

Highlights in the Discovery of Hepatitis A Virus

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Miami Beach, Florida

Known Characteristics of the Two Forms of Viral Hepatitis - 1947

Characteristic	Hepatitis A	Hepatitis B
Incubation Period	15-40 days	60-160 days
Mode of infection	Fecal/oral	Parenteral
Immunity	Homologous	Homologous
Value of IG prophylaxis	Good	None
Heat resistance	56°C X 30 min	56°C X 60 min
Ether resistance	10% X 2hr @40°C	Triple extraction @ -20°C

The Search for the Hepatitis A Virus

- **Human transmission** → **Multiple Studies**
- **Cell culture** → **Detroit 6 cells**
- **Marmoset Inoculation** → **GB Virus/Berlin Agent**
- **Gel diffusion** → **Milan Antigen/Fecal Ag**
- **IEM** → **Fecal antigen**
- **IEM** → **HAV**

Human Transmission Studies

- **MacCallum et al. – 1944: Transmission to volunteers,**
- **Neefe and Stokes et al. – 1945: Separation of A and B, homologous immunity, stool infectivity, protection by gamma globulin, disinfection of infectious hepatitis**
- **Havens – 1945: Fecal excretion, period of infectivity, homologous immunity, viral characterization**
- **Krugman et al. – 1958: Natural history of viral hepatitis and separation of two forms of hepatitis, development of standardized reagents, MS-1 & MS-2**

Saul Krugman



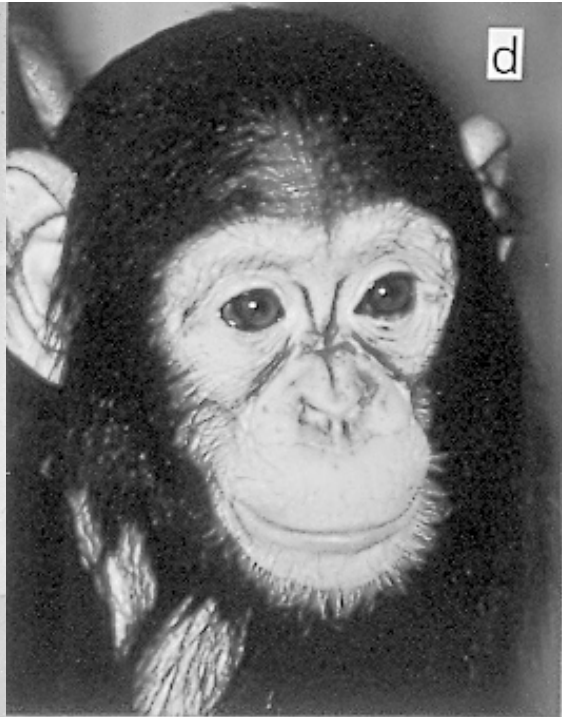
Detroit 6 Cells

- **First studied by Rightsel and McLean at Parke-Davis who observed CPE in cultures inoculated with plasma from icteric patients. 1956-1966**
- **Lack of specificity of the system was demonstrated by a series of experiments in Australia by Cole, Cross and Marmion et al. 1965-1966**
- **Melnick, Boggs et al. Using MS-1 material identified the “Kirk hepatitis virus” In D-6 cell. They ultimately showed this to be a parvovirus cell culture contaminant. 1971**

Animal Models

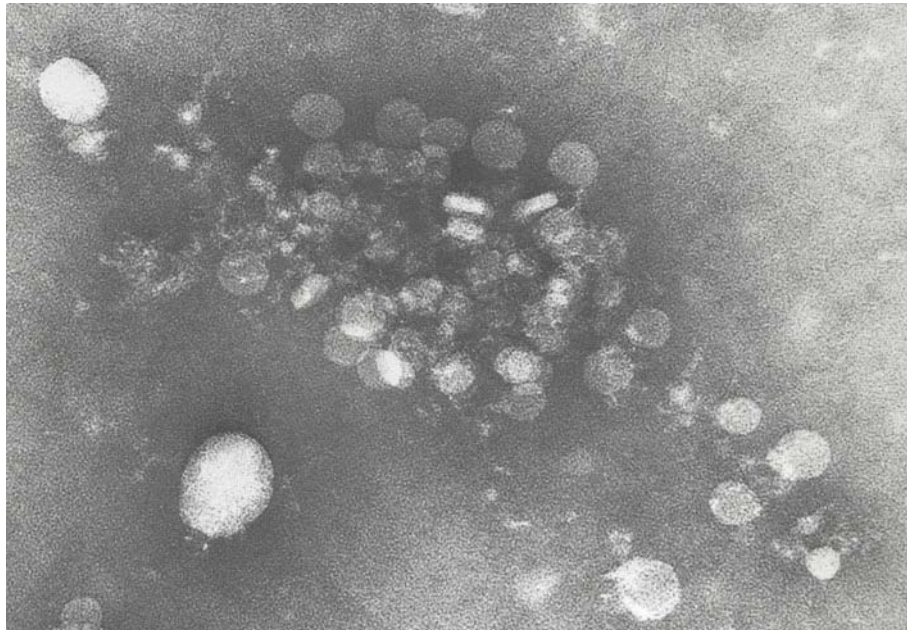
- **Deinhardt-1967, began a series of marmoset (Saguinus sp) inoculations using sera from icteric patients.**
- **GB, a 34 yo surgeon developed hepatitis and his serum on the 3rd day of jaundice transmitted hepatitis to 4/4 marmosets and was passaged several times**
- **Parks and Melnick later showed that this was likely an indigenous marmoset agent.**
- **A similar virus was identified in Germany termed the Berlin Agent**
- **Later - 1969 Deinhardt used MS-1 serum and showed true transmission to marmosets.**

Fritz Deinhardt & Friends



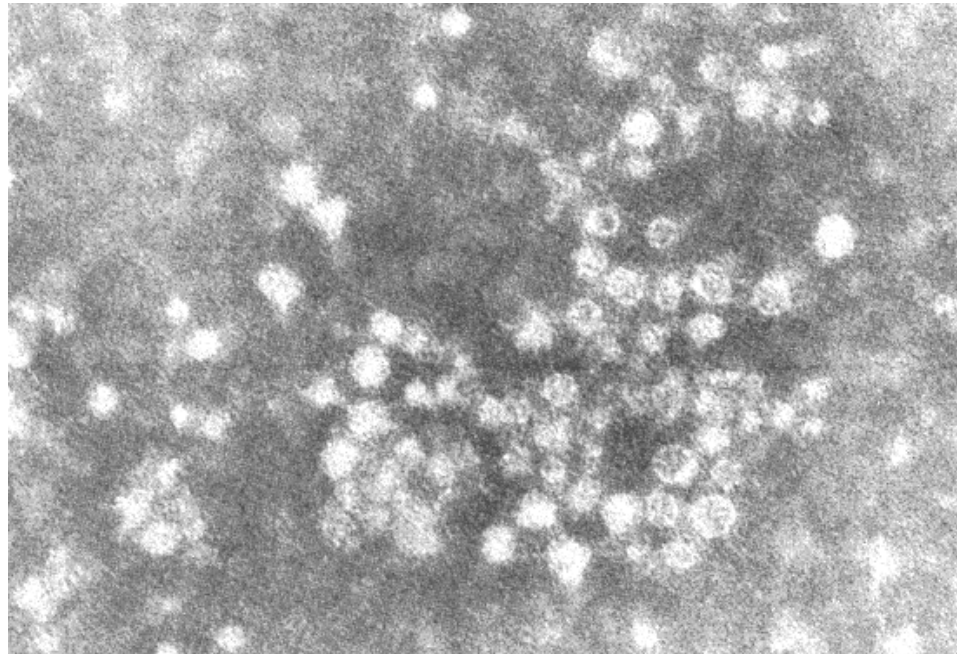
Gel Diffusion Immunoprecipitation

- **MILAN ANTIGEN** – Salvatore Del Prete et al. 1970
- Identified an antigen in the serum from patients with short incubation hepatitis using serum from multiply-transfused patient - reacted with both AuAg+ serum and AuAg- sera
- Shown by Taylor et al. to be abnormal serum lipoprotein

