Effects of Anesthesia on Irradiated Animals

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WITH the increasing use of nuclear power in industry, in shipping, in the laboratory, and for military purposes, accidental exposure to ionizing radiation is likely to become more frequent. Burdick[1] first pointed out the importance of knowing the effects of anesthetic agents on previously irradiated patients who require operations and hence anesthesia for either accompanying trauma or coincidental disease. Since radiation has a profound effect on bodily processes, it is interesting to consider the interrelation of its effects with the pharmacological action of anesthetic drugs and with the physiological disturbances sometimes associated with anesthesia. A drug usually nontoxic or a maneuver relatively harmless under normal conditions might be lethal to an irradiated organism. Some work has been done showing that certain depressant drugs *e.g.*, pentobarbital,[2] phenothiazines,[3] morphine,[4] ether,[5] alcohol,[6] may protect animals against radiation. This protection is generally thought to be due to a reduction of oxygen in the tissues. In another study ether was without a protective effect,[7] and the effects of alcohol[6] and morphine[8] have been differently explained. Hypothermia has no protective effect, merely deferring an identical outcome of the irradiation[9] unless it leads to anoxia,[10] while exposure to cold after radiation is harmful.[11]

In the event of wartime atomic disaster, the needs of expediency may overcome all other considerations, and in areas near the blast any agent or technique at hand will be employed to cope with the large number of casualties to be treated. It seems likely that, owing to the preponderance of thermal injuries and radiation over blast effects,[12] the wounds would be mainly superficial lacerations, with a low intrinsic morbidity. Therefore pejorative effects of anesthesia might be relatively high. The question arises whether some agents, and particularly the nonflammable liquids, which are logistically convenient, are less harmful than others to irradiated subjects. In the comparative calm of base hospitals the usual range of agents and techniques will still be available, but some stockpiling of suitable drugs may be desirable.

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Methods

Three hundred and fifteen animals were used in some experiments. With these large numbers it was practicable to employ small animals such as mice or rats which could be irradiated and anesthetised in groups. Stillgrowing albino animals were chosen. The mice were females of the Carworth strain (weight approximately 25 Gm.) and the rats Sprague-Dawley (weight approximately 150 Gm.) of both sex. Animals were housed in uniform cages, fed dry chow and water, and allowed to acclimatize to their surroundings for one week before treatment. Control groups received anesthesia alone, radiation alone, or neither. After treatment the animals were weighed singly or in groups daily for thirty days. Dead animals were noted and removed.

The mice were irradiated with a Westinghouse unit at 20 ma. 200 kv. 1.0 mm. aluminum + 0.25 mm. copper filtration giving 3 roentgens/minute in air, some in a fiber box divided into compartments, and later ones in individual plexiglass tubes bound together in groups of 10. The rats were treated two by two in a plexiglass box using a GEC Maxitron at 20 ma. 300 kv. 2 mm. copper filtration giving a dose of 150 r./minute. Radia-

tion was applied to the whole body, but the animals were not rotated since the field was uniform when dosage and scatter were checked with a Victoreen roentgen meter.

The course of events in man following irradiation not immediately lethal may be divided into four parts: malaise, latent period, gastrointestinal upset, and bone marrow depression.[13] In mice and rats, deaths induced by radiation in the LD_{50} range follow a bimodal pattern,[14] some occurring at about 4–6 days due to intestinal upset and others due to marrow depression grouping around the eleventh day. In these studies there was maximum weight loss in rats in three days following irradiation, and anesthesia was induced either about three hours after irradiation in the latent period, at three days in the maximum gastrointestinal period, or at three weeks when all but hematologic effects were over.

In addition to anemia, infection, and dehydration in the subject, the duration and depth of narcosis might be expected to affect the result. The small size of the animals employed prevented the use of artificial respiration. Moreover, nitrous oxide in subhypoxic concentration does not anesthetize these creatures. The technique of light anesthesia balanced with relaxants, so popular in clinical work, and likely by virtue of its low toxicity to be valuable in the irradiated human being, could not, therefore, be studied. However, to obtain the most nearly applicable information, the anesthetic level was kept as light as possible at the point where all the animals became just unresponsive to forceful tactile stimulus.

