the mast cells may be of neural crest origin. The sites where the neural crest cells were present in the tissues and organs in the mice embryos and suckling mice[4,5], corresponded with the sites where the mast cells were present[6]. According to SELYE[6], the following is known: accumulation of melanine in the lesions of urticaria pigmentosa has long suggested some relationship between the mast cell and pigmentation, and an unusually large number of mast cells has also been noted in xeroderma pigmentosa. A certain parallelism between the local mastocytosis and hyperpigmentation also exists in the experimentally produced pigmented dermatoses, and all these findings suggest a relationship between mast cells and melanin production. On the other hand, it is thought that xeroderma pigmentosa may be related to the neural crest cells[7]. It was found in the authors' laboratory that the neural crest cells in mice showed a specific sensitivity to the alkylating agents, and the DNA and protein of the neural crest cells disappeared[7], and furthermore in the suckling mice injected with mitomycin C, excessive cell proliferation, hyperplasia or tumor occurred. It is thought that these phenomena may be brought about by the dysdifferentiation of the neural crest cells[1]. Furthermore in suckling mice injected with mitomycin C, hyperkeratization of the skin and heterotrophic melanin pigmentation were seen[8]. From the above, it is speculated that the mastocytoma or mastocytosis in suckling mice injected with mitomycin C may be brought about by the dysdifferentiation of the neural crest cells, and that the mast cells may be of the neural crest origin.

Summary. In suckling mice injected intraperitoneally with mitomycin C on the 1st to 5th day after birth and sacrificed in the course of 24 or 48 h after injection, mastocytosis occurred in the oral mucosa membrane, skin of the trunk or extremities and bone marrow of extremities.

T. NOZUE, M. SHIKIMORI and T. KAYANO

Department of Anatomy, Tokyo Medical and Dental University, 5–45, 1-Chome, Yushima, Bunkyo-ku, Tokyo (Japan), 20 May 1975.

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Nuclear Bodies in the Hepatic Parenchymal Cells in Acute Viral Hepatitis

Nuclear bodies are a morphological entity with whorl-like configuration, reported in a variety of pathological conditions and also in normal plant and animal tissues[1,2]. BOUTEILLE et al.[2] have proposed a classification of these structures based on their ultrastructural appearance. The nature and function of these nuclear inclusions remains obscure. Some evidence supports the possibility of their being normal cell organelles related with cellular hyperactivity, increasing in number and assuming different degrees of differentiation and structural arrangement. In the course of an ultrastructural study of liver tissue from patients with acute viral hepatitis, nuclear bodies of simple and complex types were frequently found. The purpose of this paper is to report the presence of nuclear bodies in the hepatocytes in acute viral hepatitis. Our observations suggest that these structures could express liver cell hyperactivity related to the cellular regeneration after hepatic necrosis, and also be a reflection of the underlying acute viral process.

Material and methods. Liver tissue was obtained by Menghini needle biopsy from 6 patients with viral hepatitis in the 4th week after the clinical onset of the disease. Hepatitis B surface antigen (HB_sAg) was found by counter electrophoresis in 3 patients. The liver-biopsy specimens were fixed in cacodylate-buffered 3% glutaraldehyde pH 7.4 and postfixed sequentially in veronal acetate-buffered 2% osmium tetroxide pH 7.4 and veronal acetate-buffered 0.5% uranyl acetate pH 5.8[3]. Following dehydratation in graded ethanol solutions, they were embedded in Epon 812[4]. Ultrathin sections were cut with a glass knife on a LKB Ultratome, stained with lead citrate and examined on a Phillips EM 300 electron microscope, operated at 80 kv.

Results. Nuclear bodies of different morphological types were present in about 15% of nucleus sections in hepatocytes of patients with acute viral hepatitis. 1 to 4 nuclear bodies were found per nucleus. There was a type 2 and 3 bodies predominance. They were usually spherical in shape and consisted of whorls of granular and fibrillar material, varying between 0.3 to 0.5 μm in diameter and occupying the interchromatinic spaces. Often vacuolar structures and electron dense granules were observed in the centre of the nuclear bodies, surrounded by concentrically arranged fibrils. Some of these granules seemed perichromatinic granules. Some highly differentiated forms, type 4, were also present. Frequently the fibrils composing type 1 and 2 bodies were loosely circulary arranged and penetrated into the halo of electron-lucent nucleoplasm which surrounded the nucelar bodies. No differences were found either in HB_sAg positive and HB_sAg negative cases in the frequence and morphology of nuclear bodies.

Discussion. The morphological nature and the functional significance of the nuclear bodies are poorly understood. It has been pointed out by several authors that they are probably discrete intranuclear inclusions related to cell hypertrophy or pathological conditions, i.e. viral infections[5]. Their number is remarkably increased in multiplying and growing cells, and they also present more differentiated arrangements[2]. To our knowledge, no references concerning the presence of these structures in the hepatic parenchymal cells in acute viral hepatitis have been previously reported.

Both rapid cell growth and virus infection are present in acute viral hepatitis, suggesting that these factors

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might be additive on producing nuclear bodies, as it occurs in human epidermal cells infected in vivo with vaccinia virus[6].

The simple nuclear bodies, types 1 and 2, might be constant nuclear organelles and striking increase of their number is probably related with hepatic protein synthesis. SIMAR[7] found a relationship between the number of the nuclear bodies and the activity of cellular protein synthesis. Moreover cytochemical studies from DUPUY-COIN et al.[8] have demonstrated the prevalent proteinaceous nature of these structures. Although normal nuclear organelles, their frequent occurrence in acute viral hepatitis may be the result of a hyperactive metabolic stage of the hepatocyte nuclei.

