

The Investigation of the Replication of Hepatitis C Virus in HepG2 Cells Cultivated in a Three Dimensional Cell Culture System

Debra Ponds

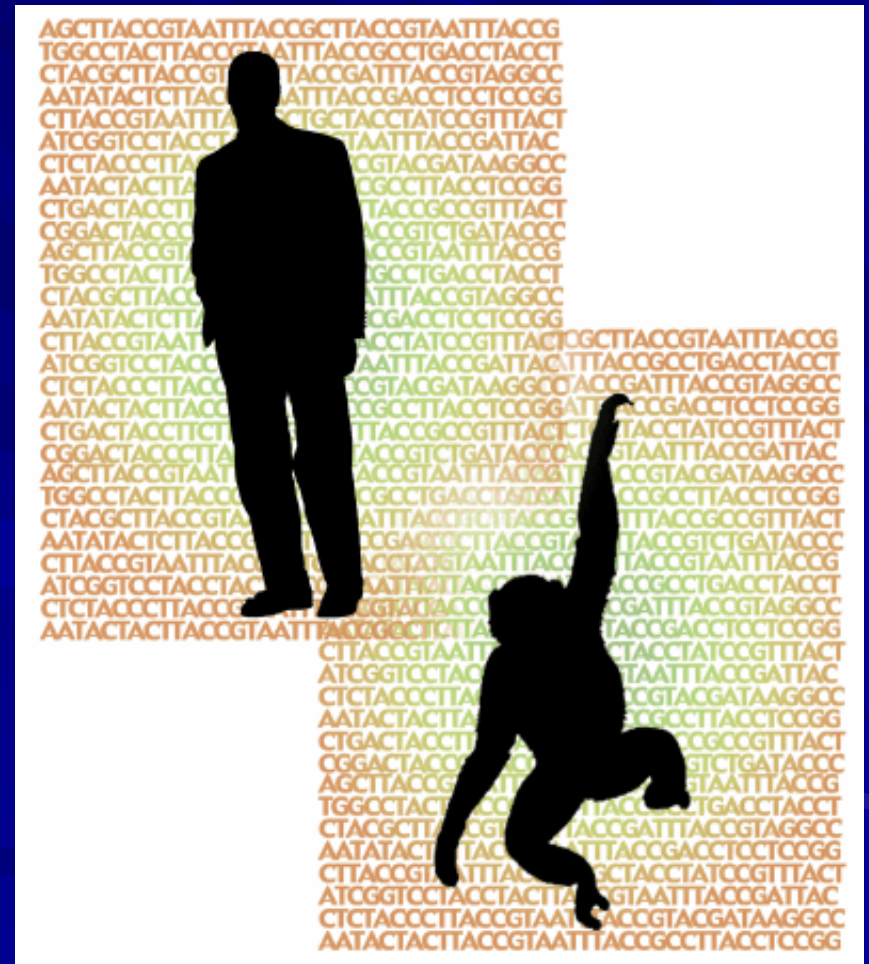
Advisors Dr. Scott Shors & Dr. Barbara Lyons

Overview

- The epidemiology of the Hepatitis C Virus
- The role the liver cells plays
- The Rotary Cell Culture System (RCCS)
- Current work

The Hepatitis C virus

- Blood-borne virus
- Humans and chimpanzees
- Hepatotropic



Hepatitis C, the silent epidemic

- 3% or more of the world population is infected ~180 Million
 - 1.8% or more of the US population is infected ~5 Million, 10,000 a year die
 - Most common chronic blood-borne infection
 - Remains undiagnosed in most patients
 - 70% of chronic liver disease
 - Leading indication for liver transplant in the US

HCV Research

- The research involving the pathogenesis, and replication of HCV has been hampered by the lack of a satisfactory cell-culture system

Therefore limits the:

- Development of vaccines and drugs
- Appropriate therapeutics

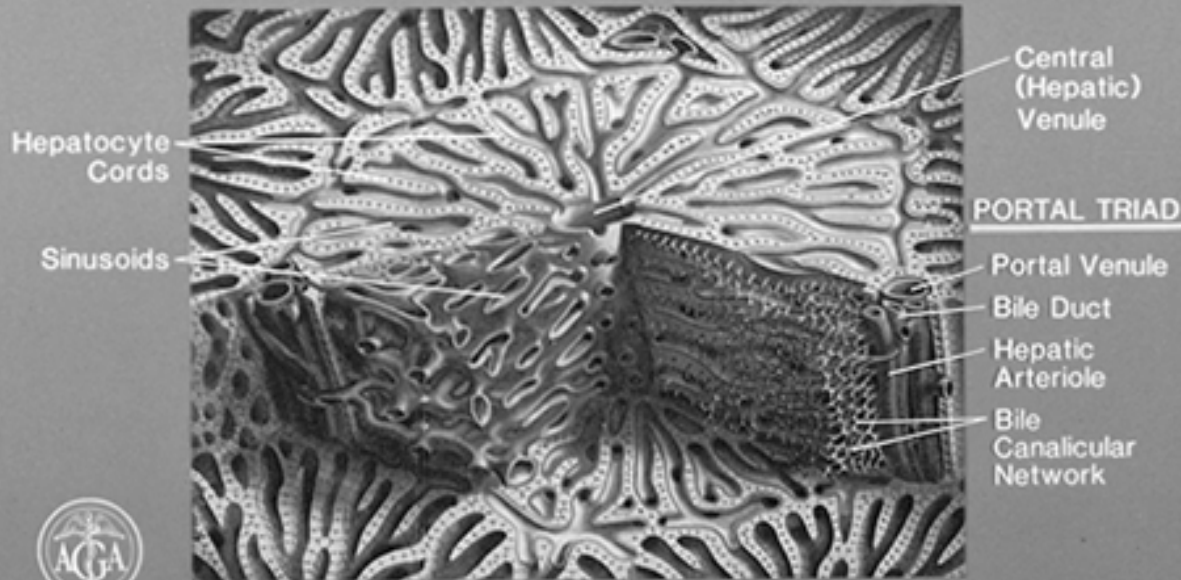
The Focus of My Research

- To create a model system to culture functional, permissive liver cells that will express and produce the Hepatitis C Virus at high enough titers to further the study of its mechanisms
- Hypotheses: Primary hepatocytes along with heptoblastoma cells are more permissive to HCV infections the more similar their morphology and metabolic functions are to that of *in vivo* cells

Liver Lobule

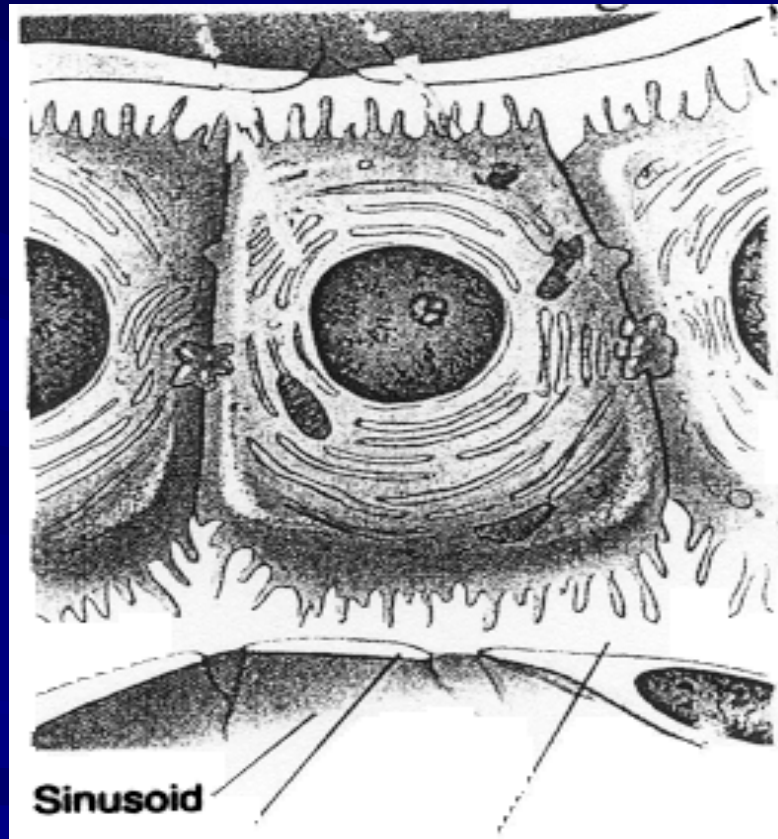
Impression: University of Washington

**THE TRADITIONAL LIVER LOBULE IS RADIALLY
ARRANGED AROUND A CENTRAL HEPATIC VENULE
WITH PORTAL TRIADS AT THE PERIPHERY**