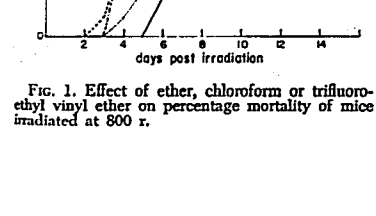

FIG. 1. Effect of ether, chloroform or trifluoroethyl vinyl ether on percentage mortality of mice irradiated at 800 r.

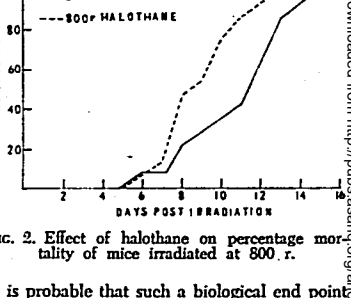

FIG. 2. Effect of halothane on percentage mortality of mice irradiated at 800 r.

It is probable that such a biological end point is more valid than any constant dose level.

Animals were color coded and equal numbers from each group were anesthetized together for one hour in an 100 liter plexiglass box resembling that used by Zauder and Orkin.[15] Continuous recirculation by a fan ensured uniform concentration of drug in all parts of the box. Oxygen concentration was monitored continuously by a Pauling meter, and that of carbon dioxide by a Liston-Becker infrared analyzer. Except in the first experiments on mice, a closed circle anesthetic system was employed using the fan to recirculate the gases through the soda lime of a standard anesthetic machine (soda lime omitted where hypercarbia was desired). Into this system, agents were delivered from a 'copper kettle' vaporizer.[16] Since some animals were more resistant than others, it was unavoidable that the more sensitive received a relative overdose, but it was hoped that this occurred evenly throughout the groups.

Results

EXPERIMENT 1: Mice, 10–15 animals per group, were irradiated at doses of 200–1,100 r. and subsequently anesthetized after a few hours with halothane, diethyl ether, chloroform or trifluoroethyl vinyl ether (Fluoromar). At 1,100 r. and 800 r. all the animals died from radiation whether anesthetized or not. As can be seen from figures 1 and 2, all the anesthetics used reduced survival time, but chloroform and trifluoroethyl vinyl ether reduced it more than did halothane or ether. The reduction with ether was not significant

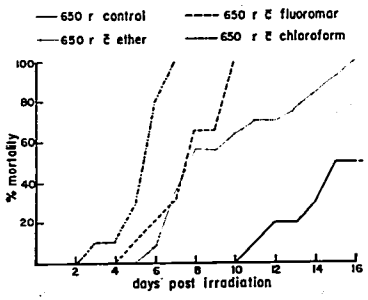

FIG. 3. Effect of chloroform trifluoroethyl vinyl ether (Fluoromar), or ether on percentage mortality of mice irradiated at 650 r.

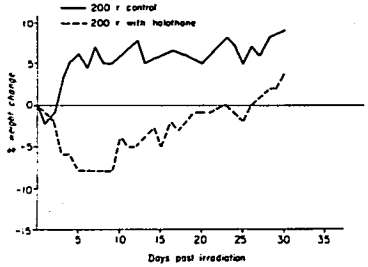

FIG. 4. Effect of halothane on percentage weight change on mice irradiated at 200 r.

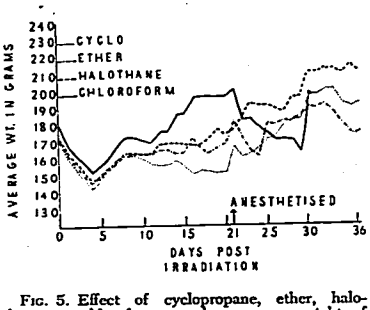

FIG. 5. Effect of cyclopropane, ether, halothane, or chloroform on the average weight of rats anesthetized 3 weeks after irradiation.

at the 5 per cent level of probability with this number of animals. The inevitable death from radiation renders the reduction in survival time from anesthesia biologically unimportant. At a dosage of 650 r., 50 per cent of the irradiated, unanesthetized control animals died before the thirtieth day following irradiation whereas there was 100 per cent mortality of those irradiated and anesthetized with chloroform, trifluoroethyl vinyl ether or ether (fig. 3). This result is significant both mathematically and biologically. Halothane was not tested at this radiation dose but figures 1 and 2 show it to resemble ether closely.

