blood plasma in converting the PE nitrates. Crandall(6) attributed the destruction of nitroglycerine by blood to the action of the erythrocytes. PE-mononitrate was attacked at the slowest rate. In general, each lower nitrate appeared to be digested at a rate slower than was its predecessor; if the situation were otherwise, sizeable quantities of the lower nitrates would not have been present. The above-mentioned influence of binding precluded the derivation of definitive information on the order of the enzymatic reactions. Such data are anticipated from enzyme studies with purified systems.

Summary. Rat blood plasma and red cells showed strong affinity for PETN. In both systems, PETN was degraded stepwise to PE-trinitrate, PE-dinitrate, PE-mononitrate and PE. PE-trinitrate was bound extensively by erythrocytes. PE, PE-mononitrate and PE-dinitrate were not bound to a significant degree by plasma or erythrocytes. Erythrocytes were more effective than plasma for the degradation of PETN. PE-mononitrate was the most resistant substrate in the series.

The authors wish to acknowledge the contribution of Mr. Edward Merrill who synthesized the C^{14}-PETN employed in this investigation.

1. DiCarlo, F. J., Hartigan, J. M., Jr., Phillips, G. E., Proc. Soc. Exp. Biol. and Med., 1965, v118, 514.

2. ———, Anal. Chem., 1964, v36, 2301.

3. Simecek, J., Collection Czechoslov. Commun., 1962, v27, 362.

4. Scholtan, W., Arzneimittel-Forsch., 1961, v11, 707.

5. Yagoda, H., von Oettingen, W. F., U. S. Pub. Health Bull., 1944, No. 282, 8.

6. Crandall, L. A., Jr., J. Pharm. Exp. Therap., 1933, v48, 127.

Received September 9, 1965. P.S.E.B.M., 1965, v120.

Thermal Separation of the Synthesis of Papovavirus SV40 Tumor and Virus Antigens.* (30632)

TSUNEHIRO KITAHARA AND JOSEPH L. MELNICK
Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas

A specific intranuclear antigen appears in hamster tumors induced by papovavirus SV40, or in hamster or human cells transformed by the virus(1-8). This tumor (T) antigen can be detected by complement-fixation or by immunofluorescence. In cells infected by SV40, synthesis of the same T antigen is induced early in the cytolytic cycle before the appearance of the virus itself(9-11). In the presence of cytosine arabinoside, the synthesis of T antigen is unimpeded, but the production of viral antigen is completely blocked(12).

Further knowledge of this T antigen is needed, particularly of the conditions under which it is synthesized. In the present study it is shown that the syntheses of SV40 tumor (T) and virus (V) antigens proceed at different rates at different temperatures. Furthermore, conditions can be manipulated so that infected cells produce either T or V antigen.

Materials and methods. Monolayer cultures of green monkey kidney (GMK) cells were grown in M-H medium and maintained in M-E medium(13). They were inoculated with SV40 at an input multiplicity of 10 TCD_{50} per cell. After adsorption for 2 hours at 37°C, the cultures were incubated at 23°, 37°, 40°, 42° and 43°. At intervals, the infected cells were collected by scraping them from the bottle with a rubber policeman. They were centrifuged and resuspended in pH 7.3 veronal buffered saline (VBS) to make a 5% suspension. The suspensions were treated for 30 seconds in a Raytheon sonic oscillator model 101 at 10KC, and then were centrifuged at 3000 rpm. The supernatant

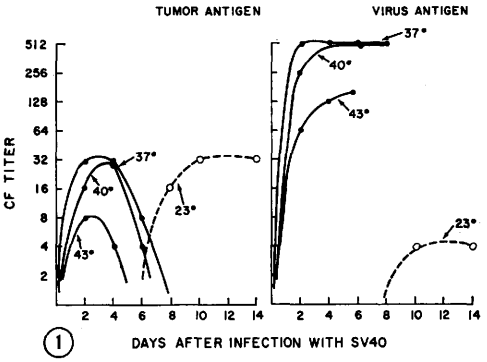
* This investigation was supported by USPHS grants CA-04600 from Nat. Cancer Inst., and AI 05382 and 5 T1 AI 74 from Nat. Inst. of Allergy and Infect. Dis., N.I.H.

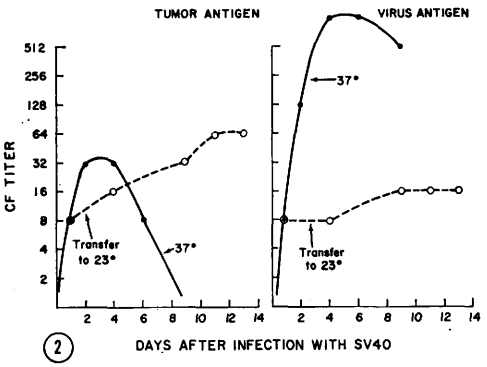
fluids were used as complement-fixing (CF) antigens. A micro CF method, using 2 full units of complement, was employed. Serum from an SV40-immune green monkey and serum from SV40 tumor-bearing hamsters were used to detect the specific V and T antigens respectively. Sera were used at dilutions which contained 4 antibody units. Antigen titer was expressed as the reciprocal of the highest dilution of antigen giving 3+ or 4+ fixation in the presence of 4 units of antibody.

