FORMATION OF MEMBRANE-GLYCOGEN ARRAYS IN RAT HEPATOMA CELLS

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INTRODUCTION

The presence of myelin figures or nebenkerns has been described in the cytoplasm of rat liver cells following a variety of experimental treatments (1–6) as well as in the cells of other tissues, such as the pancreas (7, 8). Myelin figures typically consist of whorls of concentric, smooth-surfaced membranes enclosing a cytoplasmic core which contains agranular endoplasmic reticulum and lipid, although their appearance in electron microscopic preparations is rather variable. Nebenkerns, on the other hand, usually are composed of concentric double membranes bearing attached ribosomes. A possible mode of formation of myelin figures is described in this report.

MATERIALS AND METHODS

The material used for this study consisted of a number of rat hepatomas which had been induced by the prior feeding of the liver carcinogen 2-acetylaminofluorene (AAF) to Leeds strain rats. The animals were fed a standard diet containing 0.05% of AAF for 8–10 months before being returned to a carcinogen-free diet. The rats, which were approximately 2 months old at the beginning of the experiment, were sacrificed at intervals from 2 to 6 months after the cessation of treatment. All animals, except those which died from other causes or had been sacrificed previously, developed liver tumors during the term of the experiment. The number of tumors per liver varied according to the stage of the experiment. During the early stages, only small numbers of discrete nodules were found, while in the latest stages the livers bore multiple hepatomas. At autopsy portions of each liver tumor nodule were removed for histological examination; adjacent portions were processed for electron microscopy. For electron microscopic examination the tissue was cut into small blocks, up to 1 mm^3 in size, and fixed for 4 hr, at 0–4°C, in 4% glutaraldehyde buffered with 0.067 M cacodylate buffer at pH 7.2 (9, 10). The blocks were washed for 16 hr at 0–4°C in 0.25 M sucrose in 0.1 M cacodylate buffer before being postfixed in Millonig's phosphate-buffered 1% osmium tetroxide (11) for 2 hr at 0–4°C. The tissue was then rapidly dehydrated in ethanol and embedded in Epon 812 (12).

Histological examination showed that a large proportion of the tumors present were well-differentiated trabecular hepatomas showing some invasion of the surrounding liver tissue. Six of these tumors were selected to provide the material for the present report and were examined in an RCA EMU-3G electron microscope at 100 kv. All sections for electron microscopy were stained with lead tartrate (13).

OBSERVATIONS AND DISCUSSION

Many cells of the trabecular hepatomas contained numbers of membranous structures derived from the granular endoplasmic reticulum. These structures varied in form according to their complexity and the plane of section, and ranged from fully developed myelin figures to simple structures such as that shown in Fig. 1. Typical, well-developed myelin figures were, however, observed only occasionally. There appeared to be a continuous spectrum of such structures which may therefore represent stages in the development of myelin figures.

The earliest stage observed was represented by the localized loss of attached ribosomes from small areas of the granular endoplasmic reticulum. This was accompanied by the accumulation of small glycogen particles in the cytoplasm between the cisternae at such sites. The glycogen in these tumor cells was scanty and was almost invariably present in the form of small rosettes as opposed to the large rosette-like aggregates which are normally found in liver cells. At a later stage (Fig. 2) several cisternae were involved. They appeared to

