ANOMALOUS STRUCTURES IN THE CYTOPLASM OF HeLa CELLS CULTURED IN THE PRESENCE OF 5-BROMODEOXYURIDINE

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INTRODUCTION

The presence of 5-bromodeoxyuridine, an analogue of the pyrimidine nucleoside thymidine, within the culture medium of tissue culture cells results in incorporation of this analogue into cellular DNA together with suppression of *de novo* thymidine synthesis (1–5). It has been suggested that incorporation of this analogue into the DNA

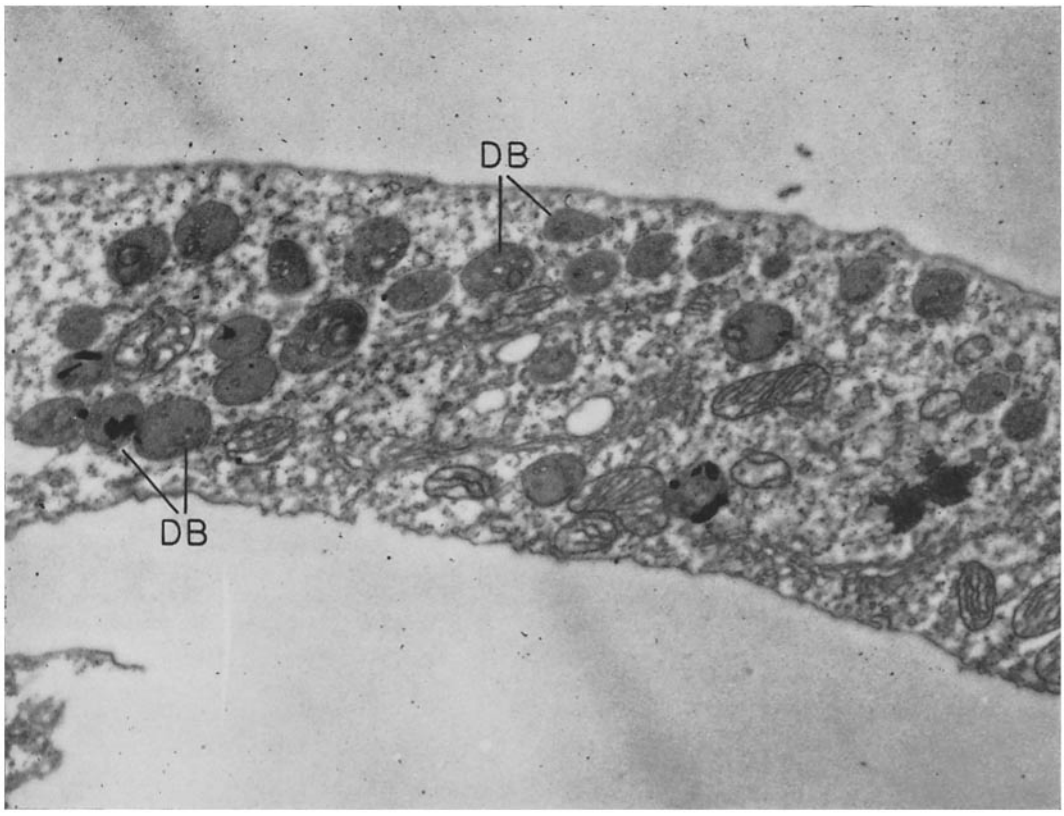

FIGURE 1

Section through a HeLa cell which has been incubated in BUDR-containing medium for 4 days. Round or oval anomalous bodies (*DB*) of uniform density are present in appreciable numbers. The internal structure of these bodies varies from an essentially homogeneous, granular matrix to a complex network of disorganized membranes. Normal-appearing structures are present adjacent to the dense bodies, although there may be some evidence of increased vacuolization of the Golgi region. Four-day incubation in 5×10^{-5} M BUDR-containing medium. × 18,000.

strand results in altered base pairing and base sequence (6–8), actions which may contribute to the decreased viability and increased radiosensitivity observed by different investigators in various analogue-labeled biological organisms (9–12). However, since the possibility exists that analogue action on other cell components may be at least partially responsible for these observations, an investigation of the influence of 5-bromodeoxyuridine on cell parameters other than radiosensitivity was undertaken. The report presented here is confined to structural anomalies observed in HeLa cells grown in medium supplemented with 5-bromodeoxyuridine.

MATERIALS AND METHODS

Stock HeLa parental cells (13) were maintained in modified Eagle's growth medium (14) as monolayer cultures grown on the surface of glass prescription flasks. Uniformity of the growth sheet and absence of heavily clumped areas were criteria for selecting cells to be subjected to experimental conditions. Selected cultures were trypsinized and placed in suspension by vigorously pipetting them into a fresh supply of growth medium supplemented with the appropriate concentration of 5-bromodeoxyuridine (BUDR). Analogue concentration ranged from 10^{-7} M to 10^{-4} M. Aliquot samples of this suspension were allowed to attach to glass coverslips. At all times, appropriate non-labeled control cells were maintained.

