previously infected animals were injected intracardially the brucellae of reinfection also disappeared rapidly from the tissues. 3. In order to meet the criticism that intracellular brucellae could not be cultivated by routine methods, all tissues were cultivated after disintegration by rapid vibration, under optimal conditions. The results obtained with this technic corroborated the findings obtained with other methods. 4. It is puzzling to see that the *Brucella* of original infection can persist for months in the tissues from which the organisms of reinoculation disappear rapidly. An explanation for this phenomenon is not apparent and these studies are being extended in an attempt to clarify this point. 5. Cultivation of disintegrated blood on the surface of solid media constituted an improvement over other methods used routinely for the isolation of brucellae from the blood.

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Received September 24, 1953. P.S.E.B.M., 1953, v84.

Relative Biological Effectiveness of Fast Neutrons, Gamma Rays, X-Rays on Grasshopper Nymph Ovarioles. (*Melanoplus differentialis*) (20704)

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In previous communications(1-3) it was demonstrated that anabolic processes are more susceptible to ionizing radiations than catabolic processes. In order to examine this phenomenon further a tissue was required in which growth, in the sense of the synthesis of cellular material, takes place, in the absence of cell division. These requirements are fulfilled by the nymphal grasshopper ovariole and its developing eggs. During early oogenesis in the ovarioles, the newly differentiated unfertilized egg is approximately 35 μ in diameter, but within 45 days this cell attains a size of 4 cu mm, increasing in volume by a factor of 1.6×10^5. Throughout this period (still before fertilization) the egg contains the diploid number of chromosomes. Meiotic divisions begin just prior to laying and are completed after the eggs are laid, as previously shown by Slifer and King(4).

In accordance with expectation the developing eggs were found to be highly radiosensitive: Whereas a total-body dose of 1500 r of 200 kv x-rays was necessary to kill 50% of the grasshopper nymphs in a 10-day period, only 350 r or its equivalent was sufficient to destroy completely most of the eggs in the ovarioles. In this paper the relative biological effectiveness of Co^{60} gamma rays and fast neutrons is compared with 200 kv x-rays for the production of this irradiation effect.

Material and methods. Fifth or sixth instar grasshopper nymphs were exposed to whole body x-radiation in doses ranging from 52 to 801 r, from a 200 kv, 15 ma, oil-cooled x-ray machine; HVL: 0.25 mm Cu; no additional filtration. The grasshoppers were exposed in a covered Petri dish made of cellulose acetate. In each dosage series 10 or 12 grasshoppers were kept as controls and an equal number were irradiated. Gamma and fast neutron exposures were all made in the gamma-neutron radiation chamber recently described by Vogel *et al.*(5). Slow neutrons from the thermal

column of the Argonne CP_3' Reactor were allowed to impinge upon a sheet of uranium in order to produce fast neutrons. The fission spectrum was modified by 3 inches of lead around the uranium. Pure gamma radiation was obtained from 18 sources of cobalt 60 located at the opposite end of the chamber from the uranium. The grasshoppers were exposed in lucite cages usually used for exposure of small mammals. These cages had been standardized as to isodose groups for both gamma and neutron irradiations. Exposures were carried out at a dose rate of approximately 13 r/min for gamma rays and 2-4 rep/min for fast neutrons. During irradiations the animals were kept at approximately 75°F and 50% relative humidity with the aid of an Aminco climatizer which circulated air to both experimentals and non-irradiated controls. Sixteen days following each irradiation the ovaries were removed and the exposed ovarioles were observed under a dis-

