Causes of Zonal Distribution of Glycogen in the Liver Acinus After a Fat-Rich Diet

Different acinar glycogen patterns are observed in rat liver after different diets[1]. These patterns are independent of the direction of the acinar blood flow[2]. This paper shows that the presence of glycogen in zone 1 of Rappaport's liver acinus after a fat-rich diet is due to (a) the disappearance of glycogen-synthesizing enzymes in zones 2 and 3, and (b) the physiological regeneration in zone 1.

Methods. Male Wistar WU rats (275–325 g) were kept on a fat-rich diet (fat) or a carbohydrate-rich diet (bread) for various periods (see Results). Livers were excised under ether narcosis. RNA was stained with methyl green-thionine (ROQUE et al.[3]). Frozen sections were stained with Sudan III-Scharlach Red. Tissue fixed in Carnoy's liquid was embedded in celloidin for staining of glycogen with carmine (Chroma Gesellschaft). Fatty acids were determined titrimetrically after saponification[4], and glycogen was measured according to the phenol-sulphuric acid method[5]. Each result decsribed in this paper was studied on 6 animals.

Results and discussion. After a 6 days' fat-rich diet RNA is mainly localized in zone 1. A similar localization was observed for glycogen in the same experiment[1]. Thus, a positive correlation exists between the localization of glycogen and RNA after a fat-rich diet. Controls, fed on a carbohydrate-rich diet, showed glycogen and RNA spread out over the whole liver acinus. Glycogen and RNA disappear completely from zone 3 if a fat-rich diet is prolonged to 50 days. The loss of RNA staining property in zone 3 is evidence for reduced protein synthesis and indicates a reduction of glycogen synthesizing enzymes.

Fatty acids determined after a 6 days' fat-rich diet amounted to about 12% of the wet weight of the liver. After 24 h of starvation the quantity of fatty acids decreased to about 3% and remained on this level if the starvation period was prolonged to 72 h. However, this disappearance of lipid (3%) is not accompanied with glycogen synthesis in zone 3, as is seen in the controls[1]. Rats were re-fed with the carbohydrate-rich diet following a 6 and a 50 day's fat-rich diet and 2 days of starvation. In both groups about 3% glycogen is synthesized within 4 h. This was mainly deposited in zone 1 after a 6 days' diet. After a 50 days' diet, however, glycogen was deposited in zone 1 only. These results show that glycogen-synthesizing enzymes diasppear from zone 3 during the fat-rich diet.

Large quantities of RNA are found in cells of zone 1 (Figure 1); this is independent of the type and the duration of the previous diet. These cells are usually small. Cells of zone 3 are larger, have reduced tinctorial qualities for RNA stain, and contain vacuoles (Figure 2), which do not contain Sudan III-Scharlach Red positive material (lipid). These data indicate that the physiological regeneration of the liver acinus is mainly localized in zone 1. The conclusion that liver parenchymal cells move towards the central vein is inevitable. In summary, RNA-rich cells in zone 1 are young and the more RNA-deficient cells in zone 3 are old. This conclusion is in line with the findings of GRISHAM[6] and LEBOUTON and MARCHAND[7] studying thymidine-^{3}H labelled cells in regenerating and neonatal rat liver respectively. The loss of RNA in zone 3 is aggravated by lipid. It is clear that the older cells of zone 3 have less 'resistance' to lipid than the younger cells of zone 1. The presence of glycogen in zone 1 after a fat-rich diet can be explained assuming that recently regener-

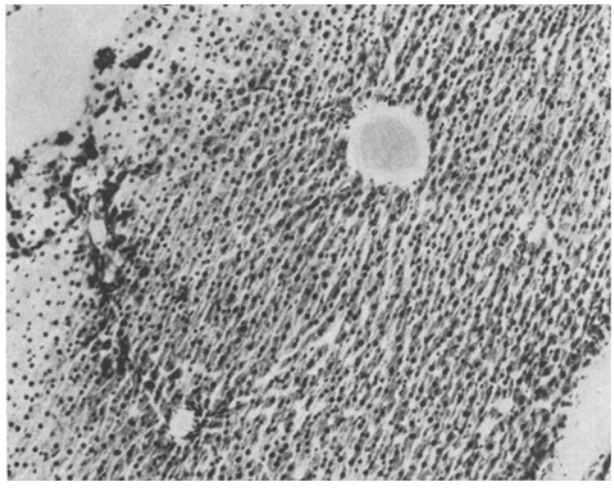

Fig. 1. Many parenchymal cells with large quantities of RNA can be seen in zone 1. Rat liver after 42 days of the fat-rich diet. Methyl green-thionine staining. 10 µm. ×125.

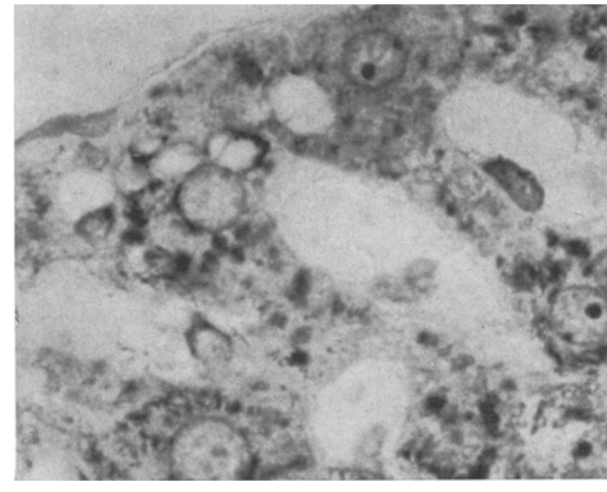

Fig. 2. Vacuoles in some cells near the central vein. Rat after 6 days of a carbohydrate-rich diet followed by 2 days of starvation. Methyl green-thionine staining, 3 µm. ×1250.

