Reprinted from RADIATION RESEARCH, Volume 25, No. 3, July 1965

RADIATION RESEARCH **25,** 514–525 (1965)

Weight and Blood Volume Changes Induced by Irradiation of the American Cockroach

D. R. A. WHARTON, MARTHA L. WHARTON, AND JOHN E. LOLA

Pioneering Research Division, U. S. Army Natick Laboratories, Natick, Massachusetts

INTRODUCTION

We have shown (*1*) that blood volume decreases markedly during the first few days after emergence of the adult American cockroach, *Periplaneta americana* L., and then levels off, whereas the total water content of the insect remains relatively stable. Starvation causes a reduction in blood volume and simultaneously increases the total water content of the organism. In view of these effects and of the anorexia that follows irradiation, quantitative aspects of the response of the cockroach to irradiation are best studied with starved rather than fed insects.

A dose of 10,000 rads induces a lethargic state which lasts several hours from which the insects never fully regain their former activity, and is lethal to 50% of the insects in 8 to 10 days. Other effects are inappetence, diarrhea, loss of weight, refusal of water during the first 2 days followed by increased water consumption, sterility, and excretion of greater amounts of nitrogenous products than of phosphorus (*2–4*). The irradiated cockroach dies sooner than the starved unirradiated one and has a marked tendency to dehydration while being at the same time quite active. It is therefore likely that disturbances of the water balance occur which affect the quantitation of body constituents. To determine this we have investigated the changes in body weight, water content, and blood volume that are caused by this dose of ionizing radiation.

MATERIALS AND METHODS

Since blood volume per cent (microliters of whole blood per 100 mg of body weight) changes greatly in the young adult cockroach (*1*), the experimental animals were selected within the age limits of about 1 to 2 weeks and were isolated individually in beakers. Three groups of insects were used: (1) fed unirradiated controls maintained on Purina Laboratory Chow Pellets; (2) starved unirradiated controls; and (3) starved irradiated insects. All were supplied with water throughout the experiment. For convenience the groups are referred to as fed, starved, and

TABLE I

WEIGHTS ± STANDARD DEVIATIONS OF IRRADIATED WHOLE INSECTS AND THEIR FED AND STARVED CONTROLS[a]

Day	1		2		3		6		8	
	No.	*Weight (mg)*	*No.*	*Weight (mg)*	*No.*	*Weight (mg)*	*No.*	*Weight (mg)*	*No.*	*Weight (mg)*
Fed	13	725.0 ± 69	12	709.0 ± 71	12	692.0 ± 74	33	711.0 ± 78	24	689.0 ± 67
Starved	24	678.0 ± 98	13	662.0 ± 56	15	683.0 ± 61	42	649.0 ± 81	17	673.0 ± 73
Irradiated	16	676.0 ± 98	5	622.0 ± 116	18	693.0 ± 101	40	632.0 ± 74	28	616.0 ± 99
S *vs.* I		Not sig.		$p = 0.1$				$p = 0.2$		$p = 0.05$

[a] Each day represents a different subsample. Irradiated and starved control cockroaches prestarved 4 days. p values given for t statistic.

TABLE II

AVERAGE WET WEIGHTS, DURING 11 SUCCESSIVE DAYS, OF INSECTS EVISCERATED AFTER IRRADIATION, AND THEIR CONTROLS[a]

Experiment	Fed		Starved		Irradiated		p	
	No.	Weight (mg)	No.	Weight (mg)	No.	Weight (mg)	F vs. S	S vs. I
1	49	522.0 ± 22.8	99	502.0 ± 47.2	105	463.0 ± 10.7	0.01	≪0.01
2	11	557.0 ± 51.7	99	554.0 ± 21.2	89	516.0 ± 24.7	Not sig.	≪0.01
3	31	575.0 ± 32.6	132	575.0 ± 19.5	127	514.0 ± 36.2	Not sig.	≪0.01
4			90	538.0 ± 11.7	88	525.0 ± 30.3		≪0.01
Total	91		420		409			

