Lauvic and stearic acids, when X-irradiated, gave similar, incompletely resolved resonances, indicating the presence of more than one radical with proton coupling. We have not yet been able to untangle the multiplets sufficiently to identify them.

Although, surprisingly, no free ethyl radical (C_2H_5) has been detected in the present work, the microwave "fingerprint" of this radical has been found by Gordy and McCormick in x-irradiated $Hg(C_2H_5)_2$ at 77° K. Interestingly, it has a symmetrical sextet with a total spread of 130 gauss. The sextet structure means that in the ethyl radical the five protons actually have equal coupling to the odd electron—a symmetry which may come about through a rapid exchange of protons at the opposite ends.

The results of these two papers with later ones obtained in our laboratory indicate that caging is a very important factor in determination of effects of radiation on molecules in solids. When a molecule is ionized, it generally breaks up. The pieces, being unable to escape, tend to react and re-react until the most stable assembly of new molecules and radicals is formed.

[1] In gamma-irradiated formic acid M. S. Matheson and B. Smaller (*J. Chem. Phys.*, **23**, 521, 1955) observed a single doublet of 15-gauss separation but did not observe the wider doublet of 135 gauss.

PHOTOREACTIVATION EXPERIMENTS ON THE NUCLEUS AND CYTOPLASM OF THE HABROBRACON EGG*

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The counteraction of ultraviolet radiation (*ca.* 2600 A) damage by radiation of a longer wave length (*ca.* 3600 A) has been called "photoreactivation." The phenomenon is widespread in nature (reactivation of inactivated fungi,[1] viruses,[2] bacteria,[3] protozoa,[4] higher plant cells,[5] salamander larvae[6]) and affects known nuclear events such as mutation induction,[7] mitotic rate,[8] and deoxyribonucleic acid synthesis.[9] The egg of the wasp *Habrobracon juglandis* Ashmead has characteristics that make it possible to irradiate the nucleus and cytoplasm[10] separately; consequently, the egg is well suited for investigating the localization of the photoreactivation phenomenon within the cell.

Fertilization of the *Habrobracon* egg is not required for normal development—unfertilized eggs become haploid males. The nucleus of the newly laid egg is in the first meiotic metaphase and is located at the anterior end on the convex surface[11] (Fig. 1). The nucleus remains in this position for 30 minutes (30° C.) while meiosis is completed. The pronucleus then migrates to the center of the egg, and development begins.

Materials and Methods.—Well-fed virgin females are removed from their host (the larva of the Mediterranean flour moth *Epthestia*) 4–12 hours before the experi-

ments are begun, in order that they may store mature eggs. At the end of this time, six females are placed in each of twelve to eighteen small Stender dishes, with two host *Ephestia* caterpillars per dish. One person can conveniently handle this number of dishes by examining the dishes cyclically for newly laid eggs. No egg will be more than fifteen minutes old when irradiated, and none will have proceeded further than the second meiotic metaphase. The eggs are slightly
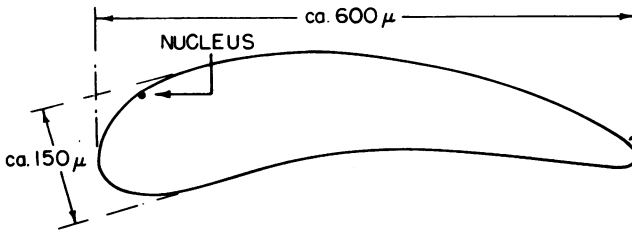

Fig. 1.—Lateral view of a newly laid *Habrobracon* egg. The nucleus is located at the anterior (wide) end near the convex surface.

adhesive; they may be removed with a dissecting needle and arranged on glass slides for irradiation. In these experiments the eggs are oriented so that either the convex (nuclear) surfaces or the concave (nonnuclear) surfaces are facing the radiation source. Under these conditions, up to two hundred eggs have been irradiated per hour. Hatchabilities are recorded two days later. Virgins from stock No. 33 were used in these experiments.

A shield used in some experiments for protecting the nucleus (Fig. 2) was constructed by stacking several cover slips on a slide. The cover slips were joined with balsam. Since the eggs adhered easily to the underside of the jutting cover slip on the top of the stack, this shield provided a method whereby the egg cytoplasm could be irradiated, while it allowed less than 5 per cent of the incident ultraviolet radiation to get to the nucleus.
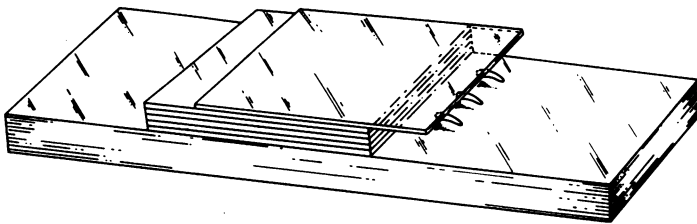

Fig. 2.—Diagram of shield used to protect the nucleus from ultraviolet radiation.

