prolonged action of mannitol hexanitrate may depend on slowness of absorption. To test this point the following experiments were carried out with glyceryl trinitrate and mannitol hexanitrate: Young female rats were starved for 48 hours and then given from 15 to 20 mg of the drug in triethylene glycol solution by stomach tube. After varying intervals of time the rats were killed and the entire gut was carefully dissected out, following the Cori technic.[3] Extraction was then carried out with 100 cc ether, after which an aliquot was evaporated to dryness and hydrolyzed with warm 1N sodium hydroxide solution. The Stieglitz and Palmer method[4] was then used with slight modifications to analyze for nitrates.

Glyceryl trinitrate was completely removed from the gastrointestinal tract within 6 hours in the case of 6 rats out of 7. In one instance removal was 60% completed. Four rats showed from 40 to 90% absorption during a 3-hour period. In the case of mannitol hexanitrate, 3 rats showed 20-30% absorption at the end of 3 hours and 6 rats showed 0-20% absorption after 6 hours. Apparently glyceryl trinitrate is taken up much more rapidly than mannitol hexanitrate.

## 13225

## Influence on Development of Certain Dominant Lethals Induced by X-rays in Drosophila Germ Cells.

B. P. SONNENBLICK* AND P. S. HENSHAW.†

*From the Department of Biology, Queens College, Flushing, N. Y., and the National Cancer Institute, United States Public Health Service, Bethesda, Md.*

The action of radiant energy in inducing gene mutations and various types of chromosomal alterations has been known for several years.[1, 2] The alterations produced have involved changes in parts of chromosomes, whole chromosomes and apparently in the composition of individual genes. In Muller's classic report[1] on

3 Cori, C. F., *J. Biol. Chem.*, 1935, **66**, 691.

4 Stieglitz, E. J., and Palmer, A. E., *J. Pharm. and Exp. Therap.*, 1934, **51**, 398.

* Fellow, John Simon Guggenheim Memorial Foundation.

† Senior Radiobiologist, National Cancer Institute, Bethesda, Maryland.

1 Muller, H. J., *Science*, 1927, **66**, 84.

2 Duggar, B. M., Editor, *Biological Effects of Radiation*, Vol. 2, Chaps. 38 and 39, McGraw-Hill, New York, 1936.

the artificial production by X-rays of alterations in the hereditary material of *Drosophila melanogaster,* evidence was presented, through breeding experiments, of the occurrence of dominant lethals. These are lethals whose presence in single dose can cause the death at various stages of development of the zygotes which contain them and thus are never transmitted to succeeding generations. Such dominant lethal genetic changes may be induced by X-rays in germ cells and then contributed to the zygote by one of the gametic nuclei. As yet, however, little is known of how the inviable nuclear alterations act to modify development. It is the purpose of this report to deal with certain aspects of this question.

The random effects of the radiations on the chromosomes of Drosophila have been reported elsewhere.[3] It was observed that the meiotic and mitotic divisions can become drastically disordered following treatment of the germ cells with X-rays. The achromatic figures as well as the chromosomes proper are frequently aberrant. In the zygotic mitoses chromosome fragmentation and elimination, asymmetrical divisions, multipolar divisions of the tripolar and quadripolar types and pycnotic, clumped chromosomal complexes with occasional chromatic bridges were noted and described. These induced abnormalities were considered to be directly responsible for the greatly increased percentage of mortality among the offspring, since the frequency of organisms showing such mitotic abnormalities was found to be about equal to the frequency of zygotic lethals.

For the present work, groups of wild type adult *Drosophila melanogaster* of both sexes, in separate gelatin capsules, were given from 2340 to 4680 r of hard (200 K.V.) X-rays. Subsequently, the treated flies were mated with untreated ones and eggs were collected and fixed at timed periods of development. Thus, each organism commenced development with one haploid chromosomal complement contributed by an irradiated parent and the other by a non-irradiated mate. All cells, germinal and somatic, are descended from the original fusion nucleus containing both the treated and untreated sets of chromosomes. Figs. 1-3 represent sections of 6 organisms which were of comparable age, all having incubated from 12 to 16 hours. Sections of 2 normal embryos are shown in Fig. 1, of 2 embryos whose mothers had been treated in Fig. 2, and of 2 embryos whose fathers had been treated in Fig. 3.

In the non-irradiated controls of our experiment (Fig. 1), the alimentary tract is differentiated into mouth, pharynx, esophagus,

[3] Sonnenblick, B. P., *Proc. Nat. Acad. Sci.,* 1940, **26**, 373.