At various time intervals between 1 and 7 days following introduction to the analogue, the cells were fixed directly on the coverslips in cold 2 per cent osmium tetroxide buffered with Dalton's potassium dichromate (15). The cells were dehydrated, carefully removed from the coverslips in a monomeric mixture of butyl and methyl methacrylate, and em-

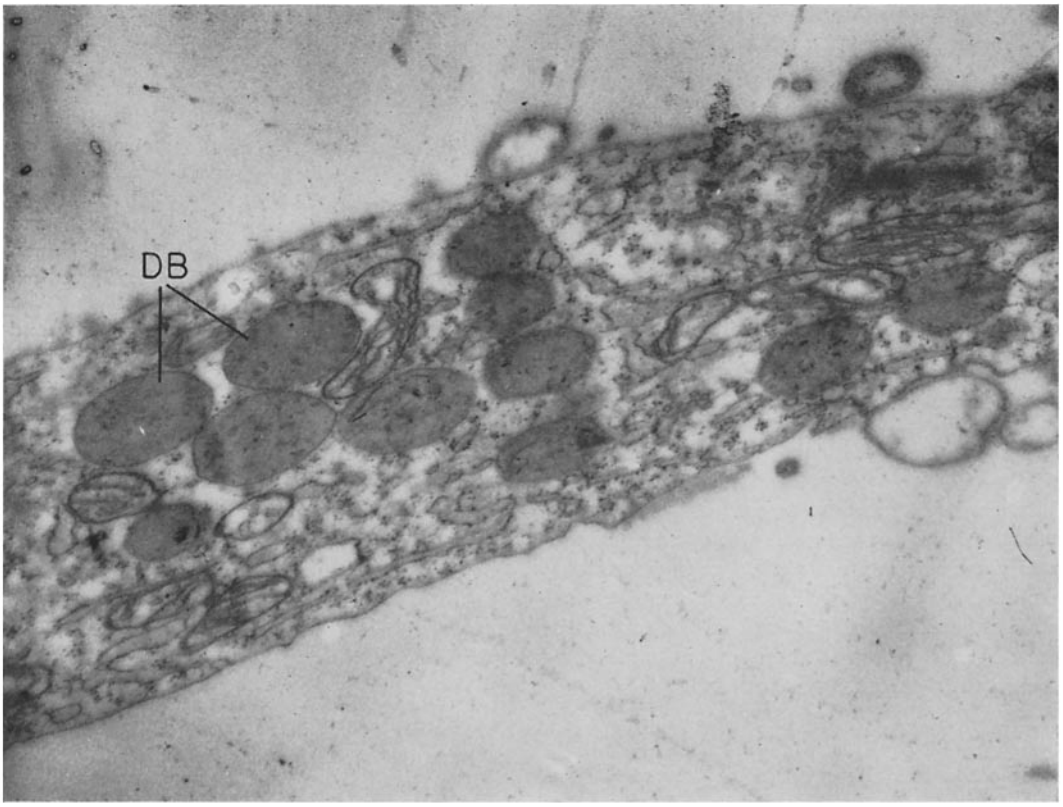

FIGURE 2

Section through another cell incubated in BUDR-containing medium, demonstrating a large number of homogeneously dense, unidentified bodies (*DB*). Although these anomalous bodies are of uniform density and resemble somewhat the dense bodies found in control cells, they are present in much greater numbers in cells which have been incubated in BUDR-containing growth medium. Four-day incubation in 5×10^{-5} M BUDR-containing medium. × 19,000.

bedded in gelatin capsules containing the same mixture. Cells were observed and photographed with an RCA EMU 3-F electron microscope. Each group of BUDR-labeled cells was compared with its own control cell group to account for morphological variations among cell samples caused by factors other than those purposefully introduced.

OBSERVATIONS AND DISCUSSION

The cytoarchitecture of representative control HeLa cells has been described by other investigators (16–20) and will not be included here. A large number of the HeLa cells cultured in the presence of higher concentrations of BUDR (10^{-5} to 10^{-4} M) are observed to contain aggregates of round or oval dense cytoplasmic bodies possessing varying degrees of internal structure (Figs. 1 to 3). The variation in internal structure ranges from an essentially homogeneous, granular matrix to a complete network of disorganized membranes. Frequently the dense bodies contain an accumulation of small, round vesicles and/or microcrystals similar to those described by Dourmashkin and Dougherty (21). The anomalous structures are usually seen adjacent to other organelles of apparently normal configuration, although there may be some evidence of increased vacuolization of the Golgi complex (Fig. 1). The dense bodies are clearly demonstrable following 72 hours of incubation in BUDR-containing medium.

At higher magnification (Fig. 3), the internal structure of these bodies is observed to consist of a complex network of membranes within a dense matrix and bound by a single, double, or multilayered membrane. However, in some cells the dense bodies show little evidence of internal structure other than a homogeneous matrix.

