GENETICALLY NONTRANSMISSIBLE NUCLEAR DAMAGE INDUCED BY ULTRAVIOLET RADIATION IN THE WASP HABROBRACON

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IN *Habrobracon*, unfertilized eggs develop normally and become haploid males; fertilized eggs become diploid females. It has been observed that when newly-laid eggs from virgin females are exposed to ultraviolet radiation on the nuclear (convex) surfaces, embryo hatchability decreases as the exposure time is increased (VON BORSTEL and MOSER 1956; VON BORSTEL and WOLFF 1955). Most of the deaths occur very early in development. However, if the eggs are fertilized before irradiation, the frequency of early deaths is markedly reduced, and preliminary experiments indicated that the fertilization-induced recovery from ultraviolet damage was indeed complete (VON BORSTEL 1961). (Operationally this is a "protection" since the modifying treatment (fertilization) takes place before irradiation, however there appear to be no interactions between egg and sperm nuclei until meiosis is complete and this occurs after the irradiation.) A similar reactivation effect seems to occur after fertilization of eggs from females treated with nitrogen mustard (LÖBBECKE and VON BORSTEL 1962).

The study reported here was undertaken to determine definitively: (1) if the fertilized induced recovery is indicative of a true repair of ultraviolet-radiation-induced damage, a postponement of death to a later stage (cf. ATWOOD, VON BORSTEL and WHITING 1956), or a large contribution of recessive lethals to the death of the unfertilized (haploid) egg, (2) the stage or stages of death in the unfertilized and fertilized egg, and (3) if the fertilization-dependent recoverable portion is identical to the photorecoverable portion of ultraviolet radiation damage.

MATERIALS AND METHODS

Wild-type No. 33 virgins were used throughout. When matings were to be made, males from the 17 *o*¹ stock (stock having sex alleles different from No. 33) were used to avoid production of diploid males. The females were fed on the host, the caterpillar of the moth *Ephestia*, for a day and then incubated overnight without the host so that the females would store eggs in the first metaphase of meiosis.

To collect large numbers of eggs quickly, groups of four females were placed with a caterpillar in small stender dishes. The eggs were collected from the dishes

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in a way so that none would be more than ten minutes old when irradiated (see VON BORSTEL and WOLFF for details of the technique). As the newly laid eggs were collected, they were oriented on slides with the convex (nuclear) surface upwards, and then the eggs were exposed to radiation. After irradiation, the eggs were either covered and placed in a humidified 30°C incubator or else were exposed to photoreactivating light before being incubated. At 30°C, the embryos hatch in 29 hours (AMY 1961). Before this time had elapsed, the embryos were transferred to stung caterpillars to enable us to score hatchability, adult survival, and time of death on the same batch of eggs (see VON BORSTEL and REKEMEYER 1959 for procedural details on scoring). Recessive lethal frequencies were determined by setting F_1 females singly and scoring hatchability of the laid eggs.

Since not every egg in the mated group is fertilized, the proportion of fertilized eggs, f , is given by the ratio of females to total adults among the eclosed animals from the mated controls. The controls were eggs collected from the same wasps that laid the eggs exposed to ultraviolet radiation.

The frequency of deaths at any given stage of development among unfertilized eggs, s_u , can be measured directly. The frequency of deaths at the same stage in development among fertilized eggs, s_f , must be estimated from $s_f = [s_m - s_u(1 - f)]/f$, where s_m is the proportion of embryos from mated females dying at this stage.

The ultraviolet irradiation was done with a General Electric germicidal lamp at a distance of 64 cm giving 5 ergs $\text{mm}^{-2} \text{sec}^{-1}$. Photoreactivating light from two General Electric BL360 lamps was passed through 2.7 cm of plate glass. At a distance of 5.5 cm it had an intensity of 86 ergs $\text{mm}^{-2} \text{sec}^{-1}$. Photoreactivating light was applied at this distance for 30 minutes at 27–28°C. Dosimetry was accomplished with a meter designed by JAGGER (1961).

RESULTS

Uncorrected data are listed in Tables 1 and 2. At least 50 eggs were irradiated at from six to ten different doses under each condition.