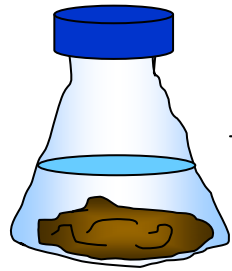


Gel Diffusion Immunoprecipitation

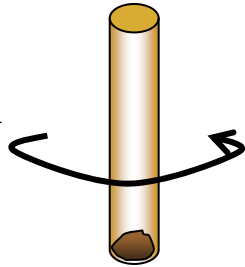
- **FECAL ANTIGEN** – Ferris, et al. 1970
- Originally described by gel immuno-precipitation using hemophiliac sera. Then fecal antigen was purified and used to raise antibody in rabbits.
- Ultimately, particles were observed by IEM using rabbit antiserum



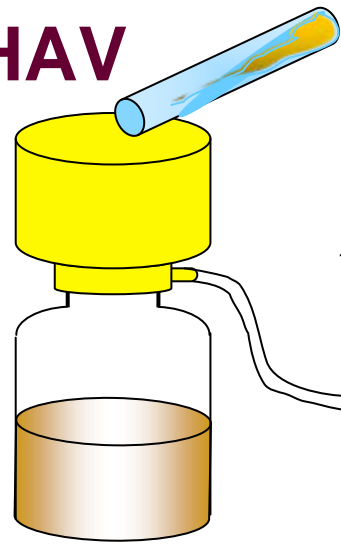
IEM for HAV



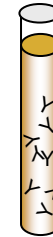
Extract stools



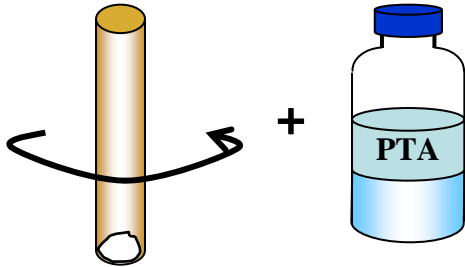
Low speed clarification



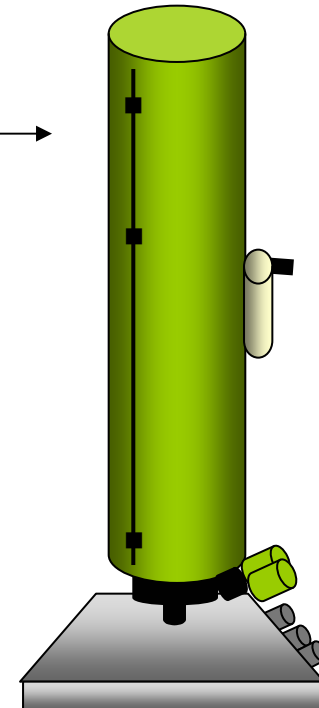
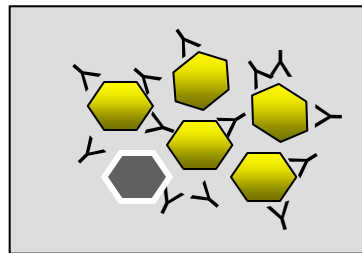
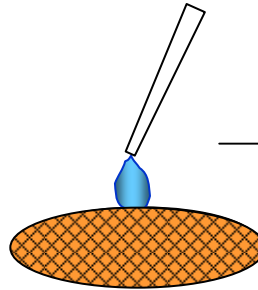
Filtration