[a] Numbers of insects sampled on successive days are indicated in Fig. 1.

irradiated, respectively. The irradiated insects were exposed to a dose of 10,000 rads of β-radiation from a 2-Mev electron accelerator, as used in the previous work (*2–4*). A known dose of C^{14}-inulin was injected into the weighed insects. Ten microliters of blood was withdrawn 4 hours later, and the C^{14} present was determined with either a liquid scintillation or a gas flow counter. The activity found was then used to estimate blood volume (*1*). A different sample of insects from each group was bled on each consecutive day after irradiation. Each insect was bled once only and then sacrificed. Total water was determined for normal and eviscerated insects as the difference in weight between the live insect and the insect after drying at 112°C (*1*). Evisceration, where indicated, was carried out prior to weighing.

RESULTS

Changes in Body Weight

The vagaries of eating and drinking are reflected in the fluctuating average weights of different samples on successive days after irradiation (Table I). After the first day the irradiated cockroaches and the starved controls both weighed less than the fed controls. The irradiated roaches weighed least of all. On the third day post-irradiation, there was an increase in the weight of the irradiated roaches, probably because of the abnormal amount of water consumed after 2 days of torpor and anorexia (*3*). The water tends to be delayed in its passage through the gut of the irradiated insects, so that on dissection many present distended crops. Because of

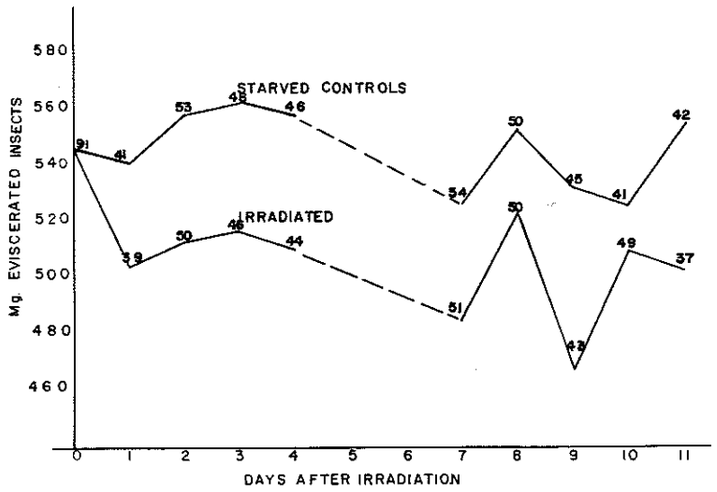

FIG. 1. Average daily weights of eviscerated, irradiated insects and of eviscerated starved controls. Total numbers as in Table II.

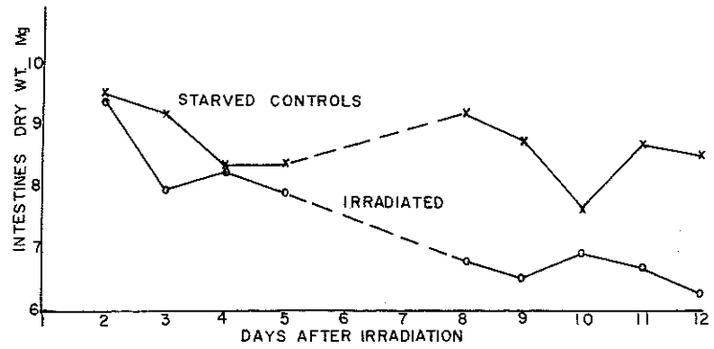

FIG. 2. Average daily dry weights of intestines of irradiated insects and starved controls. Total numbers 88 and 90, respectively.

