J. gen. Virol. (1981), **53**, 145–155
Printed in Great Britain

Coronavirus JHM: Intracellular Protein Synthesis

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(*Accepted 5 November 1980*)

SUMMARY

Coronavirus JHM contained six major proteins, four of which were glycosylated. The proteins were gp170, gp98, gp65, p60, gp25 and p23. Sac(−) cells infected with JHM shut off host cell protein synthesis, and the synthesis of three major (150K, 60K and 23K) and three minor (65K, 30K and 14K) polypeptides was detected by pulse-labelling with ^{35}S-methionine. Antiserum directed against purified virus proteins specifically immunoprecipitated the three major intracellular species and also the 65K minor species. During a chase period, species 150K and 23K were processed and three new immunoprecipitable species, 170K, 98K and 25K appeared. The intracellular species 170K, 98K, 65K, 60K, 25K and 23K co-electrophoresed with virion proteins.

Two-dimensional gel electrophoresis of infected cell polypeptides showed that the 60K, 23K, 25K and 14K species were relatively basic polypeptides whilst the 98K and 170K were relatively acidic and heterogeneously charged polypeptides. Additionally, a charge-size modification of the 23K species during processing was detected, which was not apparent using one-dimensional gel analysis.

INTRODUCTION

Coronaviruses are associated with a variety of diseases in animals and humans (McIntosh, 1974; Robb & Bond, 1979; Tyrrell *et al.*, 1978). Coronavirus JHM (JHMV) induces a variety of central nervous system disorders in mice and rats and can be used as a model for virus-induced demyelination (Nagashima *et al.*, 1978, 1979). Amongst the murine coronaviruses only the virions of A59 and JHM have been examined in detail. The emerging pattern is a single-stranded infectious RNA genome which is non-segmented, polyadenylated and has a mol. wt. of at least $5{\cdot}4 \times 10^6$ (Lai & Stohlman, 1978; Wege *et al.*, 1978). The virion also contains four to six proteins, including high and low mol. wt. glycoproteins and a non-glycosylated nucleocapsid protein of about 50×10^3 to 60×10^3 (50 to 60K) mol. wt. (Bond *et al.*, 1979; Sturman, 1977; Sturman & Holmes, 1977; Wege *et al.*, 1979).

In comparison, there is relatively little information on the synthesis of polypeptides in cells infected with murine coronaviruses. Intracellular pulse-labelling of JHMV-infected cells reveals a high mol. wt. polypeptide which is glycosylated, a 50 to 60K non-glycosylated polypeptide, and one or two low mol. wt. polypeptides (Anderson *et al.*, 1979; Bond *et al.*, 1979). Additional polypeptides designated as specific to JHMV-infected cells have also been identified during longer pulse-labelling experiments or during chase periods (Anderson *et al.*, 1979; Bond *et al.*, 1979), but the relationship of these polypeptides to each other, to the viral primary gene products or the virion structural proteins has not been established.

The experiments reported here describe JHM virion proteins and the synthesis of polypeptides in JHMV-infected cells.

0022-1317/81/0000-4388 $02.00

METHODS

Cells and virus. JHM virus and virus stocks were obtained and propagated in Sac(−) cells as previously described (Siddell *et al.*, 1980). Virions were labelled with ^{35}S-methionine (SJ 204, Amersham/Buchler, Braunschweig, F.R.G.) and purified as described previously (Siddell *et al.*, 1980). Virions from identical infections were also labelled between 12 and 20 h after infection in a complete spinner medium containing 10 μCi ^{3}H-glucosamine (TRK 398, Amersham/Buchler) per ml, and purified as described previously (Wege *et al.*, 1979). The growth of the virus was measured by titration of clarified tissue culture medium using an endpoint dilution procedure and the calculation of $TCID_{50}$ according to Reed & Muench (1938).

Labelling of intracellular polypeptides. Monolayers of cells on 5 cm Petri dishes were infected with JHMV, or mock-infected as previously described (Siddell *et al.*, 1980) using a multiplicity of infection (m.o.i.) of 5 $TCID_{50}$/cell. At the times shown in the figure legends, the medium was replaced by medium without methionine or serum (minus methionine medium). After 15 min this medium was replaced by minus methionine medium containing either 10 μCi/ml or 100 μCi/ml ^{35}S-methionine. The cells were pulse-labelled for the times indicated in each experiment. At the end of the pulse-labelling period cell lysates were either prepared immediately for one- or two-dimensional gel electrophoresis or in the experiment shown in Fig. 4 the radioactivity in the cells was chased by removing the labelling medium, washing the cells twice in complete medium containing an additional 1 mM-methionine and maintaining the cells in this medium at 37 °C for the times indicated. All cytoplasmic cell lysates were prepared at 4 °C.

One-dimensional lysate preparation and gel electrophoresis. At the end of the labelling period the cells were washed twice in phosphate-buffered saline (PBS), and lysed in 50 mM-tris-HCl pH 6·8, 2% SDS, 4 M-urea and 2% 2-mercaptoethanol. The cell lysate was immediately passed through a syringe needle 10 times, heated to 100 °C for 2 min and stored at −20 °C. Samples were mixed with an equal volume of electrophoresis sample buffer (Siddell *et al.*, 1980) and electrophoresed on 15% discontinuous SDS–polyacrylamide gels as described by Laemmli (1970). The procedures for staining and drying the polyacrylamide gels and exposure of autoradiographs have been described by Smith *et al.* (1974). The gel shown in Fig. 4 was impregnated with PPO (2,5-diphenyloxazole) and subjected to fluorography at −80 °C (Laskey & Mills, 1975).

