Table II. Data from rabbits killed on gestation day 6

| Group | No. of animals | No. of corpora lutea | No. of blastocysts | Chromosome abnormalities | | Chromosome abnormality | Sex chromosome complement | |
|---|---|---|---|---|---|---|---|---|
| | | | | No. | % | | *XY* | *XX* |
| Control | 9 | 90 | 81 | 1/74 | 1.4 | (44/45) | 41 | 32 |
| Nembutal ($^1/_4$ h pc) | 5 | 51 | 49 | 3/42 | 7.1 | 40/43/44; *n*/2*n*; 45 | 20 | 19 |
| Nembutal (6 h pc) | 5 | 66 | 59 | 7/55 | 12.6 | 3 (2*n*/4*n*); 2 (43/44); (44/45); (45) | 25 | 23 |
| Total | 19 | 207 | 189 | | | | 86 | 74 |

did not differ significantly amongst the 3 groups. The number of chromosomally abnormal blastocysts, however, was greater in animals injected with Nembutal. Only $^1/_{74}$ blastocysts from rabbits in the control group was chromosomally abnormal to give a frequency of 1.4%. This compared favourably with the 1 to 2% chromosomally anomalous blastocysts that have been found in control series from other experiments in our laboratory. Animals that had been injected with Nembutal $^1/_4$ h after mating provided 42 blastocysts that could be analyzed chromosomally. 3 or 7.1% of these were chromosomally abnormal. This was not significantly different from the control group. Blastocysts from rabbits injected 6 h pc had the greatest number of chromosome abnormalities $^7/_{55}$ of 12.7%. This was significantly different from the control group ($p < 0.01$).

The types of chromosome abnormalities are also listed in Table II. The diploid complement of the rabbit is 44. The chromosome abnormality found amongst blastocysts from the control group was a mosaic with 2 cell lines, one having 44, or the normal number of chromosomes, and the other line with an extra chromosome present. The 3 chromosome abnormalities found among blastocysts from rabbits injected with Nembutal $^1/_4$ h pc were varied in nature. There was 1 mosaic blastocyst with 3 cell lines, 1 mixoploid with haploid and diploid lines and 1 trisomy in which all cells had 45 chromosomes. Mixoploidy was the most numerous anomaly found amongst blastocysts recovered from rabbits injected 6 h pc. 3 of the abnormalities were mixoploid with diploid and tetraploid lines present. There were also 2 chromosomally mosaic blastocysts with $^{43}/_{44}$ cell lines and one $^{44}/_{45}$ mosaic. Again a trisomic blastocyst was found.

Pentobarbital sodium administered at $^1/_4$ h or 6 h post coitum did not totally inhibit oocyte maturation in the rabbit. Although a delay in maturation was apparent in 2 of the 5 animals injected at $^1/_4$ h pc, 3 other rabbits had ovulated by 17 h pc and by 24 h pc zygote development was comparable to that found in control animals.

A significantly greater number of chromosomally abnormal blastocysts was recoverd from rabbits treated with pentobarbital 6 h postcoitum than in untreated animals. Errors that could be attributed to the first cleavage division, such as mosaics and mixoploidy, predominated. Trisomy may have arisen by non-disjunction or anaphase lagging during meiosis II. It may be that pentobarbital had a direct effect on meiosis and/or mitosis.

*Summary.* Female rabbits were injected with pentobarbital sodium at $^1/_4$ h or 6 h post coitum. A slight delay in oocyte maturation was evident in animals killed at 17 h pc, however, zygote development appeared normal by 24 h pc. At 6 days pc, a greater frequency of chromosomally abnormal blastocysts was found in animals injected with pentobarbital than in control rabbits.

EVELYN L. SHAVER

*Department of Anatomy, Health Sciences Centre, University of Western Ontario, London (Ontario, Canada), 18 June 1975.*

## Effects of $\gamma$-Rays on the Developing Embryos of *Calotes versicolor*

The developing embryos of animals after their exposure to ionizing radiations show numerous types of anomalies, for example, increased embryonic mortality, decrease in weights of internal organs, exencephaly, haemorrhage in heart and other body organs, and limb defects[1-6]. The different types of anomalies obtained are among other factors dependent on the dose given, mode of exposure, the stage of development and the animal species being exposed. The present communication reports the effects of $\gamma$-rays on the developing embryos of the garden lizard, *Calotes versicolor* and describes some unusual effects on the eyes.

