## Possible Effect of Radiation Produced Hydrogen Peroxide on Post-Irradiation Aversion in Mice

Mice have been known to prefer saccharin sodium solution to tap water, but when it is offered just before the animals are exposed to ionizing radiations, post-irradiation avoidance is induced. We have reported that irradiation of the saccharin solution itself with surprisingly small dosages also resulted in avoidance behavior of non-irradiated mice[1,2].

As early as the beginning of this century (1900–1925), hydrogen peroxide has been found as a radiolytic product of water and other aqueous solutions[3–8]. Quantitative measurements of $H_2O_2$ produced in irradiated water and other solutions have been done by many investigators. So far no tests have been performed to determine whether hydrogen peroxide is produced when either sodium saccharin solution, sucrose solution, or plain water is irradiated at low dosages. However, we verified experimentally that mice also avoid both saccharin and sucrose solutions when small amounts of $H_2O_2$ were added to either solutions.

In this paper we are presenting a striking similarity of avoidance behavior caused by irradiated saccharin sodium solution, irradiated sucrose solution and irradiated water, and that of hydrogen peroxide added to these same non-irradiated liquids.

*Methods and results.* Three experiments were performed. Each was divided into 2 parts. The first part involved irradiated saccharin, sucrose and water. In the second part, hydrogen peroxide was added to the non-irradiated liquids before offering them to the animals. Thus, 6 groups of 180 male CFW mice were divided randomly into 18 cages containing 10 mice each.

*Experiment 1.* The first group of mice (3 cages of 10 mice each) had both saccharin sodium solution (1% per weight) and tap water available. The liquids were contained in graduated glass bottles placed on the right and left side of each cage and alternated daily to compensate for any possible positional preference. As soon as saccharin preference was definitely established (Figure 1a), saccharine from the same stock solution was exposed to 2000 R of X-irradiation. Radiating techniques were described throughly in reference[1]. It was observed that the normal amount of daily saccharin consumption dropped from 70–30 cm³ on the following day. The daily water intake increased from 38–65 cm³. On the next day (2nd day after irradiated saccharin was offered), recovery of saccharin preference seemed to take place. The second part of the experiment was run for a longer period (35 days after saccharin preference was established). Again 10 mice in each of 3 cages were used. On the 13th day of the experiment (Figure 1b), hydrogen peroxide (0.03% per volume) was added to the same saccharin stock solution. Daily saccharin consumption on the next day decreased from 60–30 cm³ while water consumption increased from 30–55 cm³. On the 22nd day of the experiment or 9 days after the addition of $H_2O_2$ to saccharin, regular saccharin solution replaced the one containing hydrogen peroxide. Saccharin recovery was observed on the next few days. On the 28th day of the experiment, after the preference of saccharin was definitely re-established, the mice were removed from their cages and exposed to whole-body X-irradiation (450 R at 50 R/min). In the following 2 days, daily saccharin consumption again dropped from 70–25 cm³ while water intake increased very moderately (10 cm³). This experimental part was terminated 1 week after irradiation. At this point recovery of saccharin preference again seemed to start.

*Experiment 2.* The same procedures as in experiment 1 were repeated here, except that sucrose solution (5% per weight) was used instead of saccharin sodium. Figure 2a shows the average daily consumption of both sucrose and water before and after sucrose solution was exposed to 5000 R of X-radiation and replaced the non-irradiated solution on the 7th day. On the 8th day, the daily sucrose consumption decreased from 240–100 cm³ while the increase in water intake was only 10 cm³. On the 10th day of the experiment (3 days after the exposure of sucrose

