Acta vet. scand. 1967, 8, 201—210.

From the Department of Pathology, Royal Veterinary College,
Stockholm, Sweden.

VACUOLATION AND GLYCOGEN DEPOSITION IN THE HEPATIC CELL NUCLEI OF RUMINANTS

By

*B. S. Rajya**) and *S. Rubarth*

Vacuolated hepatic cell nuclei and glycogen deposition in these nuclei are known in man normally and in different diseases. They are mostly formed in diabetes mellitus but occur in other human diseases too. *Ehrlich* (1883) postulated that the vacuolation in hepatic cell nuclei in diabetes patients was a consequence of glycogen deposition and later on this has been histochemically established. *Warren & LeCompte* (1952) showed a reciprocal relationship between the amounts of intranuclear and intracytoplasmic glycogen. In the veterinary literature these changes are only described in cattle (*Rubarth* 1962, *Holtenius* 1963).

In the routine post-mortem in the Department of Pathology at the Royal Veterinary College in Stockholm, vacuolated liver cell nuclei and glycogen deposition in these nuclei are often seen in cattle, but no case has been found in the other domestic animals (horse, dog, cat, swine, sheep and goat).

The present communication is concerned primarily with the incidence and morphology of vacuolated hepatic cell nuclei in cattle and buffalo, with particular reference to the location of these in hepatic lobules. Attempts have also been made to investigate the relationship between the morphologic variants of these nuclei and their distribution in the liver lobules.

---

*) Address: Department of Pathology, U.P. College of Veterinary Science and Animal Husbandry, Mathura (U.P.) INDIA.

MATERIAL AND METHODS

Liver material from 150 cattle and 30 buffaloes were studied histopathologically. All animals were adult except 12 cattle calves, 14 days to 2 months old. Pieces of liver from cattle were obtained from the routine autopsies in the Department of Pathology, Royal Veterinary College, State Veterinary Medical Institute and Uppsala Slaughterhouse, and the buffalo materials were taken from slaughterhouses in India.

The pieces of liver were fixed in 10 % formalin, Bouin's and Carnoy's fluids, and a counterpart of each in absolute alcohol. The paraffin sections were stained by hematoxylin and eosin stain. For the demonstration of glycogen alcohol or Carnoy fixed sections were stained by Best carmine and with periodic acid-shiff method (PAS). DNA was demonstrated by Feulgen nuclear reaction. Frozen sections prepared from each specimen (formalin fixed) were stained for fat by Scharlach R and hemalum.

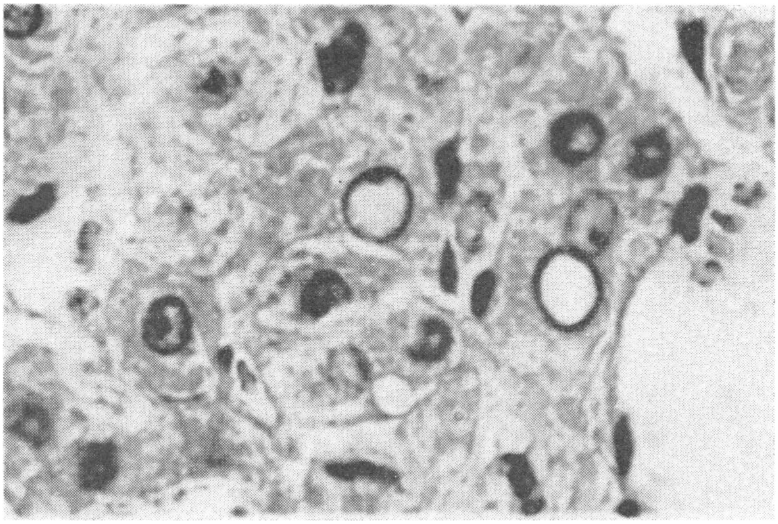
RESULTS

Morphology

From the microscopic picture of the vacuolated nuclei three different morphologic types could be distinguished and for the purpose of description these have been designated as α-, β- and γ-types.

The α-types measured about 10 to 12 μ and were spherical. These nuclei had the chromatin and nucleolus at the periphery, giving a thick appearance to the nuclear membrane (Fig. 1) thicker than in the other two types. Nucleolus and small chromatine fragments gave the inside of the membrane a rough surface. In the centre of the nucleus was a homogeneous faint basophilic mass.