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ated liver cells receive a 'normal' enzyme content. Becoming older and moving to the vena centralis, this enzyme content changes, depending on age and diet.

Zusammenfassung. Nachweis, dass nach fettreicher Diät Glykogen hauptsächlich in der Zone 1 des Rappaportschen Leberacinus abgelagert wird, was durch den Abbau von Enzymen, die in den Zonen 2 und 3 am Glykogenaufbau beteiligt sind und durch die physiologische Regeneration in der Zone 1 verursacht wird.

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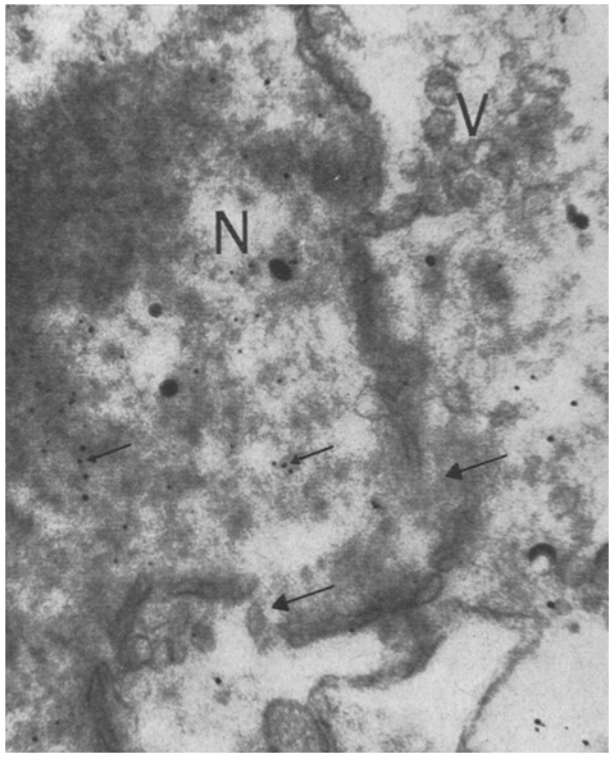
The Effect of Temperature on Nuclear Permeability

It has been shown that the transfer of RNA from the nucleus to the cytoplasm is a temperature dependent process[1–3]. These studies, however, do not distinguish between the effect of temperature on the processing of RNA within the nucleus and its effect on the permeability of the nuclear envelope to macromolecules. In this investigation the action of temperature specifically on the exchange process was studied by injecting colloidal gold particles, coated with polyvinylpyrrolidone, into the cytoplasm of the multinucleated amoeba *Chaos chaos*. The injected cells were incubated at different temperatures and the intracellular distribution of the particles determined with the electron microscope. Since these particles are inert, and not altered by temperature dependent changes in cell metabolism, their ability to enter the nucleus should depend primarily on the characteristics of the nuclear envelope, assuming that diffusion within the cytoplasm is not rate limiting. Furthermore, both colloidal particles and ribonucleoproteins cross the nuclear envelope through central channels within the nuclear pores[4,5]. Thus, variations in the uptake of gold particles should reflect changes in the properties of the pathways used for naturally occurring substances.



An electron micrograph of a permeable nucleus following incubation at 2 °C. The gold particles (small arrows) are concentrated in the nucleoplasm (N). Breaks in the nuclear envelope are apparent (large arrows). Small vesicles (V), possibly fragments of the envelope, are frequently associated with permeable nuclei. In this instance the vesicles are restricted to the cytoplasm, but they have also been seen in the nucleoplasm.

The experiments were performed on well-fed, interphase amoebae. The prodecures for culturing the cells, preparing colloidal gold, microinjection, and electron microscopy have been described in previous reports[4,6]. Two gold fractions were used; one contained particles ranging from 30–170 Å in diameter (L-fraction), and the second contained 25–55 Å particles (S-fraction). The amoebae were injected at room temperature (approximately 25 °C) and left at that temperature or rapidly transferred to an incubator set at 34°, 10°, or 2 °C. The cells were fixed in OsO_4, 30 or 50 min after injection, and subsequently sectioned and examined with the electron microscope. Gold particles were counted and measured in adjacent regions of nucleoplasm and cytoplasm according to the methods described earlier[7].

The results of the 50 min experiments performed with the L-fraction are shown in Table I, A. The percent of the total particle count present in the nucleoplasm, decreased as the temperature was lowered from 34° to 10 °C. The decrease from 34° to 25 °C is statistically significant ($P < 0.025$), as is the decrease from 25° to 10 °C ($P < 0.001$). Surprisingly, when the temperature was dropped to 2 °C there was an increase in the concentration of particles in the nucleoplasm, and the results are not significantly different from those obtained at 25 °C ($P > 0.5$). Closer examination of the cells incubated at 2 °C showed that there were 2 separate populations of nuclei. This was not the case at higher temperatures. In one population (non-permeable nuclei), consisting of approximately 70% of

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