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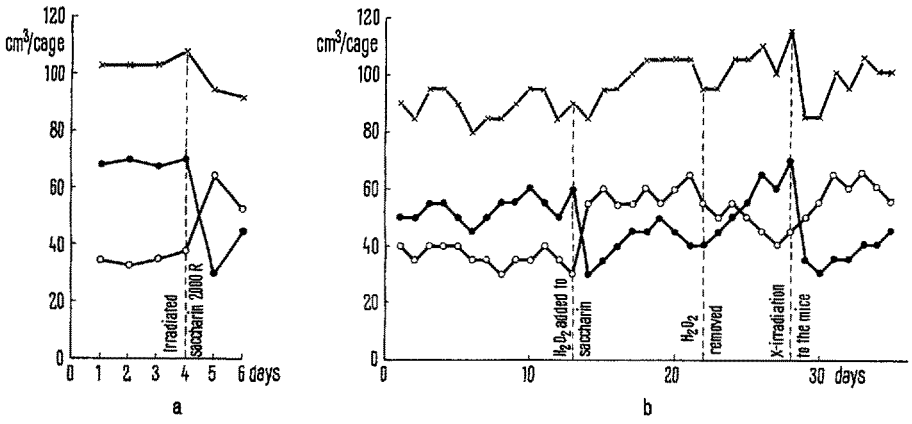


Fig. 1. (a) Average daily consumption curves for saccharin, water, and both liquids before and after the exposure of the saccharin solution to 2000 R —×— total liquid, —●— saccharin, —o— $H_2O$. (b) Average daily saccharin, water, and total liquid consumptions before and after the addition of $H_2O_2$ ($3 \cdot 10^{-4}$) to saccharin solution, and before and after whole-body exposure of the subjects to X-irradiation (450 R). —×— total liquid consumption, —●— saccharin, —o— $H_2O$.

solution to X-irradiation), regular non-irradiated sucrose solution from the original stock solution replaced the irradiated one. It was observed immediately on the following day a complete recovery of sucrose preference. In Figure 2b (30 mice in 3 cages) the daily sucrose consumption dropped from 230–120 $cm^3$ on the day after hydrogen peroxide (0.03%/volume) was added to this sweet solution. On the 22nd day of the experiment this solution was removed and replaced with regular fresh sucrose from the same stock; one observed gradual sucrose preference recovery up to the 28th day. On this same day, the mice were exposed to whole-body X-irradiation (450 R). A sharp drop in the daily sucrose intake was immediately shown on the next day.

*Experiment 3.* Two bottles of plain tap water were offered to 3 cages of 10 mice each, one placed on the right side and the other on the left. It was normally observed that the animals would drink more water from either the right or the left bottle and thus established positional preference. This preference was definitely shown for the right side water bottle in Figure 3a. On the 6th day, after the mice preferred the water from the right bottle, irradiated water (water exposed to 5000 R of X-irradiation) replaced the normal water. On the 7th day it was observed that the daily amount of water consumed from the preferred right-side bottle dropped from 75–35 $cm^3$ while that from the left side increased from 40–60 $cm^3$. Figure 3b shows the results obtained from the second part of this experiment. Again 3 cages with 10 mice in each were used. In this case, the left-side bottle was preferred to the one on the right side. On the 11th day after this positional preference was well established, hydrogen peroxide (0.03%/volume) was added to the water in the left-side bottle. No change was observed in the daily amount of water consumption on either the right or the left bottle. On the 16th day of the experiment, $H_2O_2$ concentration in water was increased to 3 times of the amount previously used (0.09%/volume). On the following day, average daily water intake on the left dropped from 60–35 $cm^3$ while the consumption on the right increased from 30–45 $cm^3$. The decrease of the daily amount of water consumed on the left side terminated 2 days after this new concentration of $H_2O_2$ was used, while the increase in water consumption of the right side bottle continued until the 4th day after this addition. On the 22nd day of the experiment fresh tap water without $H_2O_2$ replaced the one containing $H_2O_2$ on the left side of the cage. Preference of the left side bottle started to

