# NEPHRON HETEROGENEITY: GLUCONEOGENESIS FROM PYRUVATE IN RABBIT NEPHRON

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## 1. Introduction

In mammals, gluconeogenesis is not a function of the liver only, but also of the kidney cortex. Although the liver plays the main role in blood glucose homeostasis, the kidney has a significant role in certain conditions such as starvation, acidosis, and liver damage [1,2]. In the normal physiological condition, the contribution of rat kidney to blood glucose is 25% and following starvation, ~45% [1]. Extensive studies were done to observe the site of gluconeogenesis using various parts of rat nephron [3,4]. These studies were based on distribution of gluconeogenic key enzymes [phosphoenolpyruvate carboxykinase (PEP-carboxykinase), glucose-6-phosphatase and fructose-1,6-biphosphatase] along the nephron. Very few data on localization of gluconeogenesis by direct analysis of glucose synthesized by various nephron segments during incubation with precursor are available [5]. Here, we measured glucose produced by various microdissected nephron segments from pyruvate, as a substrate, in order to determine gluconeogenic sites in rabbit nephron. Gluconeogenic activity was found to be the highest in the straight portion of the proximal tubule.

## 2. Materials and methods

All reagents were of analytical grade obtained mainly from Sigma Chemical Co. (St Louis, MO) and Boehringer (Mannheim). [$\gamma$-$^{32}$P]ATP was obtained from New England Nuclear (Boston, MA).

### 2.1. *Preparation of slices*

Following perfusion with Hanks' solution [6], the kidney was removed and a rectanglular block along the corticomedullary axis was cut. Then seven 1 mm slices were cut from this block from the surface inwards using a special slicer (Hayashi, Tokyo). Each slice was incubated separately in Erlenmeyer flasks containing 1 ml incubation medium in the same way as for tubules as described below.

### 2.2. *Preparation of isolated tubules*

Male white New Zealand rabbits (1.5–2 kg) starved for 48 h were used. Isolated tubules were prepared by the method in [6].

### 2.3. *Incubation*

Individual nephron segments were collected together in separate groups. Each group was then divided and 4–6 $\mu$g tissue was transferred with a micropipette to each Terasaki's tissue culture plate. Incubation was done in 5 $\mu$l Krebs-Henseleit bicarbonate buffer (NaCl 138 mM, KCl 5.6 mM, $CaCl_2$ 1 mM, $MgCl_2$ 1 mM, $NaH_2PO_4$ 1 mM, $NaHCO_3$ 11 mM) containing 10 mM pyruvate for 60 min under controlled humidity in a shaking water bath with 100 osc./min. The tissue culture plate was gassed with mixed gas (95% $O_2$, 5% $CO_2$) and sealed before incubation. Following incubation, tubules with incubation medium were quickly sucked into siliconized capillary tubes, which were then sealed and boiled to stop the reaction. These were stored at 4°C until analyzed. Supernatant obtained by centrifugation at 10 000 × $g$ for 10 min was used for glucose assay, and the pellet was used for determination of protein.

### 2.4. *Glucose assay*

Glucose synthesized by slices was measured with HK/G6PDH [7] and that by the tubules was assayed

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by the ultramicro-method using [$\gamma$-$^{32}$P]ATP in [8]. Protein was determined after [9], slightly modified for micro-assay.

## 3. Results

Table 1 shows gluconeogenic activity in 1 mm kidney slices cut from the surface inwards. Gluconeogenic activity was observed within 5 mm of the capsule. Activity in the first 4 slices was high, with differences between them being statistically insignificant, activity decreased significantly in slice 5 (~50%), and was very low in slices 6,7. Exact location of gluconeogenic activity along the nephron cannot be well defined from the slice experiment, although a gluconeogenic zone may be conjectured. Therefore, in order to definitively study gluconeogenesis in the nephron, experiments with isolated tubules are necessary.

Gluconeogenic activity could be detected only in the proximal tubule (fig.1). Negligible amounts of glucose were synthesized in the glomerulus and the distal tubule, but no measureable glucose was synthesized in the thin ascending limb of Henle's loop and the cortical collecting tubules. Within the proximal tubule, the highest concentration of glucose was measured in $PT_4$ (113.9 pmol . $\mu g^{-1}$ . $h^{-1}$) and the lowest concentration in $PT_3$ (27.3 pmol . $\mu g^{-1}$ . $h^{-1}$). The difference between $PT_4$ and $PT_5$ is not statistically significant, but activity in $PT_4$ is significantly higher than that in $PT_1$ ($p < 0.05$), $PT_2$ ($p < 0.001$) and $PT_3$ ($p < 0.01$).

