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On the Inoculation of the HST (Hamazaki) Virus Isolated From Yoshida Tumor on Emb-ryonated Eggs

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Abstract

HST virus which was isolated in 1950 from the Yoshida ascites tumor by Hamazaki and his associates is a pantropic virus which creates a unique inflammatory granulation in mice. When virus of an acute infectious disease was inoculated on embryonated eggs, not only the egg membrane but also the chick embryo were infected more or less, and when the number of virus increased the chick embryo died, terminating the development of the egg. However, the tumor inducing virus which represents the Rous virus does not cause heavy disturbances in the embryo and it is well known that chick hatched from this egg can long maintain health unless it is subjected to a provocative factor. HST virus is no exception to this example and though it is inoculated on an embryonated egg it does not cause any serious disturbance on the embryo. The tissue changes of the chorio allantois infected by the "Virus were the focal proliferation and necrosis of ectodermal epithelium, the proliferation of the mesenchymal cells of the mesodermal layer adjacent to these foci, accompanying infiltration of lymphoid cells and leukocytes with edema, especially eosinophilic leukocytes. By these tissue changes a terrace-shaped thickening of the membrane was the result. In the viscera of the chick embryo a special change in the liver was seen, i. e., along the edge of the liver greyish white nodules submacroscopic to miliary in size appeared. The principal pathologic change of the foci is the coagulation necrosis of the liver parenchyma and only a slight infiltration about the periphery of the foci was observed. Moreover, proliferation of mesenchymal cells occurred next to the walls of the large blood vessels of the liver (principally, the portal veins) and with the added infiltration of a small number of lymphoid cells and leukocytes sharply defined nodular foci were formed. Though this was a rare instance, similar pathologic changes were seen also in the walls of the blood vessels of the cerebrum stem of the embryo and along the periphery local gliosis was observed.

**ON THE INOCULATION OF THE HST (HAMAZAKI)
VIRUS ISOLATED FROM YOSHIDA TUMOR
ON EMBRYONATED EGGS**

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The three classical characteristics of virus are that it is invisible, filtrable and cannot be purely cultured, but with the advance of science these characteristics have been gradually lost. Nevertheless, there is no indication yet that pure culture will be successful. Instead of pure culture an attempt (*Steinhardt, Israeli and Lambert*)¹⁾ was made to conduct a culture of a tissue infected with virus and moreover an inoculation of virus on chorio allantois of an embryonated chick egg was successful (*Woodruff and Goodpasture*)²⁾. The latter has contributed greatly to the progress in the morphology of virus, in the immunoserological study, in the estimation of virus amount, for in this case a large quantity of thick virus emulsion was obtained.

HST virus was isolated from the *Yoshida* tumor in 1950 by *Hamazaki* and his associates and in a previous report a comprehensive coverage was made on the experiment conducted upon an immunological study, especially on the relation between this virus and the tumor immunity responsible for *Yoshida* tumor³⁾⁴⁾⁵⁾

The study to determine whether HST virus can be inoculated on an embryonated egg is an extremely important one not only in ascertaining the character of this virus but also in formulation of research procedure for the future.

Materials and Methods

A decimal diluted emulsion of a normal saline solution made from the cerebrum of mice subjected to an intracerebral inoculation of HST virus was used as inoculation material. The eggs used were fertile ones of the Leghorn chicken. The method

of incubation and inoculation technique followed were all based upon *Tanaka's* method⁶⁾.

On the blunt end of an egg sterilized with sublimate alcohol an opening 0.7 mm square in size is made and the outer and inner layers of the shell membrane surrounding the air sack are punctured so that the chorio allantois is exposed. On it is dropped 0.1 cc of virus emulsion prepared from infected mice brain, after which the opening is sealed by a concave cover glass shaped like a watch glass, fitting against the blunt end of the egg. The cover glass is kept in alcohol and before its use the alcohol on its surface is burned off.

The number of eggs used in Series No. 1 is 39, and in Series No. 2 is 76. Eggs were successively inoculated for five generations and 30 eggs were used. In the re-inoculation of mice, eggs No. 16, No. 17, No. 27, and No. 29 were inoculated into the brain of three mice respectively.

After the inoculated eggs are incubated at 37 degrees C for a given period, the embryo is removed and observed by the naked eye. The chorio allantois and viscera are then fixed in 10% formalin solution and paraffin sections are prepared. After hematoxylin-eosin and *Giemsa* staining, they are inspected under the microscope.

