The Ultrastructural Localization of the G-6-P Hydrolyzing Enzyme Activity in Kupffer Cells

Earlier electron microscope studies have shown that a G-6-P hydrolyzing enzyme activity is present in hepatocytes and polymorphonuclear leukocytes[1]. In both cell types the reaction product is localized exclusively in the nuclear envelope and endoplasmic reticulum, sites characteristically associated with glucose-6-phosphatase (G-6-Pase) activity[2-7]. Since both cell types are able to hydrolyze G-6-P and show similar type of localization and distribution of reaction product, it was suggested that the activity observed in leukocytes was either similar or identical to G-6-Pase observed in the liver, kidney or intestine.

The present electron microscopic observations indicate that the enzyme activity is also present in Kupffer cells and is similar in its ultrastructural distribution to that found in liver, kidney and intestinal cells[4], as well as leukocytes[1].

Livers were excised from 24-hour-old C_3H mice and fixed for 2 h in 4% glutaraldehyde solution (pH 7.4). Subsequent tissue processing for histochemistry has been described in previous publications[1,7]. In brief, the blocks of tissue were washed in 0.35 *M* sucrose/cacodylate buffer solution, pH 6.5 for 2 h and then frozen and sectioned using a freezing microtome. Sections of liver were then incubated in a media containing the following: 3 m*M* lead nitrate; 0.35 *M* sucrose; and 0.05 *M* sodium cacodylate and G-6-P. Media to be used for control incubations either lacked G-6-P or contained other substrates, namely fructose-6-phosphate, ribose 5 phosphate, fructose 1, 6 diphosphate, adenosine monophosphate and B-glycerophosphate. Incubation periods ranged from 5–15 min. Test and control sections observed by light microscopy were immersed in ammonium sulfide following incubation, mounted and observed with a light microscope. For electron microscopy, the sections incubated in either control media were post-fixed in 1% osmic acid, dehydrated with ascending concentrations of alcohol and embedded in maraglas resin. The embedded tissues were then sectioned with an LKB ultrotome.

Thin sections for electron microscopy were observed either stained or unstained with lead citrate using an Elmiskop I electron microscope.

As previously reported, 24 h post-natal mouse liver reveals heavy deposition of reaction product when observed by light microscopy[5]. The reaction product is distributed almost exclusively in the cytoplasm with dense concentrations of reaction product around the nucleus. On the other hand, control sections revealed no activity or only sparse activity.

Under electron microscopy, sections incubated in G-6-P medium clearly reveal enzyme activity in the endoplasmic reticulum and nuclear envelope of hepatocytes (Figure). Areas of the cytoplasm containing Golgi vesicles and lamellae, and fully-formed microbodies show no deposits; the plasma membranes mitochondria also show no deposits.

In addition to the localization of G-6-Pase in hepatocytes, activity is also observed in Kupffer cells (Figure). The distribution of reaction product is similar to that observed in hepatocytes.

The present electron microscopic observations of Kupffer cells confirm an earlier light microscope study revealing G-6-P hydrolyzing activity in the cytoplasm of Kupffer cells[8]. In addition, the present observation indicates that the activity is localized in the nuclear envelope and endoplasmic reticulum, similar to the organellar distribution found in the post-natal[7] and adult liver, kidney, intestines[4] as well as leukocytes[1].

The presence of a G-6-P hydrolyzing enzyme activity in Kupffer cells suggests that this cell type is capable of excercizing a glycogenolytic function via G-6-Pase.

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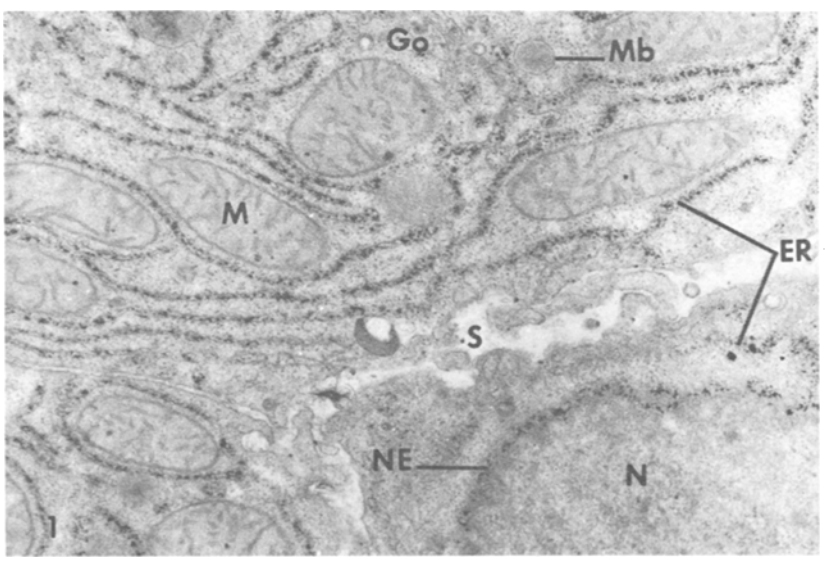

24 h post-natal mouse liver section incubated in a G-6-P medium. 2 hepatocytes occupy the major portion of the field. Both show lead reaction product in the nuclear membrane (NE) and endoplasmic reticulum (ER). Golgi bodies (GO) to the left of a fully-formed microbody (Mb), and randomly dispersed mitochondria (M), show no reaction product. At the lower right, a Kupffer cell inclusive of a nucleus (N), also exhibits reaction product in the nuclear envelope and the endoplasmic reticulum.