*Materials and methods.* Eggs of *Calotes versicolor* were obtained from the uteri of gravid females by laparotomy. The embryos were staged according to the descriptions by MUTHUKKARUPPAN et al.[7]. Since all the embryos in a cluth are always at the same stage of development the stage of the development of the embryos was observed at

[1] L. B. RUSSELL and W. L. RUSSELL, J. cell. comp. Physiol., Suppl. 1, *43*, 103 (1954).
[2] L. B. RUSSELL and C. S. MONTEGOMERY, Int. J. Radiat. Biol. *10*, 151 (1966).
[3] C. PIEAU and J. VASSE, C. r. hebd. Séanc. Acad. Sci., Paris *271*, 2023 (1970).
[4] A. K. GAJEWSKI, M. G. SLOWIKOWSKA and W. L. GRZYMALA, Roczn. państ. Zakl. Hig. *22*, 221 (1971).
[5] M. T. CASE and J. SIMON, Am. J. vet. Sci. *33*, 1223 (1971).
[6] J. VASSE, Annls Embryol. Morph. *7*, 283 (1974).
[7] V. MUTHUKKARUPPAN, P. KANAKAMBIKA, V. MANICKAVEL and K. VEERARAGHAVAN, J. Morph. *130*, 479 (1970).

Effects of $\gamma$-ray irradiation on the embryos of *Calotes versicolor*

| Experiment serial | No. of irradiated embryos | No. of surviving embryos [a] | Relative weight of the surviving embryos [b] | Anomalies among survivors | | |
|---|---|---|---|---|---|---|
| | | | | Haemorrhage | Axis defects | Microphthalmia |
| A | 8 | 7 | 0.69 | 3 | 1 | 2 |
| B | 13 | 9 | 0.67 | 6 | 2 | – |
| C | 9 | 6 | 0.54 | 6 | 5 | 2 |

[a] The control embryos (4 in each experiment) were always alive, except one in the experiment C, and had no malformations. [b] Relative weight of the surviving embryos = Average weight of the irradiated embryo / Average weight of the control embryo.

the begining of experiments by sacrificing 2 eggs. The irradiation doses were measured using Fricke ferrous sulphate dosimeter solution[8]. A pilot experiment was done wherein 11 eggs were exposed to 20,500 rads and observed after a further 7 days of development. The mortality rate was high, up to 82%, and all the surviving embryos showed haemorrhages. In the following experiments, therefore, different doses combined with shorter post-irradiation periods of development were used. In experiment A, 10,250 rads were delivered in a single exposure at the embryonic stage 30, and the embryos were observed 1 day after irradiation. In experiment B, 20,500 rads were delivered in a single exposure at the stage 29, and the embryos were observed 2 days after irradiation. In experiment C, 20,500 rads were delivered in 2 doses of 10,250 rads each, at an interval of 2 days; the first irradiation was done at the stage 30 and the embryos were observed 4 days after the first dose. The control embryos were handled like the experimental embryos but for the lack of exposure to the $\gamma$-rays. All the embryos were fixed in buffered glutaraldehyde solution at 4 °C, embedded in paraffin wax, sectioned at 8 µm and stained with Delafield's haematoxylin and eosin.

