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Author(s)	田中, 紀元
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# The Protective Effects of some Narcotic Substances Against X-irradiation on Aquatic Animals

## (II) Protective Effects of Ethyl Alcohol

Norimoto Tanaka

Biological Laboratory

(Director: Department of Radiology: Professor Dr. med. Hiromu Kaneda)

Kyoto Prefectural University of Medicine., Kyoto.

両生類に対する麻酔剤の放射線保護効果

## (II) Ethyl alcohol の保護効果

京都府立医科大学生物学科 (指導: 放射線医学教室: 金田弘教授)

田 中 紀 元

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Ethyl alcohol の生体に対する放射線保護効果については今までに数多くの文献があり、とくに Bacteria や哺乳類を材料として示されている。Alcohol の放射線保護作用に関しては2・3の機構が考えられており、放射線照射に対してCatalase の作用をして保護がえられるとか、Alcohol 投与により組織・細胞の代謝が活潑となり酸素含量の低下にともなう酸素効果ともいわれている。著者は Rana nigromaculate 幼生に5%溶液を照

射前20分間、照射中にも浸漬し、照射後の生残曲線をもつて、本剤の保護効果をたしかめた。照射方法には分割照射を加えた。その結果、両生類幼生に対しても、あきらかに本剤の放射線保護効果がみとめられた。なお分割照射に関しては、さきの著者の本幼生でえられた結果と一致し、哺乳類でみとめられる回復はみとめられないことが本研究でもしめされた。これらの結果について検討した。

### Introduction

It is a well-known fact that ethyl alcohol shows protective effects on many biological systems against X-irradiation. For example, the protection in *E. coli* was demonstrated by Holleander ('53<sup>(1)</sup>) and the protection in mice was observed by Cole et al ('52<sup>(2)</sup>), Paterson and Matthews ('51<sup>(3)</sup>) and others. Furthermore, using nucleated red cells of *Rhacophorus* larvae, Takamoto ('59<sup>(4)</sup>) demonstrated that the frequency of chromosome aberration was reduced by the treatment of ethyl alcohol prior to exposure to 140 R X-rays at 22°C.

But, the protective effect of ethyl alcohol in amphibian larvae has been little studied.

It is known that ethyl alcohol employed in the present experiment is one of the substances having a narcotic effect in animals. In the previous paper, the author has shown that 5% chlorobuthanol-treated animals (*Rhacophorus* larvae) were protected against X-irradiation, as indicated by a highly-significant increase in the number of surviving animals, by a slight increase in the body weight and by a tail regeneration in the *Branchiura sowerbyi* which were preserved in 20% chlorobuthanol solution prior to

and during the X-irradiation (600 R) (Tanaka)<sup>(5,7)</sup>. It is known that ethyl alcohol and chlorobuthanol solution have been mentioned as narcotic substances of aquatic animals.

### Material and Methods

The animal employed in this experiment were larvae of *Rana nigromaculata*, 28–33 mm in body length from the same egg block and each group consisted of 25 animals. Irradiation was carried out under the following conditions: 80 kvp, 4 ma, no-filtration, target-animal distance 10 cm, dose-rate 300 R per minute.

The experimental method was as follows;

- (1) 1200 R single exposure
- (2) Prior to and during irradiation, treatment in 5% ethyl alcohol (Total treatment time 24 minutes) 1200 R single exposure
- (3) 400 R 24 hr 400 R 24 hr 400R 3 fractionated exposure
- (4) Prior to and during irradiation treatment in 5% ethyl alcohol (Total treatment times 21 minutes) 400 R 24hr 400 R 24hr 400R 3 fractionated exposure

### Experimental Results

The experimental results are shown in Fig. 1. 5% ethyl alcohol is close to the stable concentration for the experimental animals. Soaking 20 minutes preirradiation and 4 minutes during irradiation provided in all cases significant protection against X-irradiation.