Two-dimensional lysate preparation and gel electrophoresis. After the labelling period cells were washed twice in PBS, scraped into PBS and pelleted at 800 ***g*** for 2 min. The cells were lysed in 50 mM-tris-HCl pH 7·5 containing 100 mM-NaCl and 0·2% NP40 (TNP) and additionally 1 mg/ml *N-p*-tosyl-L-lysine-chloromethyl ketone HCl (TLCK), 200 μg/ml phenylmethylsulphonyl fluoride (PMSF) and 500 Kallikrein inhibitor units/ml Aprotinin. The cytoplasmic lysate was centrifuged at 10000 ***g*** for 2 min and the supernatant was stored at −70 °C. Before electrophoresis the lysate was incubated for 20 min at 22 °C with pancreatic ribonuclease (final concentration 200 μg/ml), then mixed with an equal volume of 9·5 M-urea containing: 4% (v/v) NP40, 10% (v/v) 2-mercaptoethanol, 3·2% (v/v) pH 5 to 8 ampholine and 0·8% (v/v) pH 3·5 to 10 ampholine. Finally, solid urea was added to a final concentration of 9·5 M.

The electrophoresis procedures of O'Farrell (1975) and O'Farrell *et al.* (1977), i.e. non-equilibrium pH gradient gel electrophoresis (NEPHGE) or isoelectric focusing (IEF) in the first dimension and SDS–polyacrylamide gradient gel electrophoresis in the second dimension, were used.

Isoelectric focusing gels (0·2 × 11 cm) were prepared according to O'Farrell (1975) using a combination of ampholine pH range 3·5 to 10 and 5 to 8 in the ratio 1:2. The pH gradient was monitored immediately at the end of each run by cutting a control gel into 1 cm pieces

and shaking them with boiled distilled water. After 30 min the pH was measured. The gradients were typically between pH 5 and 7·5. Gels were loaded with 30 μl of sample and run at 400 V for 19 h. Other details (e.g. prerunning) were according to O'Farrell (1975). After running, gels were stored at −70 °C.

NEPHGE was as described by O'Farrell *et al.* (1977). Tube gels (0·2 × 11 cm) and ampholines, pH range 3·5 to 10, were used. Gels were loaded with 30 μl of sample and run at 400 V for 2·5 h. The pH gradient, monitored as described above, was typically between pH 4·5 and 8·5. After running, gels were stored at −70 °C.

First dimension gels were prepared for running in the second dimension by equilibration for 1 h in 18 ml buffer containing 62·5 mM-tris-HCl pH 6·8, 2·2% SDS and 5% (v/v) 2-mercaptoethanol. SDS–polyacrylamide slab gels with an exponential gradient of 10 to 30% acrylamide were made as described by O'Farrell (1975). The high percentage acrylamide (30% acrylamide/0·15% bisacrylamide) was stirred in a 20 ml constant volume mixing chamber, into which the lower percentage acrylamide (10% acrylamide/0·27% bisacrylamide) was pumped. A 2 cm 3% acrylamide stacking gel was poured on top of the main slab gel and the equilibrated first dimension gel was fixed on top with 1% agarose in 62·5 mM-tris-HCl pH 6·8, 0·2% SDS coloured with 0·1% bromophenol blue. Electrophoresis was for 4·5 h at 20 mA. Radioactively labelled mol. wt. marker proteins were run in a slot at the side of the gel. Gels were stained, dried and exposed for autoradiography as described above.

Immunoprecipitation of cytoplasmic lysates. At the end of the pulse and each chase period the cells were washed twice in PBS, scraped into PBS using a rubber policeman and pelleted at 800 ***g*** for 5 min. The cell pellet was resuspended in TNP, the lysate centrifuged at 10000 ***g*** for 2 min and the supernatant stored at −70 °C. One vol. cytoplasmic lysate was incubated at 22 °C for 2 h with an equal volume of a 1/100 dilution (in TNPE, see below) of hyperimmune serum raised in rabbits against purified virus (Siddell *et al.*, 1980), together with 2 vol. 50 mM-tris-HCl pH 7·5 containing 150 mM-NaCl, 5 mM-EDTA, 0·05% NP40 and 1 mg/ml bovine serum albumin (BSA) (TNPEB). The immune complexes were adsorbed to a suspension of protein A-bearing *Staphylococcus aureus* (Kessler, 1975) and the mixture washed twice in TNPE (TNPEB without BSA) by pelleting at 10000 ***g*** for 2 min and resuspension. The final pellet was resuspended in electrophoresis sample buffer, incubated for 5 min at 37 °C, then centrifuged at 10000 ***g*** for 2 min. The supernatant was heated to 100 °C for 2 min and electrophoresed as described above for one-dimensional gel electrophoresis.

Materials. The ^{14}C-labelled mol. wt. markers were purchased from Amersham/Buchler (CF 262). These markers are myosin (200K), phosphorylase b (92·5K), BSA (69K), ovalbumin (46K), carbonic anhydrase (30K) and lysozyme (14·3K). Ultrapure urea was purchased from Schwarz/Mann, Orangeburg, N.Y., U.S.A. Ampholines were purchased from LKB, Frankfurt, F.R.G. Aprotinin was purchased from Sigma.

