Release of Deoxyuridine by Shope Virus Induced Papilloma and Vx-2 Carcinoma Cells into the Incubation Medium

In a study to screen the nutritive pattern of the tumor cells for the nucleic acid precursors, it was discovered that Shope virus induced papilloma and Vx-2 carcinoma cells release deoxyuridine into the incubation medium. Studies related to this finding are reported here.

Papilloma growths were induced on wild and domestic cottontail rabbit skin with Shope virus[1]. Vx-2 tumor initially derived from papilloma[2] was in its 162–167th generation. Rabbit embryos used in this study were in their late gestation period. Slices of young and vigorously growing tissue from papilloma and Vx-2 tumor was hashed and incubated in TC 199 medium containing specific nucleic acid precursors. Incubation of 100 mg hashed tissue in 3 ml medium at 37 °C for 6 h was found most satisfactory[3].

The medium after incubation was run on Dowex anion exchange resin column devised[4] for separation of bases, nucleosides and nucleotides in a single run. A single molarity of 0.14 *M* acetate buffer at pH 4.4 was used. Effluents were led into a quartz flow cell of a Zeiss spectrophotometer with 0.28 ml capacity and wavelength was adjusted at 260 nm. The area under each peak was calculated and concentration of the nucleic acid precursor determined before and after tissue incubation. In case of radioactive precursors, count of the affluent was done in a Tri-carb liquid scintillation spectrometer. The radioactive count of the labelled nucleic acid precursor before and after tissue incubation gave the amount of the labelled material taken up or given off to the medium by the cells under study. Cysteine-sulfuric acid reaction[5] and spectral characteristics were used to identify the deoxyuridine released into the medium by the cells.

Domestic rabbit (DR) and wild rabbit (WR) papilloma and Vx-2 carcinoma hashed tissue was incubated in TC 199 medium supplemented with all deoxyribonucleosides at 10^{-5} molar concentrations. The tissue was then spun down and the medium chromatographed to see to what relative extent these nucleosides are taken up. While all the nucleosides were taken up to some extent or other, the peak area of deoxyuridine on the tracing was more than in the media without incubation. In the next experiment (Table I), the above tumor tissues were incubated without any deoxyribosides. When such media was chromatographed there was a clear peak where deoxyuridine elutes on the column. Since elution of uracil, uridine and deoxyuridine was very close, further studies were done to confirm that the eluent in the peak is deoxyuridine. The spectral characteristics of the eluent at pH 2 were 280/260 nm = 0.36 and 250/260 nm = 0.89 indicating that the eluent is uridine or deoxyuridine. When cysteine sulfuric acid reaction was carried out, 90% of the eluent accounted for deoxyuridine of the amount calculated from optical density at 262 nm.

Embryonic, hyperplastic and normal skin of the rabbit were incubated under the same conditions. No deoxyuridine was given off indicating that deoxyuridine is released only by the tumor and not by normal cells, even when they have a rapid growth rate.

Further, aminopterin (0.25 mg/l) was added to the medium to see if any more deoxyuridine will be released by blocking deoxyuridine conversion into thymidine. No such increase in deoxyuridine release was seen. Similarly folic acid and vitamin B_{12} at 5 mg/l concentrations in the medium had no influence on deoxyuridine released by papilloma cells.

Radioactive orotic acid and cytidine (Table II and III) were added separately to the medium and the tumor tissues were incubated. In both cases, labelled deoxyuridine was released into the medium. With the same

Table I. Release of deoxyuridine by DR and WR papilloma and Vx-2 carcinoma

Tissue	Deoxyuridine released (nmoles/ml)
DR papilloma	
1	46.24
2	50.53
2[a]	48.51
2[b]	49.63
WR papilloma	
1	36.34
2	32.85
Vx-2 carcinoma	
1	10.11
2	8.08

[a] 0.25 mg/l aminopterin added. [b] 5 mg/l folic acid and vitamin B_{12} added. Conditions of the experiment were those described in the text. Embryonic or normal rabbit skin did not release any deoxyuridine under the same conditions.

Table II. Conversion of cytidine-2-C^{14} into labelled deoxyuridine in DR and WR papilloma and Vx-2 carcinoma

Tissue	Cytidine-2-C^{14} uptake (nmoles/ml)	Labelled deoxyuridine released (nmoles/ml)
DR papilloma		
1	24.28	18.27
2	36.61	26.18
WR papilloma		
1	32.69	23.10
2	30.00	21.34
Vx-2 carcinoma		
1	31.88	18.61
2	18.43	13.42
DR-embryo skin		
1	21.04	0.83
2	17.14	0.96

Cytidine-2-C^{14} used had specific activity of 1.2 μc/mg. While embryonic skin took up considerable amounts of labelled cytidine, only negligible amounts of labelled deoxyuridine were given up to the medium unlike tumor tissues.