Hepatocyte

Impression: Dr. Sue Whittle Leeds University



Cell cultures in the rotary cell culture system versus traditional monolayer flask

RCCS



Flask



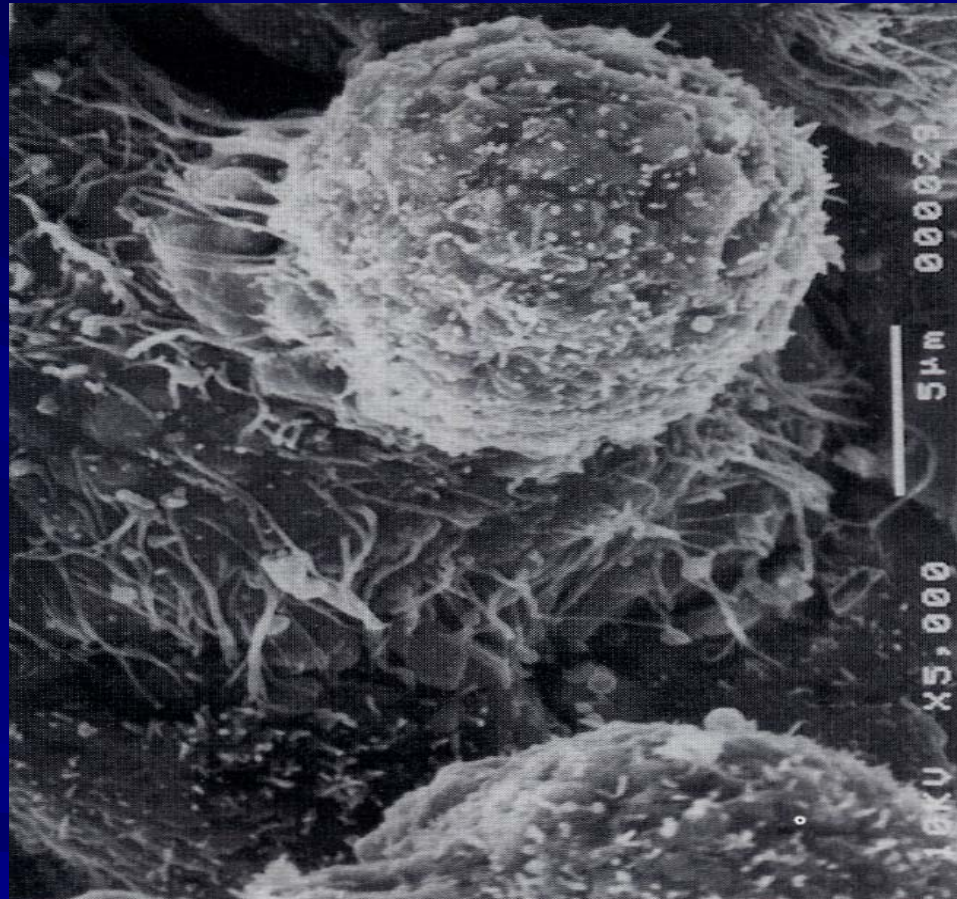
RCCS

- Retain natural 3D architecture
- Retain structurally intact microvilli on surface
- Maintenance of tight junctions and normal intercellular space
- Formation of bile duct like structures
- Produce and maintain numerous plasma proteins

Monolayer Culture

- Flat due to gravitational force
- Lack of structurally intact microvilli on surface
- Do not maintain tight junctions and normal intercellular spacing
- Many plasma proteins undetectable after 48 hrs.

HepG2 Cell Cultured in The RCCS



Vladimir Khaoustov, 1999 Baylor College of Medicine

The Focus of My Research

- To produce functional, permissive liver cells for HCV infections
- Hypotheses: the more similar cell are to *in vivo* cells, the more permissive they are to HCV

Current Work

- HepG2 Cells in RCCS
- Infected with HCV
 - VA-RNA₁ INF alpha knock down
- Cultured Infected cells 60 days

Current work

- 1. SDS PAGE on cell media 43 days out
 - Results: Four protein bands
 - Mass spec analysis revealed >90% HCV Proteins
- 2. Working on immuno labeling cell media and infected cell samples
 - Viewing on the TEM
- 3. Western on Claudin 1 and CD81 membrane proteins
 - Results: both proteins expressed in HepG2 cells
 - Working on Flow cytometry & Confocal microscopy
- 3. Working on Real Time RT PCR
 - Looking for the replicative strand of HCV

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