The ultraviolet radiation source was a G.E. germicidal mercury-arc lamp at 60 cm., which produced a flux of 504 ergs/mm²/min, as measured by a thermopile calibrated against an NBS standard lamp. Approximately 80–85 per cent of the energy produced by the mercury lamp is at a wave length of 2537 A. The photo-reactivating source was a G.E. 360 BL lamp at 4.8 cm., with a flux of approximately

7,000 ergs/mm²/min, as measured by a thermopile and standard lamp; most of the incident energy is at a wave length of 3600 A. The air under the lamps was in constant circulation. Eggs were kept at a constant temperature of 24°–25° C. during the exposure periods and for at least 24 hours afterward. The slides containing the eggs were then placed in an incubator at 30° C., so that the eggs would start hatching in the morning of the second day after irradiation.

Results and Discussion.—The curves at the left in Figure 3 indicate that dose-hatchability values give an exponential curve when the convex (nuclear) side of the egg is irradiated. The data are listed in Table 1. Approximately one-third

TABLE 1

HATCHABILITIES OF EGGS IRRADIATED ON CONVEX (NUCLEAR) SURFACES

| DOSE (ERGS/MM²) | HATCHABILITY AFTER TREATMENT | | | | x^2 | $P(x^2)$ |
| | Ultraviolet | | UV + Photoreactivating Light for 15 Minutes | | | |
	Larvae/Eggs	Per Cent	Larvae/Eggs	Per Cent		
0	126/127	0.992	122/125	0.976	...	...
42	77/99	.778	82/88	.932	8.6831	<0.01
84	51/106	.481	76/102	.745	15.2327	< .01
126	54/266	.203	68/100	.680	74.4108	< .01
252	15/95	.158	33/102	.323	7.3226	< .01
378	55/367	.150	20/90	.222	2.7585	< .01
504	12/214	.056	16/109	.147	7.3516	< .01
630	21/300	.070	16/116	.138	4.7640	.03
882	7/103	0.068	16/140	0.114	1.4861	0.23
					P (total)	<0.00001

of the eggs appear to be especially resistant to the irradiation; this is evidenced by the concave character of the curve and by extrapolation to zero dose of the second exponential component. This resistance is probably caused either by meiotic stage sensitivity differences or by differences in location of the nucleus within the egg. Figure 3 shows also a maximum photorecovery curve from ultraviolet damage induced by irradiation of the convex side of the egg. The dose reduction of approximately 2.2 is in general agreement with that obtained on other materials.[12]

A dose-effect curve for photoreactivation at a constant ultraviolet radiation exposure of 126 ergs/mm² is shown in Figure 4. It is apparent that photorecovery begins with very short exposures of reactivating light. Since the nucleus is in the process of meiosis at this time, it is likely that active synthesis of chromosomal material is not requisite for photorecovery. This conclusion can be inferred also from data on photoreactivation of inactivated sperm.[13] Controls placed under the reactivating light without prior exposure to ultraviolet radiation showed normal hatchability even after two hours of exposure. Relative to the inactivation dose at 2537 A, the dose at 3600 A after two hours (*ca.* 10⁶ ergs/mm²) is still below the exposure required to produce a striking lethal effect in bacteria.[14]