[1] W. F. FRIEDEWALD, J. exp. Med. *80*, 65 (1944).
[2] J. G. KIDD and P. ROUS, J. exp. Med. *71*, 813 (1940).
[3] S. ROGERS, *Molecular Basis of Neoplasis* (University of Texas Press, Austin 1962), p. 180.
[4] N. G. ANDERSON and F. C. LADD, Biochim. biophys. Acta *55*, 275 (1962).
[5] S. A. BRODY, Acta chem. scand. *7*, 502 (1953).

labelled procursors and under identical conditions, embryonic or normal skin did not release any labelled deoxyuridine into the medium, although comparable amounts of the labelled precursors were taken up by the embryonic skin from the medium. Normal skin took up only negligible amounts of the label.

Conversion of deoxyuridine into thymidine involves several factors and these studies do not clearly indicate what causes deoxyuridine release into the medium. The observation that either aminopterin or B_{12} and folic acid do not change the magnitude of deoxyuridine released into the medium, however, suggests that the enzyme which converts deoxyuridine into thymidine is lost in these tumor cells.

In chemotherapy of tumors only those antimetabolites can be used whose normal counterparts are extensively utilized by the tumor but not by normal cells. A search for such agents can be done by a chromatographic system which has been adopted in this study and which is simple and rapid. A study of alternate pathways and evaluation of their relative importance is vital for selecting a step in a biosynthetic pathway where the chemotherapeutic agent will be most effective. For example, the folly of use of deoxyuridine or uridine antimetabolites for chemotherapeutic purpose in case of tumors which release or do not use these precursors for DNA synthesis is demonstrated by these studies.

Table III. De novo formation of labelled uridine from orotic acid-2-C^{14} in DR- and WR-papilloma and Vx-2 carcinoma

Tissue	Orotic acid-2-C^{14} uptake (nmoles/ml)	Labelled uridine released (nmoles/ml)
DR-papilloma		
1	4.67	0.359
2	4.48	0.307
WR-papilloma		
1	5.73	0.703
2	5.82	0.89
Vx-2 carcinoma		
1	5.72	0.042
2	6.34	0.019

Orotic acid-2-C^{14} used had specific activity of 7.2 μc/mg.

Zusammenfassung. Virusinduzierte Tumorzellen geben im Gegensatz zu normalen Hautzellen Deoxyuridin ins Medium ab.

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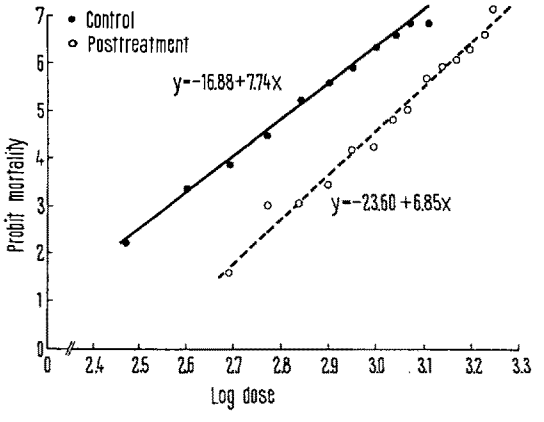
Recovery Effect of Serotonin-Creatinine Sulfate Complex on X-Irradiated Planarians

Several hypotheses have been proposed to explain the radio-protection afforded by serotonin-creatinine sulfate complex (5-HT)[1,2]. Since radiosensitivity in organisms depends as much on the alteration of critical structures as on the restoration of recovery mechanisms, in this communication it is shown that 5-HT may play an important role not only in radio-protection, but in the restoration of recovery mechanisms. The planarians (*Dugesia dorotocephala*) used in the experiments were collected from a pond on the grounds of the botanical garden of the Universidad Nacional Autónoma de México. Planarians were maintained at room temperature in dishes containing electro-purified water and were fed fresh liver twice a week. No natural deaths were recorded.

The control (C) and posttreated (P) groups were irradiated in small petri dishes containing electro-purified water. After irradiation, P-planarians were submerged for 1 h in a solution of serotonin-creatinine sulfate complex (Hycel, Houston, Texas) at a concentration of $3.14 \times 10^{-5} M$ and were then transferred to electro-purified water in regular dishes. After irradiation, C-planarians were returned to electro-purified water in regular dishes too.

Total exposures from 300 to 2000 Roentgens (R) were applied to groups of 40 planarians. Mortality data were recorded daily for 60 days. A Siemens Stabilipan apparatus was used as a source of X-irradiation. Operating at 250 KV and 15 mA and with a 0.5 mm Cu filter at a distance of 40 cm from the subject, it gave a dosage rate of 117 R/min.

The mortality data of C and P groups are illustrated in the Table. The usual sigmoidal dose-mortality curve was obtained. The dose giving 50% mortality in 60 days ($LD_{50/60}$) for group C was 676.1 R (limits 646.5–707.1) and for group P was 1140 R (limits 1104–1177), obtained by Probit analysis (Figure).



Probit analysis of X-rays irradiated planarians with and without posttreatment of serotonin-creatinine sulfate complex (5-HT).

[1] R. Villalobos-Pietrini and A. Laguarda-Figueras, Radiation Bot. 7, 369 (1967).

[2] A. Laguarda-Figueras and R. Villalobos-Pietrini, Proc. Soc. exp. Biol. Med. *126*, 667 (1967).