RESULTS

Under our conditions cells infected with JHMV at an m.o.i. of 5 $TCID_{50}$/cell start to release infectious virus 4 to 6 h after infection. The cytopathic effect of JHMV is characterized by the formation of syncytia, leading to a totally fused monolayer which is evident by approx. 10 h after infection. After 12 h post-infection the cell monolayer starts to detach from the Petri dish.

Virion proteins

The ^{35}S-methionine-containing proteins of purified JHMV are shown in Fig. 1. The virion is comprised of six major proteins which are designated by their mol. wt. and whether or not they are glycosylated. The pattern shown here is essentially as described by Wege *et al.*

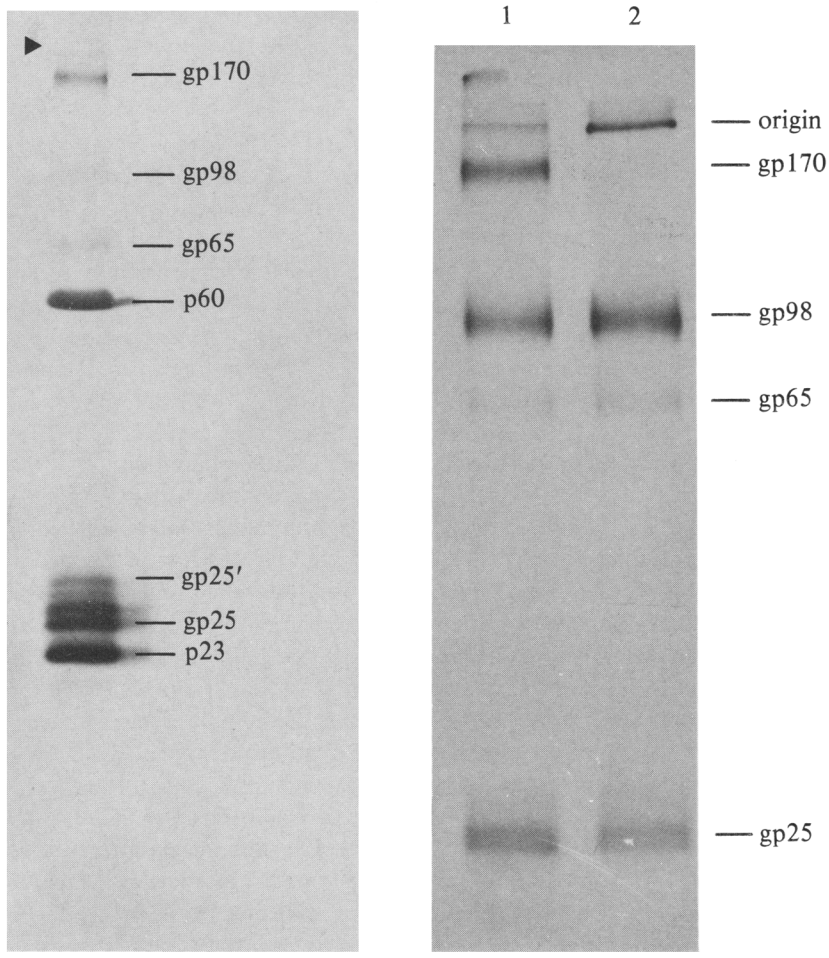

Fig. 1. Structural proteins of JHMV. JHM virions were labelled with ^{35}S-methionine and purified as described in Siddell *et al.* (1980). Virus was mixed with an equal volume of electrophoresis sample buffer and heated to 37 °C for 2 min before electrophoresis.

Fig. 2. Effect of heating on the polypeptides of JHMV. JHM virions were labelled with ^{3}H-glucosamine and purified as described in the text. Virus was mixed with an equal volume of electrophoresis sample buffer containing dithiothreitol and heated to 37 °C (track 1) or 100 °C (track 2) for 2 min before electrophoresis.

(1979), but differs in showing a previously undescribed protein of 65K (gp65), and the 125K glycoprotein (GP2 of Wege *et al.*, 1979) was not detected. Secondly, whereas Wege *et al.* (1979) reported two low mol. wt. virion proteins (GP5 of 25K and VP6 of 23K), by using a uniform 15% polyacrylamide gel with a low bisacrylamide content we can now resolve several species in the 25K region. These species vary both in number and in relative proportion in different batches of purified virus (Fig. 1).