At 400 r. and 200 r. with halothane there were no deaths but the anesthetized mice showed a significant weight loss and delay in weight gain compared to the controls (fig. 4).

EXPERIMENT 2: In mice irradiated with 340 r. and 680 r. and given halothane at 24 hours, 3 days, 7, 14, and 28 days, an effect on the mortality was not seen.

EXPERIMENT 3: Rats, 15 per group, were irradiated to determine the 30 day LD_{50} (table 1). From these results this value was therefore considered to be between 675 and 725 r. for the strain. Although not clear cut this result is comparable to that in other studies, *e.g.*, Bond and Robertson[17] who found 640 r. in rats.

EXPERIMENT 4: Since the chief clinical interest lies in radiation doses unlikely of themselves to cause death, the effects of cyclopropane, ether, chloroform, and halothane were studied in rats irradiated with 525 and 600 r., 15 in each group. Anesthesia was induced at 3 hours, 3 days, or 3 weeks after radiation. The group exposed to 600 r shows a mortality in controls and those about to be anesthetized on the twenty-first day (also controls in effect) of 12, 6, 10, 12, and 12 out of 15 which is an average of 10.4 with a standard deviation of 2.6. At the 5 per cent level of probability the range, outside which effects of anesthesia are not attributed to dose, is 5.2 to 15.6 deaths per group. Since there are only 15 animals in each of these groups it is not possible conclusively to demonstrate a harmful effect of anesthesia on mortality. Similar reservations must be made at 525 r. However, in both experiments there was an

obvious weight loss after chloroform given at 3 weeks (fig. 5). This was noted to a lesser extent with ether and cyclopropane but not with halothane. No such effect was seen with any agent at 3 hours or 3 days. Chloroformed rats took longer fully to recover normal activity and appearance of well being. Some looked sick even on the following day whereas those anesthetized with halothane appeared completely recovered in a few minutes. The effect of ether lasted a few hours. Moreover, rats tended to die earlier after chloroform than other agents; this effect had also been noted in the mice of experiment 1. Anesthesia might therefore be adding another stress, the degree of which varies with the agent, since from the postanesthetic clinical appearance it could have been foretold which drug would produce increased morbidity (seen here as weight loss).

EXPERIMENT 5: Male and female rats were treated separately with 525 r. and anesthetized with halothane at 7 or 14 days afterwards. No effect was seen in either sex on mortality or morbidity other than a weight loss of a few grams only on the first postanesthetic day. This result was noted in the nonirradiated controls also.

EXPERIMENT 6: The effects on rats, 15 per group, of hypercarbia and hypoxia during anesthesia with halothane at 3 hours, 3 days, or 3 weeks after irradiation with 525 r. were noted. A group irradiated and anesthetized at normal O_2 and CO_2 tensions was added to the other controls. Levels of 15 per cent CO_2, 13 per cent O_2, or both together were attained by introducing CO_2 or N_2 in appropriate amounts after induction of anesthesia. The effect of these measures on the 30 day mortality may be seen from table 2. Five rats were irradiated but not anesthetized—none died. Graph of the animals' weight changes run parallel; *e.g.*, figure 6 shows the group anesthetized 3 weeks after radiation. It can be seen that at none of these times did these abnormal atmospheres exert any effect on mortality or morbidity following radiation and anesthesia.

EXPERIMENT 7: The effect of burns on rats irradiated with 525 r. and anesthetized with halothane was investigated. Whilst momentarily anesthetized, rats were dipped for five

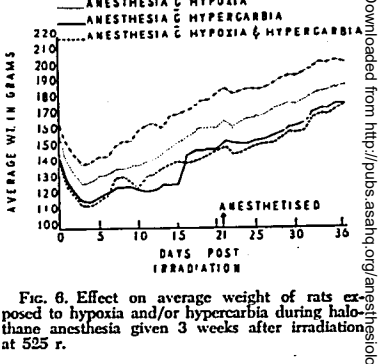

FIG. 6. Effect on average weight of rats exposed to hypoxia and/or hypercarbia during halothane anesthesia given 3 weeks after irradiation at 525 r.

