BIKEN JOURNAL Vol. 22 81-89, 1979

STUDIES ON THE POLYPEPTIDES OF VARICELLA-ZOSTER (V-Z) VIRUS
1. DETECTION OF VARICELLA-ZOSTER VIRUS POLYPEPTIDES IN INFECTED CELLS

YOSHIZO ASANO[1] and MICHIAKI TAKAHASHI

Department of Virology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565, Japan

(Received April 23, 1979)

SUMMARY Virus-induced polypeptides in cells infected with varicella-zoster virus (VZV) were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography. When human embryonic lung (HEL) cells infected with the Oka strain of VZV were labelled with ^{35}S-methionine or ^{14}C-glucosamine from 40 hr to 46 hr after infection, at least 18 VZV-induced polypeptides and 10 glycoproteins could be identified in the infected cells. The molecular weights of the polypeptides and glycoproteins ranged from about 145,000 to 23,000, and from about 105,000 to 48,000, respectively.

Lysates of VZV-infected cells were treated with specific antisera prepared in green monkeys or guinea-pigs, and analysed by SDS-PAGE and fluorography. In all, 33 polypeptides (with molecular weight of about 145,000 to 22,000) and 13 glycoproteins (molecular weight, about 105,000 to 38,000) were found in the immunoprecipitates. None of these polypeptides and glycoproteins were detected when infected cells cultured in the presence of phosphonoacetic acid (PAA) were treated in the same way.

INTRODUCTION

We have developed a live varicella vaccine and a varicella skin antigen (Takahashi et al., 1975, Kamiya et al., 1977). Humoral antibody to varicella virus evoked by natural infection or by vaccination can be determined reliably by the neutralization test (Asano and Takahashi, 1978) and cell-mediated immunity can be conveniently assessed by the skin test with that antigen. To identify VZV-specific proteins responsible for the neutralizing antibody and the cell-mediated immunity, we have attemped to detect VZV specific polypeptides in VZV infected cells by the SDS-polyacrylamide gel electrophoresis (SDS-PAGE). It was difficult to identify viral components in preparations of whole cells because of the high back-

1 Pressent address: Department of Pediatrics, Nagoya Hoken-Eisei University School of Medicine, Toyoake, Aichi 470-11, Japan.

ground radioactivity of host cell components in infected cells, but using high-titered specific monkey or guinea-pig antisera we could detect many viral polypeptides by immunoprecipitation followed by SDS-PAGE and fluorography.

MATERIALS AND METHODS

1. *Cell culture*

HEL cells at the 3rd to 10th passage level were grown in a mixture of equal volumes of Eagle's MEM and Medium 199 supplemented with 10% fetal calf serum (FCS) in Falcon 100 mm dishes. Cultures were maintained in similar medium but with 3% FCS.

2. *Virus*

The Oka strain of varicella virus isolated in Osaka (Takahashi et al., 1975) was used in this study.

3. *Radioactive labelling of infected or mock-infected cells*

Monolayer cells of HEL were inoculated with cells infected with the Oka strain of varicella virus at a ratio of about 1 infected cell to 5 uninfected cells. After adsorption for 2 hr at 37 C, cells were washed three times with phosphate buffered saline (PBS) and incubated for 40 hr, in the presence or absence of phosphonoacetic acid (PAA), (ICN pharmaceuticals, Inc., Plainview, NY), which inhibits VZV replication in vitro. Then cells were labelled for 6 hr with 10 μCi/ml of ^{35}S-methionine (1,105 Ci/mmole; Amersham) in MEM containing one-tenth the normal concentration of methionine and 3% dialyzed FCS, or with 6 μCi/ml of ^{14}C-glucosamine (60.8 mCi/mmole; Amersham) in MEM containing half the normal concentration of glucose and 3% dialyzed FCS.