The effect of boiling purified virus preparations in electrophoresis sample buffer containing dithiothreitol is shown in Fig. 2. With ^{3}H-glucosamine-labelled virus the disappearance of gp170 and the appearance of material which remains at the origin of the gel is seen upon boiling. This suggests therefore that the material remaining at the origin of the gel is an

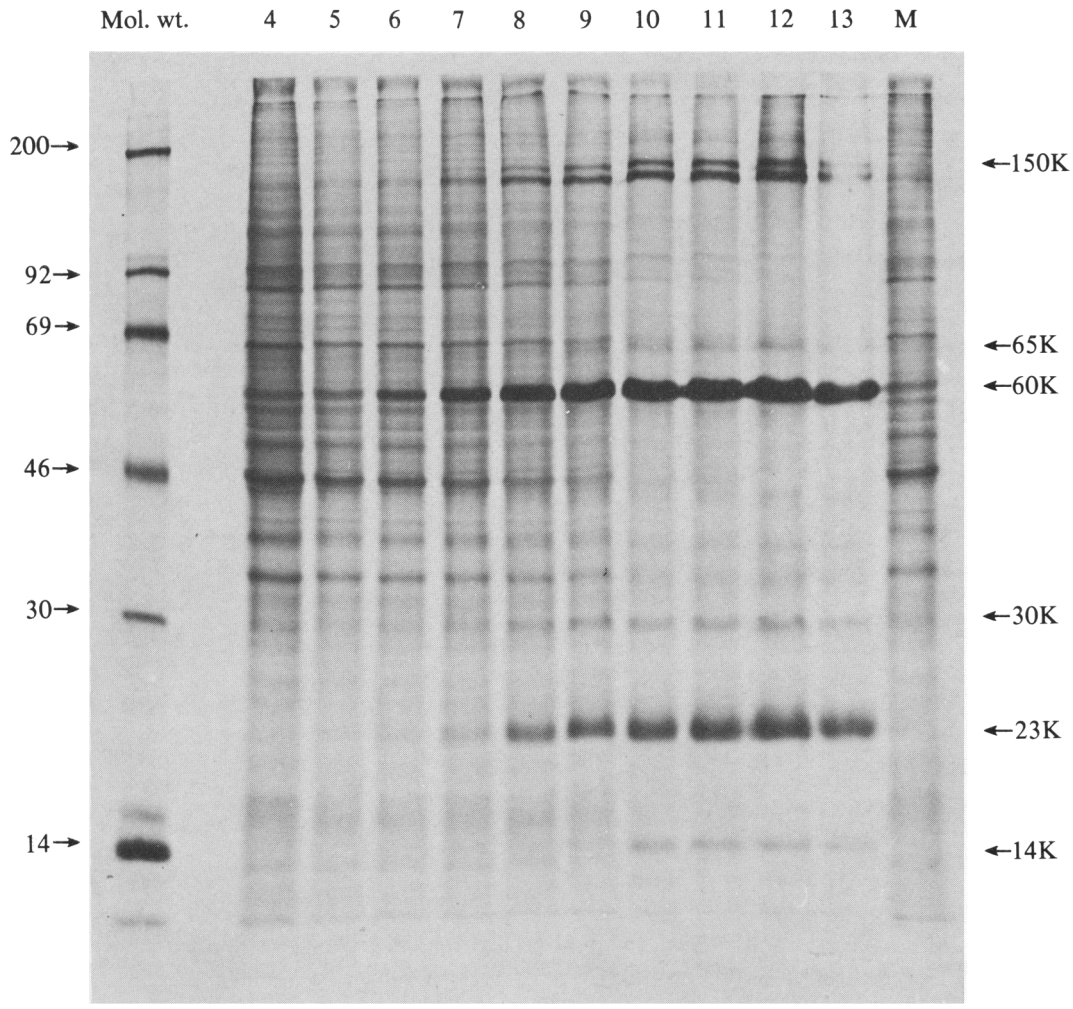

Fig. 3. Polypeptides synthesized in JHMV-infected cells. Sac(−) cells were infected with JHMV and labelled at the times indicated for 15 min in medium containing 10 μCi/ml ^{35}S-methionine, and lysates were prepared as described in the text. Samples of each lysate (80 000 ct/min) were electrophoresed as described in the text. M, Mock-infected.

aggregated form of gp170. Fig. 2 also confirms that JHMV has four major glycoprotein bands (Wege *et al.*, 1979).

Intracellular protein synthesis

One-dimensional electrophoresis

Fig. 3 shows the result obtained by pulse labelling of cells with ^{35}S-methionine at different times after infection. At an m.o.i. of 5 $TCID_{50}$/cell a clear shut off of host protein synthesis can be seen by 9 to 10 h after infection. Coomassie blue staining of the gel shown in Fig. 3 showed that at all time points approximately equal quantities of cellular polypeptides were

