Intrinsic Interference Caused by Hepatitis Sera[1] (37394)

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Study of the viruses involved in human hepatitis would be aided by the multiplication of these viruse *in vitro*. Production of cytopathic changes in tissue culture by these hepatitis viruses has not been described. To demonstrate possible noncytopathic multiplication of these viruses new methods had to be sought. These were found in the "Nuclear Antigen in Virus Infection" [Berthold and Luthardt (1, 2)] as well as in the system of "Intrinsic Interference" described by Marcus and Carver (3, 4) and Carver and Seto (5, 6). The following is a report of the results obtained with regard to hepatitis virus assay by means of the latter system.

Materials and Methods. Tissue culture. Human diploid fibroblast cells developed in our laboratory from human embryonic lungs were used as a cell system.

Hepatitis sera. Australia antigen (Au) antigen-positive and Au antigen-negative sera from the acute phase of disease were obtained in a sterile manner and stored at $-22°$.

Three milliliters of cell suspension containing about 1.5×10^6 cells in Eagle's medium were placed into each culture flask. After formation of a monolayer 3.0 ml of a 1:300 dilution of each hepatitis serum (in galactose–Eagle BHK medium) was added to the appropriate culture flasks. The cell cultures were then incubated at 37°. After 24 hr the medium was changed. Forty-eight hours later the incubation temperature was reduced to 28°. This temperature was maintained until 12 hr prior to challenge with Newcastle disease virus (NDV) when the cells were again incubated at 37°. NDV challenge was usually carried out on Day 20 after infection with hepatitis sera. Medium changes were only undertaken if the medium was too acid or too alkaline.

Challenge with Newcastle disease virus. The Herts Newcastle disease virus (NDV) was used. This NDV had been grown in the chorioallantoic sac of the embryonated chick egg.

Challenge of the tissue culture with NDV was done with approximately 100 PFU/cell. This was contained in 0.5 ml of medium. Adsorption of the NDV was carried out at 37° for 60 min. During adsorption the cultures were gently agitated twice. Subsequently they were washed twice with galactose–Eagle medium and 3.0 ml of this medium were added. Hemadsorption was done after incubation at 37° for 16 to 17 hr.

Hemadsorption. For hemadsorption a 1% guinea pig erythrocyte suspension in phosphate buffered saline (PBS) was used. Adsorption of red cells was done for 40 min at 4°. Subsequently the cultures were rinsed carefully three time with PBS at 4°.

Results and Discussion. Cultures which had been infected with hepatitis sera developed intrinsic interference. Twelve days after infection with hepatitis sera these cultures showed hemadsorption-negative plaques following NDV challenge. These plaques increased in size following NDV challenge until Day 20 after infection with hepatitis sera. At this time large hemadsorption-negative areas were often found.

Figure 1 shows hemadsorption on cells infected only with NDV virus. Homogeneous hemadsorption was found.

Figure 2 shows the hemadsorption which

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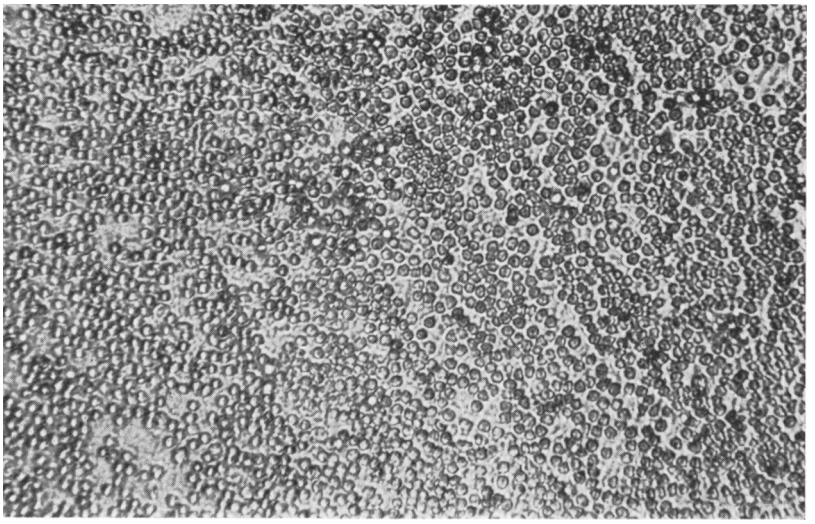

FIG. 1. Red cell hemadsorption after NDV challenge of previously uninfected human diploid fibroblast cells.

occurred after challenge with NDV of cells preinfected with a hepatitis virus. Large hemadsorption-negative areas were found. The cultures proved resistant to NDV infection. The phenomenon of intrinsic interference was induced by Australia antigen (Au-Ag)-positive as well as by Au-Ag-negative hepatitis sera.