4. *Fractionation of cells*

Infected or mock-infected cells ("whole cells") labelled with ^{35}S-methionine or ^{14}C-glucosamine were solubilized in 4 ml of "TD buffer" (1% Triton X-100, 0.5% sodium deoxycholate, 0.15 M NaCl, 1 mM phenylmethylsulfonyl fluoride, 10 mM sodium phosphate buffer, pH 7.2) per 100-mm dish, and then sonicated for 3 min in an ice bath. The preparations were allowed to stand for 1 hr on ice, and then centrifuged for 2 hr at 30,000 rpm in a Hitachi RPS 40T rotor at 4 C to separate the supernatant (TD-lysate) and pellet (Ikuta, et al., 1978b).

5. *Antisera*

Antisera were prepared as follows. The Oka strain of varicella virus was adapted to guinea-pig embryo cells or green monkey kidney cells and cultivated serially 5 to 10 times. Guinea-pigs or green monkeys were given 3 or 4 intramuscular inoculation of equal volumes of infected cells and complete Freund's adjuvant at weekly intervals and sera were collected 7 to 10 days after the last inoculation. The neutralizing antibody titers of these sera were approximately 1 : 1024.

6. *SDS-PAGE*

SDS-PAGE was done by the method of Maizel (1971). Whole cells and TD-lysates were concentrated by treatment with cold 10% trichloroacetic acid (TCA) and washed twice with cold 5% TCA and once with cold acetone. The concentrated samples were suspended in "sample buffer" (1% SDS, 1% 2-mercaptoethanol, 10% glycerol, 0.001% phenol red, and 50 mM Tris-HCl buffer, pH 8.2) and solubilized by heating at 100 C for 90 sec. Electrophoresis on discontinuous SDS-slab gel (4% stacking and 9% separating gel) was carried out at a constant voltage of 100 V for 6 hr. After electrophoresis, the gel was stained with Coomassie-blue R250 and destained as described by Ikuta et al. (1978a). Autoradiograms were prepared by exposing the dried gels to Kodak "X-Omat" R film.

7. *Indirect immunoprecipitation*

Indirect immunoprecipitation was done essentially by the method of Kessler (1975). A 400 μl portion of TD-lysate was incubated with 10 μl of antiserum for 16 hr at 4 C, and reincubated with 100 μl of a suspension of *Staphylococcus aureus* (Cowan 1 strain) for 3 hr at 4 C. The mixture was centrifuged at 5,000 rpm for 6 min and the immunoprecipitate was washed three times with TD buffer and once with 10 mM sodium phosphate buffer, pH 7.2. Immune complexes were eluted by heating the preparation for 3 min at 100 C in the "sample buffer" used for SDS-PAGE.

8. *Fluorography*

After electrophoresis, the gels were treated with 2,5-diphenyloxazol in dimethylsulphoxide by the method of Bonner and Laskey (1974).

RESULTS

1. *Identification of VZV-induced polypeptides and glycoproteins in " whole cells "*

Mock-infected HEL cells and cells infected with the Oka strain of varicella virus were labelled from 40 hr to 46 hr after infection, with ^{35}S-methionine (10 μCi/ml) and ^{14}C-glucosamine (6 μCi/ml), in the presence or absence of PAA (200 μg/ml). The infected and mock-infected cells were concentrated by TCA treatment, dissolved in " sample buffer ", and applied to a slab gel. The autoradiograms of the samples are shown in Fig. 1. Because of the high background radioactivity in the host cells, it was not easy to determine whether the polypeptides were virus-induced or not. However, at least 18 polypeptide bands found in infected whole cells labelled with ^{35}S-methionine could not be found in mock-infected cells. The molecular weights of the 18 polypeptides, calculated by comparison of their mobilities with those of standard proteins, ranged from about 145,000 to about 23,000. These polypeptide bands were not detected in infected cells cultured in the presence of PAA (Fig. 1A). When infected cells were labelled with ^{14}C-glucosamine, 10 glycoprotein bands with molecular weights of about 105,000 to about 48,000 were detected in the absence of PAA (Fig. 1B). The number and molecular weights of these glycoproteins could not be determined accurately, because the glycoprotein bands were diffuse.