From these data and the equation, a dose-response curve for deaths at stage 1 (Type I lethality) was constructed (Figure 1). Over the early part of the curves of both the nonreactivated and photoreactivated groups, the fertilized eggs are considerably less sensitive to ultraviolet radiation than the unfertilized eggs. This difference, as we shall demonstrate, corresponds to the genetically nontransmissible portion of the radiation damage. At higher doses the slope of the curve for the fertilized eggs changes markedly and intersects the curve for unfertilized eggs. It has been postulated (VON BORSTEL 1961) that as the dose increases, dominant lethal damage accumulates in both the sperm and egg nucleus of the fertilized eggs and results in a crossing of the two curves at approximately 40 percent survival. The doubling of the target size, by this argument, would appear to be the determinant of sensitivity. This curve was not extended because approximately 15 percent of the egg nuclei are protected from the radiation by cytoplasmic shielding (AMY and VON BORSTEL 1958; VON BORSTEL and MOSER 1956).

TABLE 1

Survival and stages of death of embryos after irradiation of Habrobracon gametes from unmated No. 33 females

UV incident dose, ergs/mm ²	Viable embryos/total	Number dying at each stage of development															
		Embryos					Larvae				Pupae				Adults		
		1*	2	3	4	5	6	7	8	9	10	11	12	13	♂	♀	
<i>Not photoreactivated</i>																	
25	46/52	2	0	0	1	3	0	2	4	0	0	0	0	0	40	0	
50	34/56	13	0	4	5	0	0	2	5	1	1	0	1	0	24	0	
75	30/55	17	0	3	2	3	0	5	1	0	1	2	1	0	20	0	
100	12/52	33	1	3	3	0	0	0	1	0	1	0	1	0	9	0	
125	23/57	29	0	0	4	1	0	1	0	0	0	2	0	0	20	0	
150	6/50	39	0	2	2	1	0	0	0	0	0	0	0	0	6	0	
<i>Photoreactivated</i>																	
50	21/25	1	0	0	1	2	1	2	0	0	0	1	4	0	13	0	
75	36/38	0	0	0	1	1	1	1	0	0	0	0	2	0	32	0	
100	80/100	9	0	2	3	6	5	0	1	2	6	0	2	1	63	0	
150	53/78	14	0	1	5	5	5	3	3	2	0	1	5	1	33	0	
200	51/101	34	0	8	4	4	0	4	1	0	5	0	2	0	39	0	
300	19/57	29	0	2	4	3	0	1	0	0	0	0	1	0	17	0	
400	18/56	29	0	4	3	2	0	3	2	1	1	0	0	0	11	0	

* These numbers refer to stages of development; for further description, see VON BORSTEL and REKEMEYER 1959.

Whether the eggs are photoreactivated or nonphotoreactivated, the ratios of dose-response of the fertilized to the unfertilized eggs remain roughly constant. Therefore the photorecoverable lesion probably is not affected by the presence of the second nucleus.

Figure 2 shows frequencies of embryos dying at different stages of embryonic and larval development at four different doses of ultraviolet radiation. For the nonphotoreactivated group, it is apparent that not enough later death occurred to account for the large dissimilarity between stage 1 deaths for the unfertilized and fertilized eggs at any one dose. For the photoreactivated group, in particular at 100, 150, and 200 ergs mm⁻², a sizable proportion of deaths later in embryonic development among the fertilized eggs that were irradiated seems to compensate in part for the discrepancy among the deaths at stage 1, but this may be due to the relatively small numbers involved. In any case, the discrepancy is not supported by sex ratio data to be presented in a later paragraph.

In the photoreactivation group at 300 ergs mm⁻² (Figure 2d) the discrepancy between Type I deaths of the unfertilized and fertilized eggs is not compensated by deaths later in development. By examining the later deaths only, it can be seen that the embryos from fertilized eggs die later than do the embryos from unfertilized eggs. These have been called conditionally delayed dominant lethals (ATWOOD, VON BORSTEL and WHITING 1956) or Type II dominant lethals and have been attributed to gross chromosomal deletions (VON BORSTEL and REKEMEYER 1959; VON BORSTEL 1960). Simultaneous induction and overlapping manifestation of the photorecoverable, fertilization-recoverable, and conditionally

TABLE 2

Survival and stages of death of embryos after irradiation of Habrobracon gametes from No. 33 females $\times$ 170ⁱ males