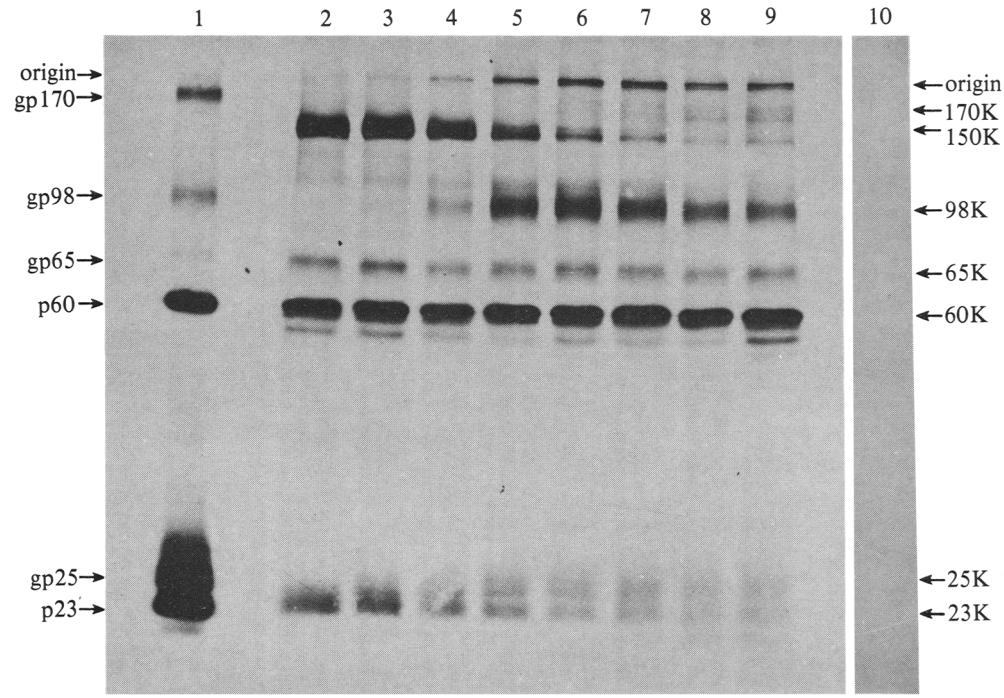

Fig. 4. Pulse–chase labelling of JHMV-infected cells and immunoprecipitation of cytoplasmic lysates with anti-JHM serum. Sac(−) cells were infected with JHMV, pulse-labelled 9 h after infection in medium containing 10 μCi/ml ^{35}S-methionine and chased with medium containing unlabelled methionine as described in the text. Cytoplasmic lysates were prepared and aliquots immunoprecipitated with anti-JHM serum or preimmune serum and electrophoresed as described in the text. Purified virus was prepared as described in Fig. 1. 1, Purified virus: cytoplasmic lysates prepared after a 15 min pulse (2) and a 15 (3), 30 (4), 60 (5), 90 (6), 120 (7), 150 (8) and 180 (9) min chase and immunoprecipitated with anti-JHM serum. Track 10, as 2 but immunoprecipitated with preimmune serum.

loaded on to each track, whilst the autoradiograph clearly shows an almost complete inhibition of, for example, actin synthesis during the experiment.

At late times in infected cells it was possible to detect the synthesis of three major (150K, 60K and 23K) and three minor (65K, 30K and 14K) polypeptides. The synthesis of the 30K species was sometimes difficult to detect and the 150K polypeptide was reproducibly resolved as a doublet. To investigate any relationships between these intracellular polypeptides and the virion proteins, a ^{35}S-methionine pulse-labelled cytoplasmic lysate was immunoprecipitated with serum directed against purified virions, and the immunoprecipitate co-electrophoresed with labelled virus (Fig. 4, tracks 1 and 2). This experiment showed that the three major intracellular species, 150K, 60K and 23K, are one minor species, 65K, are specifically immunoprecipitated (immunoprecipitation of an infected lysate with control serum is shown in track 10). These species are therefore related to virion proteins. The 65K, 60K and 23K species co-migrate with the virion proteins gp65, p60 and p23. The 150K species does not co-migrate with a virion protein. The non-precipitated species of 30K and 14K are assumed to be polypeptides which are not present in purified virions. The minor band migrating slightly faster than the 60K species is generated during the immunoprecipitation procedure and is presumed to be a degradation product, most probably of nucleocapsid protein.

Fig. 4 also shows the immunoprecipitation with the same antiserum of cytoplasmic lysates pulsed with ^{35}S-methionine and then chased in the presence of an excess of methionine for various periods of time. During the chase period, the 150K and 23K species were processed