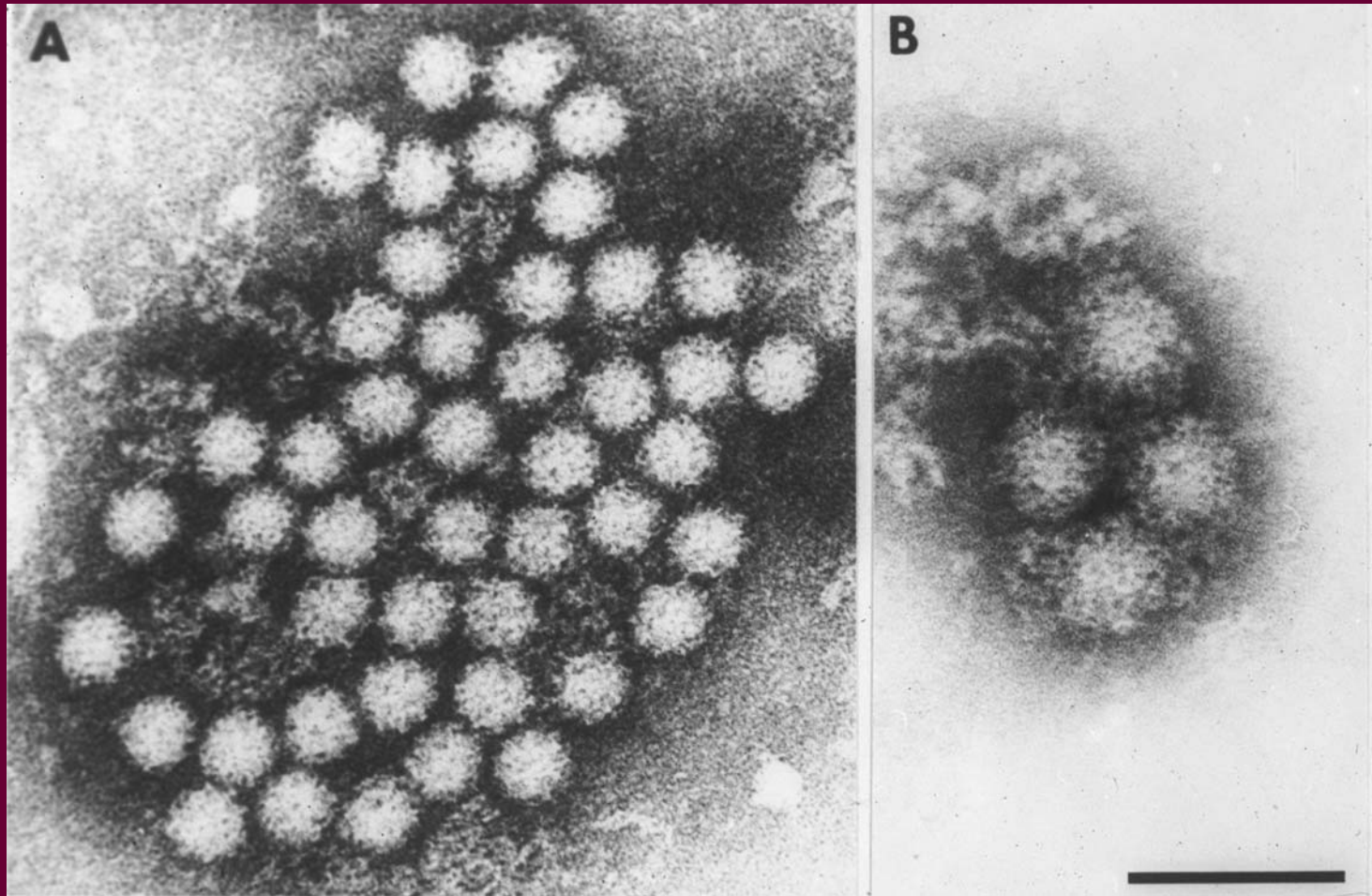
Incubate stool filtrate with antibody



**High speed centrifugation
Resuspend pellet and stain**

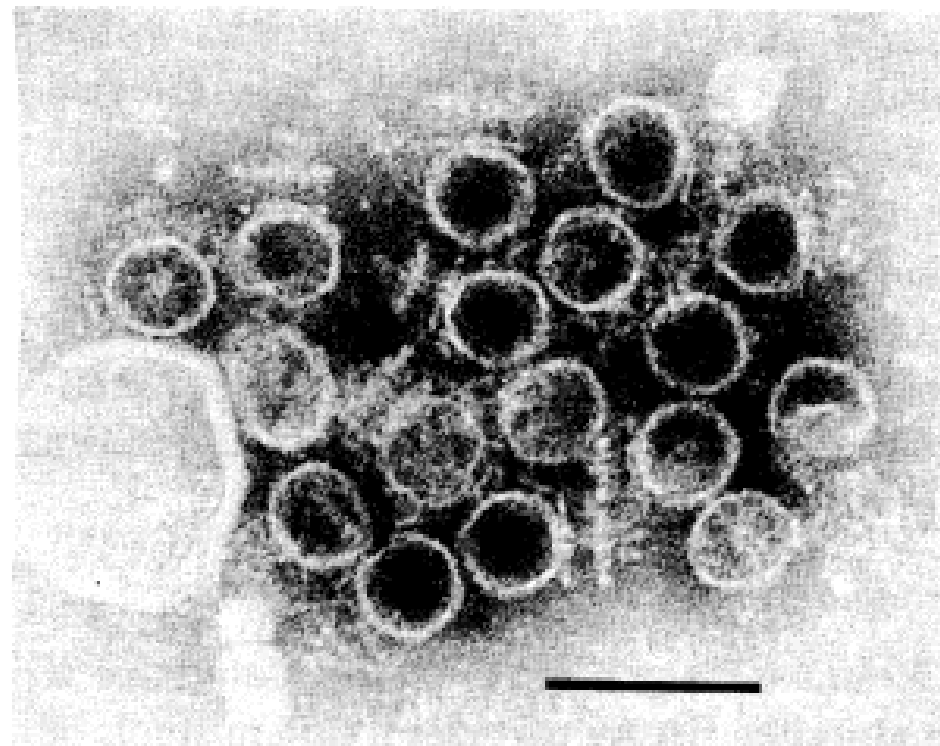
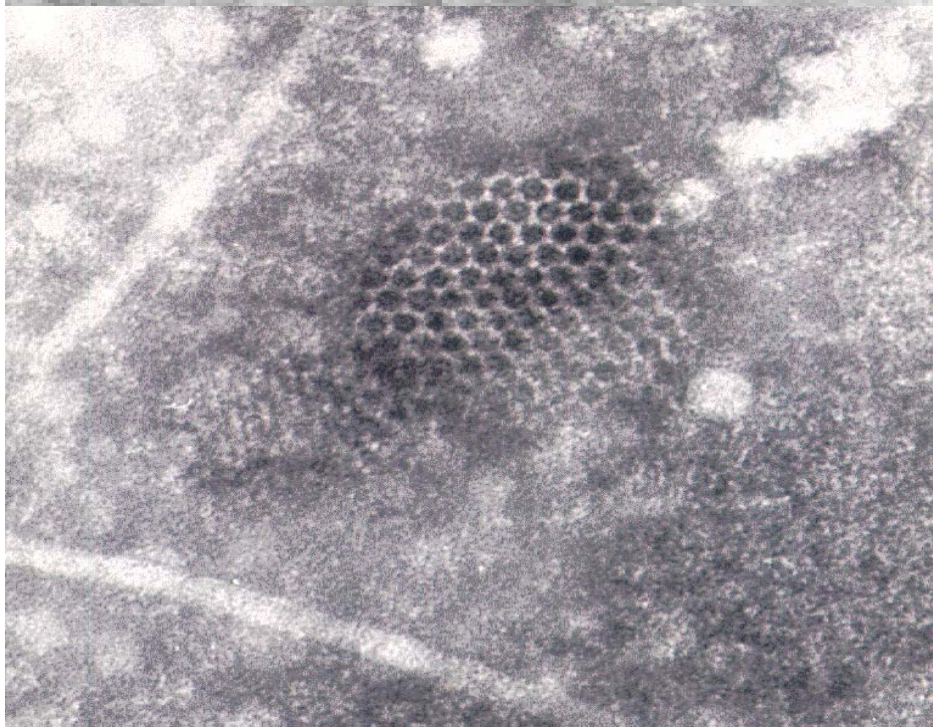
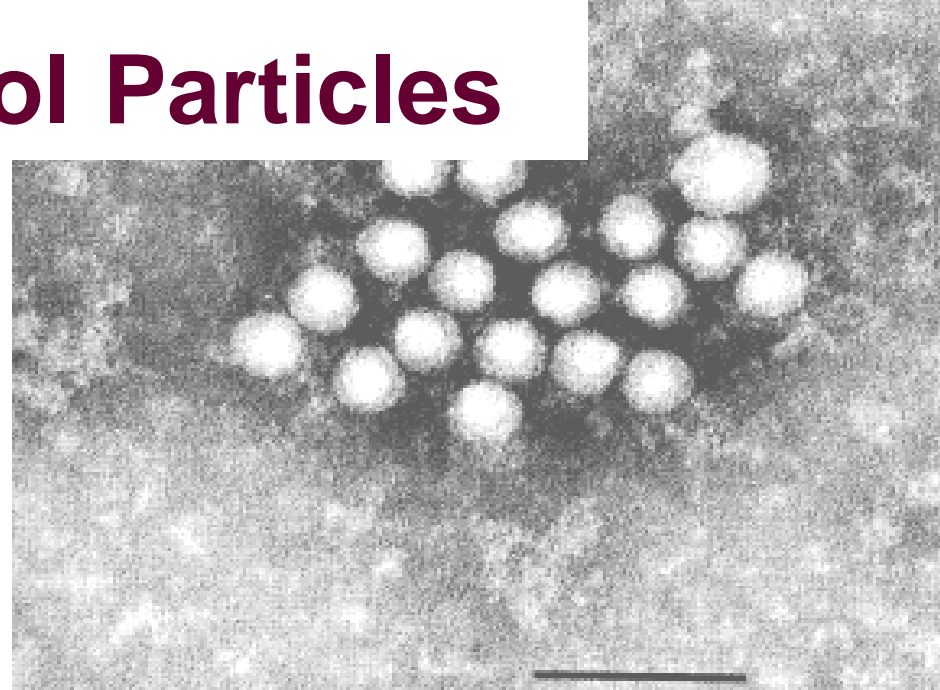
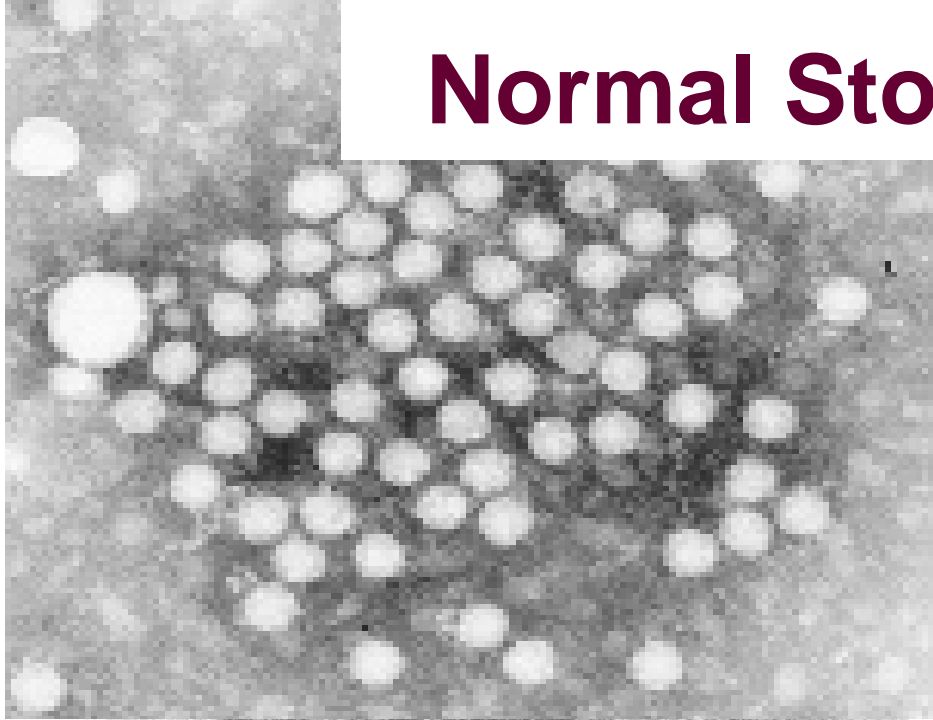


