Absence of Circulating Interferon in Patients with Infectious and Serum Hepatitis* (32989)

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Hepatitis, both infectious (IH) and serum (SH), has a markedly variable clinical course. The basis for the fluctuations in clinical severity is unknown. Since hepatitis is a disease of probable virus etiology, fluctuations of interferon production *in vivo* might be responsible for, or at least reflect, the varied clinical course. *In vitro* studies of certain virus-cell systems have revealed that virus replication and cell destruction are inversely related to interferon production in the cell cultures (1,2). Interferon is a small protein produced in cells in response to viral infections as well as to exposure to various nonviral agents and has been shown to have strong virus-inhibitory properties *in vitro* and *in vivo* (3). Interferon has been detected in a variety of human viral infections including both naturally occurring infections (4–7) and those produced by inoculation of live virus vaccines (8–11).

The present communication reports our attempts to detect interferon in the sera of 34 patients hospitalized for hepatitis between May and December 1965 at the University of Texas Southwestern Medical School Hospitals.

The diagnosis was established clinically in all 34 patients and confirmed by liver biopsy in 11. As shown in Table I, all the patients were hyperbilirubinemic on admission and showed considerable elevation of serum SGOT, but the course was variable with hospitalization ranging from 1–45 days (mean 16.6 days) and death in 2 individuals. Details of some of the clinical findings and data regarding the time of interferon sampling with respect to hospitalization are given in Table I.

Interferon was assayed, according to a previously described procedure (9), by determining the ability of serum dilutions to protect human fetal lung cell monolayer cultures against the cytopathic effects produced by subsequently inoculated Sindbis virus.

Interferon assays of sera were conducted in general at dilutions between 1:20–1:80. Most of the serum samples produced marked toxicity in human lung cell cultures at dilutions lower than 1:20 and it was therefore not possible to test them at these low dilutions.

No virus-inhibitory activity was detected in any of the 226 serum samples. These negative results are considered to be significant in that the interferon assay employed was identical to that used previously to detect circulating interferon in titers of 1:10–1:80 following vaccination with the 17D strain of yellow fever virus (9) and active Asian influenza virus (11).

The absence of detectable circulating interferon in the serum of the patients with hepatitis lends itself to several possible interpretations. (i) The viruses that presumably cause hepatitis may not stimulate the formation of interferon, at least to detectable levels. Although no single virus has been isolated with any consistency from patients with hepatitis, it may be pertinent that adenoviruses, which are poor inducers of interferon, have been found in serum, stool and urine of some hepatitis patients (12). Deinhardt *et al.* (13) have recently demonstrated that inoculation of serums or plasmas obtained from patients during the acute phase of hepatitis can induce chemical and morphological hepatic disease in marmosets. The production of hepatitis in marmosets may provide a good experimental model in

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TABLE I. Patient Material.

	Hepatitis patients				
	IH[a]		SH		
Sex	White	Negro	White	Negro	Total
Male	10	5	1	1	17
Female	6	8	2	1	17

Onset icterus before admission to hospital (days)[b]	
Mean	4.8
Range	0–14
SGOT on admission (Karmen Units)	
Mean	1138
Range	48–3692
Mean peak during hospitalization:	1414
Bilirubin on admission (total bilirubin in mg/100 ml)	
Mean	9.1
Range	2.0–20.8
Mean peak during hospitalization:	12.2
Interferon assays	
Total no.	226
Mean no. of assays/patient	6.6
Mean duration of assay period (days) from onset of hospitalization:	28.7
First specimen obtained (days after admission to hospital)	
Mean	2.4
Range	0–7

[a] Four subacute, 2 deaths.

