

ELECTRON MICROSCOPY OF INTRANUCLEAR INCLUSIONS  
FOUND IN HUMAN AND RAT LIVER  
PARENCHYMAL CELLS\*

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PLATE 145

Intranuclear inclusions generally concluded to represent a heterogeneous class of bodies are readily distinguishable from nucleoli, fat droplets, crystals, or melanin also found within nuclei. The intranuclear inclusions associated with virus infection have been broadly classified into two types by Cowdry, type A and type B (1). Both A and B inclusions exhibit morphological variations and the few histochemical studies indicate that not all A nor all B inclusions are chemically alike (1-3). The type B inclusion associated with poliomyelitis, Borna disease, Rift Valley fever (1), and salivary gland virus disease (cytomegalia) (4), often appears in cells where no apparent association with virus etiology could be established. This led Cowdry in 1934 (1) to state, "Consequently with these type B inclusions the existence of a virus should not be taken for granted. They may be simply the expression of nuclear modifications occurring not only in some virus diseases but also in many conditions for which viruses are probably not responsible." Typical intranuclear inclusions have been reported to occur spontaneously in liver and kidney cells of several wild mammals and birds (5), in liver cells of "normal stock" mice (6-8), and in tissue culture cells of foetal leptomeninges (3). Similar nuclear inclusions have been experimentally induced by injections of aluminum hydroxide, ferric hydroxide and carbon (9), by lead poisoning and irradiation (10), and treatment with thioacetamide (11, 12).

In a cytochemical study of nuclear inclusions of human, rat, and mouse liver with the light microscope (13) the inclusions were found to be round and to vary in size. They were acidophilic, Feulgen negative, and contained variable levels of basophilic material identified as ribonucleic acid, protein, and occa-

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sionally some lipide and glycogen. The inclusions were often enclosed by a basophilic boundary. These inclusions correspond to those reported by Wilson in mouse liver (8), and resemble Cowdry's type B virus inclusions.

The study here reported was designed to examine intranuclear inclusions by means of electron microscopy in order to clarify their morphological characteristics and determine, if possible, their origin.

#### *Materials and Methods*

A specimen of human liver was obtained by punch biopsy from a 65-year-old female suffering from biliary cirrhosis.<sup>1</sup> Samples of rat liver were taken from six male Wistar rats subjected to daily subcutaneous injections of thioacetamide (5 mg. per 100 gm. body weight), for 14 days.

Liver samples were fixed for 1 to 4 hours in a 1 per cent solution of OsO<sub>4</sub> buffered at pH 7.3 with acetate-veronal buffer. Further processing was carried out according to the procedure described by Palade (14). Sections 0.05  $\mu$  thick were observed in a RCA EMU-2A electron microscope.

#### RESULTS

The intranuclear inclusions found in both the human and rat liver cells were often enclosed within a dense membrane-like boundary as illustrated in Fig. 1. The nucleoli were often found in close association with the nuclear membrane and the boundary of the inclusion. Fig. 2 is an electron micrograph of a human liver cell nucleus. The nuclear membrane has invaginated into the nucleus, engulfing and trapping a portion of cytoplasmic material and thus forming the intranuclear inclusion. The trapping of cytoplasmic material often included endoplasmic reticulum, which in the human liver cell (Fig. 2), maintains its form. The close association of the nucleolus to the invaginated boundary of the nuclear membrane is clearly evident (Fig. 2). The inclusions found in the human liver cells generally have a large opening leading from the forming inclusion to the surrounding cytoplasm, suggesting a process of formation similar to the formation of a food vacuole by the amoeba.

Similar inclusion bodies were observed in electron micrographs of thioacetamide-treated rat liver cells, (Figs. 3 and 4). The nuclear membrane appears to invaginate at a certain region to form a narrow opening which connects the center of the developing inclusion to the surrounding cytoplasm. Two or more inclusions sharing a common opening were often found within a single nucleus (Fig. 4). The opening connecting the inclusion to the surrounding cytoplasm was always found to be narrow, and differed in this respect from the wide-mouthed invaginations observed in the human liver material. This suggests that a flowing in of cytoplasmic material accompanied by growth of the nuclear membrane in that area occurs as the intranuclear inclusion enlarges. The contents of the forming inclusion in the rat liver cell were generally disor-

<sup>1</sup> We are grateful to Dr. D. B. Brown for making this material available to us.

ganized. Organized cytoplasmic components (endoplasmic reticulum or mitochondria) could not be identified. Many inclusions appeared to lie free within the nucleus. It remains to be determined by studies of serial sections whether the opening of the inclusion body truly closes, thus isolating the trapped cytoplasm.

#### DISCUSSION

The results of this investigation indicate that the nuclear inclusions found in the hepatic cells of the human and rat liver samples studied are formed by invaginations or inpocketings of the nuclear membrane with a subsequent trapping of cytoplasmic material within the nucleus. In the human liver cell, the cytoplasm appears to be encircled by a wide-mouthed opening of the invaginated nuclear membrane. The narrow opening to the inclusion body of the thioacetamide-treated rat liver cell plus its disorganized contents suggest a somewhat different mode of formation.

The intranuclear inclusions reported as spontaneously appearing in tissue culture cells of foetal leptomeninges were thought to be of cytoplasmic origin by Fischmann and Russell (3). They reported that the staining properties of the inclusions were identical to those of the surrounding cytoplasm and that deep indentations were frequently observed within the nuclear membrane.

