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THE EFFECT OF
2-DEOXY-D-GLUCOSE ON VESICULAR STOMATITIS
VIRUS GROWN IN VERO CELLS USING PYRUVATE
AS THE ENERGY SOURCE

by
Carol E. Telasco

A Thesis
Submitted to the Faculty of Graduate Studies through the
Department of Biology in Partial Fulfillment
of the Requirements for the Degree of
Master of Science at the
University of Windsor

WINDSOR, ONTARIO, CANADA

1977

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ABSTRACT

The effect of 2-deoxy-D-glucose (DOG) on vesicular stomatitis virus (VSV) grown in Vero cells in the presence of pyruvate as an energy source has been reported. 10 mM DOG, in the presence of pyruvate, was found to reduce the infectivity of VS virus by 99%. In contrast, 10 mM DOG in the presence of glucose reduced the infectivity of VS virus 49%.

Other cell lines showed the same general trend although the difference between glucose- and pyruvate-grown virus treated with DOG was not as apparent.

The results suggest that DOG acts to inhibit VS viral RNA production thereby resulting in reduced viral glycoprotein synthesis as well as a reduction in the synthesis of other viral proteins. Therefore fewer viral particles or particles containing aberrant glycoproteins are produced resulting in decreased infectivity.

ACKNOWLEDGMENTS

The author wishes to express sincere appreciation to Dr. L. R. Sabina, Department of Biology, University of Windsor, for his guidance throughout the course of this research and the writing of this thesis.

The author is grateful also to Dr. A. H. Warner, Department of Biology and Dr. D. E. Schmidt, Department of Chemistry, both of the University of Windsor, for their aid in the reviewing of this thesis.

A note of thanks is extended to Mr. T. D. Edwards and Mrs. B. Abson for their assistance with research materials.

To my mother a very special thanks for understanding, patience and encouragement given during this research.

This investigation was supported by grant A 2904 from the National Research Council of Canada.

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INTRODUCTION

In 1959, Kilbourne reported that the production of influenza B (Lee) virus in chick embryos was markedly inhibited by 2-deoxy-D-glucose (DOG), a potent glucose antimetabolite.

Although the inhibiting activity of DOG was not reversible by glucose in the intact chick embryo, he could readily reverse the inhibition by using in vitro cultures of chorioallantoic membranes. In assessing the toxic effect of DOG, Ball et al., (1957) and Landau et al., (1958) found the antimetabolite possesses relatively low toxicity in both experimental animals and man. These early findings in different biological systems have prompted subsequent in vitro studies to define DOG-induced changes during the replication of viruses.

Klenk et al., (1972) demonstrated that DOG and D-glucosamine affect different biological activities of fowl plague (FP) virus, a member of the influenza A group. High concentrations of D-glucosamine and DOG were shown to inhibit the formation of

hemagglutinin, neuraminidase and infectious virions. Further analyses by polyacrylamide gel electrophoresis (PAGE) revealed that under these conditions virus-specific, carbohydrate-free polypeptides were still being formed. However, all viral glycoproteins were missing. In their place a novel, viral-specific protein was found and this is believed to be a precursor of the hemagglutinin accumulating in infected cells when glycosylation is blocked. Apparently, the antimetabolite interferes with the sequence of events for correct glycosylation and with the processing of the precursor to hemagglutinin I and II. In the influenza virus system normal amounts of viral RNA and nucleocapsid proteins were synthesized in the infected cells.

DOG was also found to have a similar effect on herpesviruses (Courtney et al., 1973; Ludwig and Rott, 1975). The envelopes of these DNA viruses contain carbohydrate components linked to viral-specific proteins. Courtney et al., (1973) found that DOG inhibited the production of infectious herpes simplex

virus (HSV) and promoted the synthesis of altered viral-specific glycoproteins recognizable by PAGE. Their data suggests that the HSV nucleocapsids were enveloped with altered glycoprotein components. These newly virus-specified alterations may affect the physical and biochemical integrity of the envelope resulting in the inability of the virus to properly attach to host cells, penetrate host cells, or be uncoated once inside host cells.

