STRUCTURAL HETEROGENEITY IN STRAND NUMBER AND DEPTH OF TIGHT JUNCTIONS IN CENTRAL AND PORTAL ZONES OF LIVER OF THE SMALL SKATE

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A quantitative analysis of tight junctional strand number and depth was performed on sections from portal and central zones of skate liver to determine if there is heterogeneity in the structure of this blood-bile barrier within the hepatic lobule. Skate livers were isolated as previously described (Reed et al., Bull MDIBL 16:83-84, 1976). After a thirty minute perfusion in Elasmobranch Ringers, the livers were fixed for ten minutes by portal vein perfusion with a modified Karnovsky fixative consisting of 2% gluteraldehyde, 4% paraformaldehyde and 2.5% calcium chloride.

Thin slices of tissue were prepared using a razor blade and a dissecting microscope. Small sections of liver were removed from periportal and pericentral zones of the liver lobule, a process facilitated by the predominance of pigmented sinusoidal cells in Zone III or central lobular regions (Boyer and Wade, Bull. MDIBL. 19:95-96, 1979). After two to three hours of additional fixation, the tissue is soaked in 25% glycerol in 0.1M cacodylate. The tissue was then frozen in Freon 22, cooled by liquid nitrogen and fractured by a Balzers freeze unit (BAF 301, Balzers-High Vacuum, Litchtenstein). Platinum-carbon replicas were then prepared and photomicrographs were obtained with a Zeiss 10B electron microscope. Measurements of tight junction strand number and depth were quantitated from photo micrographs at 40,000-70,000 magnification using a sonic digitizer (Graf-Pen, Scientific Accessories Corporation, Southport CT) and a micro computer (PDP11-03, Digital Equipment Corporation, Maynard, MA). Strand number was defined as the number of strands intersecting a line perpendicular to the long axis of the junction, while tight junction depth represented the shortest distance between the outermost and innermost strands. Abluminal strands were excluded from the determinations of junction depth. 439 measurements were made from 14 photographs of the central zones of the lobule from two skates while 217 measurements were obtained from 13 photographs of periportal zones. As noted in Table 1, there were no differences in these parameters between portal and central zones of the

Table 1.--Strand Number and Junction Depth in Portal and Central Zones of Skate Liver

	# of Animals	# of Measurements	Strand # (mean + S.D.)	Junction depth (mean + S.D.)
Portal zone	2 skate	217	5.0 $\pm$ 1.9	0.182 $\pm$ .099
Central zone	2 skate	439	5.1 $\pm$ 1.6	0.178 $\pm$ .100

lobule. Figure 1 illustrates a representative freeze fracture replica. The junctional structure is qualitatively similar to that observed in rat and human liver. Figure 2A and B illustrate the frequency distribution of strand number and junction depth in portal zones of the lobule. Similar data were obtained from central lobular zones.

Figure 1.--Freeze fracture replica of skate liver bile canaliculus (BC) illustrating the zonula occludens (ZO) or tight junction. The lumen of the BC runs from the top to bottom of the figure and is bordered by the ZO. The heterogeneity in strand number and depths can be seen. In some areas 2-3 strands are noted (arrowheads), whereas 7-8 strands are viewed in other areas (arrows). The fracture represents a P-face replica where the elements of the junction appear as strands rather than as grooves. Magnification x 25,560

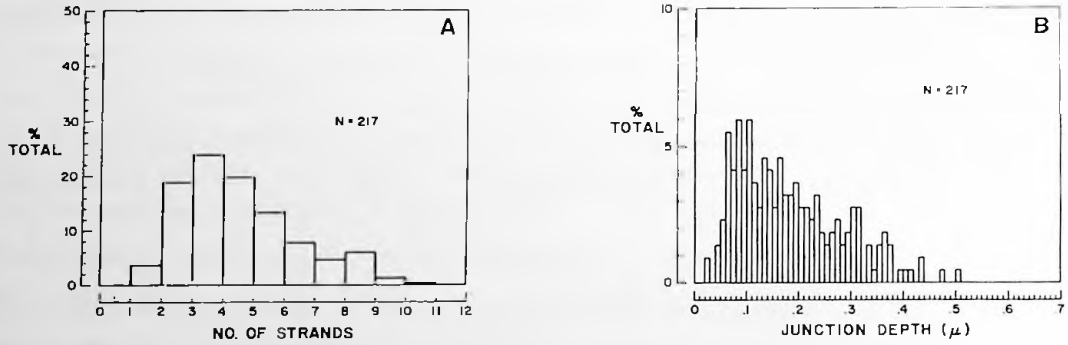

Figure 2.--Frequency distribution of strand number (A) and depth (B) in tight junctions in portal zones of skate liver. This analysis quantitates the variation in these structurral barriers bordering the canalicular lumen.

This structural analysis of the tight junctions from skate liver demonstrates the heterogeneity of this structure with respect to both strand number of depth, but does not detect significant differences within portal and central areas of the hepatic lobule. If junctional structure as viewed by freeze fracture replicas bears any relationship to the permeability of the blood bile barrier then permeability differences should be present at multiple points along the surface of the individual hepatocyte rather than in different areas of the hepatic lobule.