Treatment	Viable embryos/total	Number dying at each stage of development														Adults	
		Embryos					Larvae				Pupae						
		1*	2	3	4	5	6	7	8	9	10	11	12	13	♂	♀	
<i>Control</i>																	
1	101/110	1	0	1	5	2	1	6	7	0	0	0	0	0	39	48	
2	47/48	1	0	0	0	0	0	0	0	0	4	0	1	0	21	21	
3	83/88	1	0	0	3	1	0	2	0	0	4	0	1	0	37	39	
Total	231/246	3	0	1	8	3	1	8	7	0	8	0	2	0	97	108	
<i>UV incident dose, ergs/mm², not photoreactivated</i>																	
25	82/94	5	0	0	4	3	4	1	0	0	0	0	2	1	18	56	
50	74/96	13	0	0	6	3	1	10	0	1	1	1	0	0	17	43	
75	79/114	28	0	2	2	3	4	6	0	0	0	0	0	0	15	54	
100	20/47	20	0	1	3	3	0	0	0	0	0	0	1	1	4	14	
125	32/98	59	0	1	4	2	1	1	1	0	1	0	1	0	3	24	
150	20/76	54	0	0	2	0	0	6	0	0	0	0	0	0	8	6	
200	14/91	72	0	0	3	2	0	3	0	1	0	1	0	0	3	6	
250	23/138	101	0	1	9	4	0	0	1	0	0	2	5	0	4	11	
300	15/103	84	0	0	2	2	0	1	1	0	0	0	1	0	5	7	
400	5/58	50	2	0	1	0	0	0	0	0	0	0	0	0	2	3	
<i>Photoreactivated</i>																	
100	41/51	4	0	0	3	3	1	1	0	0	0	0	0	0	7	32	
150	36/55	9	0	0	8	2	0	2	1	1	2	1	1	1	8	19	
200	66/120	30	0	0	9	15	0	10	0	0	2	0	1	0	12	41	
300	67/144	56	0	1	12	8	4	5	5	8	5	1	3	0	8	28	
400	23/97	63	0	0	8	3	0	0	0	0	0	0	0	0	2	21	
500	17/91	62	0	1	6	5	0	2	0	1	0	0	1	0	1	12	

* These numbers refer to stages of development; for further description, see VON BORSTEL and REKEMEYER 1959.

delayed sectors of induced lethality complicate interpretations from hatchability data (Tables 1 and 2) and time of death data (Figure 2). Nevertheless, the major discrepancy between frequencies of early death in Figures 2a and 2b is not compensated in nonphotoreactivated embryos.

If the effect is that of selective survival of fertilized eggs, then it would be expected that the female/total adult ratio would increase with dose. It can be seen in Figure 3 that such a response was induced. The points were fitted by a linear regression line after an arc sine transformation of the ratio. The P value for the significance of regression for each set taken separately is 0.2, therefore taken together the increase seems to be real. Since the data are few, and since a theory of expected dose-response has not yet been developed, no attempt will be made to discuss apparent departures of some of the points from the regression line. As in Figure 1, the curves were not carried further because the resistant fraction of eggs brings the curves back to the control level.

The frequencies of recessive embryo lethality induced at several different doses of ultraviolet radiation are shown in Table 3. At doses higher than 100 ergs mm⁻²

FIGURE 1.—Dose-response curves for *Habrobracon* embryos dying during early development that were irradiated as newly laid eggs. ● unfertilized; ▲ fertilized; ○ unfertilized photo-reactivated; △ fertilized, photoreactivated.

in the nonphotoreactivated group and 200 ergs mm^{-2} in the photoreactivated group, the number of females at each dose was small, varying from three to eleven. These were summed; no increase was large enough to account for the sex ratio increase seen in Figure 3 nor for the reduction in frequency of early deaths seen in Figure 1. Postembryonic recessive lethality was also scored, but mutant frequencies are less than one third of those estimated by scoring hatchability. All but one of the recessive embryo-lethal mutations were expressed in postblastula stages of embryonic development. This stands in contrast to the deaths caused early in development from which fertilization-dependent recovery is obtained (Figures 1 and 2.)

The data on recessive lethals from Table 3 were plotted on a log log scale (Figure 4). The slope indicates that induction by ultraviolet radiation of recessive lethality in the newly laid egg follows one-hit kinetics even though the photo-reactivation data are few and subject to being otherwise explained.

FIGURE 2.—Frequencies of death at different stages of development of embryos irradiated as newly laid eggs.