TABLE 1. Thirty Day Mortality in Irradiated Rats (15 Animals per Group)

Dose Roentgens	30 Day Mortality (Per Cent)
0	0
600	20
625	0
650	0
675	40
700	60
725	40
750	100

TABLE 2. Effects of Hypercarbia and Hypoxia During Halothane Anesthesia on Irradiated Rats (15 Animals per Group)

Type of Anesthesia	Nonirradiated	Irradiated and Anesthetized at		
		3 Hours	3 Days	3 Weeks Thereafter
Normal	0/10	0/12	0/14	0/13
Hypoxic	0/9	1/12	1/14	0/12
Hypercarbic	0/12	1/14	0/14	0/10
Hypoxic and Hypercarbic	0/11	1/10	0/15	1/10

Denominator represents the number of rats surviving from the period of radiation to the end of the period of anesthesia, and the numerator the number dying between the end of the period of anesthesia and the thirty-first day.

TABLE 3. Effect of Burns on Rats Irradiated with 325 r.

	Number	Dead at 30 Days	Maximum Weight Loss (Per Cent)
Untreated control	5	—	4.7
Dummy burn	10	—	3.1
Dummy burn + anesthesia	15	—	3.2
Dummy burn + radiation	15	—	7.3
Dummy burn + anesthesia + radiation	15	—	6.1
Real burn	15	—	9.3
Real burn + anesthesia	15	—	8.6
Real burn + radiation	15	3	12.8
Real burn + anesthesia + radiation	15	7	17.6

seconds into water heated to 62° C., which covered them from tail to axilla. This produced edema with occasional ulceration especially of the feet. Other rats were dipped into tepid water to produce a dummy "burn." Other controls were completely untreated. Radiation was given immediately after immersion. A full one hour's anesthesia was given 24 hours after immersion. Weight loss occurred over the next three days as seen in table 3, with subsequent weight gain. It seems then that the pejorative effect of burn trauma considerably enhances that of anesthesia.

EXPERIMENT 8: The effect of previous radiation on the acute mortality produced in mice by the intravenous injection of a local anesthetic drug (lidocaine) was investigated. Two groups of animals were irradiated with 660 r. Subsequently 1 per cent lidocaine was injected into the tail vein as quickly as possible. Dosage, radiation–injection interval, and results are shown in table 4. The increase in mortality at 3 hours is significant in both series. It is noteworthy that the animals appeared healthy at this time, but at 3 days, when they were obviously sick, administration of lidocaine caused no significant rise in mortality.

Discussion

The large numbers of animals needed for significant results and the likelihood that with such numbers other factors will intervene to alter the results prompts caution in interpretation. These animals must be kept for relatively long periods in a state of low resistance due to diarrhea, bleeding, ulceration, and a depressed bone marrow, with infection almost inevitable.[18] Sick animals tend to be attacked by their cage mates, killing off possible survivors. A death often raises the mean weight of a group since the sickest animal is usually, though not always, the lightest. It is not, therefore, surprising that results are indefinite. All the studies reveal how much less does anesthesia cause illness than radiation itself, although a higher radiation dose may make an anesthetic effect more obvious.[15] Indeed, dosage of radiation must be carefully adjusted to demonstrate the effects of anesthesia and this may be the reason for the differences in findings between one worker and another.

In experiments 1, 2 and 3, halothane and ether had an adverse effect on irradiated mice if given within a few hours, but halothane, at least, had no effect when given after 24 hours. Zauder and Orkin,[15] on the other hand, found increasing effect in the second two weeks, and considered halothane slightly more harmful than ether. Neither halothane, ether, nor cyclopropane had any effect on rats at 3 hours, 3 days, or 3 weeks (experiment 4). Wilson[19] found ether given after radiation had no effect on mortality. He did find cyclopropane at 7 days to increase mortality, but this group had several preanesthetic deaths and all of them died by the thirtieth day, neither of these latter findings being seen in any other group. Possibly, some other factor such as unrelated infection was responsible for this isolated result. Chloroform had a markedly harmful effect in mice (experiments 1 and 2), *i.e.*, at 3 hours, but not in rats until the third week after irradiation (experiment 4). Wilson found marked scatter of survival time with chloroform which he considers may be due to liver damage in some individuals. The possible importance of this mechanism is emphasized by the finding[15] that divinyl ether induced a uniform high mortality in mice after radiation, whereas nonirradiated mice were unaffected by it. Another compound with a vinyl group, trifluoroethyl vinyl ether, likewise appeared harmful to mice and was not further investigated. Experimentally, 15 per cent CO_2 added to the anesthetic atmosphere did not appear to affect morbidity or mortality subsequent to anesthesia with halothane in irradiated rats. Similarly, hypoxia of 13 per cent O_2 likewise had no effect