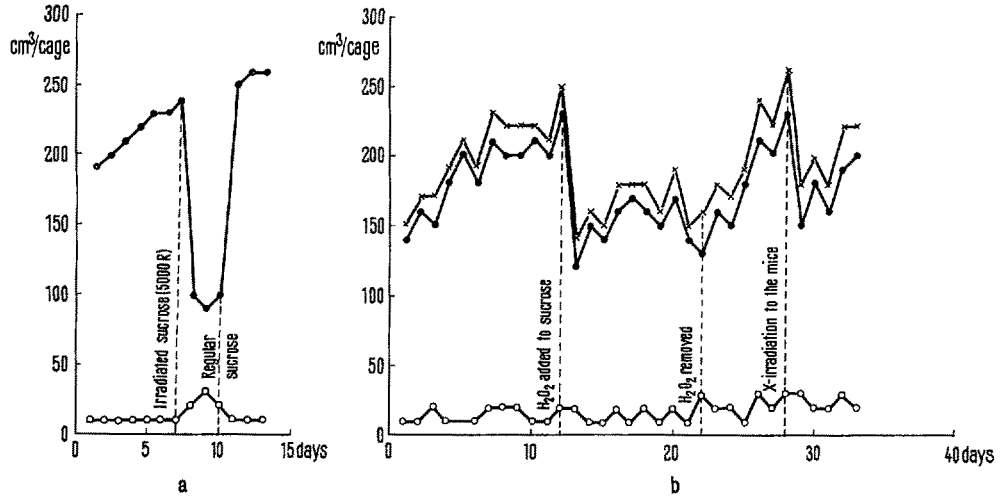


Fig. 2. (a) Daily consumption curves for sucrose solution and water before and after the exposure of sucrose to 5000 R. —●— sucrose, —o— $H_2O$. (b) Average daily sucrose, water and total consumptions before and after the addition of $H_2O_2$ ($3 \cdot 10^{-4}$) to sucrose, and X-irradiation (450 R) to the subjects. — × — total liquid, —●— sucrose, —o— $H_2O$.

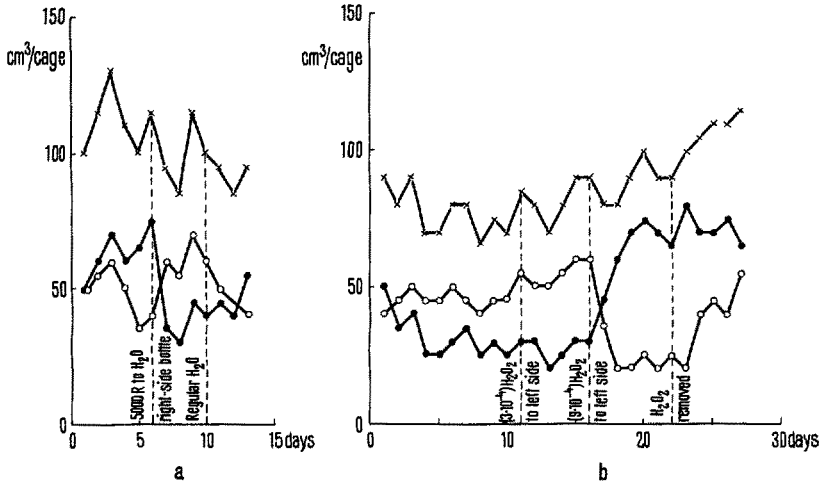


Fig. 3. (a) Average daily water consumptions as measured from the right and left-side bottles before and after the exposure of the water in the right-side bottle of 5000 R. (b) Average daily water consumption from the right and left bottle before and after the addition of $H_2O_2$ ($3 \cdot 10^{-4}$ and $9 \cdot 10^{-4}$) to the left-side bottle. — × — total consumption, —●— right side bottle, —o— left side bottle.

recover on the following days. The experiment was terminated on the 27th day.

*Discussion.* The mechanism of post-irradiation saccharin avoidance behavior is virtually unknown to investigators in this field. The question whether the effect is a result of a patho-physiological change or a chemical change in the taste perception is still unanswered.

