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was lower than the O-agglutination titer. No correlation was observed between the state of the carrier and the titers.

6) It was effective in the control of the disease to keep newly introduced calves separately in small groups.

POSSIBILITY OF REVERSION IN AN INFECTIOUS CANINE HEPATITIS VIRUS-INDUCED HAMSTER TUMOR CELL LINE

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It has recently been reported that the properties of tumor cells are not irreversibly fixed but changeable. RABINOWITZ & SACHS reported that they isolated the variants which have a reduced tumorigenicity by seeding polyoma virus-transformants on the fixed cell layers. SIRAGI et al. isolated the non-tumorigenic variants from a mouse melanoma cell line by culturing the cells in a medium containing non-growth inhibiting concentrations of 5-bromodeoxyuridine (BUdR). In the case of adenovirus-transformants or adenovirus-induced tumor cell lines, however, no such report has been presented. Attempts were made by the author to determine whether reversion occurs in an infectious canine hepatitis virus-induced tumor cell line (HT-7 cell). Prior to the experiments, the HT-7 cell line was cloned three times.

The monolayers of hamster embryo cells, mouse embryo cells, dog kidney cells, HeLa cells and HT-7 cells were fixed by 1% glutaraldehyde solution and HT-7 cells were seeded onto these fixed layers to give rise to cell colonies originating from single cells. The cloning efficiency was 0.7~15%, which was lower than that of polyoma virus-transformants. The total of 60 clones isolated showed as high a tumorigenicity as the parental cells. The HT-7 cells grown in the medium containing 1 to 5 $\mu\text{g/ml}$ of BUdR and 5-iododeoxyuridine at $41\pm 0.5^\circ\text{C}$, and in the presence of a conditioned medium of normal cells, were also shown to be highly tumorigenic. A clone (Clb) obtained in a soft agar medium and showing a rather epithelioid shape also produced tumors in hamsters, but the number of days required for the forming of tumors, with the inoculum of 10^5 cells, was about 10 days longer than in the case of the parent cell. Subclones of Clb showed tumorigenicity similar to either Clb or the parent cell, except one which formed a relatively slow-growing tumor. The slow-growing

tumor was finally regressed. From the above results it is concluded that the tumorigenicity of the infectious canine hepatitis virus-induced hamster tumor cell line is stable but the reversion of tumorigenicity is possible.

ON THE COMPONENTS OF TROPONIN

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1) Native tropomyosin was precipitated between 40 and 45% ammonium sulfate saturation from the crude extract (EBASHI et al. 1963) of the rabbit skeletal muscle. This fraction had a higher purity and physiological activity and also gave a better yield than other fractions. Troponin as the supernatant was separated by the subsequent isoelectric fractionation of native tropomyosin at pH 4.5.

2) All the troponin preparations contained four components. TN-1, TN-2, TN-3 and TN-4, having molecular weights of 40,000, 24,000, 19,000 and 14,000 respectively, as determined sodium dodecyl sulfate gel electrophoresis. These components were present in the purified troponin and the natural actomyosin, but not in the desensitizing actomyosin.

3) Fractions containing variously relative amounts of each components were obtained from the purified troponin by chromatography on SP-Sephadex in 6 M urea-33 mM citrate buffer pH 6.0.

4) Reconstitution experiments using all possible combinations indicated that Fraction D containing TN-1 and TN-2 had TN-B-like activity, and the mixture of Fraction D and Fraction A containing TN-3 restored the troponin activity. The mixture of Fraction E containing mainly TN-1 and Fraction B containing mainly TN-2 restored the troponin activity when these fractions were mixed prior to the removal of urea.

5) Both TN-1 and TN-2 were essential components for troponin activity. However, clear results were not obtained on the necessity of TN-3 for troponin activity.