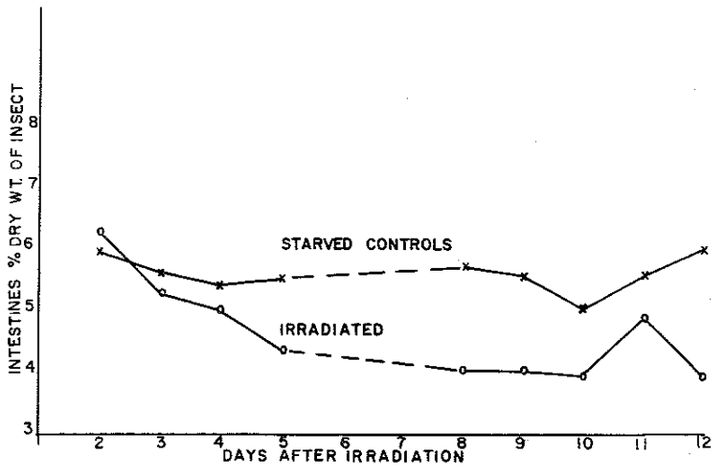

FIG. 3. Average daily dry weights of intestines as per cent of body weight after irradiation. Numbers as in Fig. 2.

this and the varied water intake, the generally lower weight of the irradiated insects suggests that the insect body itself is losing weight. During 11 days, when daily subsamples of the three groups were eviscerated, the mean wet weights of the irradiated insects were significantly less ($p = < < 0.01$) than those of the starved controls (Table II). Since starvation causes as much as 4% increase in the total water content of the cockroach (*1*), it is evident that the dry weight loss of both the irradiated insects and the starved controls is greater than is indicated by the wet weights.

TABLE III

WEIGHT LOSS SIX DAYS AFTER STARVATION OR IRRADIATION

Groups	*Number of roaches*	*Body weight (mg)*	*Loss (%)*	*Weight of fat body (mg)*	*Loss (%)*	*Weight of gonads (mg)*	*Loss (%)*
Fed controls	10	685.2 ± 53.9		32.1 ± 8.5		8.34 ± 1.48	
Starved controls	10	654.4 ± 60.2	4.5	17.8 ± 5.0	45.6	7.50 ± 1.22	10.1
Irradiated	19	587.6 ± 65.0	14.2	20.3 ± 8.39	36.8	6.44 ± 1.48	22.8

Loss of weight in eviscerated insects is found on the first day postirradiation (Fig. 1) and persists for the duration of the experiment. However, the relative loss is not uniform. During the first few days the weights of the irradiated cockroaches parallel those of the starved controls but show a definite tendency to diverge after the eighth day. The weights of both groups become irregular and uncorrelated, indicating that irradiation has superimposed disturbances upon those caused by starvation. It should be recalled (*2*), and will be illustrated later, that this period corresponds approximately with that of the highest mortality rate after exposure to the given dose of radiation, at which time the irradiated insects are likely to show evidence of dehydration.

Further evidence indicates that there is a greater weight loss by the intestines of the irradiated insects than by those of starved controls (Fig. 2). Moreover, the weight of the intestines of the irradiated roaches is smaller when it is plotted as a percentage of the dry weight of the whole insect, taken as the sum of the dry weight of the intestines plus the dry weight of the eviscerated insect (Fig. 3). This suggests that the intestines have been more severely damaged by irradiation than the body tissue generally. The gizzard and other parts of the intestine show tissue disintegration and fragility and contribute to the observed shedding of the cuticular layer, remnants of which may be seen in the feces.

The gonads of the irradiated insects and the starved controls were smaller than those of the fed, and the fat bodies also weighed much less than those of the fed insect after 6 days of starvation (Table III). The fat body did not lose as much weight in the irradiated cockroaches as in the starved controls, possibly because irradiated insects are less active than starved unirradiated ones and have a lower metabolic rate (*1*), or because metabolic water may be produced and retained by the fat body.