The β-types measured about 12 to 15 μ and in one calf a few were nearly 20 μ. The shape of these nuclei was oval or spherical with a marginating of the chromatin. The nucleus membrane was uniformly thick, sometimes locally thickened by the nucleolus. The remaining part of the nucleus was filled by a homogeneous basophilic (in E-H-stain faintly bluish) mass, in some cases with a diffusely defined but distinct vacuole in the centre (Fig. 2). Some nuclei had a fragmentary or lytic membrane in the last mentioned case diffusely merging into the cytoplasm.



F i g u r e 1. In the centre an α-type with the nucleolus on the nuclear membrane, liver, cattle, 200 $\times$.

F i g u r e 2. Mainly γ-type and a few of the β-type, liver, cattle, 160 $\times$.

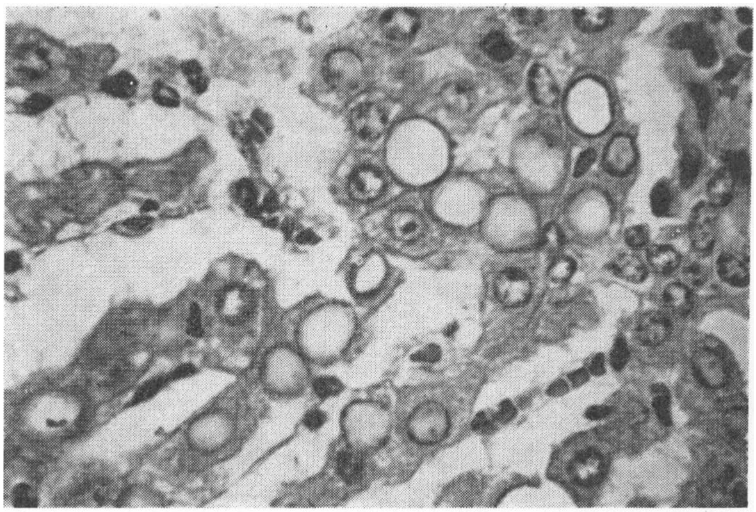


Figure 3. All the three nucleus types, liver, cattle, 200 ×.

The γ-types were the largest and measured in general 12 to 20 μ and were of oval or spherical shape. These could be differentiated from the β-type by the highest grade of ballooning with a clear, large vacuole without stainable (Figs. 2 and 3) content and a prominent sharp-limiting nuclear membrane.

The most common of these three types was the β-type (see Table 1).

Intranuclear glycogen

In most sections where vacuolated nuclei were present, the intranuclear glycogen granules could be demonstrated by PAS and Best carmine stains. In addition, the three morphologic types exhibited an appreciable difference with regard to the quantity and size of the glycogen granules. The γ-type had 2 to 3 minute granules at the periphery close to the nuclear membrane. In the β-forms similarly sized glycogen granules were present at the periphery, but comparatively large, round and light pink stained granules were also scattered throughout the vacuole. The vacuole in the α-type contained a round body of the glycogen in the centre surrounded by a clear space. Besides this, a few minute granules could also be noticed in some instances at the periphery of this type of nucleus. The intracytoplasmic glycogen was also present in cells with normal and vacuolated nuclei. But the intra-

Table 1. The number of the three nucleus types and their distribution in the lobules.