Norwalk Virus IEM



Kapikian et al., J. Virol. (1972) 10:1075

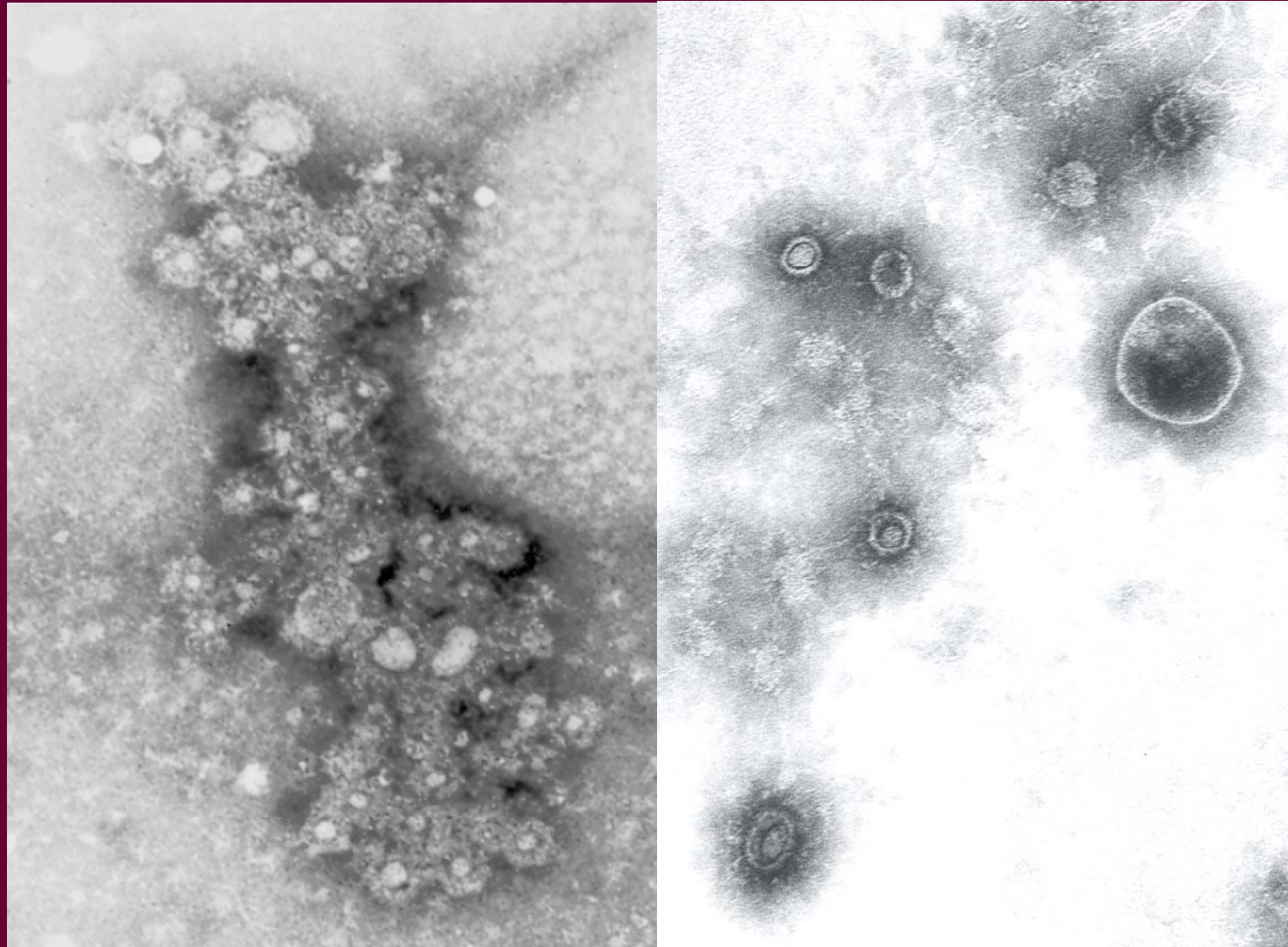
Normal Stool Particles



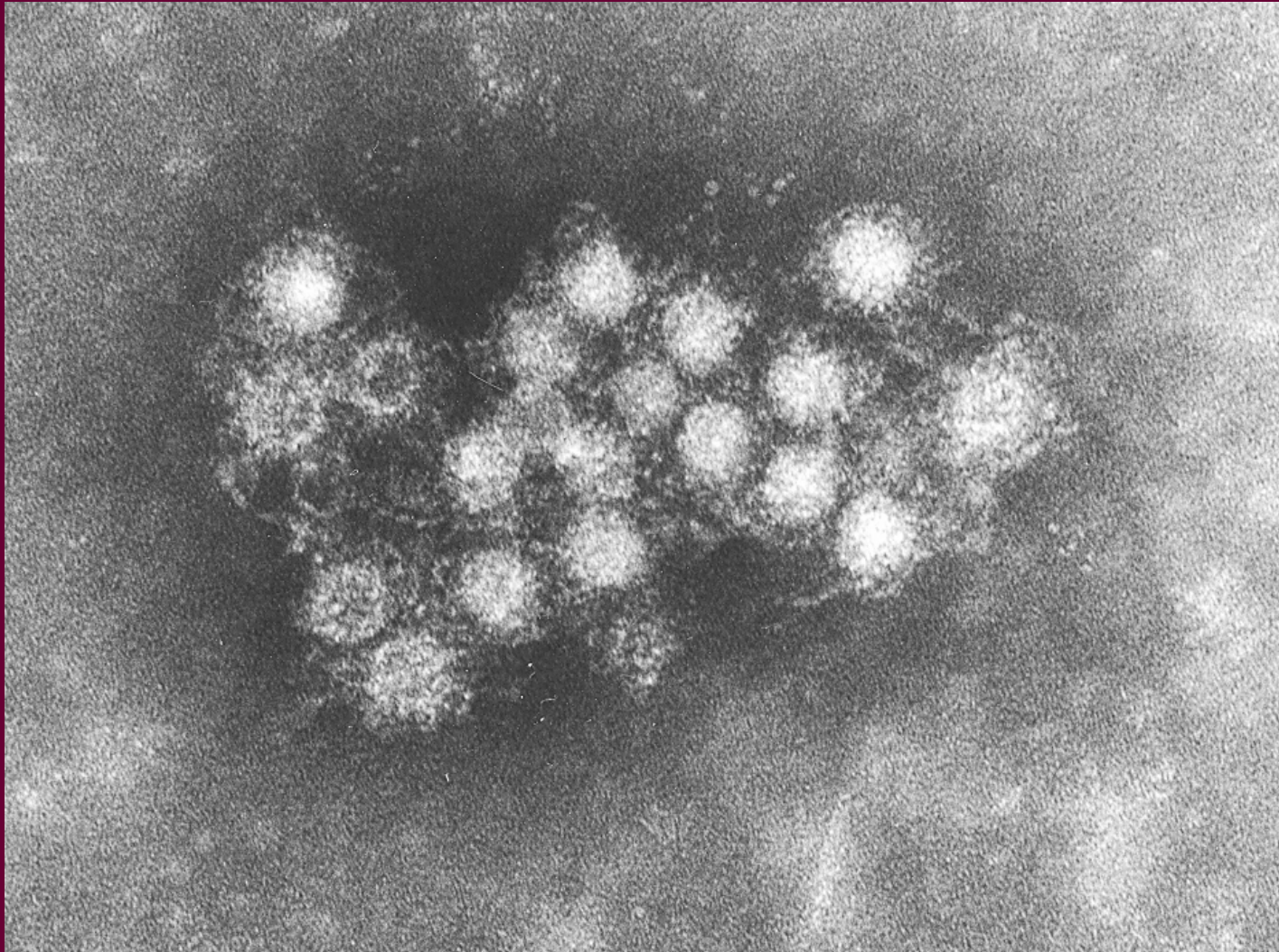
IEM Approach to the Search for HAV in Purcell's Lab