2. *Identification of VZV-induced polypeptides and glycoproteins in cell lysate (TD-lysate)*

To distinguish virus-induced polypeptides band more clearly from bands of host protein, the infected and mock-infected whole cells were fractionated as described in the Materials and Methods. Cells labelled with ^{35}S-methionine or ^{14}C-glucosamine were solubilized with TD buffer, sonicated and centrifuged and the supernatants (TD-lysates) were analyzed by SDS-PAGE. About 80% of the TCA-insoluble radioactivity of whole cells was consistently recovered in the TD-lysates. Figures 2A and B show the autoradiographic patterns of TD-lysates labelled with ^{35}S-methionine and ^{14}C-glucosamine, respectively. The number of VZV-induced polypeptide bands and the molecular weights of these polypeptides were the same as those identified in infected whole cells.

3. *Identification of VZV specific polypeptides and glycoproteins by immunoprecipitation*

Evidence for the viral specificity of the polypeptides and glycoproteins detected in infected whole cells was obtained by immunoprecipitation test. Antisera of high titer could be obtained from immunized monkeys and guinea-pigs. The TD-lysates of infected cells were treated with the antisera and the resulting immunopreipitates were analysed by SDS-PAGE and fluorography. Figure 3 shows the fluorographic patterns of the immunoprecipitates obtained with green monkey antiserum. Thirty polypeptide bands were found by immunoprecipitation in TD-lysates of cells labelled with ^{35}S-methionine. The molecular weights of the 30 polypeptides ranged from about 145,000 to 22,000. Thirteen of these polypeptides (115,000, 79,000, 68,000, 58,000, 35,500, 33,000, 32,500, 31,000, 29,000, 26,000, 24,000, 22,500 and 22,000) were not detected in whole cells or TD-lysates. Ten bands of glycoproteins with molecular weights of about 105,000 to about 56,000, were detected when labelled with ^{14}C-glucosamine. The 30 polypeptides and 10 glycoproteins were not detected in immunoprecipitates of TD-lysates from cells cultured in the presence of PAA.

Almost the same patterns were obtained by fluorography when immunoprecipitation was done with guinea-pig antiserum, as shown in Fig 4. Thirty polypeptides and 13 glycoproteins were detected in the immunoprecipitates and they were again not found in the presence of PAA. The molecular weights of these proteins ranged from about 145,000 to 22,000 and from about 105,000 to 38,000,

