The Protective Effects of some Narcotic Substances Against X-irradiation in Aquatic Animals
(1) Effects of Chlorobuthanol in Larvae of Rhacophorus arboreus

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両生類に対する麻酔剤の放射線保護効果
(I) Rhacophorus arboreus 幼生に対する chlorobuthanol の効果について

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生体の放射線感受性は呼吸代謝の強度をいろいろにかえることによつて影響されるであろうことはよく知られている．本研究において，水生動物の麻酔剤として周知のCHLOROBUTHANOLの5%溶液を用い，1800RのX線照射に対する放射線保護作用を生残率，体重を指標としてしらべた．本剤に照射前5分，ひきつづいて照射中に動物を浸漬した結果，CHLOROBUTHANOL非処理の対照群と比して，生残率においては大きな保護効果がみとめられ，そして体重においても対照群よりも体重増加がみとめられた．これらの放射線保護効果について，討論した．

Introduction

It is well known that the radiosensitivity of organisms may be influenced by various treatments modifying the intensity of the respiratory metabolism. This effect on radiosensitivity is probably caused by the change of the oxygen content of the cells. 2,4-dinitrophenol, which stimulates the respiratory metabolism when administered before exposure to radiation, has been shown as a protective agent (Tanaka[1]., Praslicka et al ('62[2])). Also, some narcotic substances and analgetics have been demonstrated to be weak protectors against radiation; i.e. ethyl alcohol, morphine, ether, urethane and others (Bacq and Alexander ('61[3])).

According to Cole et al ('52[4]), the highest degree of radiation protection, in terms of 30-days survival and body weight recovery, was observed in mice which received 25 per cent ethanol. Paterson and Matthews ('51[5]) have reported a similar protective effect of ethyl alcohol in X-irradiated 'A' mice.

In the studies of Cole et al ('61[6]), radioprotective effect in mice was observed by injection of an aqueous urethane (10%) 24 hours prior to X-ray expoure. However, its protective effect was not seen when the drug was administered 30 minutes before irradiation nor when the mice were irradiated 7 days after the last urethane injection.

Using nucleated red cells of amphibian larvae, Takamoto ('59[7]) demonstrated that the frequency of

chromosome aberration was reduced by pretreatement of 3 per cent ethyl alcohol prior to exposure to 140 R X-rays at 22°C.

On the other hand, during hibernation the metabolism of organisms is reduced to a minimum, and despite it, the animals are not protected against the effect of radiation but its manifestations are only delayed (Smith et al ('51[8])., Doull et al ('53[9])).

It is known that aqueous chlorobuthanol employed in the present experment is a narcotic substance of an aquatic animals.

However, only a few investigations have been carried out on this substance against X-irradiation (Tanaka[10]).

The experimental findings, described below, show that suitable treatment with 5 per cent chlorobuthanol (chloretone) prior to and during whole body X-irradiation has a protective effect in terms of 27 days survival and body weight at radiation dose levels ordinarily 100 per cent lethal.

Material and Methods

The animals employed in this experiment were larvae of Rhacophorus arboreus, 26–27 mm in body length and were selected from the same egg block. Each group consisted of 25 animals.

The physical factors were :80 kvp, 4 ma, no filtration, animal-to-target distance 10 cm, dose rate, 300 R/minute. The animals were irradiated by single dose exposure of 1800 R in water and in 5 per cent chlorobuthanol solution. This concentration of chlorobuthanol is far removed from the lethal level for Rhacophorus larvae.

The following experiments were done:

(1) Single dose exposure in water 1800 R

(2) Treatment with 5 per cent chlorobuthanol 5 minutes prior to and during (6 minutes) X-irradiation 1800R

(3) Drug-only (5 per cent chlorobuthanol) treatment (11 minutes) 0R

The measurements of body weight were taken 6, 10, 14, 18, 22 and 27 days after X-irradiation and were of individuals selected at random.

No difference was found between the volume of dissolved oxygen in water and in 20 per cent chlorobuthanol solution, as measured by Polarography in another experiment.

Experimental Results

Body Weight

Figure 1 illustrates the variation in average body weight per 10 individuals after X-irradiation. The effect of radioprotection was detectable from the measurements after X-irradiation. Unfortunately, the values immediately before or after X-irradation were not observed, however, it will be seen an other experiment.

As shown in figure 1, in the irradiated-only control group, the increase in body weight was slight between the 6th day and 27th day after irradiation (the average body weight per 10 individuals 18 days after irradiation was measured from 7 individuals and later measurements were taken of those surviving at the time.

On the other hand, body weight of the 5 per cent chlorobuthanol-treated animals was twice that of the irradiated-only control group, at the last observation (27 days after irradiation), but the degree of

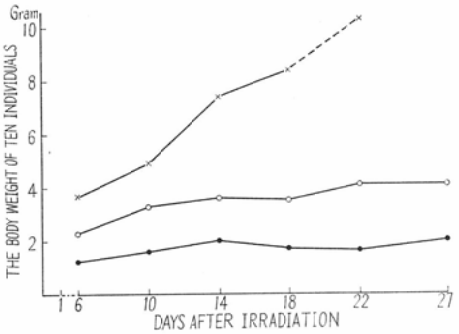

Fig. 1. The body weight of ten individuals after X-irradiation.

———— Reference records (the animals metamorphosized)
×——× unirradiated control (treatment of drug alone)
●——● irradiated controls (1800R)
○——○ 5per cent chlorobuthanol and irradiation (1800R).

body weight increase was quite lcw compared with the nonirradiated control group.

Metamorphosis of the drug-only treated animals began 18 days and continued to occur for several consecutive days.

