Laser irradiation of the chick embryo germinal crescent

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SUMMARY
The germinal crescent of head-fold and one-somite-stage chicken embryos was irradiated with multiple pulses of a microbeam ruby laser. Primordial germ cells were not detected in the gonad primordium of six laser-irradiated 5-day embryos; ten laser-irradiated embryos had varying numbers of primordial germ cells. Ten control embryos had gonad primordia populated with many primordial germ cells.

INTRODUCTION
Primordial germ cells have an extra-gonadic origin in the chick embryo. They may be found in the primitive streak or head-process stage, in an area known as the germinal crescent, located in the endoderm anterior and antero-lateral to the primitive streak at the junction of the area opaca and area pellucida (Swift, 1914; Willier, 1937; Reynaud, 1967; Clawson & Domm, 1969). Primordial germ cells are distinctively large and thus are easy to identify (Goldsmith, 1928). Primordial germ cells are migratory. They begin to separate from the endoderm at the definitive primitive streak stage (Clawson & Domm, 1967), are soon found in the lumina of blood vessels (Abdel-Malek, 1950), and are in the tissues of the embryo proper by the cranial flexure stage (Meyer, 1961, 1964). Most primordial germ cells become situated in the gonad primordium, some appear to degenerate, and others are thought to form extra-gonadic islands of germ tissue (Clawson & Domm, 1968).

The fate of these migratory giant cells has been in dispute because extirpation of the germinal crescent has generally resulted in the death of the host embryo. The fate of primordial germ cells would be revealed if they could be selectively removed with negligible or no damage to embryonic tissues. The purpose of the present study is to report preliminary results that suggest laser irradiation of the

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germinal crescent may in some instances be an effective method of preventing the appearance of primordial germ cells in the gonad primordium without causing the death of the host embryo.

**MATERIALS AND METHODS**

*The laser*

A Hadron/TRG Model 513 Biolaser System mounted on a Leitz Ortholux microscope was the source of laser radiation. The instrument produces a high-intensity monochromatic source of energy that is focused through the microscope to the target area. The site to be irradiated is aligned with cross-hairs in the optical system of the microscope. The laser has a pulse length of 150 μsec with a wavelength of 6943 Å. The diameter of irradiation used in the present study was approximately 100–150 μm, utilizing 120–180 J energy input with the ×3.5 scanning lens of the microscope.

*Chicken embryos*

Head-fold and one-somite embryos (stages 6 and 7, Hamburger & Hamilton, 1951) were obtained by incubating fertile chicken eggs for 24 h at 37 °C. Windows were cut above the embryos in the shell with a rotating carborundum disk (Drexel moto-tool). The shells of control embryos were opened in the same manner. The windows were closed in experimental and control embryos with two layers of cellophane tape. Experimental embryos were supported on a small platform on the substage condenser during laser irradiation. The germinal crescent area was irradiated with an average of 18 laser pulses to adjacent areas. The number of laser pulses depended upon the size and clarity of the crescent and varied from 8 to 28. Pilot experiments indicated that energies higher than 120–180 J resulted in a high mortality of the experimental embryos. The experimental embryos had a mortality of 55% after 5 days, which was about the same mortality (53%) as that of the control embryos. Mortality in experimental and control embryos was high probably because the experiments were conducted during the hot New Orleans summer. Mortality in chicken embryos is highest during warm weather. Control embryos were maintained at room temperature for the same time as the laser-irradiated embryos and both were returned to the incubator at the same time.

*Histology*

Live 5-day-old embryos were fixed whole in Bouin's solution and were sectioned serially. The sections were stained with Harris hematoxylin and eosin. Slides were labelled and arranged so that the experimenter was unaware whether or not the embryo had been laser-irradiated. The sections were examined for the presence of primordial germ cells in the gonad primordium.
RESULTS
Serial microscopic sections of ten unirradiated control embryos were examined. The gonad primordia of the control embryos appeared normal. The germinal epithelium was populated by many primordial germ cells that were recognized by their large size, round shape, and hyaline cytoplasm (Fig. 1).

Sixteen laser-irradiated experimental embryos were studied histologically. We were unable to find primordial germ cells in the germinal epithelium in 6 of the 16 experimentally irradiated embryos (Fig. 2). Primordial germ cells were found in varying numbers in the other irradiated embryos. Although some of the irradiated embryos appeared to contain the normal number of primordial germ cells, at least four of them had primordial germ cells in substantially reduced numbers. Presence or absence of primordial germ cells did not appear to be related to the laser energy used within the range of 120–180 J. Laser-irradiated embryos that contained no, or few, primordial germ cells were histologically normal except for a reduction in the size of the germinal ridges.