For confirmation of the earlier findings of Courtney, evidence was obtained on the formation of altered glycoproteins in another herpesvirus replicating under the influence of DOG (Ludwig and Rott, 1975). Like HSV, pseudorabies virus (PsV) particles were also formed in the presence of DOG. Although they found viral particles were noninfectious as a consequence of the incorporation of nonfunctional proteins, the production of viral DNA was not impaired by the action of the deoxy sugar.

Kaluza et al., (1972) studied the inhibition of

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multiplication of various enveloped RNA viruses by glucosamine and DOG. Glucosamine inhibited the formation of infectious fowl plague, Sindbis, and Semliki Forest (SF) viruses but had little or no effect on Newcastle disease (ND), polio and vesicular stomatitis (VS) viruses. DOG had a somewhat stronger effect than glucosamine. Only the production of viral glycoproteins seemed to be affected. In all cases almost normal amounts of viral RNA and RNA polymerase were synthesized.

Subsequently, Kaluza et al., (1973) suggested that DOG was incorporated into viral glycoprotein probably by substituting for mannose. Using tritium-labelled DOG, it was established that label was incorporated into viral glycoproteins.

Although the presence of glucose interfered with the incorporation of DOG more severely than mannose, the effect of the antimetabolite on the yield of infectious SF virus in the presence of equimolar concentrations of mannose was negligible when compared to that with glucose. Since DOG interferes with

the conversion of glucose-6-phosphate to fructose-6-phosphate (Barban and Schultze, 1961), it would therefore interfere with the conversion of glucose to mannose. A reasonable conclusion was that mannose competes more effectively with the action of the antimetabolite than does glucose. It is likely that DOG in substituting for mannose would cause incorrect glycosylation of the viral glycoproteins which otherwise have a correct backbone. These fraudulent viral components, possibly in mixture with correct ones, might prevent the assembly process or lead to the production of fragile particles.

The production of VS and ND viruses had earlier been found to be resistant to treatment with glucosamine or DOG (Kaluza et al., 1972). Scholtissek et al., (1974) found that if viruses grown in pyruvate-containing medium or dialyzed overnight in phosphate-buffered saline were used for infecting cells the multiplication of VS and ND viruses could be greatly inhibited by DOG in a pyruvate-containing medium.

It was assumed that glucose in the media would be interfering with the uptake and incorporation of DOG into cells.

Therefore, the use of pyruvate as the energy source allowed DOG to be activated.

Scholtissek et al., (1975) obtained evidence that in glucose-containing medium the effect of glucosamine is only on the synthesis of glycoproteins of FP and SF viruses, whereas the synthesis of viral RNA and protein was similar to that observed in control cultures. However, in pyruvate- and fructose-containing media glucosamine caused suppression of viral RNA and of all viral protein synthesis. Under these latter conditions protein and RNA synthesis in non-infected cells was almost normal.

This thesis is concerned with studies on the inhibitory effect of DOG on the production of infectious vesicular stomatitis in cell cultures maintained in glucose- and pyruvate-containing media. It was shown that VS virus need not be

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grown in a pyruvate-containing medium or pre-dialyzed against phosphate-buffered saline before use in infection in pyruvate-containing medium to be inhibited by DOG. In fact, in most cell lines tested, the effect of DOG on VS virus production was very similar in glucose- and pyruvate-containing media.

A greater reduction of infectivity was experienced when VS-infected Vero cells were incubated in pyruvate medium than in glucose medium. It was shown that the inhibition was reversible by the addition of glucose and, to a somewhat greater extent, mannose.

The polypeptide peaks obtained on PAGE of purified VS virions grown in Vero cells in medium containing pyruvate and DOG were greatly reduced. This reduction was also apparent in the virus-infected cell layer. Of special interest was the occurrence of two novel peaks in place of the viral G polypeptide. These could represent precursor, non-glycosylated proteins similar to that found in FP virus (Klenk et al., 1972)

and Herpesviruses (Courtney et al., 1973; Ludwig and Rott, 1975).