For comparison of inoculation materials, the brain emulsion of mice with Japanese "B" encephalitis and the brain emulsion of normal mice were inoculated on the eggs. These results confirmed that they were completely different from the results of other groups of experiments with HST virus. Moreover, the chorio allantois emulsion of a normal chick embryo was inoculated on the brain of mice, but under observation there were no change either clinical or histological.

Series 1

On the 6—8th day of incubation the inoculation of virus material was made, after which culture was continued for 5—7 days. As discussed hereunder, when the virus was inoculated considerable changes occurred in the chorio allantois and the liver of the embryo, but they did not cause the embryo to die.

By the naked eye there could be observed in the chorio allantois a development of many milky clouded small spots

roughly 0.5 mm in size especially in the local and near-by area of inoculation. Moreover, the white spots sometimes fused into a diffused thickening. Such change to occur throughout the surface of the chorio allantois was rare, but a thickening of the adventitia of blood vessels and their congestion was observed to be widespread (Fig. 1).

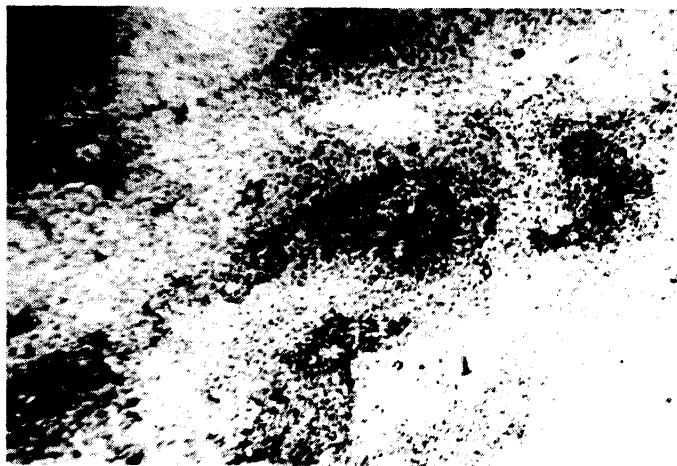


Fig. 1. Extended preparation of chorio allantois of chick embryo. On the 8th day of incubation the HST virus was inoculated, which followed by culture for 5 days. Note foci consist of stratification of fibrin, infiltration of leukocytes and proliferation of the ectodermal layer. Hematoxylin-eosin staining.

By a microscopic examination the changes in the chorio allantois appeared selectively in the ectoderm. The epithelium proliferated and was in many strata. An outstanding thickening was noted and in one section it appeared papillary. In the mesodermal tissue in contact with this section congestion was strong and sometimes an infiltration more or less of lymphoid cells, polymorphonuclear leukocytes and eosinophile leukocytes occurred with haemorrhage. The neighboring mesodermal tissue thickening to a shape of a terrace due to the proliferation of the mesenchymal cells and blood capillaries and to edema (Fig. 2).

Sometimes the thickening was led to hyaline degeneration and necrosis. In the entodermal tissue there could hardly be noted any histological change. If a *Giemsa* staining is performed, eosinophile leukocytes could be observed distinctly. These cells appeared in greater number when culture was conti-

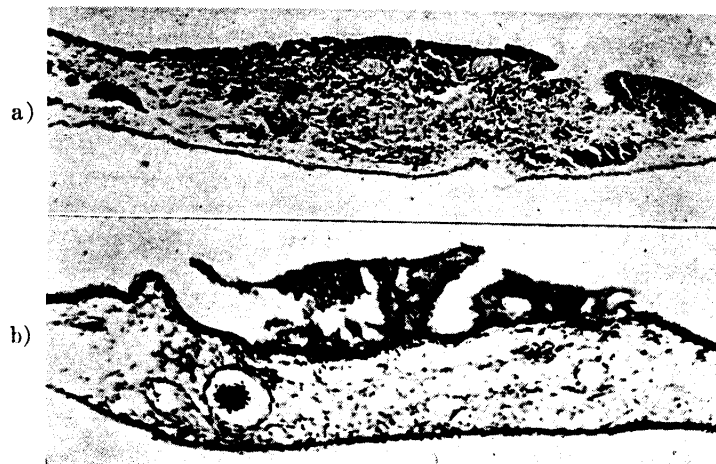


Fig. 2. a) Chorio allantois of chick embryo.

