The ribonucleoprotein-containing granules of some nuclear bodies[8] and the unknown nature of the hepatitis A virus leave unanswered the question of the significance of the nuclear bodies in acute viral hepatitis. More conclusive ultrastructural cytochemical studies and further observations in other clinical stages of the disease are required for a better understanding of the nature and the functional role played by nuclear bodies in this situation.

[13] Acknowledgments. The authors are indebted to Dr. MOURA-NUNES for helpful advice and criticism and Miss T. VAREJÃO for technical assistance.

Résumé. La présence de corps nucléaires est observée dans environ 15% des hépatocytes dans 6 cas d'hépatite virale aigue. Leur présence fréquente et leurs formes parfois complexes peuvent être en rapport soit avec l'hyperactivité métabolique des hépatocytes au cours de la régénération hépatique, soit avec la synthèse des acides nucléiques et des protéines spécifiques du virus de l'hépatite humaine.

J. O. SOARES and M. C. DE MOURA[13]

Unidade de Microscopia Electrónica, Faculdade de Medicina de Lisboa, Lisboa (Portugal), 12 May 1975.

Pentobarbital Sodium and Chromosome Abnormalities in Rabbit Blastocysts[1]

In the rabbit coital stimulus via a neural pathway causes the release of luteinizing hormone (LH) from the pituitary. It has been postulated the release of LH must continue for 1 h in order that a sufficient amount will be present to cause ovulation 10 h after mating[2]. Pentobarbital reportedly prevents ovulation from occurring in estrogen-primed rabbits after electrical stimulation of the posterior hypothalamus[3]. Given shortly after mating, the drug reduces the capacitation of spermatozoa to the level found in non-mated does[4]. Also, the progestin level of peripheral blood fails to increase. In mice, mitotic aberrations and an increased number of degenerating nuclei were reported in the epidermis of animals treated with sodium pentobarbital[5].

The following study was done to determine whether the administration of sodium pentobarbital to rabbits would affect the time of ovulation, fertilization and/or the chromosome complement of maturing oocytes.

Female rabbits were injected i.v. with sodium pentobarbital (Nembutal, Abbott) at either 1/4 or 6 h after mating. Mated rabbits not injected with the drug served as controls. Some females were killed at 17 or 24 h post-coitum (pc), the oviducts flushed and the developmental stage of the zygotes recorded. Other females were killed on gestation day 6, the blastocysts recovered from the uterus and examined for chromosome abnormalities according to the method published by SHAVER and CARR[6].

The oocytes recovered at 17 h from 3 control rabbits showed that fertilization had occurred with 2 pronuclei and 2 polar bodies visible in all oocytes examined. This same stage was found among oocytes recovered from rabbits injected with Nembutal 6 h pc. Rabbits receiving Nembutal $^1/_4$ h after mating varied in response. 2 animals had not ovulated by 17 h pc and serial sections of the ovaries revealed follicular oocytes that had begun maturation with activation comparable to that usually found at 4 to 8 h after mating. In some oocytes the 1st polar body was evident, a stage of development that occurs approximately 8 h pc in the control animal. However, 3 rabbits receiving Nembutal at 17 h ovulated and oocytes with a few adhering cumulus cells were recovered from the oviducts.

At 24 h all animals in the control and experimental groups had ovulated and the majority of the zygotes were in the 2 cell stage. Table I shows the total number of rupture sites in the ovaries and the developmental stage of the zygotes recovered from the animals in each of the 3 groups. The number of rupture sites and the total number of zygotes recovered are almost identical when the groups are compared. The majority had reached the 2 cell stage with 1 zygote in each group in the 4 cell stage. Surprizingly, zygotes that had not undergone first cleavage and thus were seen as single cells were most numerous in the control group.

Data from rabbits killed on gestation day 6 are shown in Table II. The number of blastocysts recovered compared with the number of corpora lutea counted in the ovaries

[1] This work was supported by a grant from the Medical Research Council of Canada.

[2] A. R. FEE and A. S. PARKES, J. Physiol., Lond. *67*, 383 (1929).

[3] A. VAN TIENHOVEN, *Reproductive Physiology of Vertebrates* (W. B. Saunders Co., Philadelphia 1968), p. 251.

[4] E. D. PLOTKA and W. L. WILLIAMS, Proc. Soc. exp. Biol. Med. *136*, 937 (1971).

[5] K. SETÄLÄ, B. LINDROOS and A. HUJANEN, Naturwissenschaften *50*, 45 (1963).

[6] E. L. SHAVER and D. H. CARR, J. Reprod. Fert. *14*, 415 (1967).

Table I. Developmental stage of oocytes

	17 h pc		24 h pc			
	No. of rupture sites	No. fertilized ova	No. of rupture sites	1 cell	2 cells	4 cells
Control	32	28	32	8	20	1
Nembutal ($^1/_4$ h pc)	27	24	33	0	31	1
Nembutal (6 h pc)	36	36	31	3	26	1

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 γ-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 γ-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).