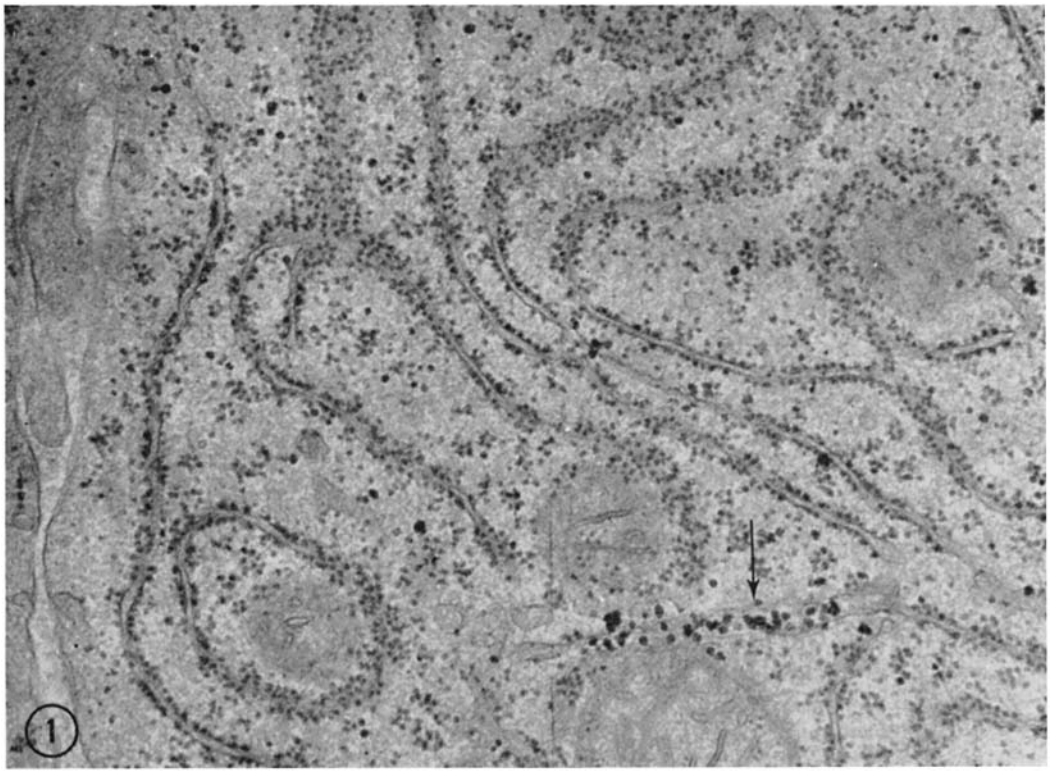

FIGURE 1 Hepatoma. Glycogen particles are present between adjacent smooth-surfaced cisternae (arrow). Continuity between these cisternae and the granular endoplasmic reticulum is shown. × 45,000.

become more closely apposed than elsewhere in the cytoplasm and the loss of ribosomes from their surfaces was accompanied by a decrease in the electron opacity of the cisternal contents and by the continued accumulation of glycogen in the intercisternal cytoplasm. This stage appeared to develop into the type of body which is shown in Fig. 3. This body consisted of concentric, smooth-surfaced cisternal membranes showing frequent fenestrations. Glycogen particles were present between the membranes. At the center the cisternae appeared to give rise to tubular membranous elements. The outermost layers of these structures consisted of concentric layers of typical elements of the granular endoplasmic reticulum, unassociated with glycogen and bearing abundant ribosomes. If the plane of sectioning was unfavorable the typical core was not included, and the appearance was then similar to that shown in Fig. 4. In a few cases it was possible to substantiate this finding by examination of serial sections.

In view of the suggested relationship between the endoplasmic reticulum and myelin figure formation, it may be apposite to describe briefly the state of the former in these tumors. The agranular endoplasmic reticulum was similar to that of normal rat hepatocytes whereas the granular reticulum tended to be elongated and sinuous in profile and to bear abundant attached ribosomes, although some parallel arrays of cisternal elements were observed. There was no evidence that the presence or absence of either myelin figures or the structures which are described in this paper was related in any way to the state of the endoplasmic reticulum in any particular cell.

From these observations it would therefore seem probable that not only the concentric, smooth membranes but also the central smooth-surfaced tubules of the typical myelin figure may be derived directly from the granular endoplasmic reticulum.

The presence of small glycogen particles entrapped between smooth-surfaced cisternae has been described in the liver cells of ethionine-intoxicated rats by Steiner et al. (6), who named the