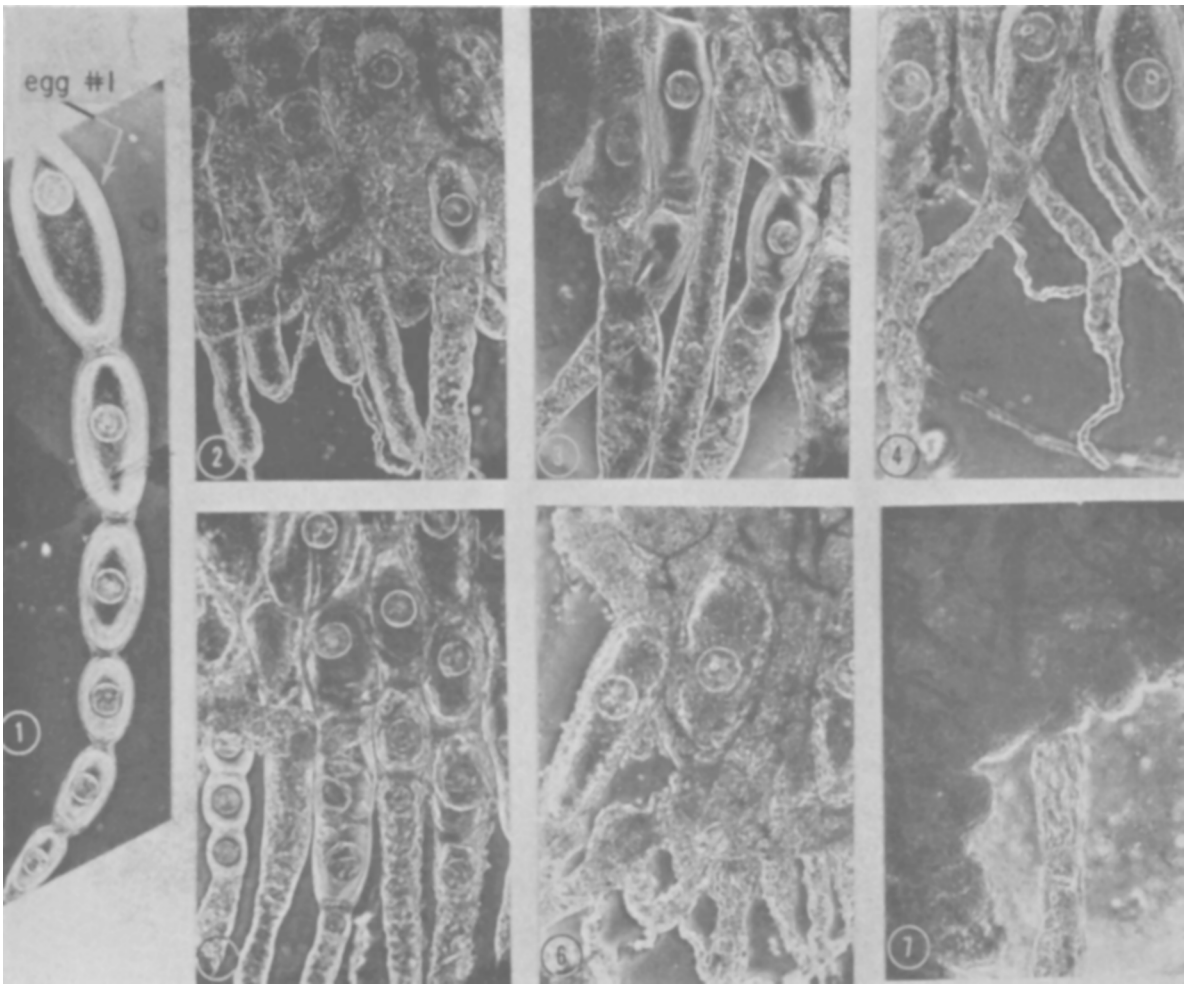

Plate 1 (67×)

FIG. 1. Normal control ovariole showing a strand of ova with egg #1 labelled.
FIG. 2. Grasshopper ovary that received total-body radiation of 350 r x-rays 16 days before it was photographed.
FIG. 3. A number of ovarioles 16 days after 407 r Co^{60} gamma rays.
FIG. 4. Ovarioles exposed to 480 r x-irradiation.
FIG. 5. Ovarioles 16 days after fast neutrons bombardment with 14.1 rep.
FIG. 6. 22.5 " .
FIG. 7. 101.4 " .