- **We knew that we should look in stools, not serum**
- **We used stool samples and sera from well characterized outbreaks and volunteer studies**
- **Stool filtrates were screened with ISG as the antibody for candidate virus-like particles**
- **All particles that were identified by ISG were then evaluated for HAV specificity by testing with paired HAV sera under code**

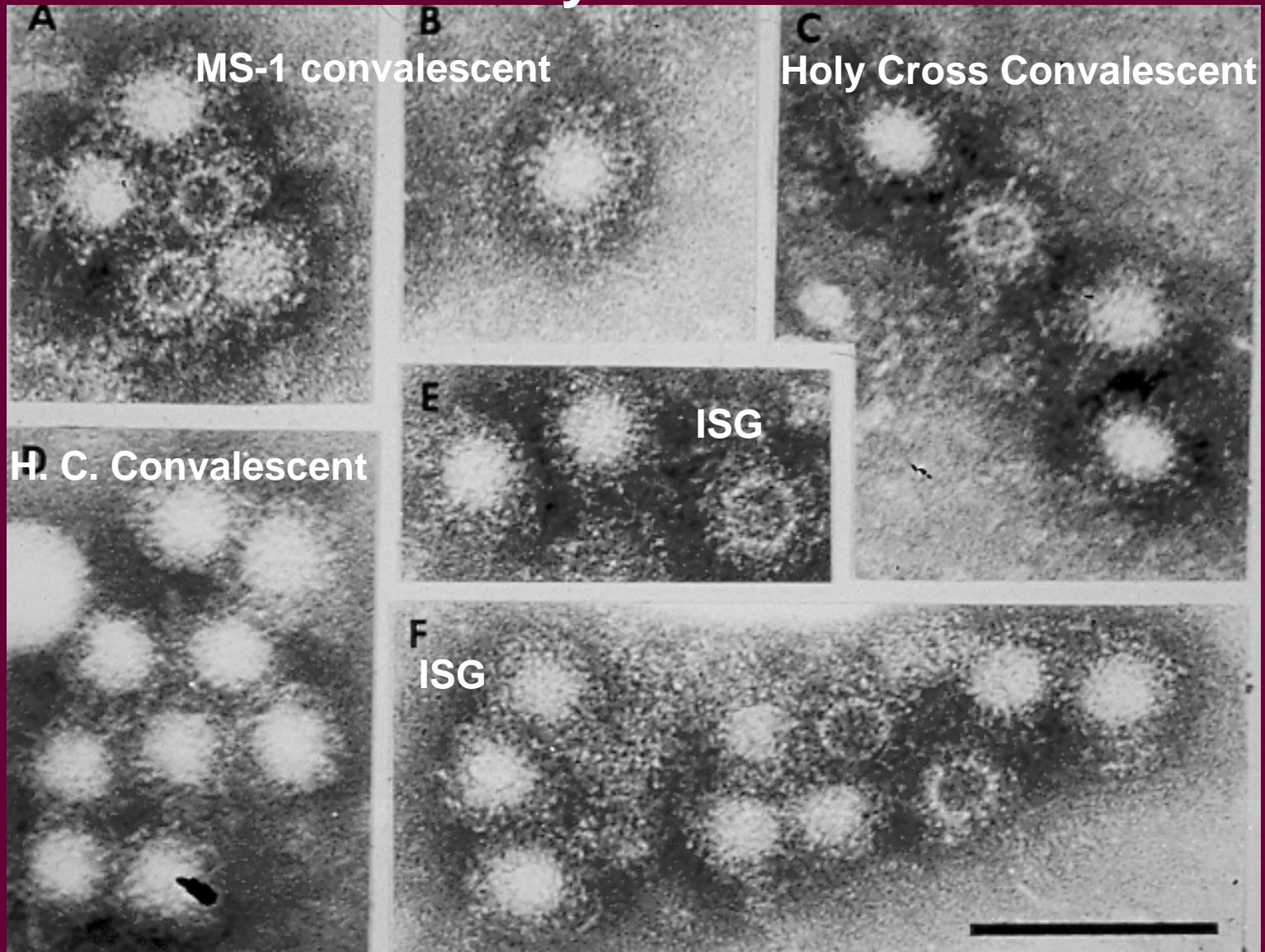
Virus-like Particles in Stool from Patient in Hepatitis Outbreak on Palau Island, Micronesia