On the 8th day of incubation the HST virus was inoculated, which was followed by culture for 5 days. Heavy proliferative inflammation accompanying leukocytes infiltration to be seen.

b) Chorio allantois of chick embryo.

On the 7th day of incubation the HST virus was inoculated, which was followed by culture for 7 days. Papillary proliferation of ectodermal epithelium is shown.

nued for a longer period. In the above specimen there could not be found any micro-organism or inclusion bodies. Successive transmission of virus was possible on embryonated eggs and a retransmission to the original host, the mouse, could be performed.

Series 2

On the 11—12th day of incubation the virus was inoculated, which was followed by culture for 3—7 days. The changes in the chorio allantois were generally greater in comparison with those of Series 1 and changes were also seen in the viscera of the embryo. That is to say, by the naked eye small milky grey nodules could be frequently seen along the edge of the liver of the embryo.

Large foci were located parallel to the capsule of the liver in the shape of a lens and the size was beyond that of a millet seed. There were various sizes which were old and new. In the new foci liver-sinus was narrow and there was an infiltration of lymphoid cells, while the liver cells were atrophic and

cytoplasm cloudy. However, the most remarkable changes were the necrosis and the necrobiosis of the parenchyma and the former was a typical coagulation necrosis. Nevertheless, in the periphery of the foci necrotic liver cells had liquified, which caused clear annular strata and a net-like necrosis of liver parenchyma to appear. The infiltration in the foci was very slight, and along the border of the intact parenchyma there was a small number of lymphoid cells and an infiltration of leukocytes (Fig. 3).

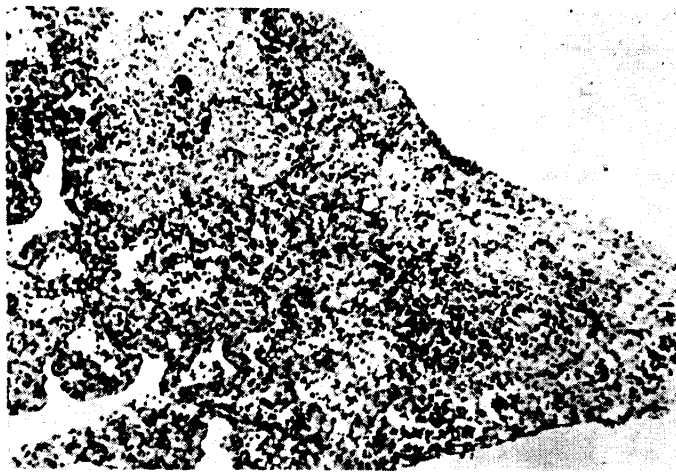


Fig. 3. On the 11th day of incubation the HST virus was inoculated on chorio allantois, which was followed by culture for 3 days. Two small necrotic foci in the edge of the liver to be seen.

As for necrotic foci, a great amount of nuclear debris appeared and due to calcification there sometimes occurred a basophile granular staining.

It was interesting to find that beside the necrotic focus nodular cell foci appeared rather diffusely along the walls of the large blood vessels of the liver and in the angle of the vessel lumen with bile duct as center. The principal element of the nodules was proliferated mesenchymal cells with a small number of lymphoid cells and they constructed a type of embryonal granulation focus. On the other hand, mesenchymal cells were proliferated diffusely along the walls of the blood vessels. These foci were mutually connected to create larger foci and sometimes to envelop a portion of liver parenchyma.

The infiltration of leukocytes was slight and was found in the periphery of the foci. Among the virus which cause liver lesions of the chick embryo, a well known one is the louping ill virus. This virus caused slight lesions in the chorio allantois and attacked the viscera of the embryo and especially the liver to cause haemolytic anemia and jaundice⁷⁾.

Though this was an unusual instance, in the cerebrum of the embryo the walls of several blood vessels located near the hypothalamus were thickened markedly by the proliferation of the mesenchymal cells and the lymphatic space of the perivascular area was enlarged, containing lymphoid cells. There was a slight diffused gliosis in the cerebrum tissue of the above mentioned perivascular area and a congestion of the capillaries was seen.

Successive inoculation with the emulsion of chorio allantois of infected chick embryo was successfully performed and from the fifth generation the retransmission of the virus into mice brain gave also a positive proof.