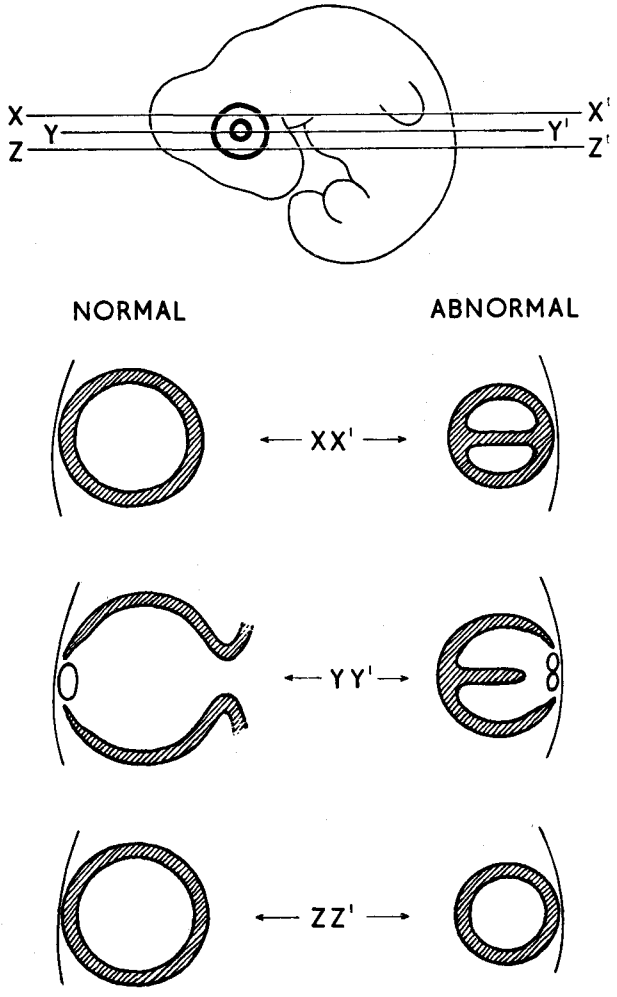


Fig. 1. Schematic representation of the sections through normal and abnormal eyes to show the pattern of the division of the optic cup and the lens.

*Results.* The experimental embryos had a moderately high survival rate, 67% to 88% (see Table), and were invariably less developed than the control embryos by up to one stage of development. Furthermore, the growth of the experimental embryos was considerably retarded as evidenced by the difference in their average weight compared with that of the control embryos (see Table).

The most common lesion produced was haemorrhage in different parts of the body, such as heart, head, tail, limbs and body wall. The heart showed varying degrees of haemorrhages, apparently increasing from slight to moderate to heavy. Some dead embryos, not yet disintegrating, also showed heavy haemorrhage in the heart. A smaller and collapsed heart tube, with or without an enlarged pericardium, but lacking haemorrhages, was obtained only in the experiment B, perhaps due to the fact that the embryos in this experiment were younger than those in experiments A and C at the time of exposure to $\gamma$-rays. The axial defects included the shortening or elongation of the axis accompanied by microcephaly and a kinky or stumpy tail.

Of particular interest were the abnormalities observed in the eye. The eye lesions were 'unilateral', on the left side, and included microphthalmia and/or unpigmented retina. Histological examination of the embryos showing microphthalmia revealed a partial division of the left optic cup and a 'double lens', except in one case. A comparison of cross sections through the normal and abnormal eyes at different levels showed the pattern of this division (Figures 1 and 2). Sections in the plane *xx'* showed a complete division of the optic cup in the left eye. Sections in the plane *yy'* through the abnormal eye showed the lens pinched into 2 closely apposed parts and a partition running in from the mesial wall of the cup incompletely divided the latter into 2 compartments.

[8] J. W. T. SPINKS and R. J. WOODS, *An Introduction to Radiation Chemistry* (John Wiley, New York 1964).

Sections in the plane *zz'* for the normal and abnormal eyes were comparable except for the smaller size of the abnormal optic cup. The total volume of the 2 lenses put together and the 2 optic cups put together was less than that of the single normal lens and single normal cup, respectively. However, the maximum circumference of the normal cup and that of the abnormally divided cup, as seen in serial cross sections, was comparable. A layer of dead cells lined the divided optic cup on its inner face while the cells on the outer side appeared healthy (Figure 2 C). Only dead and degenerating cells were seen in the wall of the abnormally divided optic cup in experiment C (Figure 2 D).

*Discussion.* Haemorrhage in the heart as observed in *Calotes* has been one of the major lesions produced in X-ray irradiation experiments with new-born pig[5]. Our observations further indicate that haemorrhage is perhaps the major but not the only factor leading to death, since we also observed dead embryos without heart haemorrhage. Death characterized by haemorrhage and general circulatory system breakdown has also been reported in chick embryos during the first day after irradiation[9].

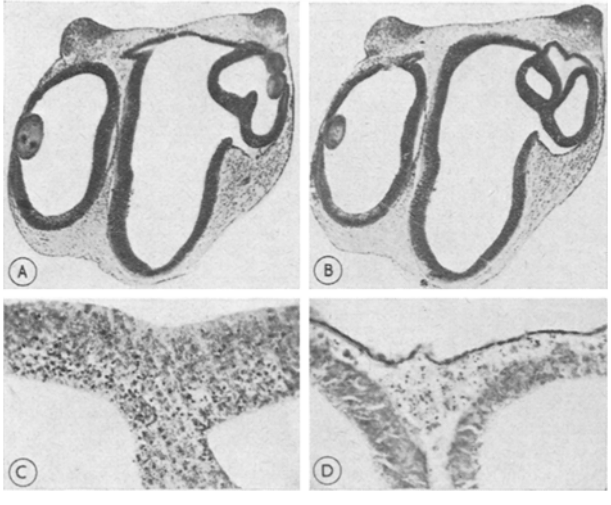


Fig. 2. Sections through experimental embryos showing abnormal optic cup and the lens. A) Partially divided optic cup and lens of the left side (experiment A, ×90). B) Complete division of the optic cup of the embryo in Figure A (×90). C) Magnified view of the mesial region of the divided optic cup of experimental embryo in Figure A showing the necrotic cells on the inner surface of the cup (×500). D) Magnified view of the mesial region of the divided optic cup of experimental embryo (experiment C) showing optic wall consisting of only necrotic cells (×500).

