. to control | | +61 | | −45 | | −51 | | +34 | | +50 |
| $p$ | <.001 | | <.001 | | <.001 | | <.001 | | <.001 | |

TABLE II. Controls (c) and Immersion in KRPG Medium with (a), and without (b), $T_3$ Addition.

| | Wt (mg) | N (mg/100 mg of wet wt) | $QO_2$ ($\mu$l/hr/100 mg of dry wt) | LDH (Bücher units/mg of N) | MDH (Bücher units/mg of N) |
|---|---|---|---|---|---|
| Controls | | | | | |
| No. of expts. | 21 | 21 | 20 | 21 | 20 |
| $X$ | 1001 | 1.71 | —16.3 | 259 | 1148 |
| SD | ±0.10 | ±0.044 | ±2.40 | ±57 | ±327 |
| Immersion | | | | | |
| No. of expts. | 5 | 5 | 5 | 5 | 5 |
| $X$ (b) | 1377 | 1.35 | —16.8 | 317 | 1197 |
| (a) | 1459 | 1.34 | —16.7 | 305 | 1207 |
| SD (b) | ±0.20 | ±0.077 | ±2.35 | ±126 | ±342 |
| (a) | ±0.29 | ±0.14 | ±2.64 | ±144 | ±361 |
| % (a) in rel. to (b) | +6 | ±0 | ±0 | —4 | +nl |
| % (b) in rel. to (c) | +37 | —21 | +3 | +22 | +4 |
| % (a) in rel. to (c) | +46 | —21 | +3 | +18 | +5 |
| $p$ (ab) | >.1 | >.1 | >.1 | >.1 | >.1 |
| $p$ (bc) | <.001 | <.005 | >.1 | =.1 | >.1 |
| $p$ (ac) | <.001 | <.005 | >.1 | >.1 | >.1 |

*Kidney perfusion with KRPG added with prednisone.* The addition of prednisone induced marked changes in the biochemical patterns studied (Table I). These were reflected in a remarkable decrease in the N total content and of the $O_2$ consumption by the kidneys, along with a high increase in LDH and MDH activities.

*Kidney immersion in KRPG medium with, or without, $T_3$ addition (Table II).* This method of tissue preservation is much more efficient than the perfusion technique in reducing the tissue edema. While a 164% mean weight increase was observed in the perfused kidney, only a 137 and 145% mean weight increase was present in the kidney immersed in KRPG with, or without, $T_3$, respectively. The decrease in N content which in the perfusion experiments was 53% of control values, was only 21% in the immersion experiments, the differences of the means being significant. With this method, tissue respiration was preserved independently of the presence or absence of $T_3$ in the medium.

The respiratory levels observed were identical to those in freshly removed kidney slices. Otherwise no changes in LDH and MDH activities were recorded.

*Discussion.* It is a well-known fact that tissue edema usually develops a short time after kidney removal. We have observed this fact in all the kidneys studied after 24 hr in our experimental conditions. However, it was apparent that this edema was less prominent when the kidney was simply immersed than when it was perfused. From a histological point of view (Drs. M. Oliva and V. Navarro, Department of Histopathology), it was observed that tubular lesions—consisting of vacuolar degeneration and cell necrosis—were more marked when $T_3$ was added to the incubation medium either in the perfusion or immersion experiments.

Tissue $O_2$ consumption capacity has been widely used by several workers to investigate the complex mechanism of action of the thyroid hormones. In our experiments, with or without added prednisone, $QO_2$ consumption very markedly decreased after 24 hr. But after adding $T_3$ to the perfusion medium, it is increased by 11% above the control values, results which are in close agreement with Barker's observations (2, 3). In our immersion experiments, it was proved that $O_2$ consumption capacity was not affected by the presence or the absence of $T_3$ in the incubation medium. Our findings support the hypothesis advanced by Eastbrook *et al.* (4)

that thyroid hormone "although able to increase the total $O_2$ consumption to more than 300 times at $5 \times 10^{-5}$ *M* concentrations of $T_4$, $T_3$ or analogues do not change the initial oxydation values, suggesting a protective effect against the loss of activity as compared to controls during the experiment."

According to our results, we could feel prone to think that the ineffectiveness of $T_3$ in our immersion experiments could be due to some inability of the hormone in getting into the cells. This is not, however, consistent with our findings that autoradiography, with $^{131}I$ labeled $T_3$ in Ringer immersed kidneys, revealed an even distribution of the radioactivity in the whole organ. As for the changes in enzyme activity in transplantable kidneys, the results obtained by different authors suggest that they are more artifactual than real (5). Doubtless, several factors like the interstitial edema, cell infiltration, etc., may introduce a dilution factor which can mimic a loss of enzymatic activity. We have see slight changes, without statistical significance, except in the prednisone experiments, the results of which show considerable increases, explanable by the well-known effect of corticosteroids on cell metabolism. In agreement with previously reported results, we also obtained a high increase in enzyme activity when prednisone was used, an effect due to the well-known inducer role of corticosteroids on cell metabolism. Wolff and Wolff (6) and Wolff and Ball (7) and others have shown that the thyroid hormones *in vitro* inhibit most of tissue enzymes (namely heart, muscle, and leukocytes). However, they also proved that certain enzymatic activity (urease, ascorbate oxidase, alanine dehydrogenase, and carboxypeptidases A and B) are stimulated.

In our experiments no change was observed on the dehydrogenases studied. The lack of inhibitory effect on MDH does not permit application of the Barker hypothesis that the *in vitro* maintainance of $O_2$ consumption capacity of renal cortex by means of thyroid hormone addition at 24 and 72 hr should be due to the lower activity of that enzyme, so avoiding oxalacetate accumulation and consequently the inhibitory effect of the latter on succinate-oxidase activity, which together with cytochrome *c* and cytochrome oxidase increase in hyperthyroidism, as is well known.

The results obtained in our experimental conditions are contrary to those seen by several workers of the transplantation field. However, two of us (J. P. and J. de O.), with wide experience in such concerns, have been able to show, in autotransplant experiments in dogs, that maximal permissible delay of stored unperfused kidneys is only 7 hr; whereas *in vitro* stored perfusion increased the organ transplantability up to 26 hr interval, which others have furthered to 40 hr by hyperbaric procedures.

There should be, therefore, other factors than those studied by us, which are more deeply or quickly affected when the kidney is not irrigated by the proper fluid during its residence outside the body.

*Summary.* Changes in kidney weight, $QO_2$ of kidney cortex slices, N content, and LDH and MDH activities of kidney homogenates were studied after several experimental procedures, aimed to preserve organ transplantability and considered as patterns of kidney preservation.

The results obtained show, from the biochemical point of view, that continuous perfusion with KRPG medium at low temperature and continuous oxygen supply, is not an adequate method, since *in vitro* $QO_2$ markedly decreases by this procedure. The use of corticosteroids is even more inadequate, because they produce high increase in enzyme activities, as expression of severe tissue metabolic or structural damage.

Thyroid hormone $T_3$, when added to the medium, however, not only prevents such a $QO_2$ decrease, but also produces an increase of the same. Tissue edema is, nevertheless, not avoided.

The most favorable procedure seems to be the simple immersion of the kidney in KRPG medium at 4° and continuous bubbling of $O_2$. In addition to causing less tissue edema, such a procedure is able to maintain *in vitro* $QO_2$ and LDH and MDH activities at the control levels. In this technique, $T_3$ addition lacks any benefit and, on the contrary, it

seems to further the histological changes observed in stored kidneys.

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