Results. When the infected cells were maintained at 37°, the pattern of production of both tumor (T) and virus (V) antigens confirmed previous findings(8-11). The T antigen first appeared in serologically detectable quantities between 8 and 16 hours after infection, a few hours before the virus (V) antigen was first detected. During the first 2 days, both T and V antigens increased in amount. The T antigen reached its maximum level 2-4 days after infection, and then began to fall. It fell below detectable levels as the viral cytopathic effect continued on to completion. During this time, the V antigen increased, reached a plateau, and remained at high level for several days. The pattern of the production of both antigens at 37° is shown in Fig. 1.

When the infected cells were incubated at 40°, the titers of both antigens and the times required to reach maximum levels were about the same as in cultures maintained at 37°. At 42° and 43° the times of production of both T and V antigens again followed the patterns of the 37° cultures, but the antigen titers were lower and the T antigen disappeared sooner. The results with the 43° cultures were somewhat irregular, because at this high temperature the cells did not do well.

At 23°, the results were quite different. The cytopathic effects characteristic of SV40 did not develop in the infected cultures incubated at this temperature, and production of V antigen was very low. In different experiments the yield of the V antigen was only 0.25-1.0% of the amount in the 37° cultures. T antigen was first detected several days later than in 37° cultures, and a much longer time was required at 23° for the T antigen to reach the high level found in the 37° cultures. Thus, 10 days were required at 23° to syn-





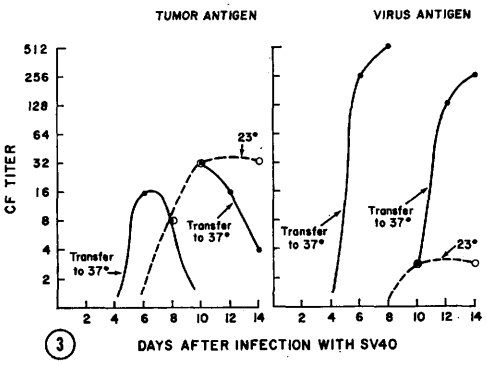


FIG. 1. Development of SV40 tumor and virus antigens in SV40-infected green monkey kidney cells incubated at different temperatures. If the titer of antigen was <2, no point is shown on the curves.

FIG. 2. Changes in synthesis and persistence of SV40 tumor and virus antigens after transfer of infected cultures from 37° to 23°.

FIG. 3. Changes in synthesis and persistence of SV40 tumor and virus antigens when infected cultures were first incubated at 23° and then transferred to 37°. When held at 23°, antigens first became detectable at 8-10 days.

thesize the same amount of T antigen as that formed after 2 days at 37°. Upon reaching the maximum level at 23°, the T antigen did not quickly decrease in concentration as it did at the higher temperature. These results are also shown in Fig. 1.

When the infected cells were first incubated at 37° and were then transferred to 23°, rates of synthesis of T and V antigens were affected in dissimilar fashion (Fig. 2). Synthesis of the T antigen continued, although at a slower rate, and reached maximum titer by the 11th day. In the control continued at 37°, the T antigen deteriorated rapidly after day 4. In contrast to prolonged and increasing synthesis of T antigen, the synthesis of V antigen virtually stopped when the cultures were transferred from 37° to 23°, but the stable V antigen persisted for the 14-day observation period with no decrease in concentration from the time of the transfer.

In another series of experiments, infected cultures were first incubated at 23°, and then at 4 days post-infection, when no detectable antigens were yet present, they were transferred to 37°. Both T and V antigens appeared within 48 hours of the transfer (Fig. 3). As in the experiments described in Fig. 1 and 2, T antigen synthesized at 37° was soon destroyed when held at this temperature, in contrast to its persistence in the 23° cultures.

When the 23° cultures were incubated for 10 days before transfer to 37°, the cells had just begun to show detectable levels of V antigen, but had made peak amounts of T antigen before this transfer. After the transfer, as also shown in Fig. 3, the T antigen was again rapidly lost, but the cells produced a 100-fold increase in concentration of V antigen, and cytopathic changes became evident.

Discussion. At temperatures above 37° GMK cells infected with SV40 show some differences in titers of T and V antigens but the production of both antigens followed patterns similar to those in 37° cultures. At 43°, the synthesis of both T and V antigens was inhibited. On the other hand, at 23° a separation of the synthesis of the T antigen from that of the V antigen was noted, in that there was hardly any development of V antigen, while the T antigen reached the same peak titers as obtained at 37°, although it took a longer time. Increasing the incubation temperature of cultures from an initial 23° to 37° reversed the effects, so that the thermolabile T antigen disappeared while the thermostable V antigen increased about 100-fold. This separation of the synthesis of the 2 antigens indicates that the formation of T antigen is less temperature-dependent than that of V antigen, and that the synthesis of T antigen proceeds without dependence upon the synthesis of V antigen. This conclusion is similar to that arrived at from studies with DNA inhibitors(8,14). As already known(9-11) and confirmed in the present study, the T antigen synthesized in the infected cells at 37° disappears as the cytopathic effect progresses and as the V antigen reaches its maximum concentration. This disappearance is probably not the result of incorporation of the T antigen into the virus particles, but it is more likely due to degradation of the thermolabile T antigen itself. At 23°, once the T antigen reached its maximum concentration, it persisted in the infected cells, even though these cells appeared normal and could be maintained as long as the uninfected control cultures. Thus the accumulation of T antigen does not cause the death of the cells, as occurs with an increase in intracellular virus.