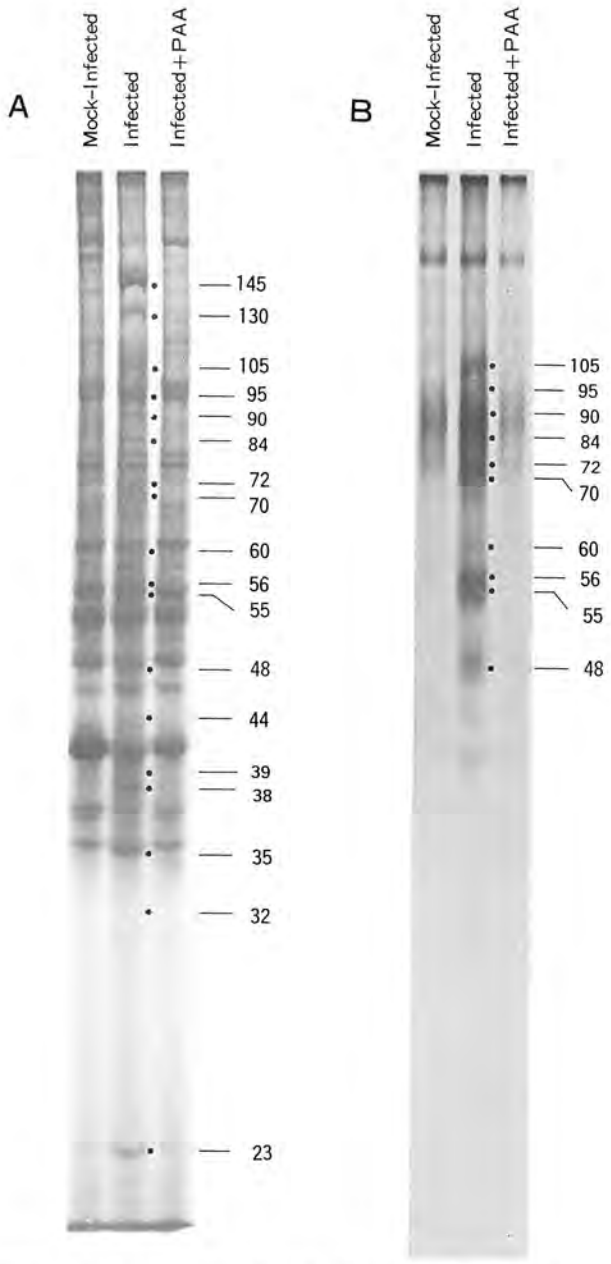

FIGURE 1. Autoradiograms of 9% polyacrylamide gels after electrophoresis of whole cells (mock-infected, infected and infected in the presence of PAA). HEL cells were inoculated with cells infected with the Oka strain of varicella virus at a ratio of about 1 infected cell to 5 uninfected cells or mock-infected in the presence or absence of PAA (200 μg/ml) and then labelled with 10 μCi/ml of ^{35}S-methionine (A) or 6 μCi/ml of ^{14}C-glucosamine (B) from 40 hr to 46 hr after infection. Whole cells were concentrated by TCA precipitation, solubilized in "sample buffer" and applied to slab gels.

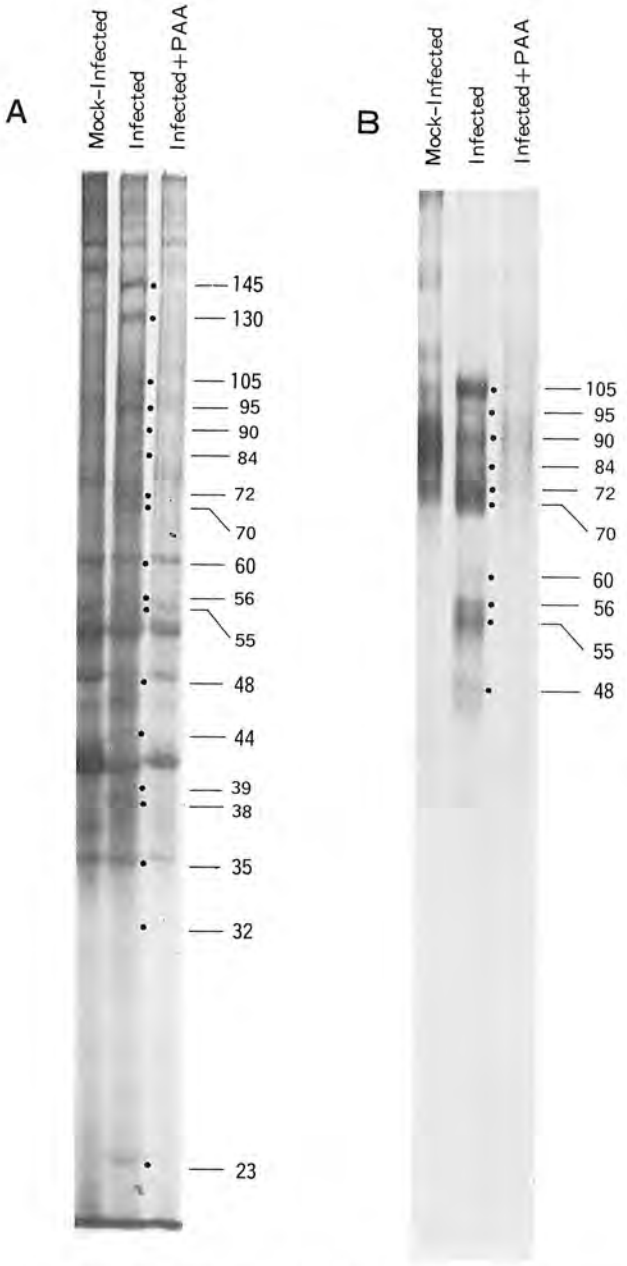

FIGURE 2. Autoradiograms of 9% polyacrylamide gels after electrophoresis of TD-lysates (mock-infected, infected and infected in the presence of PAA). HEL cells were infected and labelled with ^{35}S-methionine (A) or ^{14}C-glucosamine (B) as described in the legend to Fig. 1. Then the whole cells were solubilized in TD-buffer, centrifuged, and the supernatant fluid was analyzed on slab gels.