When the side of the egg opposite the nucleus (concave side) is exposed to ultraviolet radiation (Fig. 3 and Table 2), the hatchability curve is sigmoid. The initial exponential component of the sigmoid curve demonstrates that less than 5 per cent of the incident radiation leaks to the nucleus when the concave side is irradiated. Contrary to results obtained from exposure of the convex surface, photoreactivation does not appear to take place when the egg is injured by exposing the concave surface to the radiation. The significant difference between the
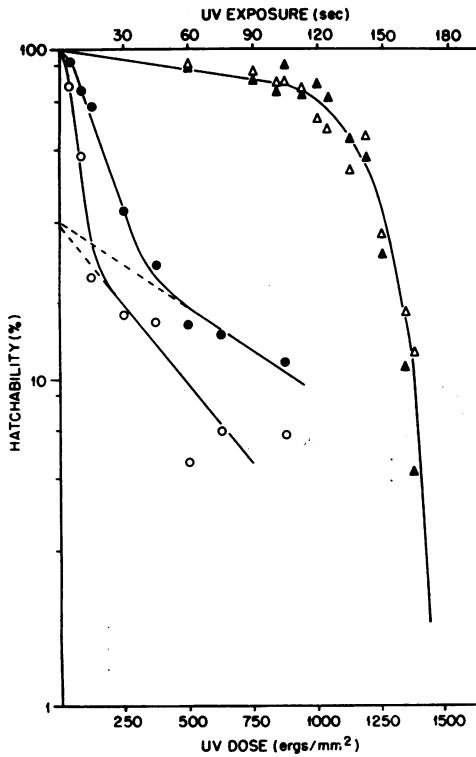
FIG. 3.—Dose-hatchability curves for *Habro-bracon* eggs irradiated on their convex (nuclear) (O, ultraviolet; ●, ultraviolet plus photoreactivating light) or concave (nonnuclear) (△, ultraviolet; ▲, ultraviolet plus photoreactivating light) surfaces.
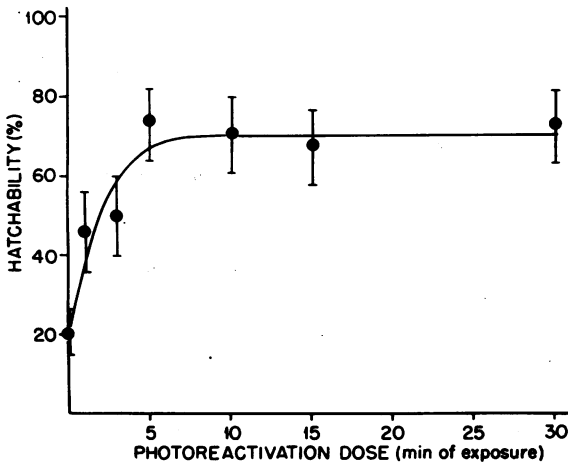

FIG. 4.—Dose-recovery curve for eggs exposed to 126 ergs/mm.² of ultraviolet radiation and to reactivating light of different duration.

inactivated and photoreactivated eggs at 1,008 ergs/mm^2 is attributed at present to leakage to the nucleus.

Experiments in which the nucleus was shielded were performed by protecting the anterior quarter of the egg from radiation (Table 3). These data show that the nonnuclear portion of the convex side of the egg has the same sensitivity to the ultraviolet radiation as the concave side, thus supporting the conclusion that the photorecoverable injury is of a nuclear nature and not merely the result of a difference between the concave and convex surfaces.

TABLE 2

HATCHABILITIES OF EGGS IRRADIATED ON CONCAVE SURFACES

DOSE (ERGS/MM^2)	Hatchability after Treatment — Ultraviolet — Larvae/Eggs	Per Cent	—UV + Photoreactivating— Light for 15 Minutes Larvae/Eggs	Per Cent	x^2	$P(x^2)$
0	81/83	0.976	142/145	0.979	. . .	. .
504	95/106	.896	96/108	.889	0.0300	0.85
756	87/102	.852	81/100	.810	0.6651	.40
850	37/47	.787	37/49	.755	0.1402	.71
882	80/100	.800	91/102	.892	3.3010	.07
950	43/56	.765	36/49	.735	0.1543	.70
1,008	128/209	.613	158/201	.785	14.6415	< .01
1,050	32/56	.572	37/52	.712	2.2942	.14
1,134	43/101	.426	61/114	.535	2.5639	.12
1,200	32/58	.552	28/59	.472	0.6968	.42
1,260	28/103	.272	23/97	.237	0.3172	.60
1,350	8/50	.160	6/55	.109	0.5874	.46
1,386	12/101	0.119	7/136	0.052	3.5642	0.06
1,512	0/103	0	0/105	0		

P (total) = 0.197

TABLE 3

HATCHABILITIES OF EGGS IRRADIATED WITH NUCLEAR ENDS SHIELDED

DOSE (ERGS/MM^2)	Hatchability after Treatment — Ultraviolet — Larvae/Eggs	Per Cent	—UV + Photoreactivating— Light for 15 Minutes Larvae/Eggs	Per Cent	x^2	$P(x^2)$
Convex:						
1,008.	39/58	0.672	30/49	0.612	0.4199	0.52
1,512	0/25	0	0/25	0		
Concave:						
1,008	42/59	0.712	37/59	0.627	0.9575	0.34

Conclusion.—Taken as a whole, these experiments indicate that, by the criterion of hatchability, photoreactivable injury is related to events within, limited to, or governed by the egg nucleus.

Summary.—The egg of the wasp *Habrobracon* is of such nature that the nucleus and the cytoplasm can be damaged separately by ultraviolet radiation by exposing the convex (nuclear) surface, on the one hand, or the concave (cytoplasmic) surface, on the other. Under these conditions, with hatchability as the criterion, nuclear inactivation data follow an exponential survival curve; cytoplasmic inactivation data follow a sigmoidal curve. The damaged nucleus can be photoreactivated; injured cytoplasm appears not to be subject to photoreactivation.

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