The thickening of the walls of the blood vessels, congestion and haemorrhage of the chorio allantois occasionally could also be observed in control experiments with brain emulsion of normal mice but they appeared intensified when virus was inoculated.

In case Japanese B encephalitis virus (*Nakayama* strain) was inoculated, embryos often perished in a few days after inoculation. As to histological changes, there could be seen a far greater evidence of exudative inflammation than productive in the chorio allantois, accompanied by a remarkable tendency toward tissue necrosis; while in the viscera, a potent inflammation could be detected in the brain, liver, and lung. Especially, meningo-encephalitis that has appeared in the brain reminds us of the cerebral lesion of adult animals.

It is noteworthy that the viral lesions by chick embryo are almost similar in their character to that of its own host animal infected with the same virus. *Peers, H. J.*, etc.⁸⁾ have infected successfully the yolk sac of embryonated eggs incubated for 6 to 18 days with a strain of group A Cocksacki virus. The principal lesion consisted of a widespread acute necrosis of striated muscle, apparently essentially identical in type and development

with that characteristically produced by the virus in suckling mice.

The principal pathological changes of the mouse inoculated with HST virus were found in the liver and cerebrum, and similar pathological changes were induced in the same viscera of the chick embryo inoculated, as mentioned above, by the virus.

Summary

HST virus which was isolated in 1950 from the *Yoshida* ascites tumor by *Hamazaki* and his associates is a pantropic virus which creates a unique inflammatory granulation in mice⁹⁾.

When virus of an acute infections disease was inoculated on embryonated eggs, not only the egg membrane but also the chick embryo were infected more or less, and when the number of virus increased the chick embryo died, terminating the development of the egg. However, the tumor inducing virus which represents the Rous virus does not cause heavy disturbances in the embryo and it is well known that chick hatched from this egg can long maintain health unless it is subjected to a provocative factor¹⁰⁾. HST virus is no exception to this example and though it is inoculated on an embryonated egg it does not cause any serious disturbance on the embryo.

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In the viscera of the chick embryo a special change in the liver was seen, i. e., along the edge of the liver greyish white nodules submacroscopic to miliary in size appeared. The principal pathologic change of the foci is the coagulation necrosis of the liver parenchyma and only a slight infiltration about the periphery of the foci was observed. Moreover, proliferation of mesenchymal cells occurred next to the walls of the large blood vessels of the liver (principally, the portal veins) and with the added infiltration of a small number of lymphoid cells and leukocytes sharply defined nodular foci were formed. Though this

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References

- ¹⁾ Steinhardt, E., Israeli, C. & Lambert, R. A.: Studies on the cultivation of the virus of vaccinia. *J. Infect. Dis.* **13**, 294, 1913. — ²⁾ Woodruff, A. M. & Goodpasture, E. W.: The susceptibility of the chorio allantoic membrane of chick embryos to infection with the fowl-pox virus. *Am. J. Pathol.* **7**, 209, 1931. — ³⁾ Hamazaki, Y. Sato, J. et al.: On meningoencephalitis caused by virus isolated from Yoshida tumor. *Fol. Psych. Neur. Jap.* **5**, 40, 1951. — ⁴⁾ Hamazaki, Y. Sato, J. et al.: Studies on the HST (Hamazaki) Virus isolated from Yoshida tumor. *Can. Research.* **13**, 119, 1953. — ⁵⁾ Hamazaki, Y. et al.: On the specificity of the HST (Hamazaki) Virus for Yoshida tumor. *Gann.* **44**, 290, 1953. — ⁶⁾ Tanaka, T.: Studies on inoculation of micro-organisms on embryonated eggs (Japanese). *Jikken-Igaku-Zasshi.* **25**, 797, 827, 939, 1069, 1082, 1941. — ⁷⁾ Burnet, F. M.: *Handb. d. Virusforsch.* **1**, 434, Wien. 1938. — ⁸⁾ Peers, H. J. et al.: The pathologic changes produced in chick embryos by yolksac inoculation of group A Coxsackie virus. *J. Exp. Med.* **96**, 17, 1952. — ⁹⁾ Hamazaki, Y.: Histological changes of mice inoculated with the HST virus. *Tr. Soc. Path. Jap.* **41**, 157, 1952. — ¹⁰⁾ Oshima, F.: Pathological studies on chicken sarcoma. *Tr. Soc. Path. Jap.* **40**, 13, 1951.
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