

period. One dog developed a superficial duodenal erosion and 2 dogs developed a rather marked antral gastritis. No definite pathologic change was noted in the other dog.

Group VII. Fundusectomy. This procedure includes excision of the acid-secreting area, as described by Connell. All dogs survived the injection period. One dog developed a 1 cm duodenal ulcer just distal to the pylorus and two dogs developed duodenal erosions with marked antral gastritis. The other dog showed a mild antral gastritis.

It would appear that these latter 2 procedures offer relatively satisfactory protection against the development of ulcer but not as

complete as the extensive gastric resection.

Conclusions. The extensive gastric resection (operations III and IVA) offer the best protection against the development of jejunal ulcer. No jejunal ulcer developed and all dogs survived the injection period.

Gastrojejunostomy, the small gastric resection and the Schmilinsky operation offer no protection. All animals developed jejunal ulcer with employment of the histamine-bees-wax technique.

The small gastric resection and the antral exclusion operations would suggest that the Edkins' "gastrin" hypothesis is invalid.

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Morphogenetic Effects of Ultraviolet Radiation.

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Holtfreter has recently described¹ the physical properties of the superficial membrane (the "surface coat") of the early Amphibian embryo, and has indicated² ways in which it may play an important role in morphogenesis. Ultraviolet radiation would seem an excellent means for further study of the surface coat, since this type of energy penetrates poorly, and methods have been worked out which permit its localization in any given region of the embryo.³

In surveying the literature on the effects of ultraviolet radiation on embryonic cells, a number of discrepant observations become apparent. On the whole, ultraviolet exerts an inhibitory effect, but various stimulatory effects have been described, for which it is difficult to account. Thus Hinrichs⁴ found

various degrees of inhibition in *Fundulus* embryos, but a few were stimulated, presumably because they were affected by only a small dose of energy. In a general discussion Hinrichs⁵ later asserts that although vertebrate embryos may be stimulated with ultraviolet, this is to be done only with difficulty. Higgins and Sheard⁶ obtained a transitory speeding up of gastrulation in *Rana pipiens* eggs, but otherwise describe no stimulatory effects. Brandes⁷ found that while most of his amphibian embryos showed inhibitory effects on the radiated side, others were stimulated, showing more rapid gastrulation movements and increase in mass on the radiated side. As a preliminary to the further use of ultraviolet energy, the present study was carried out with the aim of ascertaining conditions under which consistent and reproducible effects may be obtained.

* The author is indebted to Mr. Alvin Sellers for aid in carrying out part of the work herein reported.

¹ Holtfreter, J., *J. Exp. Zool.*, 1943, **93**, 252.

² Holtfreter, J., *J. Exp. Zool.*, 1943, **94**, 261; 1943, **95**, 171.

³ Schechtman, A. M., and Baronofsky, I., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 209.

⁴ Hinrichs, Marie A., *J. Morph.*, 1925, **41**, 239.

⁵ Hinrichs, Marie A., *Proc. Soc. Exp. Biol. and Med.*, 1928, **26**, 175.

⁶ Higgins, G. M., and Sheard, C., *J. Exp. Zool.*, 1926, **46**, 333.

⁷ Brandes, J., *Bull. Acad. Roy. Belg. (Bull. de la Classe des Sciences)*, 1938, **24**, 92; *Ibid.*, Seance du 5 Nov., 1938.

Over 600 eggs of the frog, *Hyla regilla*, were used. The egg-jelly absorbs ultraviolet energy very effectively therefore great care was taken to remove as much jelly as possible by rolling individual eggs in powdered carmine and then stripping off the carmine-jelly mixture by means of fine forceps.

Use of therapeutic cold quartz lamp. In a first group of experiments an instrument sold under the name of "Braun Fluoroscope" was used. A prospectus supplied with the instrument states that about 95% of its energy is emitted at λ 2537 Å. During radiation, the eggs were kept in a small quartz Petri-dish, 2.5 cm from the source of energy, and with just enough solution to cover them. Thermometers placed in the solution near the eggs showed no appreciable rise in temperature.

Late blastulæ and early and middle gastrulæ were irradiated. The blastulæ were first stained on one side of the presumptive epidermal region with Nile blue sulfate and then the opposite (unstained) side was irradiated. Gastrulae were treated on the right or left side without use of vital stain. Exposure times, in minutes, were: $\frac{1}{4}$, $\frac{1}{2}$, 1, $1\frac{1}{4}$, $1\frac{1}{2}$, $1\frac{3}{4}$, 2, $2\frac{1}{4}$, $2\frac{1}{2}$, $2\frac{3}{4}$, 3, 4, 5, 7, and 10. After treatment the eggs were removed from the quartz chamber to stender dishes containing 0.30% Ringer solution and observed at various intervals during a period of 8-10 days. As criteria for stimulation or inhibition the following aspects of development were compared on the radiated and non-radiated sides of the embryo: (1) The progress of gastrulation, (2) formation and closure of the neural plate, (3) development of the gill filaments and adhesive glands, (4) curvature of the body, (5) length of body and tail.

Results. No effects on development could be observed in eggs exposed for less than 2 minutes. This is doubtless to be attributed to absorption of the ultraviolet by the thin layer of egg-jelly and by the chorionic membrane. On the exposed side of the egg the jelly is dissolved away completely and the chorion loses its toughness and elasticity. Near the end of gastrulation the embryo must be removed from the membrane in order to permit the elongation of the embryo, which becomes pronounced in the tail-bud stage.