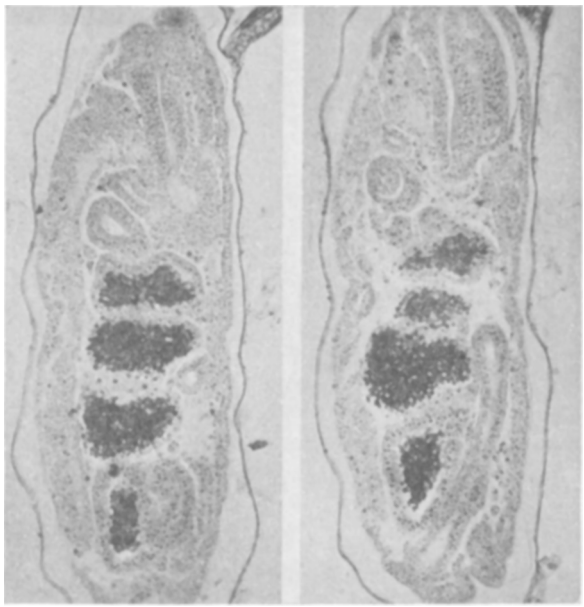

FIG. 1.
Sections of 2 normal embryos. Differentiation far advanced; hypodermis present, nervous tissue becoming concentrated, fore-gut and hind-gut wall well developed, deeply staining yolk spheres enclosed in loops of mid-gut.

proventriculus and gut, loops of which are shown in the sections to enclose the deeply staining yolk spheres. Nervous tissue is becoming more concentrated and nerve fibers are present. The hypodermis is delimited and the frontal sacs from which the optic discs are to be derived have made their appearance. These relatively complex normal organisms differ strikingly from those which contain the treated chromosomes (Figs. 2, 3). In the latter there is no gut and the yolk is free, concentrated in one or another part of the embryo and unincorporated in the mid-gut. Part of the cytoplasm may remain non-cellular, taking a deep stain (Fig. 2). Cell formation, which occurs normally over the entire surface of the early egg, has obviously been interfered with. In none of these organisms were germ cells, the first differentiations evident in the developing egg, to be noted. Masses of small cells, with occasional large spherical cells interspersed among them, make up the remainder of the embryos. Inasmuch as no differentiation or structural organization is to be observed, one may safely conclude that such animals will not hatch from the embryonic envelopes as active larvae. It is an extraordinary fact that the powers of differentiation can be so completely destroyed without at the same time destroying the powers

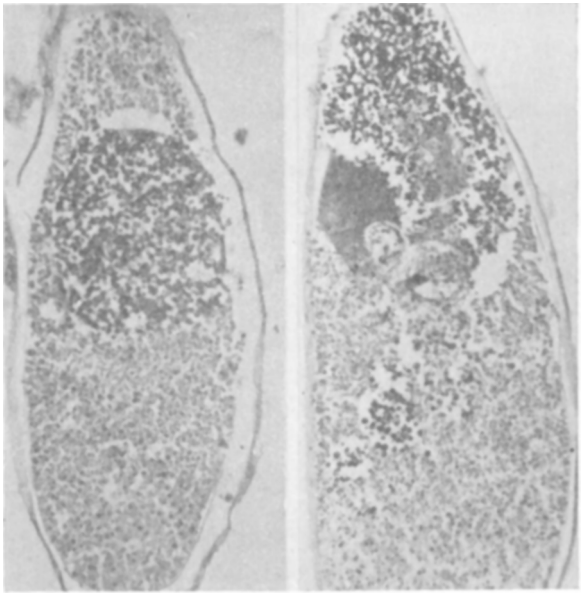

FIG. 2.
Sections of embryos derived from treated mothers and untreated fathers. No structural organization present although of same age as embryos shown in first figure. Except for the unenclosed yolk masses, the organism is virtually a sac of cells. A portion of the cytoplasm may remain non-cellular.

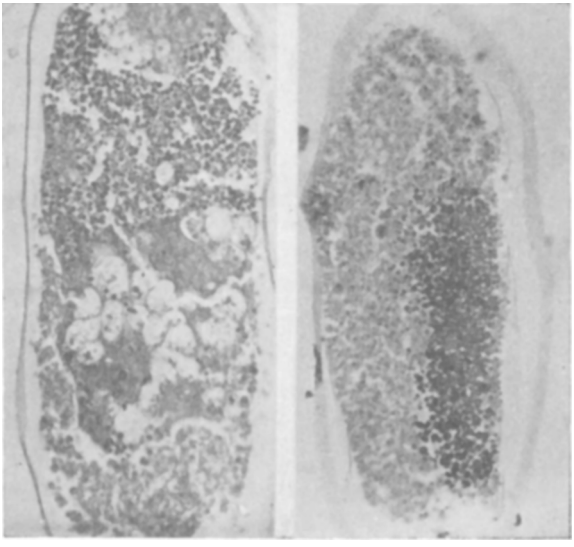

FIG. 3.
Sections of embryos derived from treated fathers and untreated mothers. No tissues or organs are evident. Unenclosed yolk inclusions, regions of non-cellular cytoplasm and huge vacuoles are present.