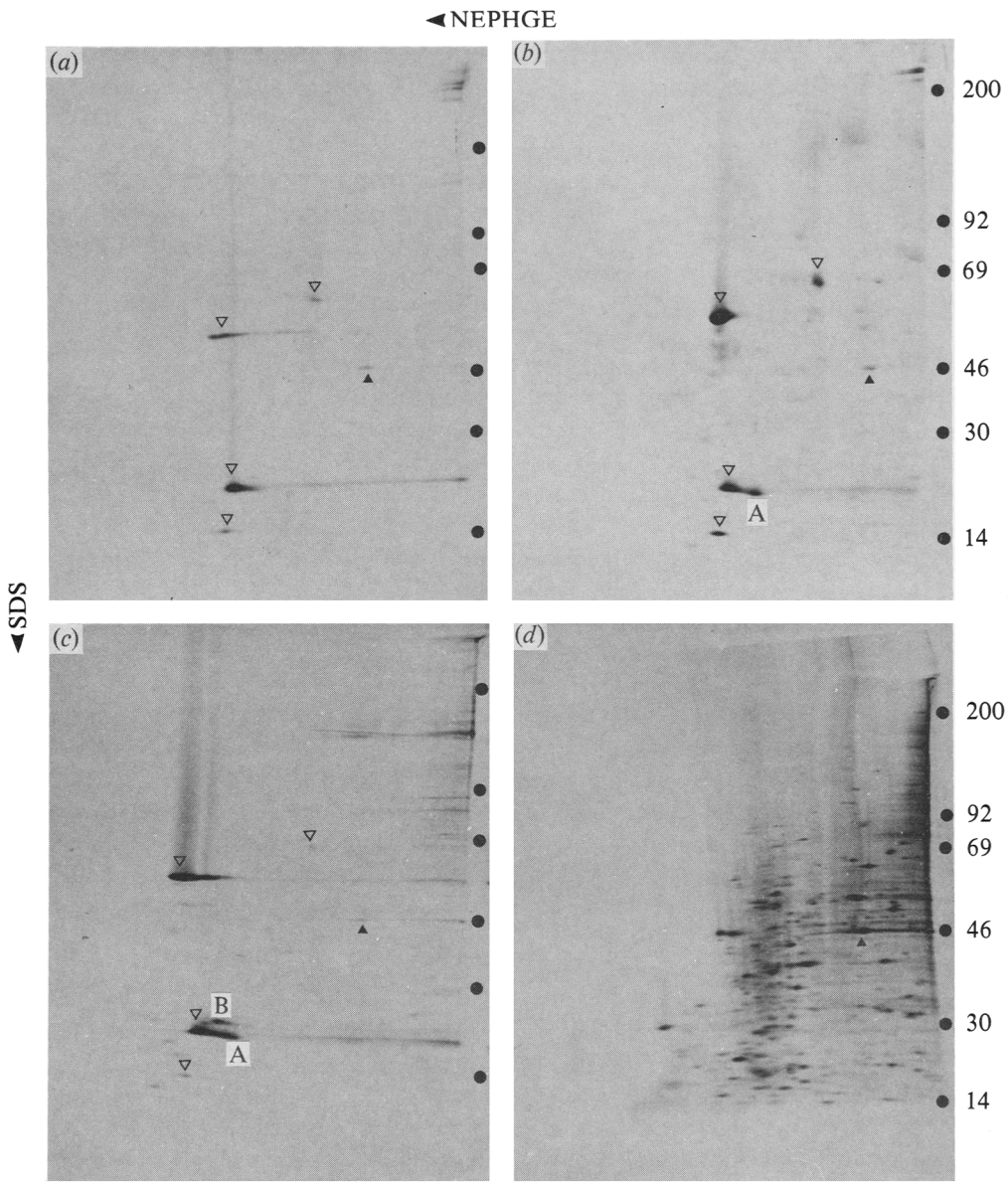

Fig. 5. Two-dimensional NEPHGE/SDS electrophoresis of cytoplasmic lysates from JHMV-infected cells. Sac(−) cells were infected with JHMV, pulse-labelled 10 h post-infection in medium containing 100 μCi/ml ^{35}S-methionine and cytoplasmic lysates were prepared and electrophoresed as described in the text. 1, 2 and 3, Infected cells pulse-labelled for 15, 30 and 45 min respectively. 4, Mock-infected cells pulse-labelled for 45 min. Actin is indicated by a closed arrow.

and two new immunoprecipitable species of 98K and 25K appeared. Also, a small amount of material of 170K and material which remained at the origin of the gel appeared during the chase period. As we have shown (Fig. 2) that boiling purified virions in electrophoresis sample buffer caused the disappearance of gp170 and the appearance of material at the gel origin, we repeated this experiment without boiling the immunoprecipitated proteins. Under these conditions no material appeared at the gel origin during the chase period but instead the intracellular species of 170K was enhanced (data not shown). The intracellular species of

170K, 98K and 25K which arose during the chase period co-migrated with virion proteins gp170, gp98 and gp25. The immunoprecipitation of all chased lysates with preimmune serum were negative, as was the immunoprecipitation of all uninfected lysates with hyperimmune serum.

Two-dimensional electrophoresis

We extended our analyses of intracellular protein synthesis in infected cells by using two-dimensional gel electrophoresis. Firstly, this increases the likelihood of detecting proteins synthesized in small amounts and secondly, provides valuable information on the properties of these proteins. Fig. 5 shows a two-dimensional gel analysis, using NEPHGE in the first dimension, of cytoplasmic lysates from infected cells pulsed with ^{35}S-methionine for 15, 30 or 45 min and mock-infected cells pulsed for 45 min.

During the 15 min pulse four polypeptides were labelled (open arrows) which are specific to the infected cells as determined by comparison with a mock-infected cell lysate. These species have mol. wt. of 65K, 60K, 23K and 14K and we believe that they are identical to the species 65K, 60K, 23K and 14K found on one-dimensional gels. Neither a 30K species nor a 150K species specific to the infected cell lysate could be found using this gel system. After a 30 min pulse the pattern was essentially the same except that a new species, 23K (A), with a slightly lower mol. wt. and slightly less basic than the 23K species, was seen. After a 45 min pulse a second new species, 23K (B), with a higher mol. wt. than the 23K species and a charge intermediate between the 23K and 23K (A) species, was detected. Although it is not possible to determine isoelectric points on the non-equilibrium system, the migration of the 60K, 23K, 23K (A), 23K (B) and 14K species, relative to actin, indicates that they are basic proteins.

We have also analysed the samples shown in Fig. 5 on the second two-dimensional gel system, i.e. using isoelectric focusing in the first dimension. The extra information obtained can be seen by examining the 45 min pulse lysate from infected and mock-infected cells and this is shown in Fig. 6. In this system we did not detect the 65K, 60K, 23K, 23K (A), 23K (B) and 14K species, which presumably do not enter the gel because of their basic charge. However, it is possible to detect a series of 30K species (open arrows) but as on one-dimensional gels, careful comparison with the mock-infected lysate is necessary. They have isoelectric points at about pH 7.