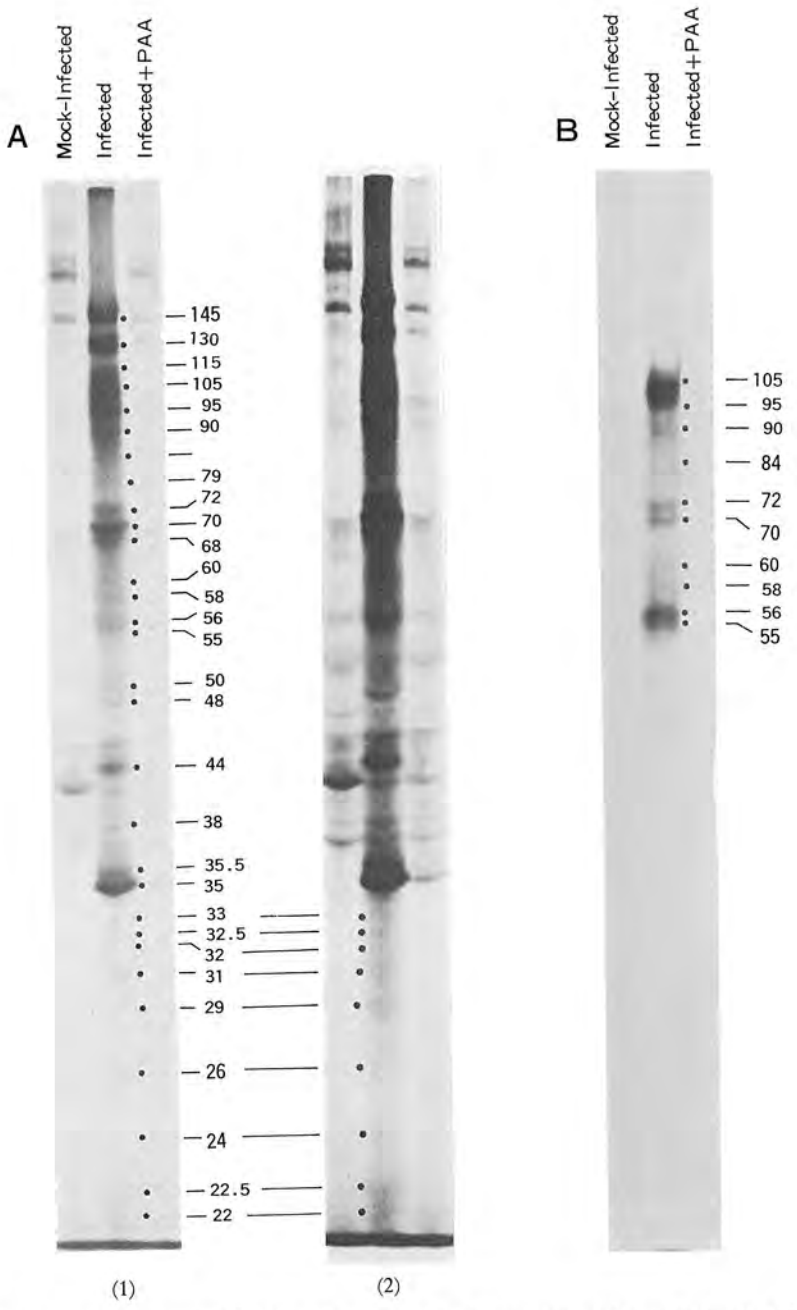

FIGURE 3. Fluorographic patterns of polypeptides (A) and glycoproteins (B) precipitated with hyperimmune green monkey antiserum. HEL cells were infected, labelled and fractionated as described in the legend to Fig. 2. Then TD-lysates were precipitated with anti-VZV green monkey serum, and the immunoprecipitates were analyzed by SDS-PAGE and fluorography. (1) 1 week exposure, (2) 3 weeks exposure.