Another possibility is that these peaks may represent aberrant

polypeptides having mobilities greater or less than that of

the G polypeptide. Reduction of viral RNA synthesis was also

evident.

MATERIALS AND METHODS

Cells and Media

Five different heterploid cell lines were used: mouse fibroblasts (L-929) and human epidermoid carcinoma of the larynx (H. Ep #2) cells obtained from the Grand Island Biological Company, Grand Island, New York; porcine kidney (PK-15), baby hamster kidney (BHK-21) and African green monkey kidney (Vero) cells obtained from Flow Laboratories, Rockville, Maryland.

Monolayer cultures were grown in Eagles' (1959) minimal essential medium (MEM) supplemented with 10% fetal calf serum (heat inactivated at 56°C for 15 minutes), sodium penicillin G (100 units/ml), dihydrostreptomycin (100 mcg/ml) and fungizone (0.25 mcg/ml). In all experiments MEM was supplemented with fetal calf serum that was heat inactivated at 56°C for 15 minutes and dialyzed for 3 hours against

0.9% NaCl (MEM-10FCS). This treatment resulted in the removal of about 85% of the glucose from serum (Appendix A). In addition overlay medium containing either 10 mM glucose (G-MEM-10FCS) or 10 mM pyruvate (P-MEM-10FCS) was used.

Virus

Vesicular stomatitis (VS) virus, Indiana serotype, was obtained from Dr. N. A. Labzofsky, Ontario Department of Health, Toronto, Ontario. The virus was plaque-purified once in L-929 cells. High-titer virus stock was prepared by inoculating L-929 cell monolayers at a multiplicity of approximately 2.5 plaque forming units (PFU) per cell. After adsorption at 37°C for 30 minutes, MEM-10FCS was added and the cultures reincubated. Twenty-four hours postinfection cultures were frozen at -20°C.

Before use in experiments the infected cultures were subjected to 2 cycles of freezing and thawing and the cell

debris was removed by centrifugation at 1,100 x g (Sorvall RC-2B; Rotor no. SS-34) for 10 minutes. The supernatant fluids had infectivity titers ranging from 5.5×10^6 to 8.5×10^7 PFU/ml.

Virus Purification

For purification of VS virus an aliquot of clarified viral cell culture fluid was centrifuged for 1.5 hours at 45,000 x g (Beckman Model L2-65B; Rotor no. 60 Ti). The sedimented viruses were resuspended in a small volume of Dulbecco's phosphate-buffered saline (PBS) minus magnesium and calcium salts, pH 7.4. The virus suspension was further purified by centrifugation through a preformed discontinuous type of sucrose gradient (Appendix B) for 2 hours at 92,500 x g in a Beckman SW 41 rotor. A 2-step gradient of 10 and 20% sucrose or a 4-step gradient of 5, 20, 30 and 45% sucrose was used. In some experiments the viral pellet was used from the 2-step

gradient. In others the bottom band of VS B-particles was collected by syringe from the 4-step gradient, repelleted and then resuspended in a small volume of PBS.

Infectivity Assays

VS virus was assayed by plaque formation in monolayers of L-929 cells grown in 60-mm plastic tissue culture dishes (Falcon Plastics, Los Angeles, California). Dilutions of the samples were made in medium MEM supplemented with 10% fetal calf serum. One-half ml aliquots of appropriate dilutions were allowed to adsorb at 37°C for 30 minutes. The virus-infected cultures were overlaid with 5 ml of agar medium (Sabina and Munro, 1969) and then reincubated at 37°C in a water-saturated atmosphere of 5% CO₂ in air. Twenty-four hours later an additional 5 ml of agar containing neutral red was added. The plaques were counted approximately 48 hours postinfection.

Protein Assays

All protein assays were carried out by the technique of Lowry et al., (1951).