Finally, no androgenetic males nor mosaic males were found among adult survivors of the irradiated eggs.

DISCUSSION

Nuclear damage inducible by ultraviolet radiation and reparable by the presence of another nucleus was first postulated by Arwood (1954) to explain non-random lack of reduction of heterokaryosis in *Neurospora* conidia, even at low survival levels. Since the effect seems to require an entire nucleus, it is reasonable that the Arwood effect would most likely be observed in haploid-diploid systems like yeast or *Habrobracon*, or haploid mononucleate-multinucleate systems such as *Neurospora*. MAGNI (1956) and MORTIMER (1961) have provided evidence for a similar effect in yeast after X-irradiation (see also discussion by MAGNI 1963, after paper of VON BORSTEL and ST. AMAND). To account for the difference between haploid and diploid survival curves in yeast, a certain frequency of

FIGURE 3.—Sex-ratio shift in response to the dose of ultraviolet radiation. ● no photoreactivation; ○ photoreactivation.

induced recessive lethality was postulated. The actual frequencies of recessive lethality were always much lower than the estimate based on the different slopes of survival curves.

The Arwood effect was first clearly recognized in *Habrobracon* by the greatly reduced frequency of early deaths in fertilized eggs after ultraviolet treatment (VON BORSTEL 1961). This interpretation also explained a small but consistent reduction of early deaths after fertilization in earlier experiments on *Habro-*

TABLE 3

Embryo recessive lethal frequencies after exposure of newly laid fertilized eggs to ultraviolet radiation

UV incident dose, ergs/mm ²	Females tested <i>N</i>	Embryo recessive lethals <i>n</i>	Percent <i>n/N</i>	Fiducial limits (STEVENS 1942) when $P=0.05$ for n =observed	
				More than	Fewer than
<i>Not photoreactivated</i>					
0	75	0	0	..	...
25	52	2	3.8	0.6	11.4
50	40	2	5.0	0.8	14.9
75	46	5	11.1	4.3	21.6
100	14	2	14.3	2.5	40.2
150-400	33	4	12.1	..	...
<i>Photoreactivated</i>					
100	31	2	6.4	1.1	19.2
150	16	2	12.5	2.6	35.4
200	31	4	12.9	4.5	27.7
300-500	19	2	10.5	..	...

FIGURE 4.—Dose-response curves for recessive lethal mutations induced by ultraviolet radiation. Δ no photoreactivation; $\circ$ photoreactivation.

bracon with X-radiation (ATWOOD *et al.*, 1956; VON BORSTEL and REKEMEYER 1959). A large effect, similar in magnitude to that found with ultraviolet radiation, has also been found after nitrogen mustard treatment of *Habrobracon* females (LÖBBECKE and VON BORSTEL 1962).

The possibility must be considered that the Atwood effect is not nuclear but is cytoplasmic in nature. Though difficult to test in other forms, this possibility can be specifically excluded in *Habrobracon*. Nuclear damage induced by ultraviolet radiation is photorecoverable to an extent whereas cytoplasmic damage is not (VON BORSTEL and WOLFF 1955). Nuclear damage causes most deaths to occur early in development whereas cytoplasmic damage kills the embryo later in development (VON BORSTEL 1957). The fertilization dependent, genetically non-transmissible damage resembles nuclear damage in both respects. Also, ultraviolet radiation does not interfere with fusion of the sperm and egg pronuclei nor inactivate specifically the egg nucleus, since no mosaic individuals nor androgenetic males were found.

Genetically nontransmissible damage is a nuclear lesion that is repaired very early in development. The very early deaths following X-irradiation have been ascribed to prolonged delay in nuclear division usually associated with a chromosome bridge (VON BORSTEL 1960, 1961). It is known that effects of radiation other than chromosome breakage also cause division delay (cf. CARLSON 1954). It seems reasonable to suppose that division may be delayed less if a wholly functional nucleus combines with a partially functional one, and together the division process occurs at a rate closer to the normal rate. This suggestion is not exclusive; it is also possible that there may be support of the system by the undamaged nucleus for a long enough time to allow spontaneous repair of the damaged nucleus.

Conclusion: A proportion of the damage induced by ultraviolet radiation in

the prezygotic nucleus of *Habrobracon* is genetically nontransmissible; it is apparently reparable in the presence of a second nucleus.