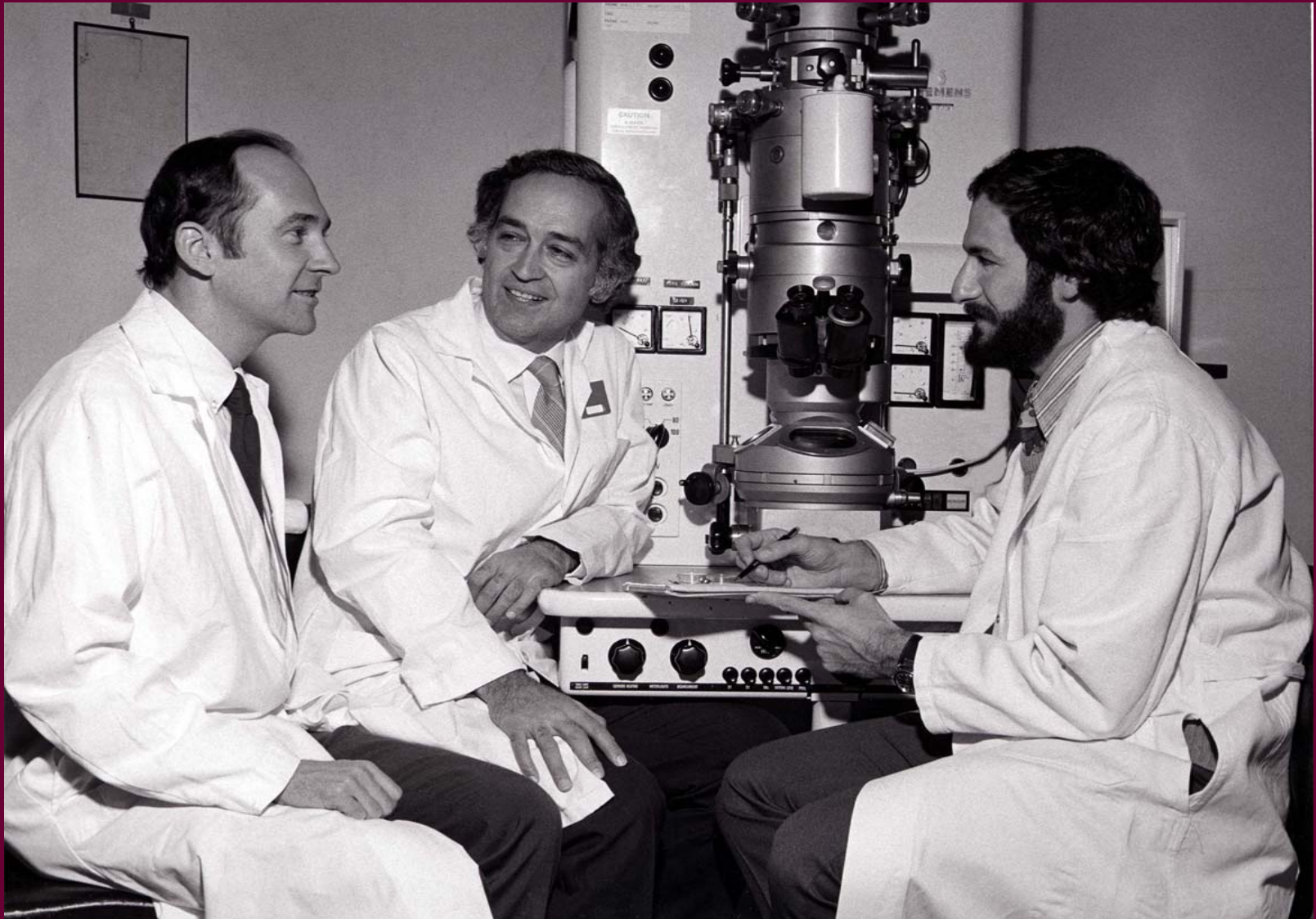
MS-1 HAV Aggregated by Immune Globulin



HAV by IEM



The Team



Anti-HAV by IEM

0+

1+

2+

3-4+

Serologic Proof of HA-Ag

Antibody to indicated antigen in first and second serum samples

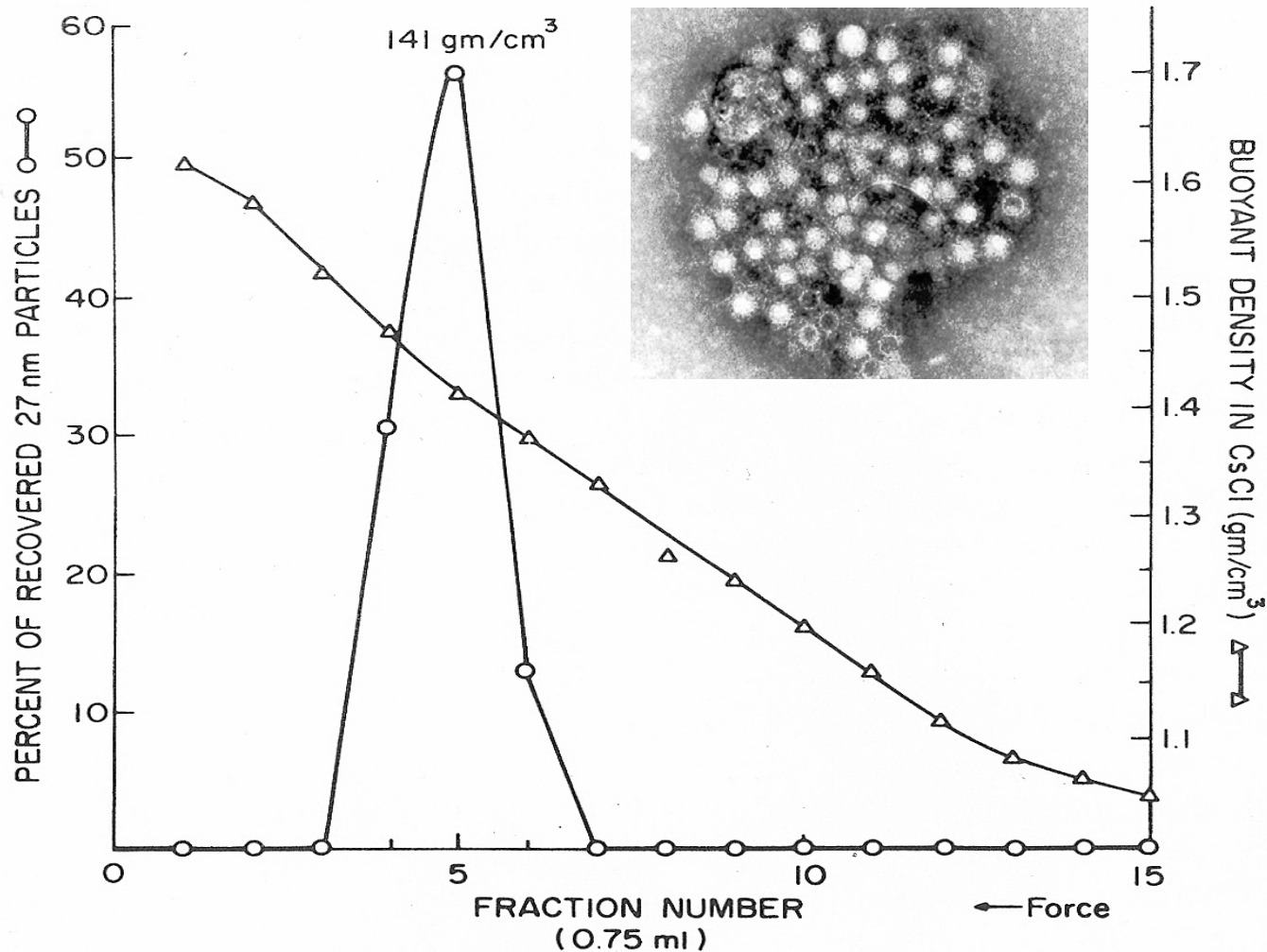
Patient No.	Hepatitis A antigen		Hepatitis B antigen		Norwalk gastroenteritis antigen	
	First	Second	First	Second	First	Second
<i>Experimental hepatitis A (MS-1), New York</i>						
1	0	1-2	—	—	NT	NT
2	0	1-2	+	+	NT	NT
<i>Experimental hepatitis A (MS-1), Illinois</i>						
3	0	3-4	—	—	1	1
4	0	3-4	—	—	1	1
5	0	1-2	—	—	NT	NT
6	0	3-4	—	—	NT	NT
<i>Naturally acquired hepatitis A, Massachusetts</i>						
7	0-1 (acute)	3-4	—	—	NT	NT
8	0 (acute)	3	—	—	NT	NT
9	0 (acute)	3	—	—	NT	NT
<i>Naturally acquired hepatitis A, American Samoa</i>						
10	0	2	—	—	NT	NT
11	0	3	—	—	NT	NT
12	1-2 (acute)	3-4	—	—	NT	NT
<i>Naturally acquired hepatitis B</i>						
13	0	0	—	+	NT	NT
14	0	0	—	+	NT	NT
<i>Experimental nonbacterial gastroenteritis</i>						
15	0	0	—	—	1	4
16	2-3	2-3	—	—	1	4

