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Studies on the Nucleic Acid Metabolism of Renal Tumour Cells in Vitro

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Summary. Renal tumours, obtained after nephrectomy, were subjected to in vitro study of the incorporation rate of C 14-8-adenine into nucleic acid bases. - In 10 examples of renal cell carcinoma, the incorporation rate into adenine and guanine were 2.01×10^{-2} (cpm/umole base) and 1.83×10^{-2} for DNA, and 3.00×10^{-2} and 2.83×10^{-2} for RNA. The incorporation rate in renal pelvic tumour was rather higher than that in renal cell carcinoma. It was demonstrated that the incorporation rate in Wilms tumour was highest. - These incorporation rates provide an index of the biological activity of the tumour cells.

Key words: Nucleic acids, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), incorporation rate, renal tumour, biological activity

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Introduction

At present the pathogenesis of renal tumours is not completely established. It is considered that growth and multiplication of malignant tumour cells are caused by some changes or disturbances of nucleic acid metabolism (1). Therefore it might be useful to assess the malignancy of tumour cells by studying their nucleic acid metabolism.

This paper presents a study of the dynamic aspects of nucleic acid metabolism of renal tumour cells in vitro, using a radio-active tracer as a precursor of nucleic acids.

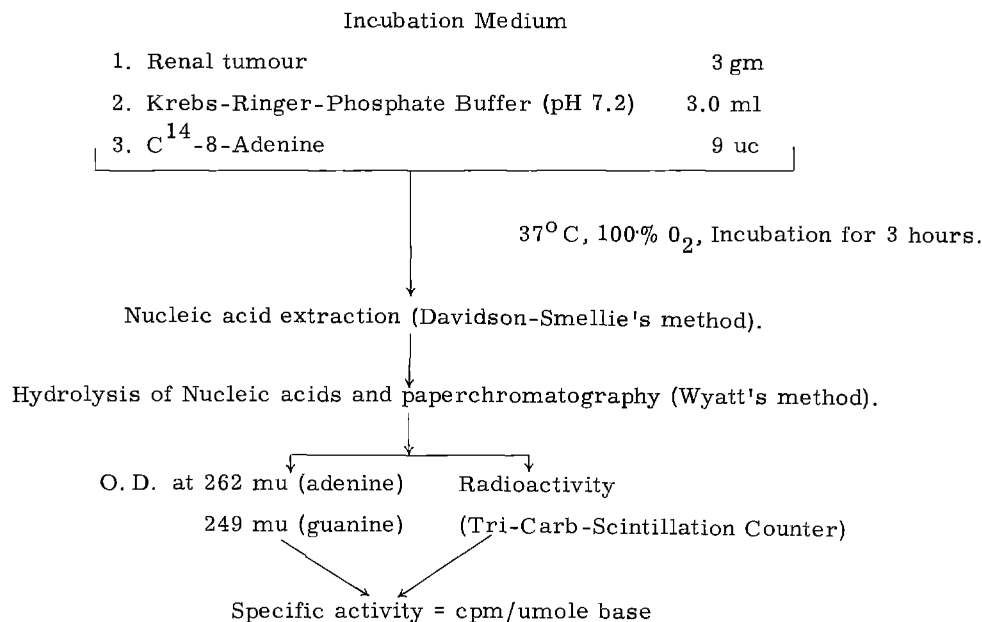
Materials and Methods

Specimens obtained from 12 patients with renal tumours (10 renal cell carcinoma, 1 renal pelvic tumour, and 1 Wilms tumour) who underwent nephrectomy at the Department of Urology, the University of Tokyo Hospital, were subjected to this study.

Experimental procedures are schematically shown in Table 1.

3 grams of renal tumour tissue were minced into small pieces, mixed and washed with Krebs-Ringer-Phosphate buffer (pH 7.2) three times. The incubation medium was 9 ml of Krebs-Ringer-Phosphate buffer, containing 9 uC of C14-8-adenine (Specific activity: 31.5 mC/mM Daiichi Pure Chemical Corp. Tokyo). Incubation was performed at 37°C under 100% oxygen for three hours. At the end of incubation renal tumour cells were separated from the incubation medium by centrifugation. The samples were washed three times with Krebs-Ringer-Phosphate buffer. Extraction of the nucleic acids from the samples was done by the method of Davidson and Smellie (2). 100 mg of the extracted nucleic acids were hydrolysed with 1 ml of NaOH under 37°C for 18 h. To this hydrolysed solution 0.2 ml of 6N HCl and 1 ml of 70% trichloric acid were added. After centrifugation of the mixture the sediment contained DNA while the supernatant was composed of RNA. To avoid contamination hydrolysis of RNA and sedimentation of DNA were repeated three times. 0.5 ml of 70% perchloric acid was added to the DNA fraction and the mixture was incubated at 100°C for 40 minutes. Hydrolysates were cooled and adjusted to pH 5.4 with 10N KOH. Potassium perchlorate was eliminated by centrifugation.

Table 1. Experimental Procedures



The supernatant contained DNA hydrolysates. To the RNA fraction 2 volumes of iced ethyl alcohol were added. Thereafter saturated barium hydroxide was added to the mixture with phenol red as an indicator. This procedure resulted in sedimentation of RNA as the barium salt. After hydrolysis as done for DNA, the supernatant was alkalinized with 10N NaOH and 10 ml of silver nitrate was added to the solution The RNA hydrolysates were sedimented as silver bases. The supernatant containing the bases of RNA was obtained after 30 min incubation at 100° C after addition of IN HCl.