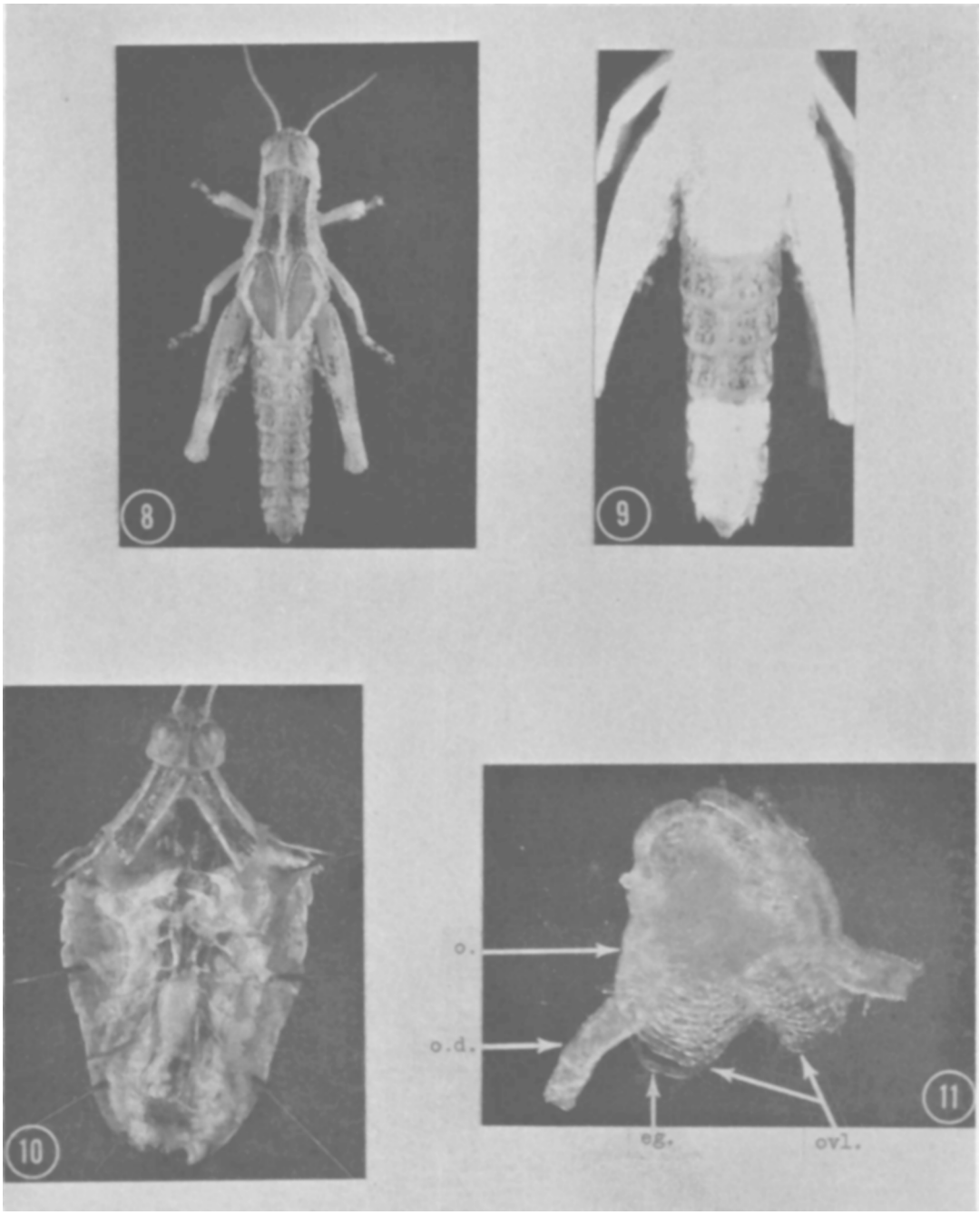

Plate 2

FIG. 8. Irradiated grasshopper nymph, fifth instar, 3×.

FIG. 9. Grasshopper showing abdominal region below which the ovary is found (shaded area indicates location of ovary), 6×.

FIG. 10. Dissected grasshopper nymph with intact ovary, 3×.

FIG. 11. Ovary dissected out (normal), 15×.

O—ovary Od—oviduct Eg—egg Ovl—ovarioles

TABLE I.
(Irradiation data.)
X-rays (200 kv 15 ma); 10 animals in each experiment.*