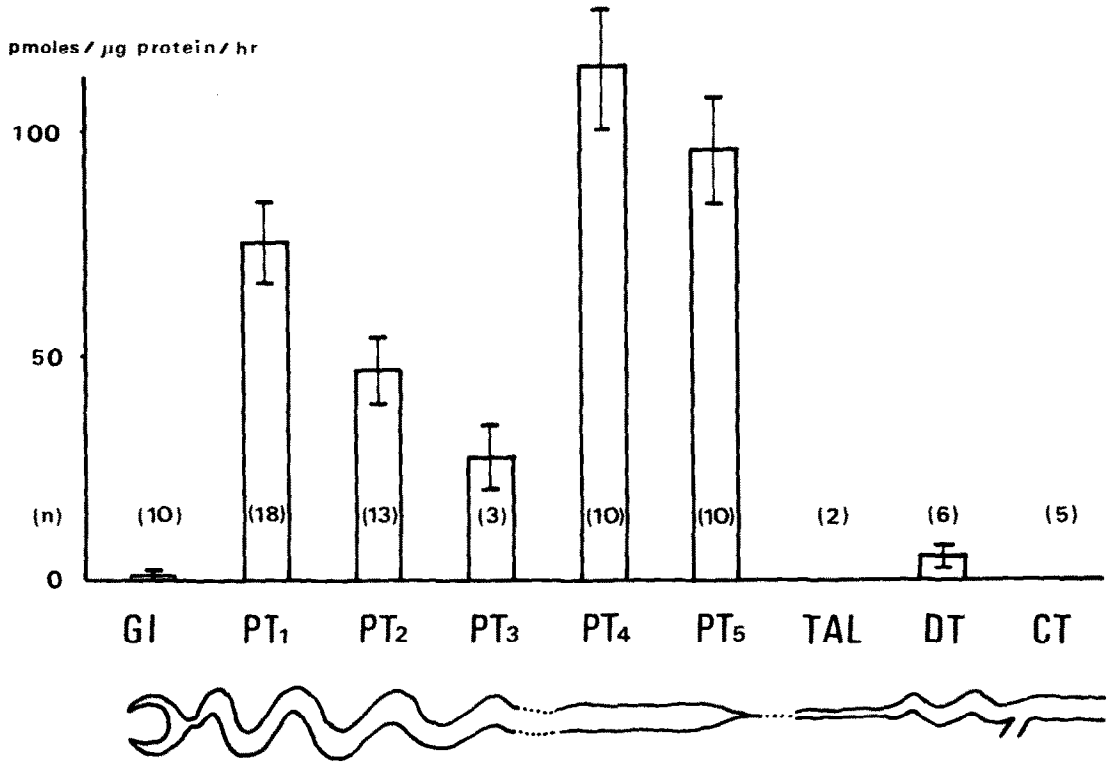


Fig.1. Gluconeogenic activities in isolated nephron segments. Results are means ± SE with the number of observation in parentheses. Glucose concentrations are expressed as pmol . $\mu g$ $protein^{-1}$ . $h^{-1}$.

*Abbreviations:* Glm, glomerulus; $PT_1$, proximal tubule (up to 3 mm from Glm); $PT_2$, proximal tubule (after 3 mm from Glm up to 6 mm); $PT_3$, proximal tubule (after 6 mm from Glm up to 9 mm); $PT_4$, proximal tubule (after 1 mm from the beginning of the thin descending limb up to 4 mm); $PT_5$, proximal tubule (1 mm from the beginning of the thin descending limb); TAL, thin ascending limb of Henle's loop; DT, distal tubule; CT, cortical collecting tubule

Table 1
Gluconeogenic activities in kidney slices

| Slice no.: | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Mean | 66.66 | 69.22 | 59.79 | 57.72 | 33.53 | 5.73 | 4.45 |
| SE | ±2.07 | ±4.09 | ±7.40 | ±5.73 | ±9.12 | ±3.02 | ±2.33 |

Values indicate means and standard errors of 6 experiments from 3 rabbits. Slice no. indicates distance in mm from the capsule. Glucose concentrations are expressed as nmol . $mg^{-1}$ . $h^{-1}$

## 4. Discussion

The metabolic relationship between gluconeogenesis and ammoniagenesis has been established for many years. Gluconeogenesis may have a role in maintaining enhanced ammonia production by disposing of 2-oxoglutarate (deamination product of glutamate) as oxaloacetate in the gluconeogenic pathway [10,11]. We have found the highest activity of phosphate independent glutaminase I in the proximal straight tubule (Shimada, Endou, submitted). Here, the highest gluconeogenic activity was also observed in the proximal straight tubule. Although a clear separation between gluconeogenesis and ammoniagenesis has been reported [12,13], from these two separate observations, we may speculate that these two metabolic processes are interrelated, and gluconeogenesis accompanies ammoniagenesis mainly in the proximal straight tubules of the rabbit nephron.

Our observation that the highest gluconeogenic activity is located in the proximal straight tubule is not consistent with the PEP-carboxykinase (thought to be rate limiting in gluconeogenesis) distribution along the nephron [3,4,14]. In these studies, the highest enzyme activity was found in the proximal convoluted tubule. This contrast of PEP-carboxykinase distribution with gluconeogenic activity may be due to involvement of pyruvate carboxylase in the gluconeogenic pathway from pyruvate. Pyruvate carboxylase may also be rate limiting in gluconeogenesis especially from pyruvate, lactate and amino acids forming pyruvate [15,16]. In [5], the highest gluconeogenic activity was not in the proximal convoluted tubule of rabbit nephron in agreement with our result. But significantly high activity was not found in the proximal straight tubule [5]. This disagreement may be due to different segmentation of the proximal tubule.

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