The complex nuclear bodies, types 3 and 4, have been observed mainly in pathological conditions and their presence in acute viral hepatitis may be related to the virus infection. It has been stressed that the occurrence of larger and more complex nuclear bodies seen frequently in viral conditions may involve some sort of relationship between nuclear bodies and virus disease. Recent data suggest that hepatitis B virus is a DNA virus. KAPLAN et al.[9,10] have postulated that the DNA polymerase activity associated with the Dane particles seemed to depend on a DNA template. PATRIZI et al.[6] have previously remarked that viruses associated with nuclear bodies, such as *Herpes zoster*, *Herpes simplex*, varicella, human cytomegalovirus, adenovirus type 12, polyoma, SV 40, Shope papilloma, are DNA viruses. LE GOASCOGNE et al.[11] have recently reviewed this subject and concluded that nuclear bodies may correspond to the morphological equivalent of one region of the DNA, based on the response of the nuclear bodies to actinomycin D and the apparent continuity of some fibrils of the periphery with the surrounding chromatinic fibrils[12]. The presence of nuclear bodies in hepatitis virus infection could express the DNA virus replication, involving the synthesis of new nucleic acid and associated specific proteins. The finding of nuclear bodies in hepatitis B as well as in hepatitis A suggest that their presence is probably more related to the viral agent as an 'inductor' factor rather than to the type of hepatitis virus.

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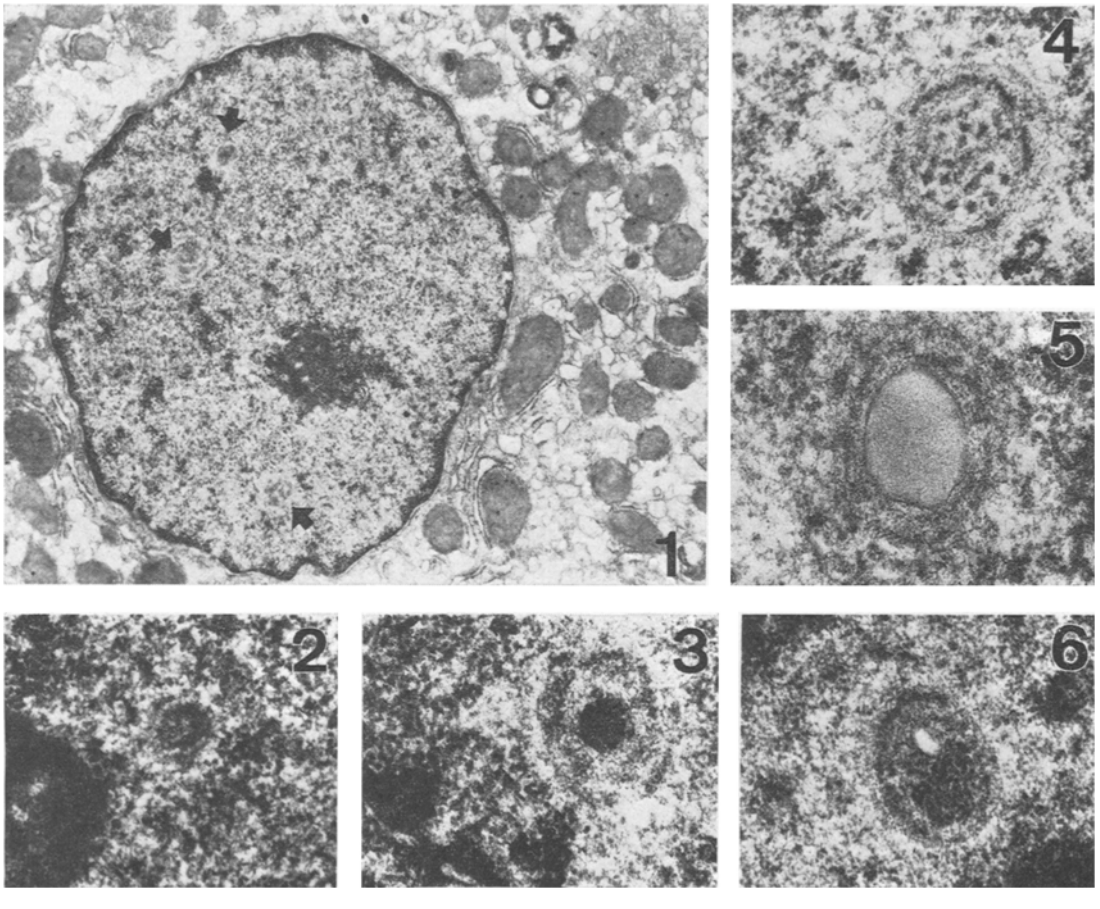

Fig. 1. Hepatocyte nucleus showing 3 simple nuclear bodies (arrows). × 8,800. Fig. 2. A nuclear body near the nucleolus. In the centre there are vacuolar filamentous structures and dense granules. × 20,700. Fig. 3. Clusters of closely packed granules in the centre of a nuclear body. × 47,500. Fig. 4. Portion of hepatocyte nucleus showing a nuclear body with granules and fibrillar material. × 32,600. Fig. 5. Fibrils concentrically arranged surrounding a lipid droplet. × 41,400. Fig. 6. A complex nuclear body which presents clusters of dense granules excentrically disposed. × 21,500.

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

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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