Case number	Types	Total number	Average size	Location: limiting plate	zone 1, 2	zone 3	near centr. vein	Approximate ratio of numb with regard t location
294	α	21	10.09 μ (9.9—11.88 μ)	15	6	—	—	2:1:0:0
	β	43	10.15 μ (11.88—14.85 μ)	11	30	2	—	5:15:1:0
	γ	36	14.18 μ (11.88—14.85 μ)	2	23	11	—	1:11:5:0
295	α	24	9.98 μ (9.9—11.88 μ)	7	16	1	—	7:16:1:0
	β	22	10.54 μ (9.9—14.85 μ)	1	10	11	—	1:10:11:0
	γ	0	—	—	—	—	—	—
298	α	14	9.9 μ (9.9 μ)	9	5	—	—	2:1:0:0
	β	21	11.46 μ (9.9—11.88 μ)	—	13	8	—	0:2:1:0
	γ	0	—	—	—	—	—	—
3	α	12	10.23 μ (9.9—11.88 μ)	8	4	—	—	2:1:0:0
	β	66	10.35 μ (9.9—11.88 μ)	16	48	2	—	8:24:1:0
	γ	35	14.85 μ (14.85 μ)	3	28	4	—	1:7:1:0
814	α	3	9.9 μ (9.9 μ)	1	2	—	—	1:2:0:0
	β	88	12.84 μ (11.88—14.85 μ)	3	29	49	7	1:9:14:2
	γ	35	13.76 μ (11.88—19.80 μ)	—	12	18	5	0:2:3:1

cytoplasmic glycogen granules were apparently smaller than the intranuclear granules. The sections stained by Feulgen reaction indicated that the nuclei which were partly clear (α- and β-types) in the hematoxylin and eosin stain, had most of the chromatin particles at the periphery and a few in the centre. But in γ-types no Feulgen positive material could be seen.

Fatty changes

The slides stained for fat revealed in some cases excessively marked to moderate fatty changes. Both cells containing vacuolate nuclei and normal cells had fat droplets in the cytoplasm. In no case could fat be seen within the nuclei.

Distribution of the vacuolated liver cells in the lobule

In cases with few vacuolated liver cell nuclei they were only found in the periportal part of the lobule. When the number was increasing these cells appeared first in the limiting plate and later on in a more or less thick perilobular zone. In one calf (no. 814), where the number of vacuolated nuclei was enormous, the distribution of these cells extended in the central part of the lobule up to the central vein.

The analysis of the total count of these nuclei in individual cases revealed a fairly significant distribution of the different types in the lobule. The data of the 5 cases represented in Table 1 indicated the presence of α-types usually in the cells of the limiting plate around the portal triangle. The β-types were mostly located in the circulatory zone 1 and 2 in terms of *Rappaport* (1958) acinar concept of liver lobule; the γ-types were invariably confined to zone 2 and 3 of the circulatory field of the acini. It was, however, no uncommon observation to find some of these types at different levels adjacent to the portal sheath especially in cases where the vacuolated nuclei were many.

Incidence

The vacuolation and intranuclear glycogen deposition in hepatic cells were observed and examined in 96 out of 138 adult cattle (69.5 %) and in 5 out of 12 calves (41.6 %). The age of the 5 calves ranged from 2 weeks to about 2 months. The histopathologic examination of most of these cases indicated the association of the vacuolated nuclei with different kinds of chronic pathologic lesions in the liver, viz. chronic passive hyperaemia, post necrotic cirrhosis, chronic interstitial hepatitis. In some cases, however, no other appreciable lesions could be detected apart from the nuclear changes. The relation of vacuolated nuclei to other liver lesions and disease conditions was studied, and most of these cases (61) were combined with acute gastroenteritis. Amongst the remaining adult cattle different kinds of disease conditions were found, viz. chronic passive hyperaemia, centrolobular necrosis, post necrotic cirrhosis, lymphadenosis. In one buffalo chronic interstitial hepatitis was present.

The morphology of the vacuoles and the distribution, fat and glycogen content of the liver cells with vacuoles in the nuclei were the same in buffaloes as in cattle.

DISCUSSION

The morphology of the vacuolated hepatic cell nuclei observed in this study revealed three distinguishable types, based on the size, distribution of chromatin and resulting vacuolation. This morphological variation was comparable with different types of vacuolated hepatic cell nuclei described in human diabetes mellitus and other diseases. The present results, however, suggested that variation in nuclear types was probably a result of the stage of gradation in the hydropic status of nucleus, rather than of some specific kind. As might be expected, the glycogen content in the vacuoles of different types of nuclei was not in correspondence with the size, but was inversely proportional to the size of the hydropic nucleus. *Baird & Fisher* (1957) pointed out that in human liver the quantity of intranuclear glycogen alone did not completely relate to the increased size of the nucleus, which is in accordance with the present observation. The Feulgen reaction in big vacuolated nuclei was stated to be negative by *Cazal & Mirouze* (1951), while *Baird & Fisher* reported that DNA by Feulgen reaction was unaltered in all gradations of nuclear swelling. Our results imply that there was a difference in the Feulgen reaction on the various types, and the largest type (γ) with a clear optical space appeared to be Feulgen negative.