Embryos which are allowed to remain within the irradiated membrane, curl upon themselves as a result of the diminished elasticity of the membrane, and thus the effects produced by irradiation may be complicated or obscured by secondary effects due to cramped quarters.

Eggs exposed for 2 minutes or longer showed a distinct difference between the radiated and non-radiated sides. In every instance the developmental processes were inhibited on the radiated side. The gastrulation movements were retarded so that a persistent yolk-plug was a common feature. The appearance of the neural fold was retarded and, in many cases, absent on the radiated side. The gill filaments and adhesive glands developed more slowly on the radiated side. The total body length was decreased but this was largely due to stunting of the tail rather than of the body proper. The unusual sensitivity of the tail to ultraviolet radiation is evident in many specimens and is of especial interest in view of the outstanding sensitivity of the tail to centrifugal force.⁸

In every case examined, in a total of 119 early gastrulæ which showed a distinct curvature of the body, the concave side corresponded to the side previously radiated.

Use of ultraviolet monochromator.[†] In this apparatus a high pressure quartz mercury arc is the source of energy and monochromatic ultraviolet light is obtained by use of fused quartz prisms and lenses. The lines of light were isolated by means of a slit in a sheet of metal and were thrown upon the right or left side of the early gastrula, which was placed close to the wall of a quartz chamber. The following lines were used, in Angstrom units: 3660, 3130, 3020, 2804, 2654, 2537, 2350. Preliminary experiments showed that no discernible effect could be produced by exposures shorter than $2\frac{1}{2}$ minutes. The exposure time of $2\frac{1}{2}$ minutes is evidently very close to the

⁸ Schechtman, A. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 430.

[†] We are indebted to Dr. Phillip A. Blacet of the Department of Chemistry for the use of this apparatus. For description of the monochromator, see Blacet, P. A., and Blacet, F. E., *J. Am. Chem. Soc.*, 1932, **54**, 3165.

minimal effective exposure since about 27% of radiated eggs were unaffected. Eggs radiated for 3 or 4 minutes showed very clear inhibitory effects. Of the various wave lengths listed above, all except 3660 showed some inhibitory effect. Somewhat more pronounced abnormalities of development occurred in the group of eggs exposed to the line at 2537, but most of the eggs showed no greater degrees of inhibition than can be found in eggs exposed to the other effective wave lengths. There is so much variation in any one group that conclusions as to relative effectiveness of various wave lengths under the present experimental conditions seem impossible. Variations in the amount of jelly which remains attached to the chorion and, possibly, variations in the degree of pigmentation of various eggs may account for the differences observed.

In this group of eggs, as in those treated with the therapeutic lamp, we have consistently observed inhibitory effects only.

Explanted material. Two types of explants (presumptive ectoderm and dorsal plastoporal lip) were removed from early gastrulæ, irradiated on both sides, and then cultured in Holtfreter solution. Non-radiated presumptive ectodermal explants normally round up, form a closed vesicle, and move over the substrate by ciliary action. Non-radiated dorsal lip explants normally elongate, forming curved finger-like structures. After radiation the explants round up more slowly than controls, or they may not do so at all. They have a strong tendency to stick to the surface of the quartz chamber, and cast off disintegrated material after they have been returned to culture dishes. As a consequence, the radiated specimens are frequently smaller than the controls. The radiated ectodermal explants move about the culture dish more slowly than controls or may be altogether quiescent. Many of them form small compact balls with little or no central cavity.

The dorsal lip explants are especially worthy of notice since their degree of elongation

permits easy comparison between radiated and non-radiated specimens. After exposure for $\frac{1}{4}$ and $\frac{1}{2}$ minutes only a slight or doubtful shortening is observed in radiated specimens. Those treated for $\frac{3}{4}$, 1, $1\frac{1}{2}$, 2, and 3 minutes are consistently shorter than controls, while those treated 4, 6, and 10 minutes do not elongate at all, or form only a short nipple-like protuberance. Disintegrated fragments are commonly present in the culture dishes of irradiated specimens.

Effect of radiation upon inductive capacity. Four groups of dorsal lip explants (10 explants per group) were irradiated on both sides, their disintegrated fragments removed by passing through 3 dishes of Holtfreter solution, and then implanted into the blastocoeles of early gastrulæ. The 4 groups were exposed to the therapeutic lamp for 2, 4, 6, and 10 minutes respectively. (Total exposure was double the values given since the explants were treated on both sides). Arranging the specimens in the order of increasing exposure to radiation, neural plates were induced in 9 of 10, 8 of 10, 9 of 10, and 6 of 10 specimens in the 4 groups. In the last group (irradiated 10 minutes), 4 of the implants were expelled from the host embryo, so that actually there was 100% neural plate induction in the successful specimens. The neural plates induced by these irradiated explants were, taken as a whole, smaller than those induced by non-irradiated implants. This is especially clear in the case of explants radiated for 6 to 10 minutes. These results are contrary to the conclusions reached by Durken,⁹ but quite in line with the results obtained by Reith.¹⁰

Summary. Entire eggs and explants from the portions of the egg of a frog (*Hyla regilla*) were irradiated for various periods of time using a (1) therapeutic cold quartz lamp and (2) an ultraviolet monochromator. Only inhibitory effects were exerted upon the several morphogenetic processes under observation.

⁹ Durken, R., *Z. Wiss. Zool.*, 1936, **147**, 295.

¹⁰ Reith, F., *Z. Wiss. Zool.*, 1937, **150**, 179.