Histochemistry of Glutamic-Oxaloacetic Transaminase in Mouse Liver During MHV-3 Infection

Mouse hepatitis virus (MHV-3) causes a hepatitis in mice that has many characteristics in common with infectious hepatitis of man. The disease develops, in the mouse inoculated intraperitoneally, in about 72–96 hours. At about the 24th h of infection, hyperaemia of the hepatic parenchyma and diffuse cell infiltration appear, while at about the 48th h of infection focal or submassive necrosis develops in the liver. Parenchymatous necrosis gets progressively worse and becomes massive from about the 72th to the 96th h in the animals that survive[1].

In the hepatitis of the mouse caused by MHV-3 virus, as in human hepatitis, characteristic modifications of the GOT and GPT[2] activities can be observed[3,4]. These modifications consist in the remarkable increase of both GOT and GTP serum levels with the inversion of their normal ratio GOT/GPT as the contemporary and proportional decrease of these activities in liver tissue homogenates has been demonstrated[4].

Even though a histochemical technique for demonstrating GPT activity is not available instead it is possible to demonstrate GOT activity in the hepatic tissue[5]. It therefore appeared of interest to study the behaviour and the localization of GOT activity in the liver of mice infected with MHV-3 virus, in order to obtain further information about the relationships between the increase of GOT activity in the serum and the behaviour of this same enzyme in hepatic tissue.

Materials and methods. Albino mice, Swiss strain (furnished by Morini, Italy) weighing 12–15 g, nourished with a balanced diet (MIL, Morini, Italy) were used. Virus: MHV-3 furnished by the American Type Culture Collection and maintained in our laboratory in receptive albino mice.

Histochemical technique: GOT activity was determined using the method of LEE and THORACK[5] which foresees the incubation of the liver sections in a medium consisting of 20 m*M* L-aspartic acid, 2 m*M* α-ketoglutaric acid, 50 m*M* imidazole and 6 m*M* lead nitrate at pH 7.2–7.4 and at room temperature (20°–22°C).

The following reagents were used: L-aspartic acid (Sigma), α-ketoglutaric acid (Sigma), imidazole (Merck), glutaraldehyde (Fischer). All other reagents were supplied by Merck. To determine GOT activity in the serum, the UV-Test (Boehringer) was used.

Experiments. The mice were inoculated i.p. with 0.10 ml of broth containing 1000 LD_{50} of MHV-3 virus. After 12, 24, 48, 72 and 96 h, the mice were killed by decapitation and their livers, rapidly withdrawn, were put into an International cryostat model CTI operating at a temperature of –25°C. The livers were cut into sections about 7–10 mμ thick which, placed on a coverslip, were fixed for 1 min in 1% glutaraldehyde in imidazole-nitric acid buffer at 0°C, and then in 4% formaldehyde in the same buffer and at the same temperature for 20 min. The successive stages were carried out as described by LEE and THORACK[5].

As a control of the specificity of the histochemical reaction, some sections were incubated in a medium

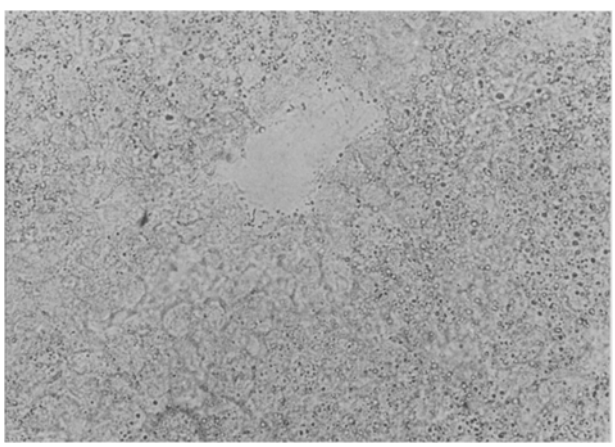

Fig. 1. Normal mouse liver showing GOT activity around a centrolobular vein.

[1] M. PIAZZA, in *Experimental Viral Hepatitis* (Ed. Ch. Thomas, Springfield, Illinois, USA 1969), p. 24.
[2] In this paper the following abbreviations are used: GOT, glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase.
[3] F. DE RITIS, M. COLTORTI and G. GIUSTI, Boll. Soc. ital. Biol. sper. *31*, 394 (1955).
[4] F. DE RITIS, M. COLTORTI and G. GIUSTI, Science *124*, 32 (1956).
[5] S. H. LEE and R. M. THORACK, J. Cell Biol. *39*, 716 (1968).

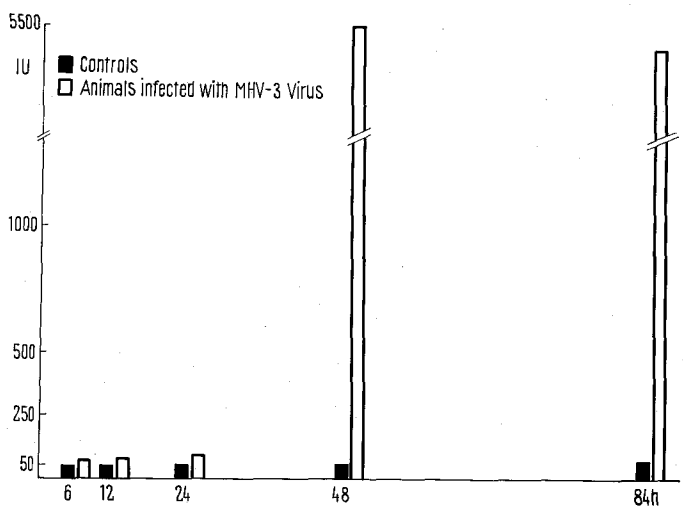

Fig. 2. Behaviour of GOT activity in the serum of mice during MHV-3 infection.