Serologic comparison of HA-Ag and Fecal Ag by IEM

ANTISERUM	Antibody Score When Incubated With	
	HA Ag	Fecal Ag
Human Convalescent (MS-1)	3+	0
Rabbit Anti-Fecal Ag		
F1	0-1+	3+
125	2-3+	3+
521	0	3+

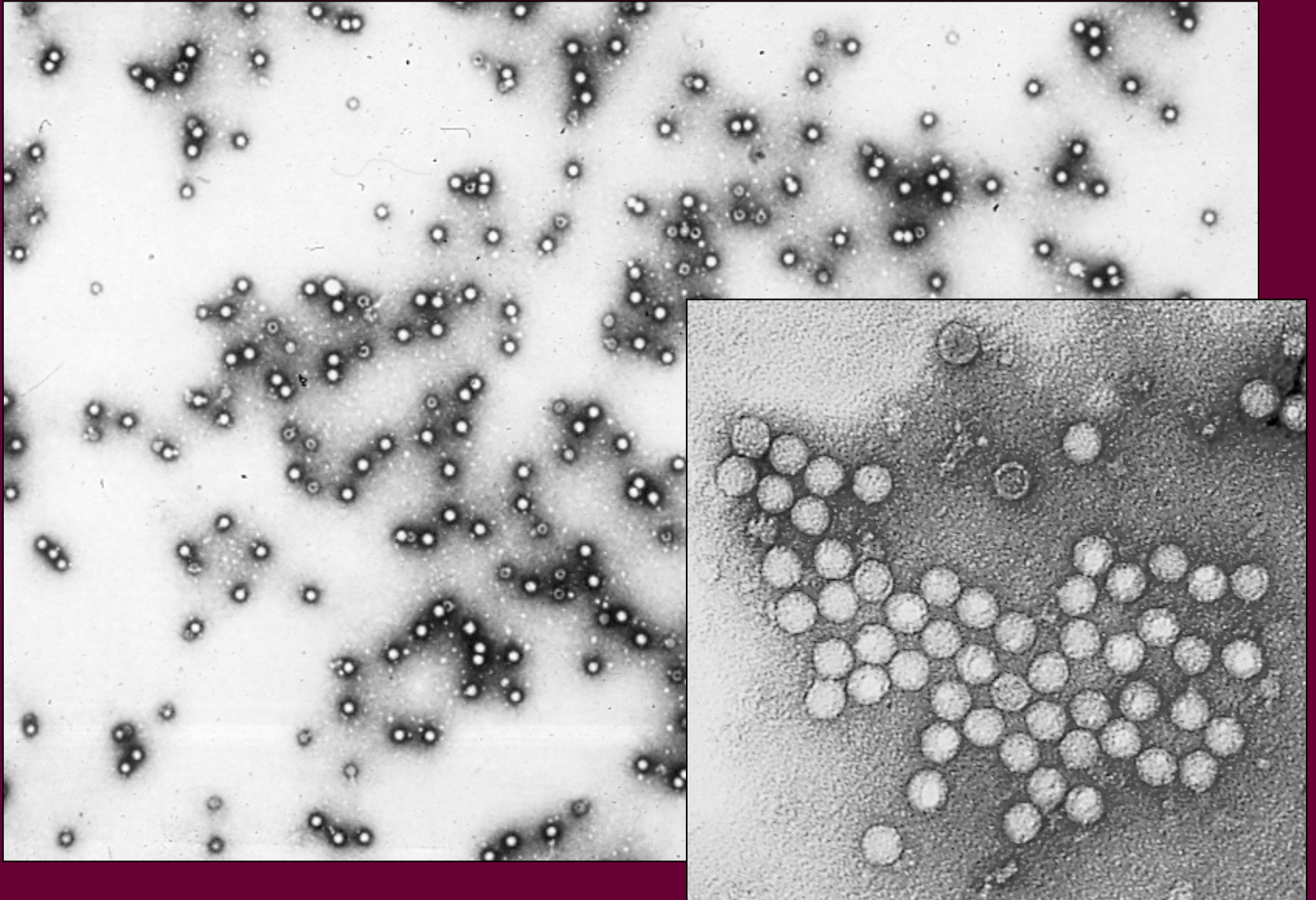
Characterization of HAV

Density of HAV by IEM