Table I shows the results of the investigation of 39 acute hepatitis sera. Twenty-eight of them caused typical plaques. Thirteen of these were Au-Ag-positive, 15 were Au-Ag-negative. Eleven sera which had been examined in related test series produced no definite results. The possible cause of these uninterpretable experiments are dealt with below.

One of 10 normal sera induced plaques. The examination of 9 Au-Ag-positive sera from healthy blood donors showed negative

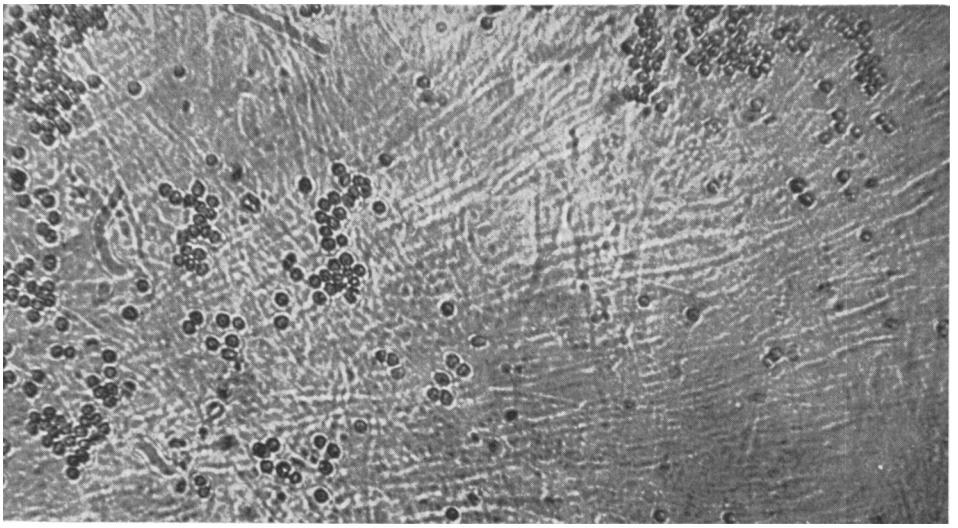

FIG. 2. Red cell hemadsorption after NDV challenge of human diploid fibroblast cells previously infected with hepatitis serum.

TABLE I. Intrinsic Interference Caused by Hepatitis and Blood Donor Sera.

Sera investigated		Intrinsic interference		No results
		Positive	Negative	
Hepatitis sera	39			11
HB[a] Ag pos	17	13	—	4
HB[a] Ag neg	22	15	—	7
Blood donors	19			
HB[a] Ag pos	9	1	8	—
HB[a] Ag neg	10	1	9	—

[a] Hepatitis B antigen.

results in 8 cases, *i.e.*, homogeneous hemadsorption after NDV challenge. One serum developed plaques which were also confirmed in a repeat test. Four hepatitis sera were titered using this system of intrinsic interference. The titers thus obtained were between 10^{-7} and 10^{-9}. In this test passage material taken from 3 Au-Ag-positive and 4 Au-Ag-negative hepatitis sera produced intrinsic interference in the same manner as did the original serum. Passage material from cells inoculated with a 10^{-5} dilution of the original sera produced intrinsic interference in dilution through 10^{-5}.

The capacity of Au-Ag-positive or Au-Ag-negative hepatitis sera for induction of intrinsic interference could not be neutralized by gamma globulin nor by hemophiliac serum containing Au antibodies.

The system of intrinsic interference and the results obtained with regard to hepatitis virology would not be adequately described if certain basic difficulties were not pointed out. As mentioned above, the examination of 11 hepatitis sera did not show definite results. In these tests the cell controls also showed nonhomogeneous hemadsorption. However, homogeneous hemadsorption of the cell controls is necessary for an interpretable experiment. The cause of the nonhomogeneous hemadsorption of the cell controls is probably an inapparent infection of the cell culture with another virus. In this connection one must mention Hallauer, Kronauer and Siegl (7). In 36 of 41 examined permanent human cell strains Hallauer, Kronauer and Siegl found contamination with PARVO viruses.

Summary. With the use of the intrinsic interference system it is possible to demonstrate in the sera from patients with acute hepatitis (both Au-Ag-positive and negative) agents which are infectious for human tissue cultures and which can be passed in them. These agents were not neutralized by gamma globulin nor by a hemophiliac serum containing Au antibodies when attempts were made to neutralize the virus in the initial serum taken from hepatitis patients.

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