Chemicals

Acrylamide, Bis crosslinker, N,N,N',N'-Tetramethylethylenediamine (TEMED), ammonium persulfate, sodium dodecyl sulfate (SDS), mercaptoethanol and Coomassie Brilliant Blue were purchased from Bio Rad Laboratories, Richmond, California. Spectrofluor PPO-POPOP concentrated liquid scintillator and NCS tissue solubilizer were obtained from Amersham/Seale Corporation, Arlington Heights, Illinois. Toluene was from the British Drug Houses (Canada) Ltd., Toronto, Ontario. Ethylene glycol monomethyl ether was from the Fisher Scientific Company, Fair Lawn, New Jersey. Actinomycin D, a by-product of Streptomyces chrysomallus and pyruvic acid were obtained from the Sigma Chemical Company, St. Louis, Missouri. 2-deoxy-

D-glucose was purchased from Calbiochem, San Diego, California.

^3H -L-Leucine (1.0 mCi/ml), ^3H -L-Tyrosine (1.0 mCi/ml) and

^3H -Uridine (1.0 mCi/ml) were purchased from New England Nuclear,

Boston, Massachusetts. The calibration kit for molecular

weight determinations was purchased from Pharmacia Fine

Chemicals, Piscataway, New Jersey.

Experimental Designs

a) VS virus growth studies

For those experiments designed to study VS virus growth in the presence of DCG, 48 hour old confluent cultures of PK-15, L-929, Vero, H. Ep #2 or BHK-21 cells in 1 oz Brockway bottles were infected with VS virus at a multiplicity of 60 to 100 PFU/cell. One hour postinfection the inoculum was removed and the cell layers washed twice with neutralized Hank's balanced salt solution (HBSS) to remove residual virus. Cultures were then overlaid with medium G-MEM-10FCS or

P-MEM-10FCS (control cultures). Experimental cultures were treated with various concentrations of DOG. At indicated times postinfection cultures were frozen at -20°C . All VS-infected cultures were subjected to 2 cycles of freezing and thawing before being assayed for infectivity.

In experiments to determine the effect of DOG added at different times following infection, cell cultures were incubated in the appropriate medium until specified times postinfection when the antimetabolite was added to a final concentration of 5 mM.

In studies on the reversal of the antimetabolite activity with glucose or mannose, the appropriate sugar was added to a final concentration of 10 mM to infected cultures containing 5 mM DOG in P-MEM-10FCS 4 hours postinfection. Cultures were frozen at indicated times postinfection. The percent inhibition was calculated according to the formula

$$100 - \frac{100A}{B} = I$$

where A is the PFU/ml in the presence of DOG, B is the PFU/ml in the absence of DOG and I is the percent inhibition.

b) Reactivity of VS virus with DOG

An aliquot of the virus pool was made 50 mM with respect to DOG and allowed to incubate at room temperature for 1.5 hours. Appropriate dilutions were made and the DOG-treated, as well as untreated virus (control), were plaqued on L-929 cells as previously described.

c) Toxicity testing of Vero cells with DOG

Medium was removed from 24 hour old cultures of Vero cells and replaced with P-MEM-10FCS containing 0, 5, 25 or 50 mM DOG. After 24 hours medium was removed and cells were infected with VS virus at a multiplicity of 60 to 100 PFU/ml. Following a one hour adsorption period at 37°C the monolayers were washed twice with HBSS and overlaid with medium

P-MEM-10FCS in the absence of DOG. Twenty-four hours later all cultures were frozen and stored at -20°C .

d) Determination of viral RNA

The medium from 48 hour old confluent Vero cell cultures was removed and the monolayers were infected at a multiplicity of 60 to 100 PFU/cell. Cell control cultures received an equal volume of HBSS. Thirty minutes postinfection all cultures were made 3 mcg/ml with respect to Actinomycin D. The inoculum-Actinomycin D fluids were removed one hour postinfection and monolayers were washed twice with HBSS. Virus-infected cultures were overlaid with medium P-MEM-10FCS containing 5 mM DOG and 3 mcg/ml Actinomycin D (experimental virus cultures) or no DOG and 3 mcg/ml Actinomycin D (virus controls). Mock-infected cells received P-MEM-10FCS containing 5 mM DOG and 3 mcg/ml Actinomycin D. Also, at one hour postinfection, duplicate cultures of virus- and mock-infected cells received