Date of exposure	Dose rate (r/min.)	Exposure (min.)	Total dose (r)	Irradiation effect†	
2/ 4/52	40.2	1.3	52	3—‡	Normal or control ovary (see Fig. 1 and 11).
"	"	2.5	101	"	
3/ 7	34.4	6.6	227	2—	No evident injury to eggs; ovary retarded in growth.
"	"	8.8	303	"	
"	"	10.2	351	+	Destruction of every egg in each ovariole, except egg #1, the most mature (as in Fig. 2, 4, and 6).
2/ 4	80.1	5	401	"	
"	"	6	481	"	
"	"	8	641	2+	Destruction of every egg in each ovariole.
"	"	10	801	3+	Complete destruction of each ovary (as in Fig. 7).
			Co^{60} gamma rays:		
2/19/53	12.9	16.26	210	—	Some destruction of eggs; ovary retarded in growth.
3/10	12.8	25.4	325	"	
"	"	30.0	384	"	
"	"	31.8	407	±	Destruction of several eggs in most ovarioles, but more than one egg evident in most ovarioles of each ovary (as in Fig. 5).
2/19	12.9	32.5	420	+	
			Fast neutrons:		
	(rep/min.)		(rep)		
7/28/52	2.5	4.	10	—	
2/10/53	4.61	3.06	14.1	±	
7/28/52	2.5	9.	22.5	+	
2/10/53	4.61	5.5	25.3	+	
7/28/52	2.5	17.	42.5	2+	
2/10/53	4.61	11.	50.7	2+	
6/30/52	1.96	41.	80.4	3+	
"	2.21	41.	90.6	"	
2/10/53	4.61	22.	101.4	"	
6/30/53	2.65	41.	108.7	"	
			Control animals:		
10 controls for each of 7 irradiation dates—(2 controls for 3/7/52 died, but ovaries appeared normal).					

* *i.e.*, number of grasshoppers whose ovaries were examined after irradiation. There were some deaths among irradiated animals, especially at higher neutron doses. For example, 11 grasshoppers out of 20 died after receiving 101 rep fast neutrons ($LD_{65/16}$ days).

† Criteria used to judge biological effects on grasshopper ovary 16 days after irradiation.

‡ With x-ray doses below 200 r, the egg primordia divide mitotically and fill the ovarioles with embryonic cells which are incapable of differentiating into eggs.

secting microscope at 40×. Cytological studies on the living eggs were carried out with the aid of the Leitz Ortholux phase contrast system.

Dosimetry. During the actual irradiation periods, Victoreen thimble chambers were inserted into standard positions in the dosimetry columns of the lucite animal cage(5). One of these chambers has been calibrated in terms of roentgens-equivalent-physical (rep) when exposed to the fast neutron field. Dr. H. H. Rossi of Columbia University used a tissue-equivalent chamber of 10.1% hydrogen composition for this calibration. Factors have been obtained to relate the response of other Victoreen chambers (25 r, 100 r, 250 r) to this calibrated chamber. Thus, after corrections for temperature and barometric pressure, the response of these chambers can be corrected to give a dosage measurement in standard units. One of the Victoreen 25 r thimble chambers with a 4 mm plastic cap was also calibrated by the National Bureau of Standards for gamma rays of Co^{60} in terms of International Roentgens. Whenever fast neutrons are used there is a problem of con-

tamination with gamma rays. By the use of films and a carbon chamber(6) it was concluded that the gamma ray contribution to the tissue dose in the neutron field was approximately 7% of the rep of fast neutrons. Slow neutrons were effectively eliminated by appropriate shields of boron carbide.

Results. As a biological criterion for the evaluation of irradiation damage by the various ionizing radiations we have taken the destruction of every egg in the 80 or more ovarioles of the grasshopper ovary with the exception of egg No. 1, the most advanced ovum, found adjacent to the oviduct (Fig. 1 and 11).

The experimental data are presented in Table I.

In order to produce similar damage to the ovarioles, the following minimum doses were required: 350 r of x-rays; 420 r of gamma rays; 22.5 rep fast neutrons. Thus, assuming gamma radiation to be 1, the relative biological effectiveness (R.B.E.) of these radiations for this result was:

$$\text{X-rays} = \frac{420}{350} = 1.2$$

$$\text{Fast neutrons} = \frac{420}{22.5} = \sim 19$$

Discussion. The state of an irradiated tissue with respect to age, metabolism, and degree of differentiation is important in considering its relative radio-sensitivity. The high anabolic rate in the formation of the grasshopper egg within the ovariole may well be a major cause for its sensitivity to ionizing radiations. It was found that the egg primordia, after low x-ray doses, were incapable of differentiation. The cells divided mitotically, but, instead of developing into eggs, they filled the ovarioles with small, undifferentiated, embryonic cells. This is in agreement with recent observations in embryonic tissue where differentiation is more susceptible to irradiation than anabolism itself(3). Invertebrates are ordinarily considered more radio-resistant than vertebrates for whole-body radiation. This apparent resistance may be due in part to metabolic rate and to the type of transport system involved. In the grasshopper two paths exist for energy transfer to any given cell: the tracheal system, supplying oxygen (directly into the cells), eliminating carbon dioxide and water, and the so-called "blood" or coelomic fluid which supplies organic foods and eliminates nitrogenous wastes from each cell through the malpighian system.