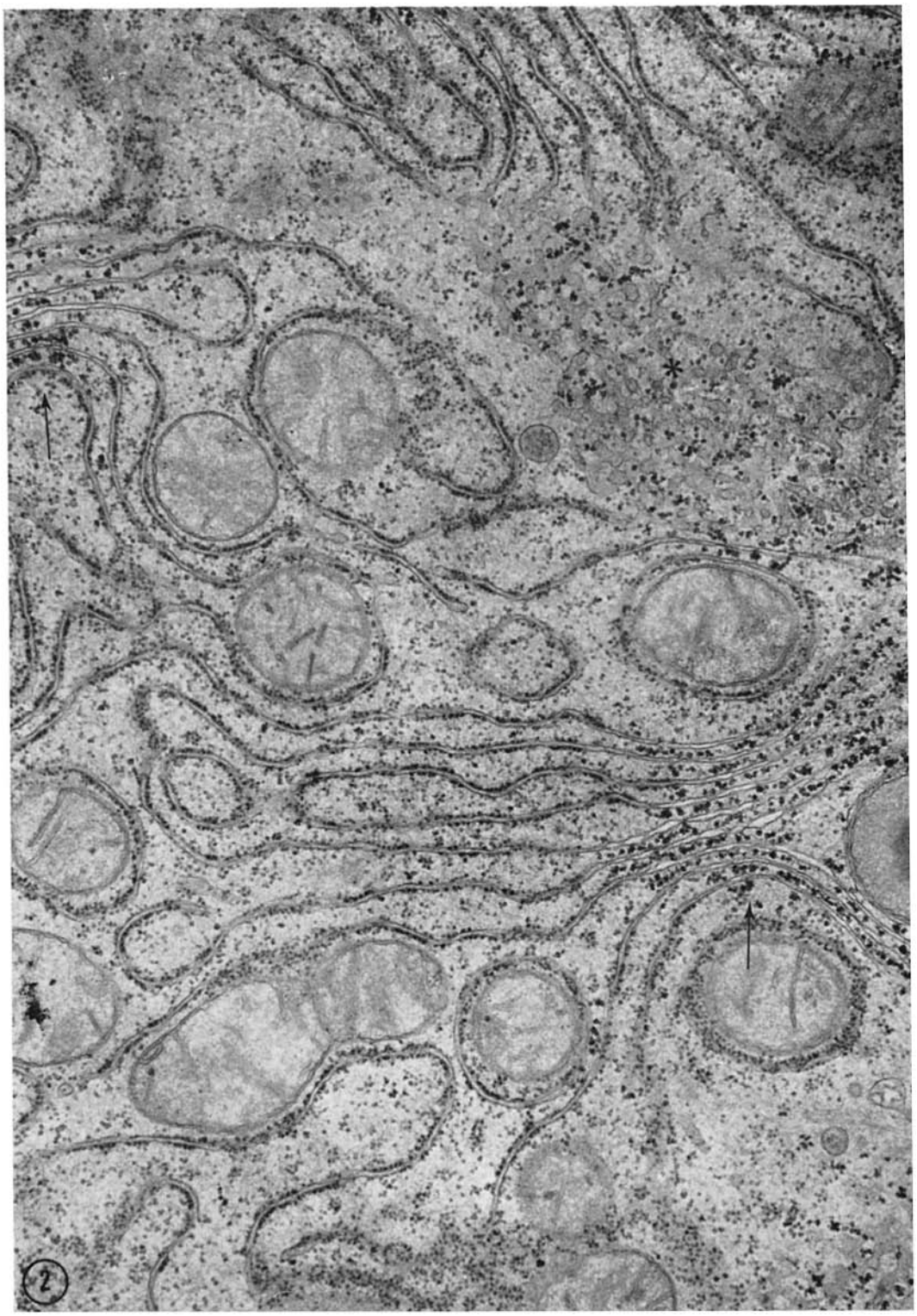

FIGURE 2 Hepatoma. The characteristic disposition of the endoplasmic reticulum in these cells is shown here. The small size of the glycogen particles is also demonstrated, both when associated with the agranular endoplasmic reticulum (asterisk) and in the membrane–particle arrays which are present (arrows). × 30,000.