Both DNA and RNA hydrolysates were subjected to paper chromatography (3). An Isopropanol: HCl: Water system was employed, as the ascending system employing Whatman No. 1 paper. The spots were identified by a ultraviolet lamp. Rf values for adenine and guanine were 0. 36 and 0. 25. Each spot was cut off and eluted in 3 ml of 0. 1 N HCl. 0. 2 ml of the sample was mixed with 15 ml of the solvent in a counting vial. Radioactivity was measured by a liquid scintillation counter (Tri-Carb. 3385. Packard Instrument Co.). Optical density was read at 262 mu for adenine and 249 mu for guanine. The amounts of these bases were evaluated by calculation using molar extinction coefficients.

Incorporation rate was expressed as specific activity (cpm/umole bases).

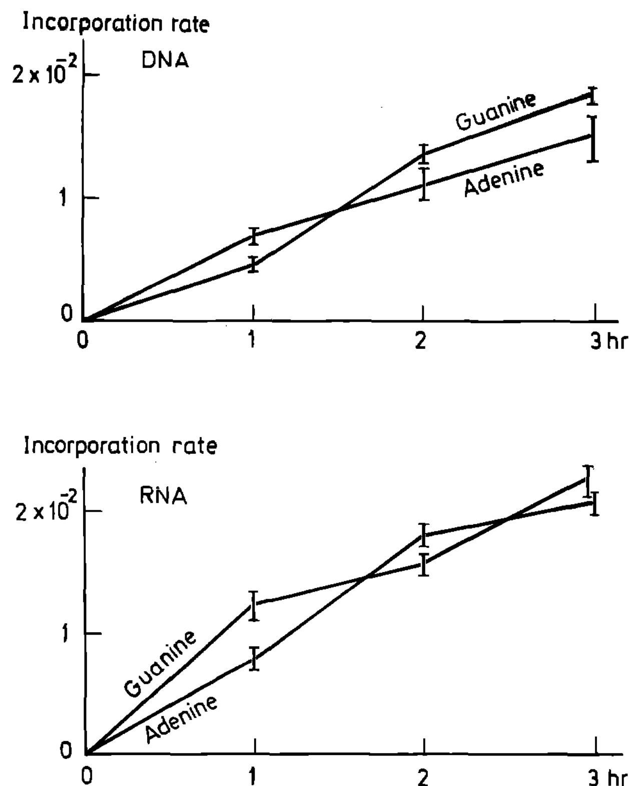


Fig. 1. Incorporation rate of C14-8-adenine into nucleic acid bases of renal tumour in vitro

Results

In one case of renal tumour the incorporation rate into nucleic acid bases was estimated at 1, 2, and 3 h after addition of the tracer.

The incorporation into both DNA and RNA bases was observed to increase with the elapse of time as shown in Fig. 1. There were no great differences of incorporation into the two bases. 3 h incubation was enough to detect the incorporation.

In renal cell carcinoma, the mean incorporation rate into DNA bases was 2.01 for adenine and 1.83 for guanine. Also the incorporation rate into RNA was 3.00 for adenine and 2.82 for guanine. Histology of case No. 8 showed a granular type of renal cell carcinoma and that of the others was clear cell type. There were no great differences in the incorporation rates between them.

In the renal pelvic tumour it was surprising that the incorporation was relatively high in both DNA and RNA bases. Histologically this tumour showed transitional cell carcinoma (grade 3) and metastases to the lungs and bones were found. Three months after the operation the patient expired. The high rate of the incorporation in this tumour corresponded well with the poor prognosis.

In the single case of Wilms tumour the incorporation rate was the highest in the series studied, and the incorporation rate into RNA bases was markedly elevated. The high rate of incorporation would be expected from the degree of malignancy of the tumour. (Fig. 2.)

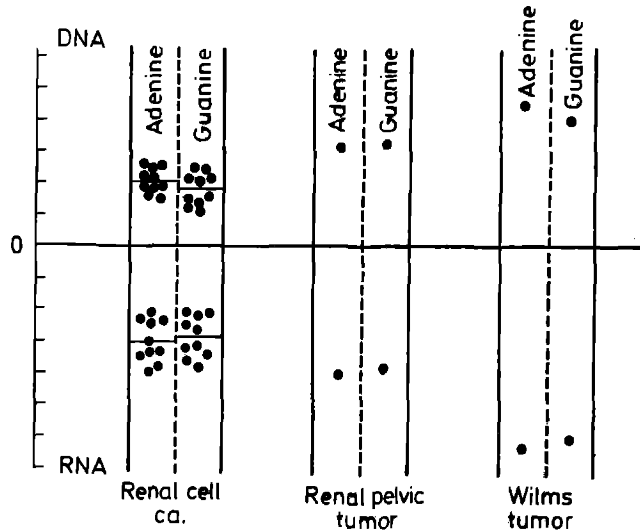


Fig. 2. Incorporation rate of C14-8-adenine into nucleic acid bases of renal tumour in vitro

Discussion

It is now obvious that nucleic acids are important substances controlling the metabolism of human cells. There are two ways of synthesising the nucleic acids in human cells. One is the de novo synthetic pathway and the other is the salvage pathway. Nucleic acid metabolism is active in proliferating cells. This active synthesis of nucleic acids is mainly supported via the salvage pathway.

In the present investigation C14-8-adenine which is incorporated via the salvage pathway, was used as a precursor of nucleic acids, to elucidate the characteristics of the nucleic acid metabolism in renal tumours.

Studies on the nucleic acids in the cells have suggested that neoplastic transformation is a result of uncontrolled or imbalanced biosynthesis of nucleic acids. In our studies the incorporation rate of radio-active precursor into nucleic acids was found to be high in all the tumours, especially in Wilms tumour and renal pelvic tumour. This proves that nucleic acids were actively synthetised and metabolised in these tumour cells.

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