While the problem of post-irradiation avoidance in mice are still unresolved pathologically, physiologically or otherwise, our results have shown that the irradiation of the solutions (saccharin and sucrose) did induce the same phenomenon of avoidance as if the animals themselves were irradiated. Many recent works have confirmed earlier results performed at the beginning of this century that hydrogen peroxide is indeed a product of irradiated water and other aqueous solutions. FREY and POLLARD[9] recently reported $3.5 \cdot 10^{-6}$ gm/ml of hydrogen peroxide produced in glucose solution irradiated with $2.7 \cdot 10^4$ R and that this irradiated medium cause a cessation of cell growth. The same effect can be obtained with reagent hydrogen peroxide. MOLIN and EHRENBERG[10] suggested that hydrogen peroxide or other peroxides produced in irradiated glucose may be responsible for the bactericidal effect. Earlier, HANNAN and SHEFFHERD[11], indicated that 'hydrogen peroxide produced from water by irradiation of a medium, and remaining thereafter the end of the irradiation, may be responsible for some of the effects seen in the systems studied'.

Although we have not determined quantitatively the amount of hydrogen peroxide produced in either irradiated saccharin, sucrose or water, we are positively certain that this reagent is produced when the liquids are exposed to low radiation dosages. We were able to verify that hydrogen peroxide was indeed a product of irradiated saccharin solution, sucrose solution and water by using the techniques of BRANDT and KESTON[12] and diacetyl 2′,7′-diachlorofluorescin from Eastman Kodak Company. The animals avoiding both the sweet solutions and plain tap water when these liquids were irradiated raises the question whether the presence of saccharin and sucrose is really necessary or only amplifying this observed avoidance behavior[13].

*Zusammenfassung.* Es wird gezeigt, dass röntgenbestrahlte Mäuse Saccharin- und Sucrose-Lösungen gleicherweise vermeiden wie Lösungen, denen minimale Mengen von $H_2O_2$ zugesetzt wurden. Es wird angenommen, dass der Effekt auf der radiolytischen Produktion von $H_2O_2$ in der Lösung zurückzuführen ist.

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## A Quantitative Histochemical Study of Succinic Dehydrogenase in the Kidney and Liver of Rats Subjected to Nephrectomy and Injected with Egg-White

The formation of large droplets in the cells of the proximal convolutions has been interpreted as a mechanism whereby either the excessive absorbed protein is metabolized by the mitochondrial enzymes or the protein is segregated by the lysosomes or phagosomes in the cell[1,2]. In this investigation, succinic dehydrogenase was studied to ascertain whether or not it plays an active part in this process. Rat kidneys in different functional states were used, and the liver also was examined.

*Methods.* Young adult male rats were treated as follows: (1) a single i.p. injection of 25 ml of strained solution of hens' egg-white in saline 24 h prior to sacrifice (2) right nephrectomy 7 days before sacrifice, and (3) a combination of the foregoing treatments. A single experiment involved 2 experimental and 1 each of untreated and sham-treated controls (saline injection or sham operation). At the end of the experiments the rats were weighed and decapitated, and the left kidney and adrenal gland were weighed individually. From the cortex of the kidney and from the liver, tissue 'punch-outs' (5 mm in diameter) were made[3] and promptly frozen in a mixture of dry ice and acetone. In a cryostat, each frozen 'punch-out' was used to obtain duplicate pairs of adjacent sections cut at 5 $\mu$. One member of each pair was used for the spectrophotometric determination of succinic dehydrogenase activity[4], in which the hydrogen acceptor was 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT). The other member of the pair was used for the spectrophotometric determination of protein nitrogen content[5], in which the reading was converted into the amount of nitrogen from a standard curve obtained from micro-Kjeldahl analyses. The final expression of enzyme activity was made by these data. In the outer part of the cortex of the kidney, the only portion used, the predominant epithelial structure positive to the enzyme reaction was the proximal convoluted tubules[6]. In the Table, any differences between the data of the untreated and the sham-treated controls were not statistically significant. Accordingly, in applying the *t*-test to the experimental results, the data for these 2 types of controls were averaged and used as a single control.

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