on rats irradiated and anesthetized with halothane. This percentage was chosen because in our experience rats tend to die at lesser concentrations. They were not anesthetized by nitrous oxide 87 per cent, and this drug was not studied further. Burdick subjected rats to more severe hypoxia by anesthetizing them with N_2O three hours after radiation using an induction concentration of 95 per cent and a maintenance of 90 per cent with only one death during anesthesia. Seventy-three per cent survived 30 days whereas only 56 per cent of the unanesthetized controls did so. He states: "Unfortunately this number was too small to permit valid statistical analysis." There was even less difference at longer radiation–anesthesia intervals. Wilson found 90–95 per cent N_2O in O_2 did not cause unconsciousness and had no effect on mortality. This is in marked contrast to the known protective effect of cellular hypoxia at the time of radiation.[20]

Toxicity of lidocaine judged by acute mortality with intravenous injection was increased immediately after radiation but not 3 days later. Russian workers believe local anesthesia to be suitable for use in irradiated subjects and that any drug tending to diminish activity of the subject, *e.g.*, morphine, will have a beneficial effect.[21] They are interested in the additive effects of trauma to the situation. Our results show that burns will further increase morbidity, enhancing the effects of irradiation and anesthesia. In contrast, blast injuries were found to have no effect on irradiated mice.[22] Although there seems to be a specific effect of all the anesthetics administered within a few hours of radiation and perhaps of some thereafter (*e.g.*, divinyl ether), late effects are mostly small. There is a parallel between immediate clinical appearance and the subsequent morbidity (experiment 4). Such changes might be seen in animals subjected to anesthesia which were, from other causes, anemic, hypovolemic, or infected. The Russian investigators believe ether to be contraindicated in the gastrointestinal period because of the increased vomiting it causes, and deprecate spinal block because of the hypotension it may produce. At this stage, therefore, more faith should be placed in meticulous attention to the general preparation of the patient for operation by way of rehydration, blood transfusion, and antibiotics, than in the virtues of any specific anesthetic agent or technique. Hypoxia and hypercarbia frequently occur by accident during the course of anesthesia. In experiment 6 irradiated and nonirradiated rats were anesthetized together under conditions of hypoxia and/or hypercarbia. In neither groups of rats was the subsequent course affected by any of these abnormal atmospheres. It therefore seems unlikely that the hazard these abnormalities entail would be particularly great in irradiated subjects.

Halothane appears to be as satisfactory an agent as any to maintain light anesthesia. Where possible it might seem wise to defer anesthesia for 24 hours after radiation.

TABLE 4. Effect of Previous Radiation on Acute Mortality in Mice by Intravenous Lidocaine

Dose of Lidocaine	Groups Injected	Percentage Mortality at Radiation Interval		Percentage Mortality in Nonirradiated Controls
		3 Hours	3 Days	
16 mg./kg.	Old mice	52.4	40	37
25 mg./kg.	Young adult mice	56.0	41	35.5

Summary

The effects of various anesthetic agents on the mortality and morbidity of irradiated rats and mice were studied and compared with controls, some of which were irradiated but not anesthetized and others anesthetized but not irradiated. All the general anesthetic agents studied caused an increased mortality or morbidity depending on the dosage of radiation. Cyclopropane and ether did not appear to have such a marked effect as did chloroform and trifluoroethyl vinyl ether, while halothane had the least effect. Hypercarbia and hypoxia during anesthesia following irradiation were investigated and did not appear to influence mortality or morbidity. Burn trauma was found to increase the pejorative effect of anesthesia following radiation. Lidocaine injected rapidly into the tail vein of mice caused a greater mortality in those injected

within a few hours of irradiation than in those injected three days following irradiation.

It is suggested that halothane appears to be as satisfactory as any agent to maintain light anesthesia following irradiation and that where possible it might seem best to defer anesthesia for 24 hours after exposure with particular attention given to rehydration, blood transfusion and antibiotics.

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