The inclusion bodies previously described in light microscopic studies of mouse (8) and rat liver (12) were thought to be derived from nucleoli for many intermediate forms were identified. Electron micrographs of liver cells known to contain large numbers of inclusions studied here indicate that the nuclear membrane is involved in their formation. The nuclear membrane was found to be continuous with the forming inclusion body or in those inclusions lying free within the nucleus; all were bounded by a double membrane identical in structure to the nuclear membrane. There were no indications of direct nucleolar transformation; however, the frequent association of nucleoli to the forming inclusions was noted.

Although the morphological and cytochemical aspects of intranuclear inclusions found in cells under a variety of physiological conditions appear to have many similarities when studied with the light microscope, finer morphological details as uncovered by electron microscopy may reveal that a wide diversity in structure and organization exists.

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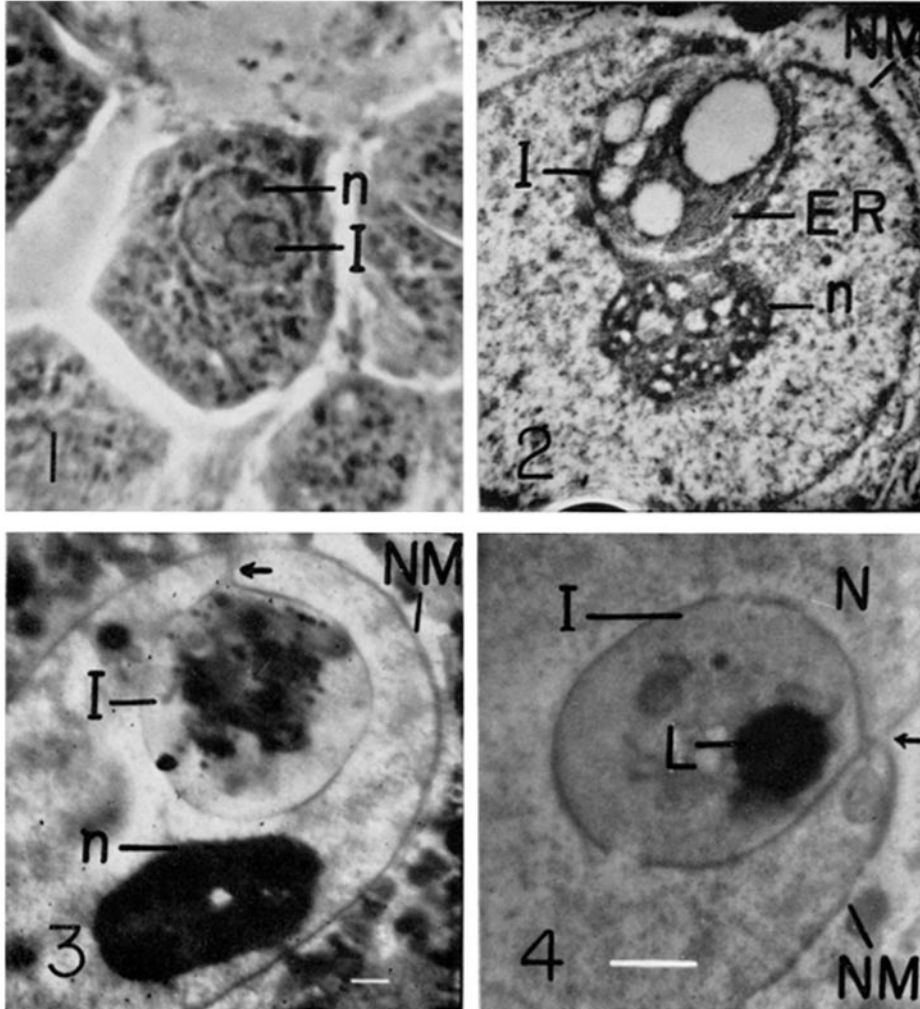
## EXPLANATION OF PLATE 145

FIG. 1. Phase contrast micrograph of a human liver cell fixed in buffered osmium tetroxide and embedded in methacrylate showing a large intranuclear inclusion (*I*) and a small nucleolus (*n*).  $\times 2,200$ .

FIG. 2. Electron micrograph of a human liver cell nucleus illustrating the invaginated nuclear membrane (*NM*) and the intranuclear inclusion (*I*). Note the close proximity of the nucleolus (*n*) to the nuclear membrane. Large vacuoles and endoplasmic reticulum (*ER*) can be identified within the inclusion.  $\times 9,000$ .

FIG. 3. Electron micrograph of a rat liver cell nucleus after 14 days of thioacetamide treatment. Note the typical dense nucleolus (*n*), the narrow neck at the point where the nuclear membrane invaginated (arrow), and the large intranuclear inclusion (*I*).  $\times 5,500$ .

FIG. 4. Electron micrograph showing a portion of a rat liver cell nucleus after 14 days of thioacetamide treatment. Two inclusions appear within a single nucleus (*N*), sharing a common neck and originating at the same region of the nuclear membrane (*NM*) at arrow. Note the large lipide droplet (*L*) within the nuclear inclusion (*I*).  $\times 12,000$ .



(Kleinfeld *et al.*: Human and rat liver parenchymal cells)