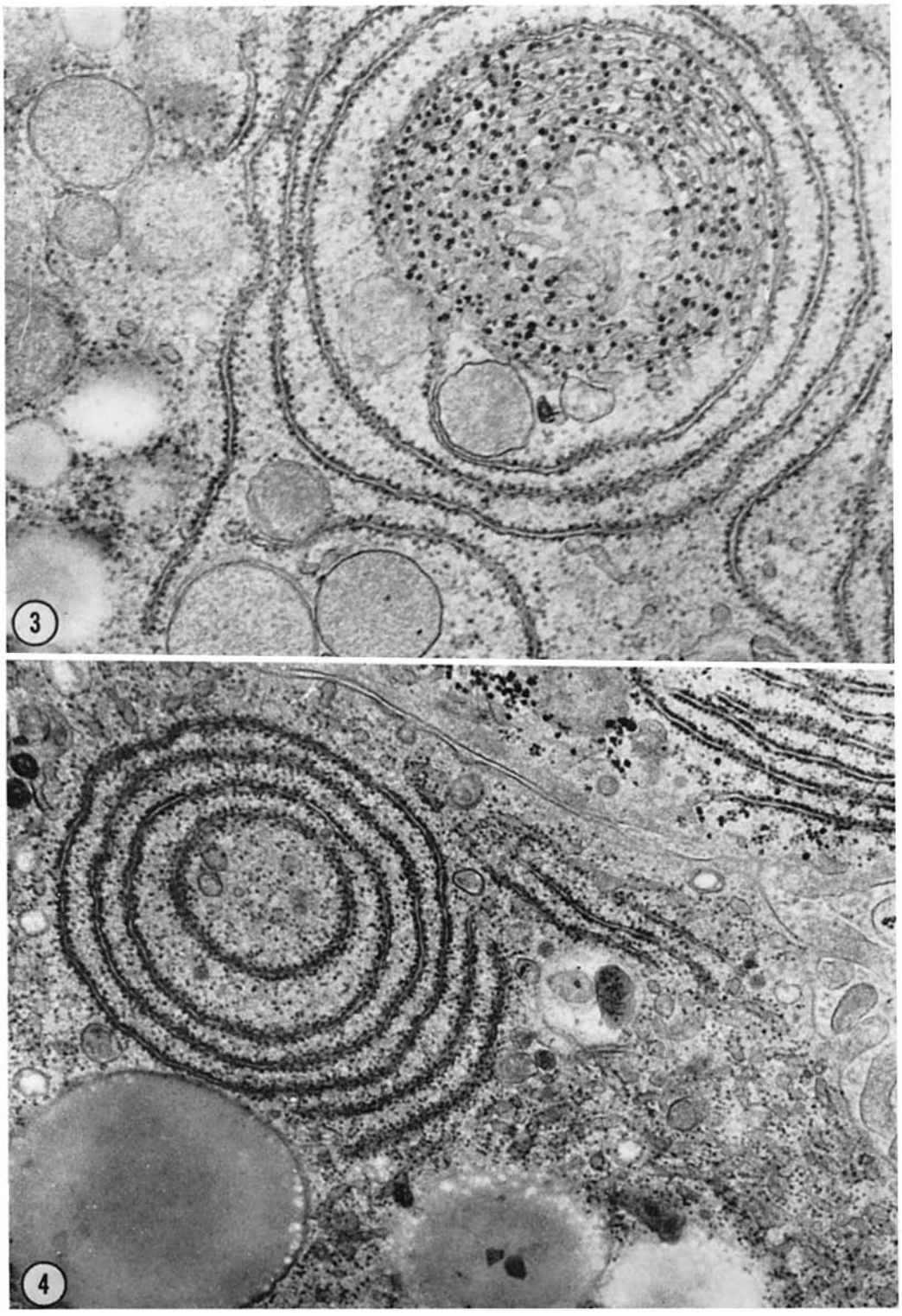

FIGURE 3 Hepatoma. A later stage in myelin figure formation is shown. Glycogen particles are still present between the smooth-surfaced cisternae, which exhibit numerous fenestrations. × 38,000.

FIGURE 4 Hepatoma. Tangential section of body similar to that shown in Fig. 3. × 30,000.

round or oval profiles which they observed "glycogen bodies." This type of structure has also been observed in human hepatoma cells (14). The significance of the formation of myelin figures or their suggested precursors is somewhat obscure but the presence of these figures in actively growing or regenerating tissues suggests that they may represent sites of cellular reorganization. Any suggestion concerning the role of the structures which have been described, with regard to degenerative or regenerative processes in the tumor cells, must necessarily be speculative in nature, since it is not possible to follow any possible changes which might occur in the tumors over a period of time. Also, in a rapidly growing tumor it may be expected that the turnover of cells is rapid and, therefore, that both degenerative and regenerative processes may be occurring in the tissue at the same time. Although the type of profile which is depicted in Fig. 4 bears some superficial resemblance to the nebenkern described by Herman and Fitzgerald (7, 8), it is probable that the two are unrelated and that the structures which have been considered here should rather be regarded as myelin figure precursors. Herdson and Kaltenbach (5) studied experimentally induced myelin figures in rat liver and speculated on the possibility that these bodies may have a functional role as opposed to being the mere end products of a degenerative process. It is difficult however, to attempt to compare a tumor with normal tissue which has been subjected to chemical action. In the case of a tumor the transformation of the granular endoplasmic reticulum into the forms described may represent an actual loss of this organelle and may, therefore, be related to loss of differentiation and progression toward greater malignancy.

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