The unilateral nature of the defects is by now a widely reported observation[3,10–12]. PIEAU and VASSE[3] attribute unilaterality of defects in their experiments with *Lacerta viridis* to the position of the embryo in the egg. However, it appears that the position of the embryo in the egg may not have a direct bearing on the unilaterality of the defects: in both *Lacerta* and *Calotes* the embryos are recumbent on the left side but in the former the defects are more pronounced on the right side[3] whereas in the latter these are more so on the left side[11].

The manner of genesis of a 'double optic cup' and a 'double lens' remains uncertain but it seems probable that the alteration in the shape and division of the optic cup results from an inwardly directed collapse of the mesial wall of the cup; the retinal layer in the eye of the right side is also detached from the outer border at about the point of collapse on the left side. This event was followed by cell death beginning along the inner face of the optic cup as noticed in experiment A, and finally resulting in experiment C in the optic cup formed of only dead and degenerating cells. The absence of the eye defect in the experiment B may possibly be due to the embryos, at the time of exposure to $\gamma$-rays, being younger as compared with those in the experiments A and C.

*Summary.* The $\gamma$-ray irradiation causes mortality, retardation in development and growth rate, haemorrhage, axial defects and unilateral microphthalmia. Histological examination of microphthalmic embryos revealed a partial division of the left optic cup and a 'double lens'.

J. M. CHIPLONKAR[13] and S. C. GOEL[14]

*Department of Zoology, University of Poona, Poona 411 007 (India), 20 May 1975.*

[9] A. P. CASARETT, *Radiation Biology* (Prentice-Hall, New Jersey 1968).
[10] M. H. FOX, Teratology *6*, 339 (1972).
[11] S. C. GOEL and J. K. MATHUR, Genetics *74*, s96 (1973).
[12] D. J. PRITCHARD, R. M. CLAYTON and W. L. CUNNINGHAM, Expl. Eye Res. *19*, 335 (1974).
[13] JMC was a Junior Research Fellow of the CSIR India during this work. Present address: Department of Zoology, Fergusson College, Poona 411004.
[14] The authors wish to thank Professors L. MULHERKAR and H. J. ARNIKAR for the laboratory facilities and to Mr. E. D. ROBERTS for drawing the Figure 1.

## Establishment of Normal Diploid and Malignant Heteroploid Cell Lines from Non-Treated and Benzo(a)pyrene Treated Hamster Embryo Cell Cultures

Various investigators have reported a limited life-span for normal cells and an indefinite growth period for transformed cells[1–6] in culture. Despite the wealth of information on the in vitro growth of hamster cells, stable diploid cell lines grown for extended periods have not been reported. During the present study, we have been able to establish two stable diploid cell lines from non-treated and a heteroploid malignant cell line from benzo(a)pyrene (a tobacco smoke component) treated hamster embryo cells.

Primary cultures were raised by growing cells from 12–14-days-old hamster embryos in Eagle's minimal essential medium supplemented with glucose, sodium bicarbonate, tryptose phosphate broth, non-essential amino acids, glutamine, Hepes and a 10% mixture of different sera[7]. Secondary mass cultures were prepared in plastic flasks, without feeder cells, and treated for 24 h with benzo(a)py-

[1] H. E. SWIM and R. F. PARKER, Am. J. Hyg. *66*, 235 (1957).
[2] L. HAYFLICK and P. S. MOORHEAD, Expl. Cell Res. *25*, 585 (1961).
[3] V. J. CRISTOFALO, Adv. geront. Res. *4*, 45 (1972).
[4] S. GELFANT and J. G. SMITH, Science *178*, 357 (1972).
[5] Y. BERWALD and L. SACHS, J. natn. Cancer Inst. *35*, 641 (1965).
[6] J. A. DIPAOLO and P. J. DONOVAN, Expl. Cell Res. *48*, 361 (1967).
[7] P. MARKOVITS, J. COPPEY, D. PAPADOPOULO, A. MAZABRAUD and M. HUBERT-HABART, Int. J. Cancer *14*, 215 (1974).