From these curves, as regards the body weight, the differences in resistance between chlorobuthanol treated animals and non-treated animals to radiation are clearly proved, but treatment of 5 per cent chlrorobuthanol alone may have no influence at all on the developemnt of the animals.

Survival of Animals

The survival curves of the experimental results are shown in figure 2.

It is evident that the animals receiving 5 per cent chlorobuthanol treatments were protected (at 27 days) against lethal X-ray exposure. The majority of the chlorobuthanol-treated larvae survived exposure to a dose of 1800 R.

The other hand, most of the animals of the irradiation-only group were dead 27 days after X-irradiation.

The final values of survival rate after irradiation were demonstrated to be 92 per cent in the chloro-

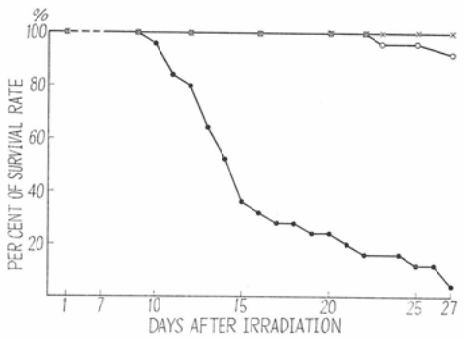

Fig. 2. The survival rate after X-irradiation.

×——× unirradiated control (treatment of drug alone)
●——● irradiated controls (1800R)
○——○ 5 per cent chlorobuthanol and irradiation (1800R)

buthanol treated animals and 4 per cent in the non-treated animals, respectively.

Discussion

As shown above, in the survival rate and body weight of 5 per cent chlorobuthanol-treated animals (amphibian larvae) demonstrated radioprotection against irradiation at a lethal dose of 1800 R.

In general, the radiosensitivity of organisms may be altered by modifying the intensity of the respiratory metabolism. The alteration of radiosensitivity probably depends on the change of intracellular oxygen tention.

It is shown by Bacq et al('61[3]), the radioprotective effect of narcotic and analgesic substances may be due to the oxygen effects.

Praslicka et al ('61[2]) presented data showing that the respiratory metabolism in mice during irradiation was increased after 2, 4- dinitrophenol by 70–80% above rest values before the administration of 2,4-dinitrophenol, and by as much as 200% above values during sleep in controls. Mice irradiated under these conditions were protected aginst radiation, as indicated by a highly significant increase in the number of surviving animals, by a smaller reduction in the number of leucocytes, etc. Furthermore, as indicated by survival response of amphibian larvae, Tanaka[1]obtained that treatment with dinitrophenol prior to and during irradiation in animals recorded a slight prolonged survival time after exposure to a dose of 1200 R.

According to Paterson et al ('51[5]), the administration of ethyl alcohol before irradiation in mice demonstrated a protective effect on survival rate and the protective effect of the alcohol is probably unrelated to its anaesthetic effect.

Cole et al ('52[4])stated that the highest degree of radiation protection, in terms of 30 days survival and body weight recovery, was observated in mice which received ethanol. The mechanism involved in the decreased radiosensitivity of mice following administration of ethanol was presented as catalase activity on irradiated tissues.

Hollender et al ('53[11]), in discussing chemical protection of bacteria against ionizing radiations, state that".....the protective ability of alcohol may be partly due to fact that, in a high concentration, it can tie up oxygen.....".

Narcosis and its effects on irradiation in mice was studied by Pomerantseva('57., '58[13]). According to his experiments, narcosis with nembutal barbamyl and ether in mice X-irradiated with 500 R increased survival rate, prolonged survival time and resulted in a weight gain. The protective effect of the narcotics was due to the inhibition of the respiratory center which resulted in hypoxemia.

As indicatted by tail regeneration in Branchiura sowerbyi, Tanaka ("66[12]) demonstrated that the treatment of chlorobuthanol on the animals reduced the radiosensitivity to X-irradiation.

Chlorobuthanol has a well-known anaesthetic action on aquatic animals.

Out As stated in a previous paper (Tanaka), since the respiratory metabolism is reduced in animals, the radioprotective effect of this drug may be caused by the low intracellular oxygen tention in the tissues.

Furthermore, no difference was found between the dissolved oxygen volume of tested water and that of 20 per cent chlorobuthanol solution. Therefore, the influence of the drug to the oxygen content of the solution need not be considered, and the reduction of the respiratory functions by anaesthetic action alone perhaps comes into question.

I do not expect, however, the sole factor (or mechanism) of the protective effects to irradiation to be the reduction of the respiratory metabolism alone; for example, for nervous system and other biological factors or the chemical properties of this drug may have caused effects.

It is not to be concluded from these experimental results alone, because during hibernation the metabolism is reduced to a minimum, and despite this, the animals are not protected against the effect of radiation (Smith et al ('51[8])., Doull et al ('53[9])).

From the above example, it has come into questin whether the intensity of the respiratory metabolism alone, regardless of other biological factors may be considered as a general criterion of the radiosensitivity of organisms.

From the results of this experiment, it is a fact that narcosis provides radioprotection to animals against X-irradiation, at least.

Therefore, as a result of reduction in the respiratory metabolism by anaesthetic action, the major factor of the radioprotective effects in this drug may be caused by the low oxygen tention in the organisms or may be related to the chemical properties of the narcotic.

Summary

The author studied the effects of treatment with 5 per cent chlorobuthaol prior to and during irradiation on the survival response and body weight in larvae of Rhacophorus arboreus. It is known that chlorobuthanol solutions have an aneasthetic action on aquatic animals.

Five per cent chlorobuthanol-treated animals were protected against radiation, as indicated by a highly-significant increase in the number of surviving animals, by a slight increase in the body weight as compared with the irradiated-only controls (1800 R).

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