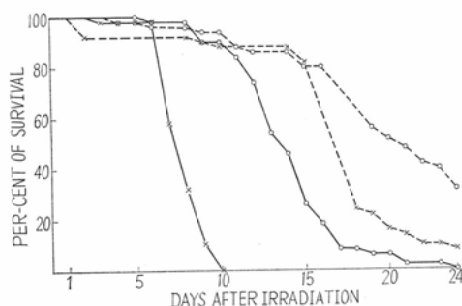


Fig. 1 The effect of fractionated X-rays on *Rana nigromaculata* tadpoles following treatment by ethyl alcohol.

- : 1200R single dose irradiation  
 ○·····○ : Treatment in 5% ethyl alcohol (total soaking time 24 minutes) 1200R single dose irradiation  
 ×—× : 400R 24hr 400R 24hr 400R  
 ×·····× : Treatment in 5% ethyl alcohol (total soaking time 22 minutes) 400R 24hr 400R 24hr 400R

#### I. Groups without treatment of ethyl alcohol

(a) There were no survivors over 10 days postirradiation in the groups of three fractionated exposures and their mean survival time was 7 days.

(b) All died within 24 days in the single exposure group and their mean survival time was 14 days.

#### II. Groups with treatment of ethyl alcohol

(c) With fractionated exposures, there was 8% survival at 24 days and the mean survival time was

17 days.

(d) In a case of single exposure, 32% of the cases survived at 24 days and the mean survival time was 20 days.

In a previous paper (Tanaka)<sup>(6)</sup>, in a survival response of *Rana nigromaculata* larvae fractionated X-irradiated was more effective than the single exposure. In this experiment the finding was the same.

### DISCUSSION

Paterson and Matthews ('51<sup>(3)</sup>), who used ethyl alcohol before lethal dose of X-irradiation (700 R) in "A" strain mice, reported its protective effect, but they considered that its effectiveness was probably unrelated to its anaesthetic action. Cole and Ellis<sup>(2)</sup> found that the highest degree of radiation protection, in terms of 30-days survival and body weight recovery, was observed in mice which received 3.76 ml. of 25% ethanol per 100 grams. A hypothesis of the protective effect of ethanol was presented that if the formation of  $H_2O_2$  (among other oxidants) contributes significantly to primary radiation damages, and catalase activity is a limiting factor preventing the accumulating of deleterious concentrations of this substance in X-irradiated tissues, it might be anticipated that chemical compounds which specifically accelerate the turnover rate of catalase would act as protective agents. On the other hand, Takamoto ('59<sup>(4)</sup>) demonstrated that the frequency of chromosome aberrations in nucleated red cells of *Racophorus* larvae was reduced with pretreatment of 3% ethyl alcohol solution prior to 140 R X-rays at 22°C. He and some other authors, suggest as the mechanism of protection that "alcohol must rapidly be metabolized by cells and therefore lower intracellular oxygen tension gives the protective effect".

In this experiment, the higher degree of radiation protection, in terms of 24 days 'survival' was observed in the *Rana nigromaculata* larvae which were soaked in 5% ethyl alcohol. This concentrations of ethyl alcohol is not far removed from the lethal level for the experimental animals. The mechanism of radioprotective action of ethyl alcohol may be connected with a low oxygen tension in the organism or may be related to the chemical properties of this narcotic.

However, during hibernation the metabolism is reduced to a minimum, and despite it, the animals are not protected against the effect of radiation but the manifestations are only delayed (Smith et al ('51)-- and other).

### SUMMARY

(1) Unlike mammals, larvae of *Rana nigromaculata* did not show any recovery phenomena with 3 fractionated irradiation doses spaced at 24 hour intervals and the survival rates smaller in fractionated irradiation than in single exposure. These results agree with the previous observation.

(2) Protective action of ethyl alcohol on *Rana nigromaculata* larvae to X-irradiation was demonstrated.

On the mechanism of protection, the author agrees with the opinion proposed by Takamoto and others that "alcohol must rapidly be metabolized by cells and therefore it lowers the intracellular oxygen tension to give the protective effect". However the protection of this drug may be partially be related to its chemical properties.

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