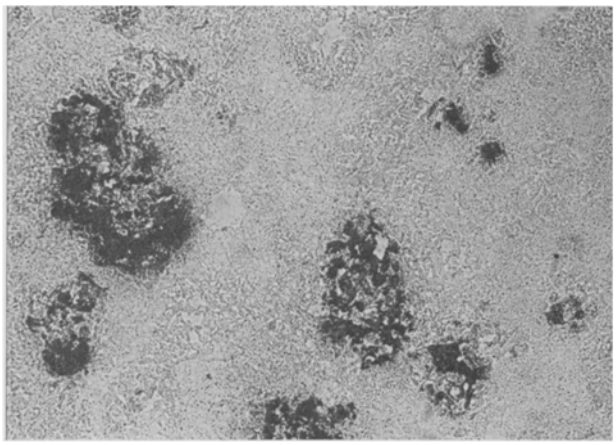

Fig. 3. Liver of mouse with focal necrosis (48 h after infection). A high GOT activity in necrotic areas is observable.

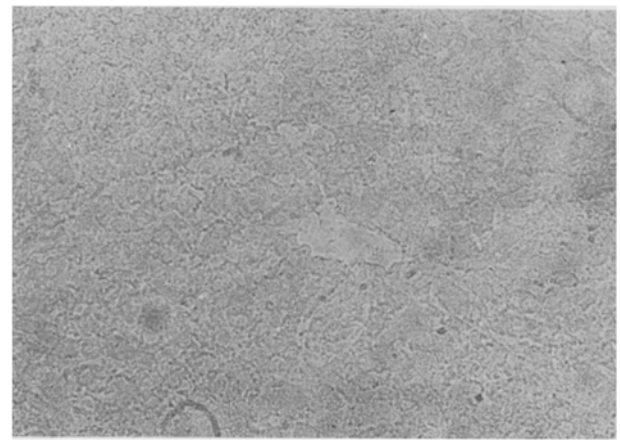

Fig. 4. Liver of mouse with massive necrosis (96 h after infection). The liver cells are almost completely devoided of GOT activity.

lacking α-ketoglutaric acid and in which the L-aspartic acid had been substituted by an equimolecular quantity of L-glutamic acid. As control animals, both untreated mice and mice inoculated i.p. with 0.10 ml of sterile broth were used.

Observations. The hepatic tissue of the control animals shows the final reaction product as fine, brownish particles in the cytoplasm. (Figure 1). In the infected animals, 12 and 24 h after virus inoculation, the hepatic tissue shows an enzymatic activity that does not vary from that of the control animals and normal values of GOT in the serum can be found. After 48 h, when the necrotic phenomena begin to manifest themselves, a sharp increase of GOT activity in the liver tissue and the serum can be observed (Figure 2). At this time, in some animals a sub-massive necrosis and in others a zonal necrosis can be found. In the former, the GOT activity in liver cells is increased uniformly, but the few areas of healthy parenchyma show an enzymatic activity not different from that of the controls. The greatest increase of GOT activity is found in the livers with zonal necrosis, but it is strictly limited to the necrotic areas (Figure 3). From 72 to 96 h after infection, in the surviving animals, a massive hepatic necrosis can always be noted. In these animals, the hepatic tissue shows a scarce or even absent GOT activity, while very high levels of enzymatic activity in the serum are found (Figure 4).

Comment. From the data given it can be deduced that the increase in GOT activity in the liver of mice infected with MHV-3 virus is strictly related to hepatic necrosis. In fact, before the appearence of necrotic phenomena, that is before the 48th h of infection, no increase in the enzymatic activity in the hepatic tissue can be observed. Moreover, in the liver of the animals in which a focal necrosis is present, the increase in enzymatic activity is strictly limited to the areas of necrosis while the surrounding tissue shows normal levels of activity. Other authors have demonstrated, by biochemical methods, that a remarkable increase of GOT serum activity during MHV-3 infection occurs together with a simultaneous rapid decrease of GOT levels in liver homogenates[3,4]. These authors have postulated that the increase of GOT activity in the serum was due to a release of the enzyme by the membranes of the damaged liver cells. Therefore, the histochemical finding of an increased GOT activity in liver cells showing sub-massive necrosis (48th h from infection) is in agreement with this hypothesis. On the other hand, the higher GOT activity in liver parenchyma exhibiting a focal necrosis is difficult to explain. This finding could be interpreted by considering that the focal necrosis is an initial stage of the liver damage, where the rapid outflow of enzymes from damaged cells into the blood still does not occur. This hypothesis, nevertheless, is contradicted by the presence of high GOT levels in the blood.

The persistence of high GOT levels in the serum during terminal stages of infection whereas the enzyme activity, from the histochemical viewpoint, is almost absent, cannot be easily interpreted. It can be hypothesized that, in the moribund animals, a high residual serum GOT activity is present after the complete outflow of the enzyme from the necrotized liver cells.

We are unable, at present, to explain completely these results and further studies are in progress on the relationships between the histochemical picture of focal and massive necrosis, respectively, and the behaviour of GOT activity in the serum in these experimental conditions.

Riassunto. Gli autori hanno studiato istochimicamente il comportamento dell'attività GOT nel fegato di topi infettati con virus MHV-3. Alla 48° ora dall'infezione si ha un notevole aumento dell'attività enzimatica, strettamente legato alla necrosi parenchimale. Tale aumento è maggiore nei fegati con necrosi focale che non in quelli con necrosi sub-massiva. Vengono discussi i reperti istochimici e le loro correlazioni con i valori dell'attività GOT nel siero degli animali infettati e con i dati biochimici riportati da altri Autori.

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