Baird & Fisher suggested that the glycogen deposition followed the hydropic change in the nucleus due to alteration in the fluid balance. *Warren & LeCompte* (1952) showed a definite reciprocal relationship between the amount of intracytoplasmic and intranuclear glycogen. There were few reports signifying the predilection of vacuolated nuclei in the area of the portal triangle in human liver, which offers support to our observation of the location of these changes in the area of the portal triangle in cattle and buffalo. Of great interest was the relationship of the vacuolated nuclei in their distribution to the circulatory field of the liver. *Rappaport* (1958) stated that there was a zonal relationship between hepatic cells and their blood supply. The cells close to the axial afferent channels were first to be supplied with blood rich in oxygen and nutrients. The further the parenchyma was from the afferent vessels, where they ramify into sinusoids, the poorer was the blood supply. Thus, this orientation permitted a better functional approach to all processes in normal and abnormal liver. Keeping in view this concept, in the present investigation the smallest type (α) was mostly located in the area rich in oxygen and nutrient blood supply, and the largest one (γ) was at the periphery of the circulatory area, which is obviously poor in blood supply and oxygen. It was therefore inferred from these distributions that the factors of blood supply play some role in the causation of vacuolated nuclei and glycogen deposition within the nucleus. Further, the largest type of nuclei (γ) was devoid of glycogen in most instances in which the location was near the centre of the lobule or at a distance from the portal sheath. Although similar views were expressed by *Chipps & Duff* (1942) the phenomenon does not exclude the probability of many other chemical factors being involved prior to the development of this change, as indicated by *Baird & Fisher*.

The findings by electron microscopy in these cells have been interpreted in different ways. *Izard* (1960) considered that the vacuole arises in the nucleolus, but this opinion is rejected by *Cossel* (1962). In accordance with *Cazal & Mirouze*'s findings, our studies by light microscopy argue against a nucleolar origin of the vacuole. *Cossel* holds that the function of the changed and insufficient liver-cell nucleus is taken over by a second nucleus, which he has often found in these cells. We have not made any such findings, however.

The findings of the vacuolated nuclei in cattle conform with the occurrence of similar changes earlier reported (*Rubarth* 1962, *Holtenius* 1963). Moreover, similar changes also manifest themselves in the allied species, the buffalo. On morphologic basis the changes appear to be identical with those found in human liver in cases of diabetes mellitus and other diseases. Diabetes mellitus has sofar not been known in cattle or buffalos. Besides, the changes of vacuolated hepatic cell nuclei were never seen in dogs, horses, cats, swine, sheep and goats. Therefore, the occurrence of vacuolated nuclei and glycogen deposition in cattle appear to be somewhat problematical. But the evidence that there was no definite correlation between vacuolation or the intra nuclear glycogen and diabetes mellitus or other diseases provides explanation of the present findings. It would also be evident from these results that the vacuolated nuclei were present in different diseases, appearing in large number in calves died of gastroenteritis. *Holtenius,* who in liver biopsies studied the occurrence of intranuclear PAS-positive masses in liver cells in cattle in different diseases, found that there was no apparent association between this nuclear change and any particular disease. It may be noticed that he found a relative low incidence of these cells in cattle with acetonemia and paresis puerperalis but a high one in chronic traumatic peritonitis and "other diseases". The other noteworthy feature of this investigation was the age range which shows that the changes can occur in calves as young as 2 months of age. In man *Chipps & Duff* observed the vacuolated hepatic cell nuclei in persons 11 months to 82 years old.

Conclusively, the necessity of further investigations on this problem in ruminants with special reference to electron microscopic and histochemical aspects would thus be obvious.

REFERENCES

Baird, W. F. & E. R. Fischer: Observation concerning vacuolation and deposition of glycogen in nuclei of hepatic cells. Lab. Invest. 1957, *6*, 324—333.

