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# ANTIBODY RESPONSE IN HEPATITIS A AND SOME OTHER ENTEROVIRAL INFECTIONS: QUANTITATIVE COMPARISON

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*Summary.* — Antibody levels produced in humans in the course of hepatitis A were compared with those in Coxsackie B2 infection and vaccination with live poliovaccine (type 1) using the method of immune-electron microscopy. It has been shown that the levels of antibody in hepatitis A were 25 to 800-times higher than in Coxsackie B2 infection and than after vaccination with the live poliovaccine.

*Key words: hepatitis A virus; enteroviruses; antibody; immune electron microscopy*

It has been concluded from a number of studies that human hepatitis A virus (HAV) shares many characteristics of genus *Enterovirus* of the family *Picornaviridae* (Matthews, 1979). Certain similarities have been observed also in epidemiological patterns of hepatitis A and enteroviral infections, including faecal-oral mode of transmission and a high rate of subclinical forms. However, in natural and experimental hepatitis A virus multiplication was found to occur only in liver cells (Mathiesen *et al.*, 1977; Murphy *et al.*, 1978) while in conventional enterovirus infections the viruses are capable to grow in a variety of tissues such as intestinal epithelium, lymph nodes, central nervous system etc.

Our study was focused on the levels of antibodies produced in humans in the course of hepatitis A as compared with Coxsackie B2 infection and vaccination with live poliovaccine (type 1 LSc 2ab, Sabin strain). Sera from rhesus monkeys hyperimmunized with wild polioviruses types 1 and 3 were also included into the study.

Antibodies to hepatitis A, Coxsackie B2 and polioviruses in corresponding sera were determined by direct visualization of virus-antibody reaction in immune electron microscopy (IEM). The technique and criteria for the evaluation of results were similar to those described by Dienstag *et al.* (1976). This method has been chosen in order to determine the content of antibodies to all viruses tested by the same procedure. In brief, the presence of single particles or small aggregates abundantly coated with antibody was interpreted as four-cross reaction (4+); small or large aggregates with excess of antibodies as 3+; large regularly arrayed aggregates with little antibody around as 2+; small aggregates with no visible antibody as 1+ (Fig. 1—4). Alternatively, antibody to HAV (anti-HAV) was measured by solid-phase enzyme immunoassay

**Table 1. Antibody titres to HAV, polio and Coxsackie viruses in human and monkey sera as determined by IEM**

| Serum source | Interval of blood sampling* | Antibody titre | Antibody titre in IEM[4] (reciprocals) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 4+ | 3+ | 2+ | 1+ | 0 |
| HA, convalescent | 32 years | 1,000[1] | 100 | | 1,000 | 10,000 | |
| HA, convalescent | 1 month | 200,000 | 500 | | 5,000 | 50,000 | |
| HA, convalescent | 1 month | 20,000 | 200 | 2,000 | 20,000 | | |
| HA, convalescent | 1 month | 20,000 | 200 | 2,000 | 20,000 | | |
| HA, convalescent | 1 month | 20,000 | 200 | 2,000 | 20,000 | | |
| Coxsackie B2, convalescent | 2 years | 256[2] | | | 25 | | 125 |
| Coxsackie B2, convalescent | 2 years | 256 | | | 25 | | 125 |
| Coxsackie B2, convalescent | 2 months | 8,000 | | | 160 | 800 | |
| Polio 1, vaccinee | 2.5 months | 600[3] | | | 25 | | 125 |
| Polio 1, vaccinee | 1.5 months | 1,500 | | | 50 | | 500 |
| Polio 1, vaccinee | 1.5 months | 3,800 | | | 200 | 2,000 | |
| Polio 1, hyperimmunized monkey | — | 40,000 | 200 | 2,000 | | 20,000 | |
| Polio 3, hyperimmunized monkey | — | 20,000 | 100 | | 1,000 | 10,000 | |

[1] Results of enzyme immunoassay, 50% blocking effect.
[2] Tissue culture end-point titration against 100 $TCID_{50}$ of the virus.
[3] Plaque reduction test, 50% dose.
[4] Rating according to serum dilutions.
* Since onset of the disease or since vaccination.

(EIA, blocking variant) and antibodies to Coxsackie B2 and polioviruses were titrated in the neutralization test by inhibition of the cytopathic effect and plaque number reduction respectively using primary vervet monkey kidney cell cultures.

From sera of children vaccinated with live poliovaccine-three specimens have been selected with antibody titre comparable to that developed in natural poliomyelitis infection. Two convalescent sera from patients with Coxsackie B2 infection showed a typical antibody titre of 256, while one convalescent serum had an unusually high antibody level of 8,000. Antibody levels in sera of recent convalescents from hepatitis A were as high as 20,000 which was also typical for this disease.

Virus suspensions of poliovirus and Coxsackie B2 virus used as antigen were infectious tissue culture fluids harvested from primary monkey kidney cells; hepatitis A virus antigen was a 10% aqueous extract of the stool from a patient with serologically documented acute hepatitis A infection. All virus suspensions were diluted to obtain 20—40 virus particles per 5 grid squares (150 mesh) when measured by conventional electron microscopy and 400—600 particles per 5 grid squares (150 mesh) in IEM. This concentration of particles roughly corresponds to $5-10 \times \times 10^7$ PFU/ml (Balayan *et al.*, 1979).

The results are summarized in Table 1. With polio and Coxsackie viruses reaction 2+ by IEM could be demonstrated with appropriate human sera at dilutions 1 : 25—1 : 200 (i.e. in the presence of 10—50 neutralizing doses). In hepatitis A convalescent sera the quantity of antibody sufficient to produce the same reaction with HAV particles was determined at dilutions 1 : 1,000 to 1 : 20,000 which seemed to depend on the duration of the convalescent period. Thereby a 25- to 800-fold difference was found between the antibody levels found after infections with conventional enteroviruses (polio, Coxsackie) and hepatitis A. Meanwhile antibodies to polioviruses in the hyper-

immune monkey sera were present at the levels comparable to those detected in hepatitis convalescent sera.

In conclusion it may be stated that in naturally infected humans antibody to HAV is present in serum at a significantly higher level as compared with conventional enterovirus infections. At present, it remains obscure whether this phenomenon is due to high immunogenic potency of HAV, or its abundant production in the body, or the replication of this virus in liver cells is closely connected to sensitive antibody producing systems.

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*Explanation of Electron Micrographs* (Plates V-VI):

*Fig. 1.* HAV particles with abudant antibody coating (4+), × 280.000.
*Fig. 2.* HAV particles aggregated with an excess of antibodies (3+), × 140.000.
*Fig. 3.* HAV particles surrounded by little antibody (2+), × 140.000.
*Fig. 4.* HAV particles aggregated with no visible antibody (1+), × 140.000.