There is a possibility that the T antigen is one or more of the early enzymes which are produced in SV40-infected cells and which are involved in DNA synthesis(15). If this is the case, these enzymes hardly seem to function at 23°, for little virus was produced at this low temperature.

Summary. The synthesis of papovavirus SV40 tumor (T) and virus (V) antigens in infected green monkey kidney cell cultures incubated at different temperatures was studied. At 37°C or higher (up to 43°), both T and V antigens were formed reaching peak titers in 2 to 4 days. However, at 23°C, there was hardly any development of V antigen, while the T antigen reached the same peak titer obtained in cultures incubated at 37°. The 23° cultures with peak amounts of

T antigen appeared normal, but raising their temperature to 37° was followed by a rapid hundred-fold increase in V antigen and the appearance of cytopathic changes. These findings indicate that the synthesis of tumor antigen is less temperature dependent than is the synthesis of virus antigen. The thermal separation of the synthesis of tumor and virus antigen confirms the finding obtained previously with DNA inhibitors—that is, formation of tumor antigen is not dependent upon the synthesis of virus.

1. Black, P. H., Rowe, W. P., Turner, H. C., Huebner, R. J., Proc. Nat. Acad. Sci., 1963, v50, 1148.

2. Sabin, A. B., Koch, M. A., ibid., 1963, v50, 407.

3. Rapp, F., Butel, J. S., Melnick, J. L., Proc. Soc. Exp. Biol. and Med., 1964, v116, 1131.

4. Pope, J. H., Rowe, W. P., J. Exp. Med., 1964, v120, 121.

5. Kitahara, T., Butel, J. S., Rapp, F., Melnick, J. L., Nature, 1965, v205, 717.

6. Sabin, A. B., Shein, H. M., Koch, M. A., Enders, J. F., Proc. Nat. Acad. Sci., 1964, v52, 1316.

7. Habel, K., Jensen, F., Pagano, J. S., Koprowski, H., Proc. Soc. Exp. Biol. and Med., 1965, v118, 4.

8. Gilden, R. V., Carp, R. I., Taguchi, F., Defendi, V., Proc. Nat. Acad. Sci., 1965, v53, 684.

9. Sabin, A. B., Koch, M. A., ibid., 1964, v52, 1131.

10. Rapp, F., Kitahara, T., Butel, J. S., Melnick, J. L., ibid., 1964, v52, 1138.

11. Hoggan, M. D., Rowe, W. P., Black, P. H., Huebner, R. J., ibid., 1965, v53, 12.

12. Rapp, F., Melnick, J. L., Kitahara, T., Science, 1965, v147, 625.

13. Melnick, J. L., in Diagnostic Procedures for Viral and Rickettsial Diseases. Am. Public Health Assn., New York, 1956, 2nd Ed.

14. Rapp, F., Butel, J. S., Feldman, L. A., Kitahara, T., Melnick, J. L., J. Exp. Med., 1965, v121, 935.

15. Kit, S., Dubbs, D. R., Melnick, J. L., Virology, in press.

Received September 9, 1965. P.S.E.B.M., 1965, v120.

Blood Group Differentiation Using Fluorescent Antibodies. (30633)

BYRON A. MYHRE (Introduced by A. J. Quick)
Department of Pathology, Marquette University Medical School and Milwaukee Blood Center, Milwaukee, Wis.

Considerable quantitative work has been performed with human blood groups using antisera labelled with radioisotopes(1-4), but the use of fluorescent labelled antibodies has been confined almost exclusively to the qualitative localization of antigens. Several recent reports have demonstrated the practicality of using fluorescent compounds for quantitating antigen-antibody reactions. Head(5) presented a study of diphtheria toxin in which diphtheria antitoxin was labelled with fluorescein and allowed to react with the toxin forming a precipitate. The precipitate was dissolved in formamide, and measured for resulting fluorescence. A similar approach was used by Fox(6) who agglutinated streptococci with fluorescein labelled anti-streptococcal serum, dissolved the agglutinated organisms with 1N NaOH, and measured the fluorescence of the solution. Tengerdy(7) employed systems of either human or bovine gamma globulin and their corresponding fluorescein labelled antibody. The fluorescence of the antibody solution was measured before and after addition of the antigen and the decrease in the amount of fluorescence of the supernatant solution was used as an indication of the amount of antibody combined with the antigen. This report deals with the application of the above procedure to human blood groups.

Materials and methods. Seventeen human antisera for various isologous blood groups of various titers have been labelled with fluorescein isothiocyanate (F.I.T.C.) on Celite (Calbiochem, Los Angeles, Calif.) using the meth-