Total Water Content

The water content of the whole fed cockroach changes only slowly after the first week of adulthood (*1*). Starvation causes a rise in the total water content which may increase over 4% by the twelfth day. Apparently, except on the second day after irradiation, the percentage of total water in the whole irradiated insect does not differ significantly from that of the starved. On the second day it is significantly less ($p = 0.01$), probably because of the relative dehydration resulting from diarrhea and the low consumption of water during the first 2 days postirradiation. The later maintenance of an equal level with the starved roaches probably results from the great increase in the consumption of water by the irradiated ones (*3*), and their retention of water in the crop. In fact, when irradiated insects are eviscerated they have a significantly lower concentration of water than the eviscerated starved controls (Fig. 4). The significant increasing gain in water content demonstrated by the eviscerated starved roaches over the fed ($p = 0.01$ from the fourth day on),

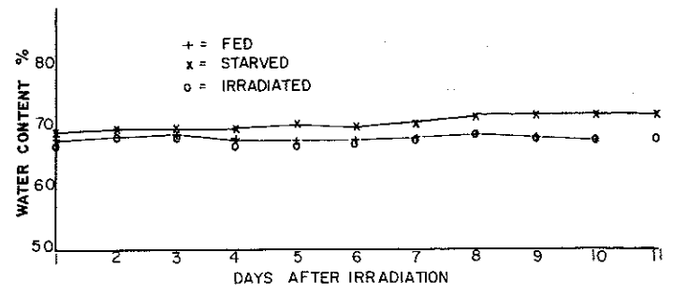

FIG. 4. Water content of eviscerated, irradiated insects and of eviscerated controls: 413 irradiated, 420 starved, 74 fed.

TABLE IV

FRACTION OF COCKROACHES FAILING TO BLEED AFTER STARVATION AND IRRADIATION

Day	*Fed*	*Starved*	*Irradiated*
1	0/17	4/19	3/16
2		1/20	3/12
3	0/16	0/9	0/11
4		0/13	0/16
5		1/16	13/35
6	0/25	5/32	30/59
7		1/16	18/39
Total (%)	0	9.6	35.6

shows that the water increase in the starved controls is systemic. In view of the tendency of starvation to increase the percentage of total water, the low level of water in the starved irradiated insect represents a considerable dehydrating effect of radiation. Moreover, the effect involves the distribution as well as the over-all content of water as shown by the fact that, although the per cent water was almost as high in the irradiated cockroaches as in the fed controls, nevertheless a large number of irradiated insects failed to bleed, or yielded little, when punctured, and their hemocoeles were dry when dissected. Thus, in a typical group of experimental adult males, during the first 7 days of the experiment all the 58 fed cockroaches bled, while 9.6% of the 125 starved controls and 35.6% of 188 irradiated failed to bleed (Table IV). In contrast with the starved controls, inability to bleed among the irradiated insects rose abruptly and greatly after the fourth day and tended to increase thereafter, so that between the fifth and seventh days 45.8% of the irradiated failed to bleed (Table IV).

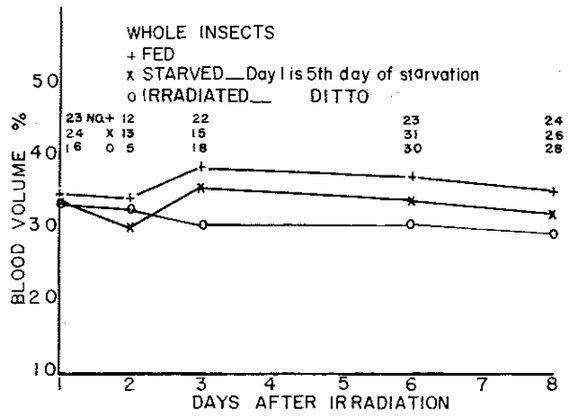

FIG. 5. Average daily blood volume per cent of irradiated and control insects after irradiation.