Moreover, since G-6-Pase is now known to have multiple enzymatic activities[9,10], cells bearing the enzyme, under appropriate physiological stimuli, may also be able to generate G-6-P and Pi[11]. On this basis one might suggest that the G-6-P splitting enzyme activity in Kupffer cells plays a complex role in the various physiological functions of this cell type. It is of interest to note that a G-6-P hydrolyzing enzyme activity is also present in a spectrum of different cell types in virtually all of the lymphoid organs[8]. However, as cautioned in previous publications[1] although leukocytes and Kupffer cells are capable of hydrolyzing G-6-P and also demonstrate an identical organellar distribution of reaction product similar to that of liver cells, further studies are required to demonstrate whether the G-6-Pase like activity in RES cells is biochemically similar to liver G-6-Pase, particularly with respect to their kinetics and multifunctional specifities[12].

Zusammenfassung. Die Aktivitätsverteilung eines Glukose-6-Phosphat hydrolysierenden Enzyms wurde in der Leber 24 Stunden alter Ratten elektronenmikroskopisch untersucht. Die Verteilung der Enzymaktivität in den Kupffer-Zellen schien mit der in den Hepatozyten gefundenen identisch zu sein.

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Vitamin E Deficiency and Chemical Carcinogenesis

There are several reports in the literature on the carcinogenic and co-carcinogenic effect of heated fat[1,2]. SUGAI et al.[2] were the first to demonstrate that a polymeric fraction of heated fat accelerates the induction of tumors in rats given a low level (5 mg/100 g of the diet) of N-2-fluorenylacetamide (FAA). It was not clear whether the synergistic effect of heated fat was directly related to the presence of oxidative polymers in the diet or whether these compounds caused in vivo peroxidation of tissue unsaturated lipids which resulted in a more favorable environment for the neoplastic transformation. In an attempt to answer this question, we have superimposed vitamin E deficiency on animals receiving a low level of FAA. No long term experiments have been previously reported to determine whether a diet deficient in vitamin E and high in polyunsaturated fatty acids would accelerate or retard the induction of tumors in rats given a low level of carcinogen.

Materials and methods. 4 groups of 18 male rats each (Holtzman, 60 g) were fed a semisynthetic diet containing casein 20%, cerelose 66.1%, salt mixture 3.5%, choline chloride 0.3%, fat 10% and all the necessary vitamins. Groups 1 and 2 received 10% corn oil without and with 0.005% FAA respectively. Groups 3 and 4 received 10% stripped corn oil (Distillation Products Industries, Rochester, New York) without and with 0.005% FAA respectively. The animals were given feed and water ad libitum and their weights were recorded periodically. The deficiency of vitamin E in the blood of animals from groups 3 and 4 were confirmed by performing the dialuric acid hemolysis test[3]. Microquantities of blood were withdrawn periodically from the tail vein and used for obtaining the serum lipoprotein patterns[4]. The tissues of the animals were fixed in formalin and paraffin sections were stained with hematoxylineosin.

Results and discussion. The rats on diet containing stripped corn oil and FAA grew poorly as compared to controls, and 5 rats died within the first year. After 14 months on the diet, many rats of this group became quite emaciated and 3 more rats died. Therefore, 6 rats were sacrificed at this time and 4 remaining rats were sacrificed along with the animals in the other groups during the 18th month of the experiment.

Almost all the rats from group 4 (10 out of 13 which were histologically examined) developed liver tumors of the adenomatous type. No ear duct tumors or other tumors were observed in rats from this group. Rats from group 3, which were fed the same diet, but without the carcinogen, did not develop any tumors and excluded the possibility of malignant transformation due to stripped corn oil alone for this long time period (18 months). The serum lipoprotein patterns indicated an increase in high density lipoproteins of animals given the carcinogen (groups 2 and 4 vs groups 1 and 3 respectively) and are consistent with similar results reported before[5].

A low level of 0.005% FAA was chosen on the basis of the observations of SUGAI et al.[2]. However, in the present experiment (and confirmed by a subsequent experiment) this level of FAA was sufficient to induce tumors of liver and other tissues in rats given fresh corn oil (14 out of 14 from group 2). Thus, this observation precludes us from concluding that stripped corn oil (or vitamin E deficiency) acts synergistically in conjunction with 0.005% FAA to induce neoplasia. It is, however, possible that a still lower level than 0.005% FAA may demonstrate this effect.

A lower level of the carcinogen would mean a more prolonged period of experimentation, and, in the case of vitamin E deficient animals, it is impractical.

Since vitamin E and intracellular antioxidants are reported to be enriched in tumor tissues as compared to normal tissue[6,7], some retardation of tumor induction or tumor growth might be expected in acute vitamin E deficiency. Since the growth of the animals in group 4

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