The results of these experiments indicate that 420 r of Co^{60} gamma rays are equivalent to 350 r of 200 kv x-rays and to 22.5 rep of fast neutrons for the criterion selected to measure the degree of destruction of the grasshopper ova. Gray(7) and Ehrenberg and Nybom(8) have published the following mean linear ion densities for these radiations:

	Ion pairs/μ
Co^{60} gamma rays	8
200 kv x-rays	80
Fast neutrons (Stockholm cyclotron, 1-11.5 Mev)	700

(The mean energy of the fast neutrons used in our experiments was approximately 1 Mev with the highest flux at about 0.7. The linear ion density is therefore, according to the above data, greater than 700 ion pairs/μ.)

It is apparent from these figures that the R.B.E. of these 3 ionizing radiations, for this particular effect, is in the same order as their respective ion densities, gamma radiation requiring the highest dose and neutrons the lowest. Zirkle(9) has emphasized the importance of specific ionization and linear energy transfer in many radiobiological effects. The R.B.E. of gamma rays to fast neutrons for this biological effect (18-19) is the highest yet observed in experiments run in our gamma-neutron chamber. For example, the R.B.E. for acute 30-day lethality in the female mouse (CF-1) after 90-minute exposures to these same ionizing radiations is 4.4 ± 0.05(10).

Although there is a 16-day interval necessary between irradiation and evaluation, it is hoped that the grasshopper ovary may serve as a useful biological dosimeter. The insects are easily maintained in the laboratory, and the removal and examination of their ovaries can be done successfully with some experience. Our results, listed in Table I, show that doses of fast neutrons in excess of 80 rep completely destroy each ovary, but, for doses in the range 10-50 rep, we believe that our criteria can be

used by other investigators to give an approximation of fast neutron doses.

It is clear from the data that the neutron dosages are less accurate than the other radiations for the criterion effect, since the separation of experimental dosages is higher in the effective range. In an attempt to test the accuracy and reproducibility of the criteria used, 5 groups of grasshoppers were recently irradiated with 14, 16, 18, 20, and 22 rep respectively, and their ovaries were later examined by an investigator who did not know the doses given to the insects. The 22 rep group was diagnosed in the single plus category and was named correctly as the group receiving the highest dose. This test substantiated the fact that 22 rep of fast neutrons was the minimum dose for the plus criterion. The grasshoppers that had been exposed to 14 rep were estimated to have received "about 15 rep" and these were placed in the ± category from a study of their ovarioles. However, there was enough variation in the ovaries of the remaining three groups so that they could not be clearly distinguished from each other and could only be termed "intermediate between 14 and 22 rep."

It is realized that this dosimeter gives at present only an approximation of the dose of fast neutrons within the rather narrow range of 10-50 rep. However, there is no reason to believe that such dosimeters cannot be improved both in quantitative accuracy and in reproducibility. Although biological materials will always possess variability, it is possible that ionizing radiations may be measured by several carefully selected biological dosimeters, each highly sensitive to a specific dose range of the radiation involved.

Summary. In order to destroy every egg in each grasshopper ovariole, with the exception of the most advanced, it was necessary to irradiate the total body with 350 r (200 kv) x-rays, 420 r Co^{60} gamma rays, or 22.5 rep of fast neutrons. Thus, the relative biological effectiveness of Co^{60} gamma rays: 200 kv x-rays: fast neutrons, for this specific effect, is 1.0: 1.2: 19. This is correlated with relative specific ionization and linear energy transfer. It is suggested that damage to the grasshopper ovariole could be used as a biological dosimeter for fast neutron doses in the range 10-50 rep.

The authors wish to acknowledge the photographic assistance of Miss Jane K. Glaser of the Division of Biological and Medical Research, Argonne National Laboratory. They also appreciated the critical reading of the manuscript by Dr. Austin M. Brues and Dr. John W. Clark of this Division.

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