Blood Volume Per Cent

The indicated shift in water balance was substantiated by the changes in blood volume (Fig. 5). Both the starved controls and the irradiated cockroaches had significantly lower blood volumes per cent than the fed controls. The irradiated cockroaches had significantly less than the starved controls, with p values of <0.01 for day 3 and <0.05 for days 6 and 8, respectively. They showed an average difference from the fed (as on the third day) of as much as 7.6%, which represents a decrease of nearly 20%, and a decrease of 14% from the starved. It should be emphasized that this is the percentage of blood volume lost by those cockroaches that were able to yield 10 μl or more of blood. The loss is greater in those that failed to do so.

DISCUSSION

Although dehydration is a usual result of exposure to a lethal dose of radiation, its effect, with that of concomitant shifts in water distribution, on the quantitative evaluation of results has been generally overlooked in insect hematology. To the observation that starvation induces changes in the content and distribution of water (*1*), it can now be added that in the starved irradiated insect changes of a different order are produced and are sufficiently great to require consideration in studies on quantitative evaluation of radiation effects. Fasting from water may cause a considerable loss of body weight, and irradiation decreases it further. The fall in weight on the second day after irradiation coincides with the animal's aversion to water, and its recovery with the consumption of excessive amounts.

Furth *et al.* (*5*) found evidence of heightened capillary permeability in the increased rate of loss of injected tagged albumin and erythrocytes in rabbits and mice

irradiated with 1000 R and 500 R, respectively. Lukin (*6*) found no indication of breakdown of capillary endothelium leading to increased loss of injected material, and could find no significant differences up to 24 hours before death in the blood volume of splenectomized dogs. However, Lukin and Gregersen (*7*) found that a drastic reduction of 25 to 35% in blood volume took place during the last 10 hours of survival, and noted that loss of fluid occurred chiefly through the gastrointestinal tract.

In the present study, failure of so many irradiated insects to bleed is a manifestation of a dearth of hemocoele fluid and occurs with increasing frequency as the death rate rises. The fact that a certain percentage of the starved unirradiated insects failed to bleed during the period of these observations when the death rate was nil indicates that starvation contributes to the dehydration of the starved irradiated cockroaches. As the data show, this dehydration takes the form of a shift in the water balance from the blood to the tissues. In the irradiated insect, other factors such as tissue breakdown, which is severe in the intestines as indicated by weight loss and tissue fragility, and diarrhea complicate the process. Among those insects yielding 10 μl of blood, the loss of some 10% does not seem drastic. However, among insects yielding only a few microliters of blood, distinctly lower blood volumes were observed than are presented here, which indicates that a terminal loss of drastic proportion may actually occur. Considering the mechanisms of water regulation, injury to which might antecede and give rise to the observations here recorded, Dethier and Evans (*8*) have shown that in the blowfly drinking is initiated by stimulation of the tarsal and labellar receptors and terminated by adaptation or by the rising pressure of the hemolymph, and may be resumed by reducing the pressure in the abdomen, as by bleeding. The hemolymph pressure stimulates receptors which transmit their message to the brain via the recurrent nerve, and the brain acts on the information (*9*). The hemolymph pressure is determined not alone by water intake but also by its excretion. This is controlled by a diuretic and an antidiuretic hormone produced by the corpora allata and corpora cardiaca (*10, 11*), the former of which is probably released during feeding (*12*). Interacting with these various mechanisms is the solute concentration, particularly of the amino acids, and tracheal respiration. We have shown (*4*) that even a nonlethal dose of 1000 rads of β-rays promptly sterilizes the female American cockroach, causing a cessation of oöthecal production and eventual involution of the ovaries, and destroys or suppresses production of the sex attractant. In view of the fact that the corpora allata control maturation of the ova, and have been shown to control attractant production in *Byrsotria fumigata* (*13*), it can be inferred that these glands have been damaged and that possibly their water-regulating functions may also have been disorganized. However, the early excessive excretion of nitrogenous products (*3*), the sloughing of the intestinal lining, and the water imbalance shown by the insect irradiated with 10,000 rads indicate quite clearly that tissue injury has occurred on a wide scale and upset the normal regulatory functions. Drinking

may be stimulated by the reduced blood volume and resulting reduced pressure postirradiation; but, with injury to its source, it seems likely that hormonal control would be usurped by the osmotic demands of the physically changing system.