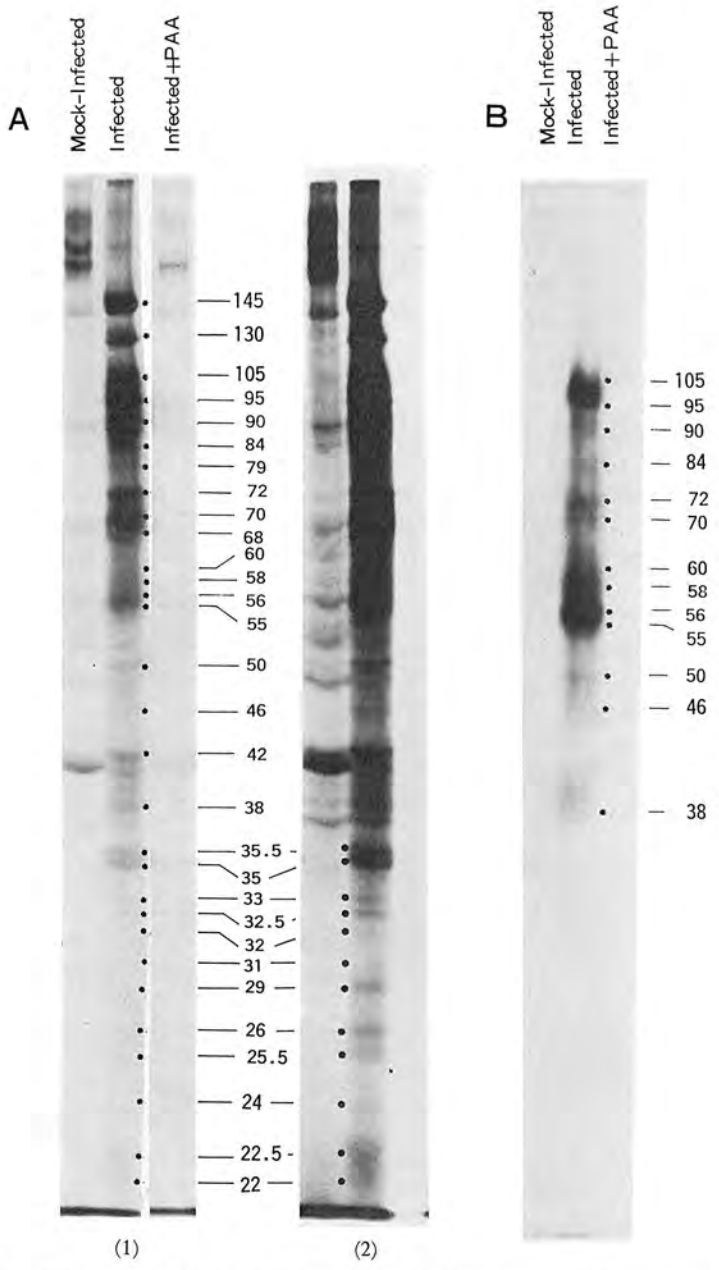

FIGURE 4. Fluorographic patterns of polypeptides (A) and glycoproteins (B) precipitated with anti-VZV guinea-pig serum. The experimental conditions were similar to those for Fig. 3. (1) 1 week exposure, (2) 3 weeks exposure.

respectively. Three polypeptides (115,000, 48,000 and 44,000) precipitated with green monkey antiserum were not detected in immunoprecipitates with guinea-pig antiserum, while 3 polypeptides (46,000, 42,000 and 25,500), not precipitated with green monkey antiserum, were prepcipitated by guinea-pig antiserum. Three of the 13 glycoproteins (50,000, 46,000 and 38,000) were not detected in immunoprecipitates with green monkey antiserum.

DISCUSSION

In the present study, infected cells were labelled for 6 hr with ^{35}S-methionine and ^{14}C-glucosamine at 40 hr after infection. This labelling time was chosen on the presumption that it should be the time when virus replication was maximal (Schmidt and Lennette, 1976). At least 18 polypeptide bands and about 10 glycoprotein bands were detected at this time in infected " whole cells " and TD-laysates. None of them were found when the cells were cultured in the presence of PAA, which is known to inhibit VZV replication by inhibiting virus-induced DNA polymerase (May et al., 1977).