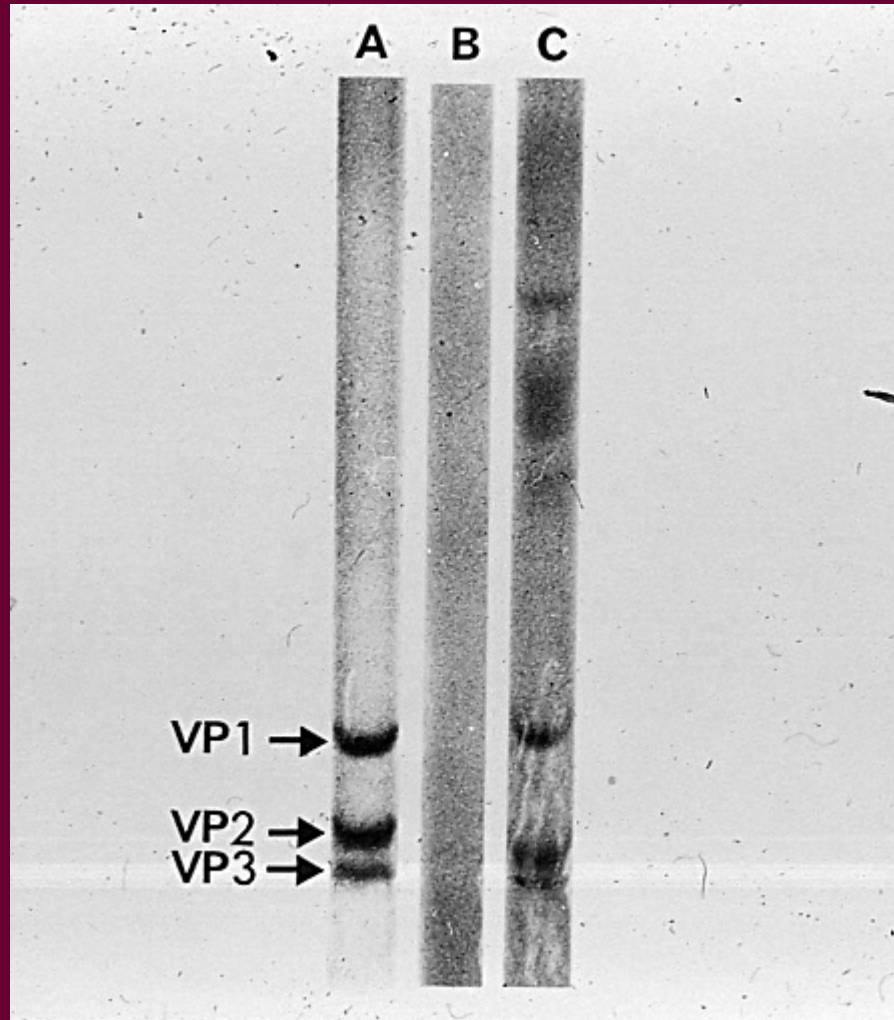
Characterization of HAV

Purified HAV

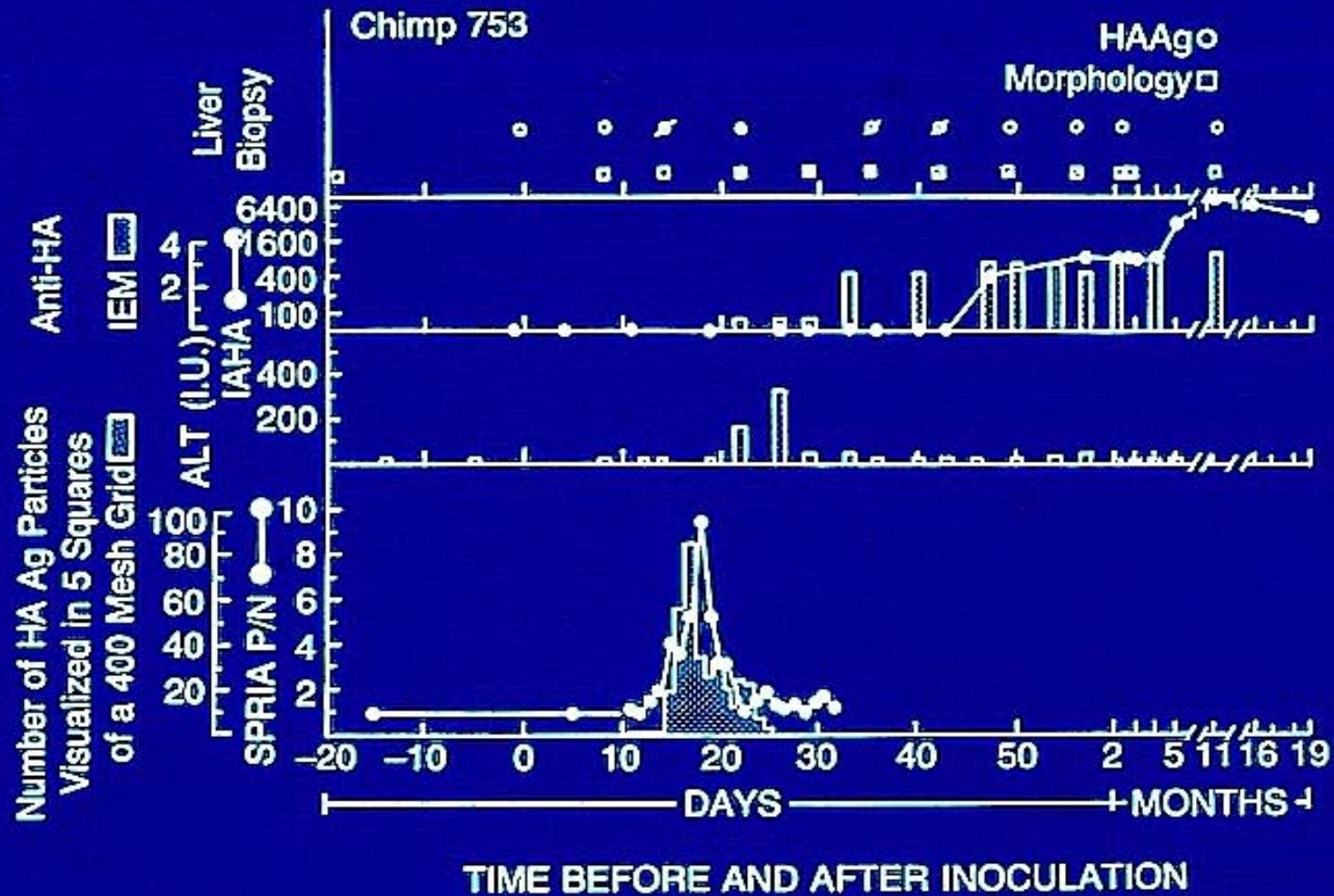


Characterization of HAV

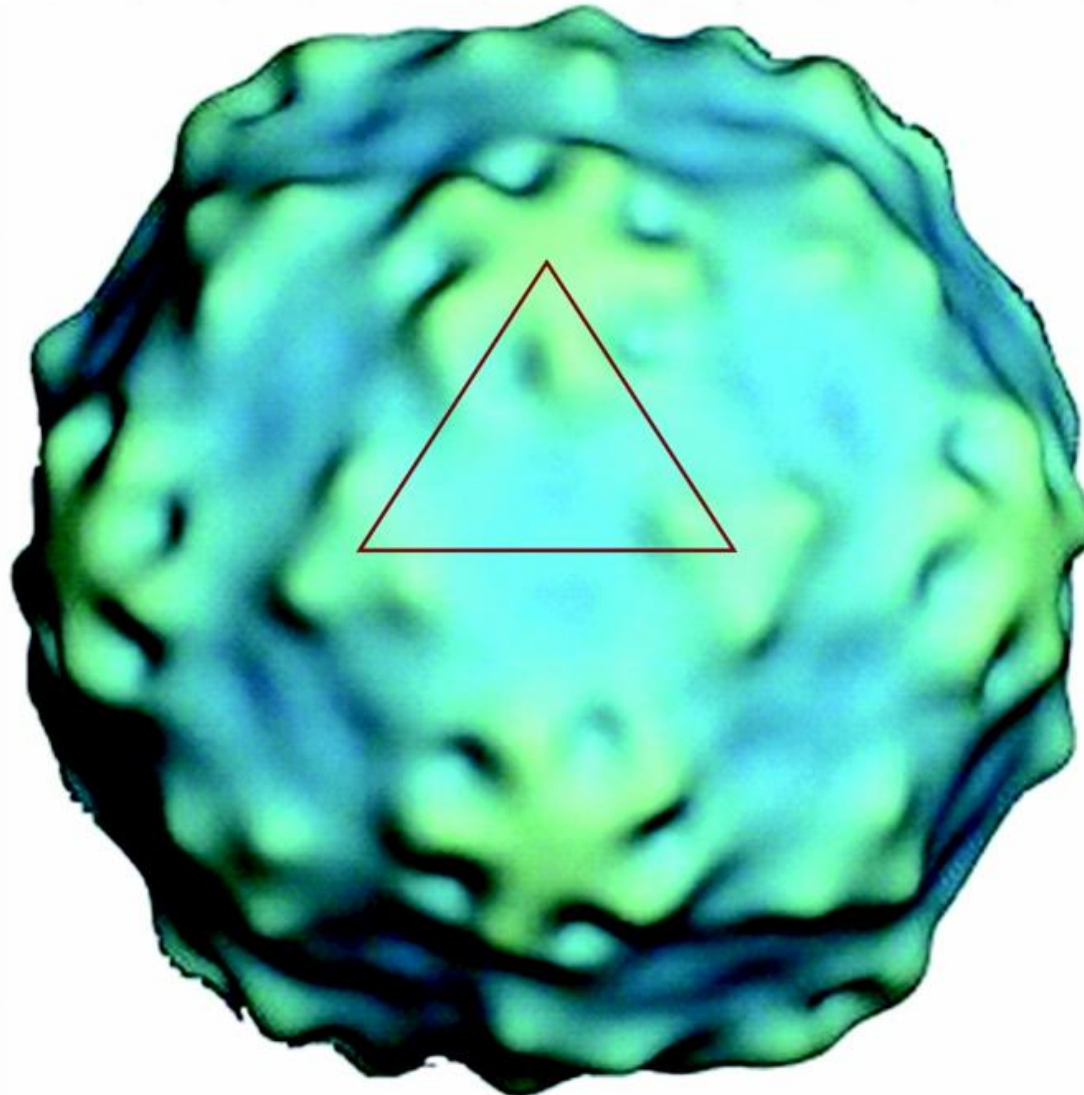
HAV Proteins – Western Blot



HAV Infection of Chimpanzee



Cryoelectron Microscopy of HAV



Holland Cheng in Martin and Lemon, 2006