SUMMARY

1. Exposure of the cockroach to 10,000 rads of β-radiation from a 2-Mev van de Graaff electron accelerator causes an early and continuous loss of body weight, except on the third day after irradiation.

2. The body weight of the whole irradiated insect on the third day after irradiation is greater than that of the fed or the starved controls because of an excessive intake of water, much of which is retained in the crop.

3. Body weight becomes more irregular with dehydration and an increasing mortality rate.

4. Per cent weight losses in the intestine, fat body, and gonads are greater than in the body at large.

5. The fat body loses less weight in the irradiated insects than in the controls, possibly because of the lower metabolic rate or of retained metabolic water.

6. The blood volume per cent (microliters per 100 mg of body weight) is much reduced postirradiation, and an increasing number of insects fail to bleed as the mortality rate rises.

7. The per cent total body water of the whole irradiated insect is greater than in the fed and similar to that of the starved controls. The similarity is due to the generally large intake of water by the irradiated insects.

8. When the intestines are removed, the body water is shown to be less and differently distributed in the irradiated insect.

9. The probable causes of water changes are discussed.

ACKNOWLEDGMENTS

We thank John Lake and Stanley Wentworth for their assistance in certain phases of this work.

RECEIVED: October 26, 1964

REFERENCES

1. D. R. A. WHARTON, MARTHA L. WHARTON, and JOHN LOLA, Blood volume and water content of the male American cockroach, *Periplaneta americana* L. Methods and the influence of age and starvation. *J. Insect. Physiol.* **11,** 391–404 (1965).
2. D. R. A. WHARTON and MARTHA L. WHARTON, The effect of radiation on the longevity of the cockroach, *Periplaneta americana*, as affected by dose, age, sex, and food intake. *Radiation Res.* **11,** 600–615 (1959).
3. D. R. A. WHARTON and MARTHA L. WHARTON, Effects of radiation on nitrogen and phosphorus excretion by the cockroach, *Periplaneta americana* L. *Radiation Res.* **14,** 432–443 (1961).
4. MARTHA L. WHARTON, and D. R. A. WHARTON, The production of sex attractant substances and of oöthecae by the normal and irradiated American cockroach, *Periplaneta americana* L. *J. Insect. Physiol.* **1,** 229–239 (1957).

5. J. FURTH, G. A. ANDREWS, R. H. STOREY, and L. WISH, The effects of X-irradiation on erythrogenesis, plasma and cell volumes. *Southern Med. J.* **44,** 85–92 (1951).
6. L. LUKIN, The effects of total-body X-irradiation on blood volume in splenectomized dogs. *Radiation Res.* **7,** 150–160 (1957).
7. L. LUKIN and M. I. GREGERSEN, Premortal blood volumes in splenectomized dogs after total-body X-irradiation. *Radiation Res.* **7,** 161–166 (1957).
8. V. G. DETHIER and D. R. EVANS, The physiological control of water ingestion in the blowfly. *Biol. Bull.* **121,** 108–116 (1961).
9. V. G. DETHIER and D. BODENSTEIN, Hunger in the blowfly. *Z. Tierpsychol.* **15,** 129–140 (1958).
10. G. ALTMANN, Die Regulation des Wasserhaltes der Honigbiene. *Insectes Sociaux* **3,** 33–40 (1956).
11. S. H. P. MADDRELL, A diuretic hormone in *Rhodnius prolixus* Stal. *Nature* **194,** 605–606 (1962).
12. J. A. NUÑEZ, Probable mechanism regulating water economy. *Nature* **197,** 312 (1963).
13. R. H. BARTH, JR., Hormonal control of sex attractant production in the Cuban cockroach. *Science* **133,** 1598–1599 (1961).