The most remarkable feature specific to the infected cell lysate is three series of polypeptides with mol. wt. of approx. 98K (series A), 170K (series B) and a value which we have not accurately determined (series C) but is significantly greater than our highest mol. wt. marker of 200K. Each series is comprised of about 15 species which have a spacing in the IEF dimension consistent with single charge differences. The polypeptides in each series are relatively acidic with pIs in the range between pH 5·5 and 6. There is also a slight increase in the apparent mol. wt. of the more acidic species in the second dimension. As a 45 min pulse-labelling period is sufficient time to allow processing of intracellular polypeptides (Fig. 4), our interpretation is that series A and series B detected in Fig. 6 correspond to the 98K and 170K species which appear during the chase period in Fig. 4. Series A and B could not be detected in cell lysates which have been pulsed for only 15 min (data not shown). Using the IEF system we did not detect any discrete polypeptide corresponding to a mol. wt. of 150K, although there is material of this size remaining at or near the top of the first-dimension gel. There is also a small amount of diffuse material of this mol. wt. which is at the bottom of the first-dimension gel.

DISCUSSION

The protein composition of JHMV described here agrees with earlier reports of Wege *et al.* (1979) with two minor exceptions. Firstly, we suggest that the glycoprotein GP2 observed by Wege *et al.* (1979) was dimeric and that the monomeric form of this protein has a mol. wt. of

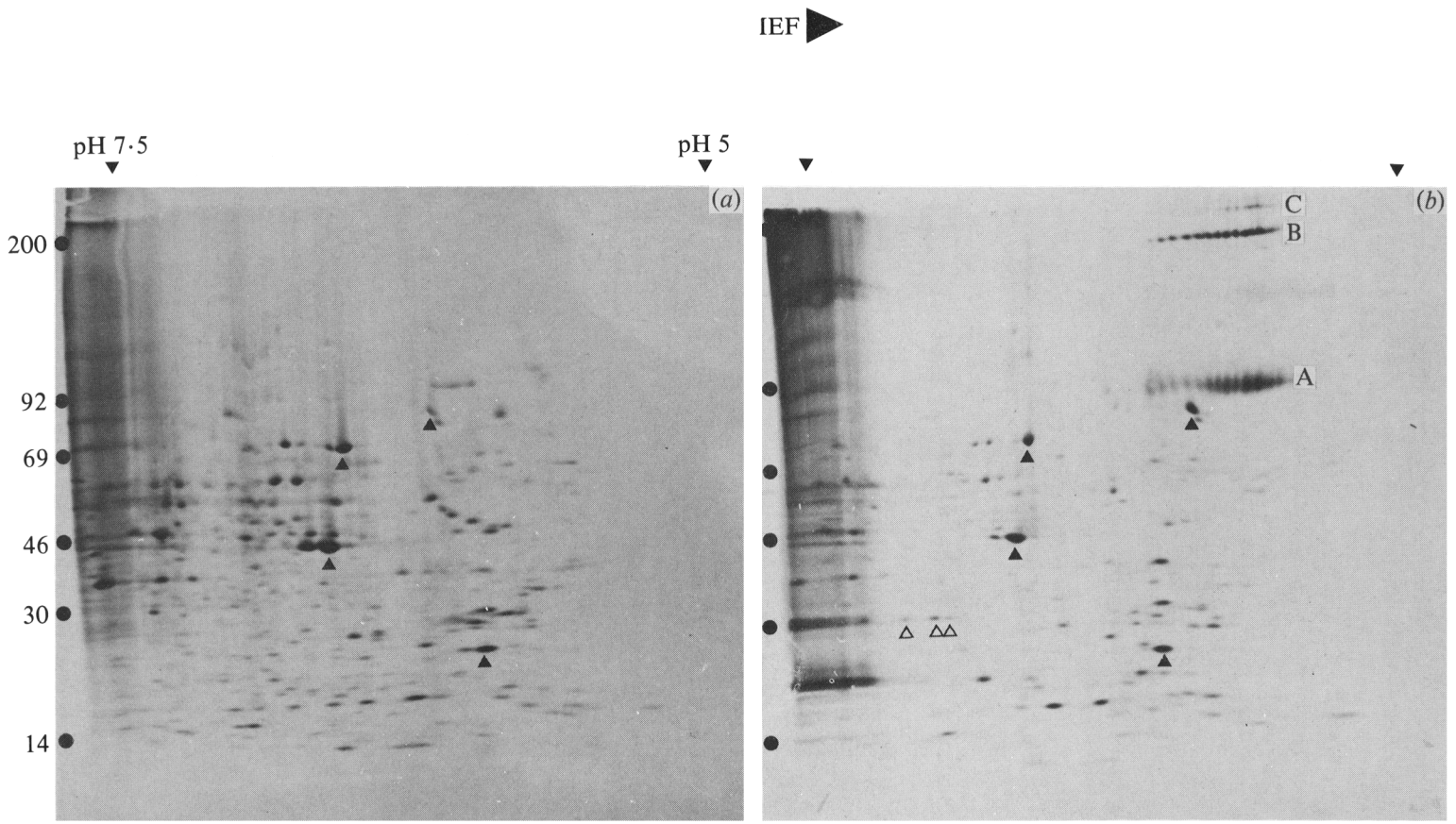

Fig. 6. Two-dimensional IEF/SDS electrophoresis of cytoplasmic lysates from JHMV-infected cells. Sac (–) cells were infected with JHMV, pulse-labelled 10 h after infection in medium containing 100 μCi/ml ^{35}S-methionine and cytoplasmic lysates were prepared and electrophoresed as described in the text. 1, Mock-infected cells pulsed for 45 min. 2, Infected cells pulsed for 45 min. Actin and other host cell proteins that serve as reference points are indicated with closed arrows.