Furthermore, 33 polypeptide bands and 13 glycoprotein bands were detected by the immunoprectipitation test using hyperimmune sera prepared in green monkeys and guinea-pigs. These components were not detected in the presence of PAA and appeared to include all the 18 polypeptide bands and 10 glycoprotein bands found in infected " whole cells " and TD-lysates described above. The absence of these bands in the samples of mock-infected cells and PAA-treated cells suggests that these polypeptides and glycoproteins were of viral origin, although this requires further confirmation.

Stinski (1977) described 12 polypeptides and 7 glycoproteins by immunoprecipitation with rabbit antiserum in cytomegalovirus-infected cells, and calculated that they had molecular weights of 155,000 to 16,000 and 145,000 to 16,000 respectively. Recently Wolff (1978) separated 14 polypeptides specific to VZV from partially purified virions by immunoprecipitation with human serum. At present his results cannot be compared with ours, because the systems used were different. In our study, we detected some difference in the numbers and molecular weights of polypeptide precipitated with green monkey antiserum and with guinea-pig antiserum, probably due to differences between VZV antigens produced in green monkey cells and guinea-pig cells.

Analysis of the polypeptides related to neutralization and cell-mediated immunity specific to VZV are under way.

ACKNOWLEDGMENTS

We are grateful to Dr. Kazuyoshi Ikuta in this Institute for valuable technical advices and to Mr. Akihisa Takamizawa (Research Foundation for Microbial Diseases, Osaka University) for providing us with highly immune monkey serum to V-Z virus. This study was supported by grants from the Ministry of Education, Science and Culture of Japan (No. 287055 and 37009).

REFERENCES

Asano, Y. and M. Takahashi 1978. Studies on neutralization of varicella-zoster virus and serological follow-up of cases of varicella and zoster. Biken J. 21: 15–23.

Bonner, W. M. and R. A. Laskey. 1974. A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. 46: 83–88.

Ikuta, K., H. Miyamoto and S. Kato. 1978a. Studies on the polypeptides of poxvirus I. Comparison of structural polypeptides in vaccinia, cowpox and Shope fibroma viruses. Biken J. 21: 51–61.

Ikuta, K., H. Miyamoto and S. Kato. 1978b. Studies on the polypeptides of poxvirus II. Com-

parison of virus induced polypeptides in cells infected with vaccinia, cowpox and Shope fibroma viruses. Biken J. 21: 77–94.

Kamiya, H., Y. Ihara, A. Hattori, T. Iwasa, M. Sakurai, T. Izawa, A. Yamada, and M. Takahashi. 1977. Diagnostic skin Test reactions with varicella virus antigen and clinical application of the test. J. Infect. Dis. 136: 784–788.

Kessler, S. W. 1975. Rapid isolation of antigens from cells with a staphylococcal protein A-antibody adsorbent: Parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. 115: 1617–1624.

Maizel, J. V. 1971. Polyacrylamide gel electrophoresis of viral proteins. p. 179–246. *In* K. Maramorosch and H. Koprowski [ed.] Methods in Virology. Vol. 5. Academic Press, New York.

May, D. C., R. L. Miller and F. Rapp. 1977. The effect of phosphonoacetic acid on the *in vitro* replication of varicella-zoster virus. Intervirology 8: 83–91.

Schmidt, N. J. and E. H. Lennette. 1976. Improved yields of cell-free varicella-zoster virus. Infect. Immun. 14: 709–715.

Stinski, M. F. 1977. Synthesis of proteins and glycoproteins in cells infected with human cytomegalovirus. J. Virol. 23: 751–767.

Takahashi, M., Y. Okuno, T. Otsuka, J. Osame, A. Takamizawa, T. Sasada and T. Kubo. 1975. Development of a live attenuated varicella vaccine. Biken J. 18: 25–33.

Wolff, M. H. 1978. The proteins of varicella-zoster virus. Med. Microbiol. Immunol. 166: 21–28.