Cazal, P. & J. Mirouze: Une anomalie mal connue des cellules hépatiques; les noyaux vacuolaires et leur signification physiopathologique probable dans le syndrome d'adaptation. Presse méd. 1951, *59*, 571—573.

Chipps, H. D. & G. L. Duff: Glycogen infiltration of the liver nuclei. Amer. J. Path. 1942, *18*, 645—655.

Cossel, L.: Elektronmikroskopische Befunde an den sog. Lochkernen in menschlichen Leberepithelzellen. Frankfurt Z. Path. 1962, *72*, 115—122.

Ehrlich, P.: Über das Vorkommen von Glykogen im diabetischen und im normalen Organismus. Z. klin. Med. 1883, *6*, 33.

Franzini, C.: Histological alteration of the liver in diabetes. Functional connections. Osped. maggiore Novara-Fegato. 1962, *8*, 170—194.

Holtenius, P.: On the occurrence of intranuclear Pas-positive material in liver cells of cattle. Cornell Vet. 1963, *83*, 322—327.

Izard, J.: Étude en microscopie électronique de l'hepatocyte humain normal et pathologique. Toulonte: R. Lion 1960.

Lagnens, R. & N. Bianchi: Fine structure of the liver in human idiopathic diabetes mellitus. II. Parenchymal cell nuclei. Exp. molec. Path. 1963, *2*, 534—540.

Lorenz, S.: Über das Kernglykogen der Leber. Zbl. allg. Path. path. Anat. 1954, *92*, 437—442.

Macary, J.: Les nucléoles vacuolaires de la cellule hepatique humaine. C. R. Soc. Biol. (Paris) 1954, *148*, 39—41.

Rappaport, A. M.: The structural and functional unit in the liver. Anat. Rec. 1958, *130*, 673.

Rubarth, S.: Leber und Gallenwege in Joest: Handbuch der speziellen pathologischen Anatomie der Haustiere. 1962, 14 Lieferung, Bd. VI, 77.

Warren, S. & P. M. LeCompte: The pathology of diabetes mellitus. Lea, Philadelphia 1952.

SUMMARY

The incidence of vacuolated hepatic cell nuclei is reported in cattle and buffalo with different diseases. The change is noticed in adult animals and also in up to 2 months old calves. There is an inverse correlation between the intranuclear glycogen content and the size of the nucleus. The morphology of three different gradations of vacuolated nuclei is described and the distribution of the affected cells in the liver lobules in relation to blood supply is discussed.

ZUSAMMENFASSUNG

Vakuolisierung und Vorkommen von Glykogen in den Kernen der Leberzellen bei Wiederkäuern.

Die Verfasser berichten über das Vorkommen von Vakuolen und von Glykogen in den Leberzellkernen beim Rind und Büffel bei verschiedenen Krankheiten. Diese auch beim Mensch, vor allem bei Diabetes mellitus, vorkommenden Veränderungen treten bei erwachsenen Tieren auf, wurden aber auch bei Kälbern im Alter von 2 Monaten wahrgenommen. Die veränderten Leberzellkerne besitzen variierende Grösse, und es scheint eine umgekehrte Relation zwischen der Grösse und dem Glykogengehalt des Kernes vorzuliegen. Das morphologische Bild der drei Vakuolisierungsgrade in den Kernen wird beschrieben und die Verteilung der veränderten Zellen in den Lobuli im Verhältnis zur Blutzirkulation wird diskutiert.

SAMMANFATTNING

Vakuolisering och glykogen förekomst i levercellernas kärnor hos idisslare.

Författarna rapportera förekomsten av vakuoliserade levercellkärnor med glykogenförekomst hos nötkreatur och buffel vid olika sjukdomar. Förändringarna, som även förekomma hos människa och främst vid diabetes mellitus, uppträda hos vuxna djur men ha även iakttagits hos kalvar i 2 månaders ålder. De förändrade levercellkärnorna ha en varierande storlek och det tycks föreligga en omvänd relation mellan kärnstorleken och glykogenhalten. Den morfologiska bilden av de tre vakuoliseringsgraderna i kärnorna beskrives och de förändrade cellernas fördelning i lobuli i relation till blodcirkulationen diskuteras.

(Received January 2, 1967).