SUMMARY

A type of nuclear damage that is genetically nontransmissible can be induced prezygotically in the wasp *Habrobracon*. The damage is expressed as death very early in development. It is not a dominant lethal event because the damage is not expressed in the presence of a second nucleus. It is not a recessive lethal event because the damage cannot be found by genetic testing of the F_1 females. It is not cytoplasmic damage because the total damage when induced by ultraviolet radiation responds to photoreactivation, whereas cytoplasmic damage in *Habrobracon* embryos does not. It is inducible in *Habrobracon* by ultraviolet radiation, apparently also by nitrogen mustard, and to a limited extent by X-radiation. The effect is that of a nuclear reactivation which seems to be independent in response from photoreactivation.

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LITERATURE CITED

- AMY, R. L., 1961 The embryology of *Habrobracon juglandis* (Ashmead). *J. Morphol.* **109**: 199-210.
- AMY, R. L., and R. C. VON BORSTEL, 1957 The effects of different wavelengths of ultraviolet light on the *Habrobracon* egg. *Atti del Cong. Intern. di Fotobiol. Minerva Med., Minerva Fisioterapica (Collana Monografica)* **2**: 419-422.
- ATWOOD, K. C., R. C. VON BORSTEL, and A. R. WHITING, 1956 An influence of ploidy on the time of expression of dominant lethal mutations in *Habrobracon*. *Genetics* **41**: 804-813.
- CARLSON, J. G., 1954 Immediate effects on division, morphology, and viability of the cell. pp. 763-824. *Radiation Biology*. I. Edited by A. HOLLAENDER. McGraw-Hill Book Co., Inc., New York.
- JAGGER, J., 1961 A small and inexpensive ultraviolet dose-rate meter useful in biological experiments. *Radiation Res.* **14**: 394-403.
- LÖBBECKE, E. A., and R. C. VON BORSTEL, 1962 Mutational response of *Habrobracon* oocytes in metaphase and prophase to ethyl methanesulfonate and nitrogen mustard. *Genetics* **47**: 853-864.
- MAGNI, G. E., 1956 Problems concerning the radiosensitivity of yeast cells. *Compt. rend. Lab. Carlsberg, Ser. physiol.* **26**: 273-284.
- MORTIMER, R. K., 1961 Factors controlling the radiosensitivity of yeast cells. *Brookhaven Symp. Biol.* **14**: 62-75.
- STEVENS, W. L., 1942 Accuracy of mutation rates. *J. Genet.* **43**: 301-307.

- VON BORSTEL, R. C., 1957 Nucleocytoplasmic relations in early insect development. pp. 175-199. *The Beginnings of Embryonic Development*. Edited by A. TYLER, R. C. VON BORSTEL and C. B. METZ. Am. Assoc. Advan. Sci., Washington, D.C.
- 1960 Sulla natura della letalità dominante indotta dalle radiazioni. Atti Assoc. Genetica Italiana **5**: 35-50.
- 1961 Induction of nuclear damage by ionizing and ultraviolet radiation. pp. 243-250. *Progress in Photobiology*, Proceedings of the 3rd International Congress on Photobiology (Copenhagen, 1960). Edited by B. C. CHRISTENSEN and B. BUCHMANN. Elsevier Publishing Company, Amsterdam.
- VON BORSTEL, R. C., and H. MOSER, 1956 Differential ultraviolet irradiation of the Habrobracon egg nucleus and cytoplasm. pp. 211-215. *Progress in Radiobiology*. Edited by J. S. MITCHELL, B. E. HOLMES, and C. L. SMITH. Oliver and Boyd, Ltd., Edinburgh.
- VON BORSTEL, R. C., and M. L. REKEMEYER, 1959 Radiation-induced and genetically contrived dominant lethality in Habrobracon and Drosophila. *Genetics* **44**: 1053-1074.
- VON BORSTEL, R. C., and W. ST. AMAND, 1963 Stage sensitivity to X radiation during meiosis and mitosis in the egg of the wasp Habrobracon. *Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells*. Edited by F. SOBELS. Pergamon Press Ltd., London (in press).
- VON BORSTEL, R. C., and S. WOLFF, 1955 Photoreactivation experiments on the nucleus and cytoplasm of the Habrobracon egg. *Proc. Natl. Acad. Sci. U.S.* **41**: 1004-1009.