65000. The 65K form is seem when dithiothreitol, rather than β-mercaptoethanol, is used as reducing agent. Two-dimensional gel electrophoresis using non-reducing conditions in the first dimension and reducing conditions in the second shows that gp65 can be derived from GP2 (S. G. Siddell *et al.*, unpublished data). Secondly, we can now resolve the low mol. wt. glycoprotein of JHMV (GP5, gp25) into a number of species all of which are glycosylated. If all these species have the same polypeptide component and represent different degrees of glycosylation, this would perhaps explain the variation in the amount and proportion of these species in different virus batches. In agreement with earlier reports (Sturman, 1977; Wege *et al.*, 1979) the use of different heating conditions alters the mobility of the high mol. wt. coronavirus glycoprotein in SDS–polyacrylamide gels.

Comparison of the proteins of MHV-A59 and JHMV reveals striking similarities. MHV-A59 is composed of two high mol. wt. glycoproteins, a non-glycosylated nucleocapsid protein and a low mol. wt. glycoprotein (Sturman, 1977; Sturman & Holmes, 1977). If the low mol. wt. protein of JHMV can be glycosylated in particular cell lines to produce a single species then the only difference in the protein composition of the two viruses is that JHMV contains gp65.

We have shown previously (Siddell *et al.*, 1980), and confirmed here, that JHMV infection shuts off host cell protein synthesis. In infected cell lysates pulse-labelled for 15 min, polypeptides of 150K, which was resolved as a doublet on one-dimensional gels, 65K, 60K, 30K, 23K and 14K were detected. The analysis of lysates on two-dimensional gels did not reveal any additional species, although it did provide interesting information on the properties of the intracellular polypeptides. Firstly, the intracellular precursor to the nucleocapsid protein (the 60K species in our system) was found to have a basic charge. Additionally, we found the 23K species (and its proposed derivatives) and the 14K species also to be relatively basic polypeptides. In contrast, the 98K and 170K species were composed of a heterogeneously charged population of relatively acidic polypeptides. The chemical basis of the charge heterogeneity apparent in the 98K and 170K species remains to be investigated, but is clearly of great interest. We did not detect a 150K species in either NEPHGE or IEF two-dimensional polyacrylamide gels. Bond *et al.* (1979) also found that the 150K polypeptide specific to infected cell lysates is not detected on NEPHGE gels; however, it did enter and remain in their IEF gels and had a pI of 7·75. This value is just above the range analysed in our IEF system and may provide an explanation of our result.

Immunoprecipitation of pulsed and pulse–chased lysates showed that four of the intracellular polypeptides, the 150K, 65K, 60K and 23K species were related to proteins found in the virion and that the processing of two of these, the 150K and 23K species resulted in the production of three more species of 170K, 98K and 25K, which were also related to virion proteins. This data and the co-electrophoresis of intracellular and virion proteins suggests that the 65K, 60K and 23K species are incorporated into virions without modifications which grossly alter their apparent mol. wt. in SDS–polyacrylamide gels, but that the 150K species is processed to produce the 98K and 170K species which are incorporated into the virion. Our experiments show that the kinetics of the production of the 98K and 170K species in the cell are identical, as is the processing of the 150K doublet. In addition, the 98K and 170K species both have identical properties when analysed by isoelectric focusing. Taken together this data suggests that gp98 is the monomeric form of the dimeric gp170. We have not, however, excluded a number of alternative pathways, for example in which the 98K and 170K species are derived independently from the two 150K components. Tryptic peptide maps of these polypeptides are needed to resolve this question.

In the low mol. wt. region we suggest that the intracellular 25K species (and the series of virion proteins gp25) are derived from the 23K species which is synthesized in the cell. Our two-dimensional gel analysis and *in vitro* translation studies (Siddell *et al.*, 1980) show that

the 23K species is a single polypeptide. Additionally, the kinetics of processing of the 23K and 25K species, and the basic charge of both polypeptides suggests to us a product–precursor relationship. As gp25 in the virion is glycosylated, whilst p23 is not, a glycosylation event could be proposed to explain the change in apparent mol. wt. Our two-dimensional gel analysis also shows that the processing event involves not only the proposed glycosylation but at least one other intermediate polypeptide modification.

Finally, our results suggest that the intracellular 30K and 14K species are polypeptides synthesized in the cell but not incorporated into virions. It is interesting to note that whilst the synthesis of the 30K species is difficult to detect in the cell, amongst the major products of the *in vitro* translation of infected cell poly(A)-containing RNA (Siddell *et al.*, 1980) is a polypeptide of the same size which is not immunoprecipitated by anti-JHM serum. If these polypeptides are related this suggests that a strong translational control is exerted on the synthesis of the 30K species in the cell.

Recent tryptic peptide mapping studies indicate that the intracellular 65K species found after pulse–chase-labelling is not the same polypeptide as that detected by pulse-labelling.

This research was supported by Deutsche Forschungsgemeinschaft Me 270/18.

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(Received 22 August 1980)