The Organ-specific Growth Inhibition of the Tubule Cells of the Rat's Kidney

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Earlier experiments [1] indicated that organ-specific growth inhibitors are released from autolyzing kidney and liver cells, respectively. The inhibition of growth appeared as an apparent depression of the mitotic rate of the tubule cells of the kidney 48 h after subtotal nephrectomy, when finely disrupted kidney tissue was spread in the peritoneal cavity. Later on this procedure proved to be equivocal as the peak of the first mitotic wave may be attained several hours earlier than 48 h after the operation. The first mitotic wave to appear after subtotal nephrectomy is by far the largest with a peak between 38 and 48 h after the operation; the peak of the small second wave is attained between 60 and 75 h after the operation. By determining the actual course of the mitotic waves in each kidney dealt with, it was possible to confirm that the first wave was specifically suppressed by autolyzing kidney cells, while the second wave increased in size. Autolyzing liver cells had no effect upon these mitotic waves. The temporary activity of the growth inhibitor(s) could be demonstrated also in the very young rat. The mitotic rate that was due to the normal growth of the kidney tubules, was suppressed for several hours both when finely disrupted kidney tissue was spread in subcutaneous pockets or in the peritoneal cavity. A third way of demonstrating the release of organ-specific growth inhibitor(s) from autolyzing kidney tissue was brought to light by a study of how the removal of perinephrium affected the mitotic rates of the tubules. Application of kidney mass directly to the uncovered kidney surface resulted in a significant decrease in the mitotic rate at this place, as compared to the rates effectuated deeper in the cortex. The mitotic rate of the tubules close to the kidney surface was not changed when this latter was covered by finely disrupted liver tissue.

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Because the kidney mass was prepared from the entire kidney, an eventual cellular specificity of the growth inhibition could not be disclosed and the term "organ-specificity" had to be used in the present context.

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The Cytidine Diphosphate Reductase System

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A partially purified enzyme system ("Fraction B") from *Escherichia coli* B catalyzes the formation of deoxyCDP from CDP [1]. The reaction shows absolute requirements for ATP, Mg^{2+} and reduced lipoic acid. Fraction B contains at least two protein fractions (B1 and B2).

A similar reaction occurs in preparations from Novikoff hepatoma [2]. An enzyme system showing requirements similar to bacterial Fraction B was prepared from the tumor.

The requirement for reduced lipoic acid was studied with both the bacterial and tumor Fraction B. With bacterial Fraction B reduced lipoic acid could be completely replaced by a heatstable protein (Factor S_2) together with TPNH. Factor S_2 was purified about 6000 fold over an extract from *E. coli*. It behaved as a single component on agar electrophoresis and during ultracentrifugation.

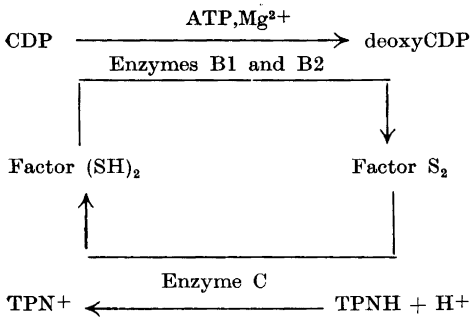
With tumor Fraction B, Factor S_2 and TPNH replaced reduced lipoic acid only in the presence of an additional enzyme fraction (Enzyme C). This was purified about 300 fold from *E. coli*. Bacterial Fraction B was heavily contaminated with Enzyme C.

Enzyme C catalyzed the stoichiometric reduction of Factor S_2 by TPNH:

Factors S_2 + TPNH + H^+ → Factor $(SH)_2$ + TPN+

It is believed that Factor $(SH)_2$ — and not reduced lipoic acid — is the physiological hydrogen donor for the reduction of CDP. Our

present knowledge of the CDP reductase system can be summarized as follows:



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Deoxyuridine Triphosphatase, an Enzyme Connected with the Synthesis of DNA

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The enzymatic synthesis of DNA requires the simultaneous presence of the four deoxyribonucleoside triphosphates [1] (deoxyATP, deoxyGTP, deoxyCTP and deoxyTTP). In the absence of any of the four triphosphates no synthesis of DNA occurs. However, if deoxyUTP is substituted for deoxyTTP, DNA containing uracil in place of thymine is synthesized [2]. So far uracil has not been found in "natural" DNA.

The absence of uracil from "natural" DNA was thought to be caused by the lack of a kinase, capable of phosphorylating deoxyUMP [2]. At that time it was believed that deoxyUMP was formed through a reduction of UMP.

With a partially purified enzyme system (Fraction B) from *Escherichia coli* it was found that the reduction of cytosine ribonucleotides occurred at the diphosphate level [3]. Therefore the possibility of an enzymatic formation of deoxyUTP was reinvestigated.

With Fraction B it could be demonstrated that UDP was the substrate for the enzyme system and that the formation of deoxyUDP required the presence of ATP, Mg^{2+}-ions and reduced lipoic acid. These requirements are similar to those demonstrated earlier for the corresponding formation of deoxyCDP from CDP.

In both crude bacterial extracts and in Fraction B a kinase was present which rapidly transformed deoxyUDP to deoxyUTP. A second enzyme catalyzed the rapid breakdown of deoxyUTP.

This enzyme is called deoxyuridine triphosphatase. The enzyme reaction catalyzed by Fraction B could be studied either by following the appearance of pyrophosphate or of labelled deoxyUMP (from tritiated deoxyUTP). The following stoichiometric reaction could be shown: deoxyUTP $+ H_2O \rightarrow$ deoxyUMP + PP. The enzyme showed no requirement for added Mg^{2+} but was inhibited by EDTA. No cleavage of UTP, deoxyTTP, CTP, deoxyCTP and deoxyUDP was observed. K_m (deoxyUTP) = 3×10^{-6} M.

It is believed that deoxyuridine triphosphatase participates in a series of enzyme reactions which prevents the utilization of deoxyuridine phosphates for DNA synthesis and instead directs deoxyuridine phosphates towards the synthesis of thymidine phosphates.

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