of cellular proliferation. It should be stated, however, that while the illustrated instances of unregulated proliferation are extreme cases, such are by no means rare. In 182 embryos which were sectioned and studied, we observed this effect 6 times, indicating that 1 out of approximately 30 treated germ cells had been so altered by the X-rays that subsequent differentiation went completely awry while the cells derived from the treated gamete could still multiply for many cell-generations. A somewhat similar result has been obtained by irradiating diploid cleavage nuclei.[4] However, in that instance a variable number of nuclei were exposed and the ensuing events could not be analyzed in terms of the effect on a single nucleus as in the observations reported here.

Technics exist whereby the Drosophila geneticist can breed strains in which identified chromosomes or sections of chromosomes are present as duplications, or are translocated, or are even absent entirely. Effects on development of certain genes or blocks of genes may thus be determined. Poulson[5] has recently studied the development of Drosophila eggs which lack the entire X-chromosome. There is an irregular disposition of the syncytial cleavage nuclei and no blastoderm is formed. Cell multiplication proceeds, however, and in sections of eggs which have incubated for more than 10 hours, a stratification of materials is observed with cellular aggregates, non-cellular cytoplasm and unincorporated yolk in the different regions. The absence of a known chromosome, or, to put it another way, the lack of the gene complex of that chromosome has resulted in an extensive but unregulated cellular proliferation and eventual death of the zygote. With this study of Poulson's in mind and recalling the effects of X-rays on Drosophila chromosomes mentioned above, it would appear that the loss of a relatively large block of genes through chromosome breakage and elimination, or through the abnormal distribution of chromosomes and chromosome fragments, has been a primary factor in the initiation of the processes leading to uncontrolled cellular divisions and failure to differentiate.

The problem is hardly confined to Drosophila for the induction with X-rays of dominant lethal nuclear alterations has been reported in other animals and in plants. Conclusive evidence for their production in mammals was obtained by Snell.[6, 7] The reduction in

[4] Henshaw, P. S., *Radiology*, 1935, **24**, 438.

[5] Poulson, D. F., *J. Exp. Zool.*, 1940, **83**, 271.

[6] Snell, G. D., *Proc. 6th Int. Congress of Genetics*, 1932, **2**, 188.

[7] Snell, G. D., *Am. Nat.*, 1933, **67**, 81.

productivity in litters sired by irradiated male mice he attributed to lethal genetic alterations in the exposed spermatozoa. Subsequent analysis by Snell of several generations of offspring indicated a high incidence in the sperm of chromosomal aberrations of the type known as translocations. Dominant lethal alterations resulting in abortion of the embryos have also been induced in maize pollen by X-rays and ultraviolet radiation.[8] No cytologic or embryologic examination of such abortive embryos has as yet been reported.

In conclusion, therefore, it should be emphasized that it is possible to demonstrate one type of dominant lethal effect whereby cells are not impaired in their ability to proliferate while their ability to differentiate is at the same time completely destroyed. The lethal effect, it would appear, is probably due to the absence or deficiency of parts of chromosomes, and therefore of gene loci, necessary for the development of the organism. Important implications arise from these considerations since somatic as well as germ cells may be affected by radiation in a manner similar to that discussed in this paper with resulting proliferative growth unaccompanied by any differentiation.

13226

## Specificity of Fowl and Mammalian Antigonadotropic Sera.*

HERBERT S. KUPPERMAN, C. H. MELLISH† AND W. H. MCSHAN.
(Introduced by R. K. Meyer.)
*From the Zoological Laboratory, The University of Wisconsin, Madison, Wis.*

Numerous investigators have found that the injections of gonadotropic extracts over long periods of time result in the production of a substance in the sera of the treated animals that neutralizes the gonad-stimulating action of the injected extract. The antisera obtained from animals repeatedly injected with gonadotropic preparations from either beef or sheep pituitary glands inhibit not only the extract used in the production of the antisera but also various other gonadotropic extracts, such as human, rat and horse pituitary;

[8] Stadler, L. J., *Proc. 7th Int. Congress of Genetics*, 1939, 269.

* Supported in part by a grant-in-aid from the Wisconsin Alumni Research Foundation administered by Roland K. Meyer. Assistance was also furnished by the personnel of the W.P.A. Official Project No. 65-1-53-2349.

† Present address: Lakeside Laboratories, Milwaukee, Wisconsin.