Fig. 1. Germinal epithelium of a control (unirradiated) 5-day chick embryo. Primordial germ cells are large, round and have hyaline cytoplasm.

Fig. 2. Germinal epithelium of a 5-day chick embryo after microbeam laser irradiation to its germinal crescent 4 days earlier.
DISCUSSION

This study shows that microbeam laser-irradiation to the germinal crescent can be an effective means of preventing the appearance of primordial germ cells in the gonad primordium of 5-day chicken embryos. The efficacy of the procedure is witnessed by the six laser-irradiated embryos whose germinal epithelium appeared to be devoid of primordial germ cells. However, the procedure as developed thus far is imperfect because ten of the treated embryos contained from a few to many primordial germ cells in the germinal epithelium.

The presence of primordial germ cells in some of the laser-irradiated embryos may be attributed to one or more of the following possibilities. (1) Primordial germ cells sometimes appear to originate in parts of the blastoderm other than the germinal crescent (Rawles, 1936; Romanoff, 1960; Fargeix, 1969). (2) Primordial germ cells may begin their migration as early as the definitive streak stage (stage 4, Hamburger & Hamilton, 1951). We irradiated first somite (stages 6-7, Hamburger & Hamilton, 1951) embryos; accordingly, some primordial germ cells may have already begun migration. (3) The area of irradiation, in some instances, may have been insufficient to affect all of the primordial germ cells in the crescent. Despite the fact that some irradiated embryos contained germ cells, it is significant that six laser-irradiated embryos were devoid of detectable primordial germ cells in contrast to their consistent presence in control embryos.

Our data are in harmony with many experimental studies of the germinal crescent in the chicken embryo. Excision (Reagan, 1916; Goldsmith, 1935; Simon, 1957), cauterization (Dantchakoff, 1932), and ultraviolet irradiation (Benoit, 1930; Reynaud, 1969) of the germinal crescent result in gonad primordia that are sterile. Unfortunately, these procedures result in trauma to the embryo sufficient to cause the death of most experimental animals.

Methods of reduction of primordial germ cells in other vertebrates include treatment with drugs, irradiation other than laser, and loss of specific cytoplasm. Treatment of frog eggs with actinomycin, an inhibitor of RNA transcription, is reported to result in larvae with reduced numbers of primordial germ cells (Wolsky & L'Estrange, 1969). Ultraviolet irradiation of vegetal hemisphere cytoplasm (Smith, 1966) and pricking with loss of germinal cytoplasm (Buehr & Blackler, 1970) are procedures that result in sterile anurans.

Abnormally small germinal ridges were observed in laser-irradiated embryos that contained no, or few, primordial germ cells. The reduced size of these germinal ridges seems to suggest an interaction between primordial germ cells and the normal development of the germinal ridge. Further studies are suggested because this result in our, admittedly preliminary, observations conflicts with the study of Reynaud (1969).

Our results do not rule out the germinal epithelium as a source of primordial germ cells in embryos older than 5 days (Firket, 1920; Asayama, 1967). Our
results are consistent with the theory that primordial germ cells originate in the germinal crescent, migrate to the gonad primordia, and persist there for at least 5 days. The site of the origin of the definitive sex cells in the chicken is not yet definitely known (Hamilton, 1952; Nelsen, 1953; Asayama, 1967). It is possible that laser-irradiated embryos that are sterile may be reared to maturity in future experiments. Sterile chickens that were laser-irradiated would comment cogently on the developmental significance of primordial germ cells.

Microbeam laser irradiation is emerging as a useful tool in experimental embryology. Parts of cells such as chromosomes and nucleoli can be selectively irradiated with significant biological effect (Moreno, Lutz & Bessis, 1969; Berns, Olson & Rounds, 1969; McKinnell, Mims & Reed, 1969; Berns & Rounds, 1970). The present study is probably the first study of laser-irradiated chick embryos in vivo although chick pigmented retinal cells have been irradiated in vitro (King & Geeraets, 1968).

No vital dye was used on the germinal crescent to facilitate absorption of laser irradiation. We sought to avoid the use of any agent that might decrease the vitality of the embryo. Evidently primordial germ cells or other adjacent structures absorb sufficient energy to result in death or altered migration of many primordial germ cells such that sterile germinal ridges ensue.

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