[b] Four subacute cases with icterus of 90, 21, 21, and 2 days prior to this admission, respectively, were not included in the calculation.

which to study the role of interferon in the pathogenesis of this disease. (ii) The interferon assay may have been carried out too late in the course of illness to detect the presence of the virus-inhibitory substance. Future studies on interferon in patients with hepatitis should therefore be conducted during the incubation period and before the onset of icterus. (iii) Liver disease may suppress interferon elaboration. This possibility is much less likely since one would have expected a spectrum of interferon levels corresponding to the severity of liver involvement and this was not found. (iv) Certain viruses have been shown to stimulate cells to produce substances which either block the synthesis (14) or action (15) of interferon. It is possible that such substances are produced in patients with hepatitis. (v) The failure to detect interferon in patients with hepatitis may reflect the relative insensitivity of the assay technique; attempts to detect interferon in this disease might thus be postponed until more sensitive assay systems are available. It is therefore evident, that by current techniques, assay for serum interferon will not permit a clinical differentiation between viral and toxic etiology in patients with hepatic necrosis of unknown origin.

Addendum: Zuckerman and Taylor of the London School of Hygiene and Tropical Medicine (personal communication), using differentiated human embryo liver cell cultures, have been unable to detect any virus-inhibiting substances in the peripheral blood of patients and known carriers of hepatitis "virus."

Summary. Multiple serum samples from 34 patients in various stages of acute and chronic hepatitis were collected during a 7-month period and assayed for interferon. The diagnosis of infectious and serum hepatitis was based on accepted clinical and laboratory criteria. No virus-inhibitory activity characteristic of interferon was detected in any of the serum specimens.

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Problems in the Detection of Rubella Virus in African Green Monkey Kidney Tissue Culture (32990)

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The isolation and study of viral agents has been dependent on innovation and new techniques. As these new methods have come into common usage, numerous difficulties have become apparent and more guide-lines and controls are required to insure the proper interpretation of results. In studies with rubella virus, the interference technique demonstrating the presence of rubella virus is in common usage. Recent studies using this technique with Coxsackie A-9 as a challenge virus have given false negative results in our laboratory. Samples previously showing high titer in AGMK demonstrate reduced titer or no detectable virus when re-titrated in different lots of this same tissue. The present study was undertaken to determine the relative sensitivity of various lots of African green monkey kidney tissue culture. At the same time an attempt was made to correlate the sensitivity of the tissue to rubella virus with complement-fixation titers for SV_5 of the donor monkeys and hemadsorption in the tissue culture system.

Materials and Methods. Virus strains. The RV strain (1) rubella virus in the fourteenth and sixteenth passage in AGMK was used to test the sensitivity of AGMK tissue culture used. Coxsackie A-9 was used as the challenge virus in the interference test.

Cell cultures for titrations. Primary African green monkey (*Ceropithecus aethiops*) kidney (AGMK) roller tube cultures grown in the presence of SV_5 hyperimmune rabbit antiserum were obtained from Microbiological Associates, Inc., Bethesda, Maryland and Flow Laboratories, Rockville, Maryland. They were maintained with 1.5 ml of a medium consisting of Eagle's basal medium Earle's balanced salt solution, 2% fetal bovine serum, 5 μg of amphotericin B, 10 μg of polymyxin, 30 μg of erythromycin, and 100 μg of streptomycin/ml.

Interference titration procedure. Infectivity titers of rubella virus in AGMK tissue cultures were determined by a modification of the enterovirus interference technique as described by Sever *et al.* (1). Briefly, serial 10-fold dilutions of virus samples were made in Hanks' balanced salt solution containing 2% fetal bovine serum, 100 μg of streptomycin, and 100 μg of neomycin/ml. Three tubes per dilution were inoculated with 0.2 ml of virus and incubated at 37°C. Eight to 10 days after inoculation, the medium was removed and replaced with fresh maintenance medium containing 100–1000 $TCID_{50}$ of Coxsackie A-9. The cultures were incubated for an additional 3–4 days and examined microscopically for the cytopathogenic effect of Coxsackie virus. The absence of cytopathogenic effect was interpreted as indicating the interfering effect of rubella virus. Fifty percent interference ($TCInD_{50}$) end points were calculated by the method of Spearman and Kaerber (2).

Complement-fixation procedure. The monkeys were bled when they were received and the sera were used in the complement-fixation test for SV_5 according to the microtechnique previously described (3).

Hemadsorption procedure. The hemadsorption test (HAd) was performed on the first 10 lots listed in Table II after the cells were held