Although the anomalous bodies bear a structural resemblance to the "globoid bodies" (22), "lysosomal granules" (18), or "lamellar bodies" (23) observed by different investigators in control HeLa cells, the increased size and much greater abundance of the homogeneously granular bodies in BUDR-labeled cells suggest that their formation is a reaction of the cell to cultivation in BUDR-containing medium. The bodies also structurally resemble to some degree the microbodies of rat liver which Rouillier and Bernhard (24) interpret as either degenerating or regenerating mitochondria. Since the dense bodies observed in this study on HeLa cells are often found adjacent to mitochondria of normal configuration, a different interpretation is suggested in this case, namely, that these dense bodies may represent structures synthesized *de novo* as a response to a substance foreign to the cell. The intense osmiophilia of the anomalous bodies suggests the presence of quantities of lipid or lipid-like substance, possibly arising from an alteration in the phospholipid or lipid metabolism of the cell.

SUMMARY

Cultivation of HeLa cells in the presence of the thymidine analogue 5-bromodeoxyuridine for periods of 72 hours or longer results in the accumulation in the cytoplasm of aggregates of round or oval, dense, homogeneously granular bodies. Although the origin of these bodies is unknown, it is suggested that they may arise as a result of altered phospholipid or lipid metabolism.

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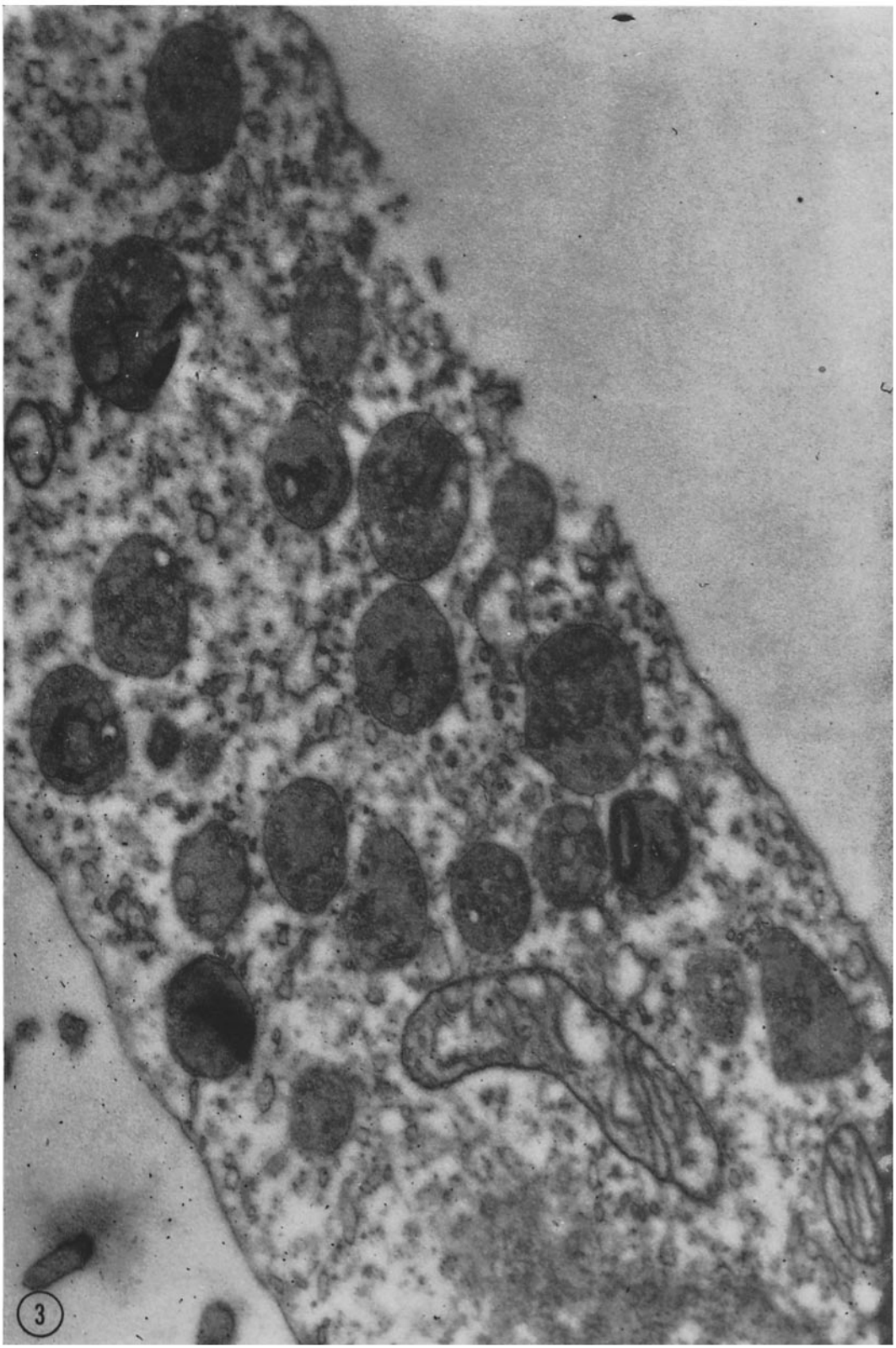
FIGURE 3

The internal structure of the unidentified dense bodies seen at higher magnification. Membranous and vesicular elements are embedded within the uniformly dense matrix. Three-day incubation in 5×10^{-5} M BUDR-containing medium. × 42,000.



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