10 μ Ci/culture of ^3H -Uridine in HBSS having pyruvate substituted for glucose (P-HBSS). Similarly, ^3H -Uridine was added to other duplicate cultures at 2, 3, 4 and 5 hours postinfection. After one hour pulses, the cell layers were washed twice with PBS, three times with 6% trichloroacetic acid (TCA), twice with ethanol and once with methanol. The dried cell layers were then dissolved in 1 ml of 0.2 N NaOH and heated at 60°C for 10 minutes. Two-tenths ml aliquots were combined with 10 ml of counting fluid and samples were counted in a Nuclear Chicago Mark II Liquid Scintillation System.

e) SDS-polyacrylamide gel electrophoresis of VS viral proteins

i) Molecular weight determination of VS viral polypeptides

The bottom band of VS particles grown in glucose medium without DOG collected from a 5, 20, 30, 45% discontinuous sucrose gradient and a combination of molecular weight protein

standards (Ribonuclease A, Chymotrypsinogen A, Ovalbumin and Aldolase) were subjected to polyacrylamide gel electrophoresis (PAGE) according to Weber and Osborn (1969) and Maizel, Jr. (1971).

Virus suspensions were solubilized in a solution containing 15% SDS and mercaptoethanol diluted in the gel buffer (0.2 M phosphate, 0.2% SDS, pH 7.0) by heating at 100°C for 2 minutes (Maizel, Jr., 1971). An aliquot was mixed with glycerol and marker dye (bromphenol blue) and approximately 50 mcg protein was applied to 7.5% polyacrylamide gels having a 3% stacker gel.

Standard molecular weight protein markers were prepared according to Weber and Osborn (1969). A combination of these proteins was mixed with the gel buffer, mercaptoethanol, bromphenol blue and glycerol and 100 mcg protein was directly applied to gels.

Gels were subjected to electrophoresis at 7 mA/gel for

6 hours.

Gel columns were stained with Coomassie Brilliant Blue and cleared according to Spear and Roizman (1972). Densitometer tracings were made of the stained gels.

ii) Electrophoretic separation of radiolabelled viral polypeptides

Forty-eight hour old confluent Vero cells in 4 oz Brockway bottles were infected with VS virus at a multiplicity of 10 to 40 PFU/cell. After virus addition cultures were overlaid with medium G-MEM-10FCS, P-MEM-10FCS or P-MEM-10FCS containing 5 mM DOG. All media were lacking in the amino acids leucine and tyrosine. Those cultures subsequently used for electrophoresis of virus-infected cell layers were treated with 3 mcg/ml Actinomycin D. At 2 hours postinfection 20 μ Ci of an equal mixture of 3 H-leucine and 3 H-tyrosine was added to all cultures. The infected cultures were harvested 8 hours postinfection and stored at -20° C until the labelled virus particles were

purified. For the electrophoresis of infected cell proteins, the monolayers were scraped into the medium and subjected to low speed centrifugation. The resulting cell pellets were resuspended in 1 ml PBS and kept at -20°C .

For isolating viral polypeptides the samples were frozen and thawed 3 times before passing virus through a 10 and 20% preformed sucrose gradient as described under the section Virus Purification. The viral pellet was resuspended, solubilized and the polypeptides were separated by polyacrylamide gel electrophoresis.

Gels were sliced into fractions 2 mm wide according to a method of Maizel, Jr., (1966). These fractions were placed in glass scintillation vials and 0.5 ml NCS was added. Swelling of gels was permitted to occur overnight at 37°C . Five ml of toluene-based counting fluid was added and samples were counted in a Nuclear Chicago Mark II Liquid Scintillation System.

EXPERIMENTAL RESULTS

The Effect of 2-Deoxy-D-Glucose on the Production of VS Virus in Various Cell Lines

Scholtissek et al., (1974) reported, as did Kaluza et al., (1972), that DOG did not significantly inhibit the multiplication of VS virus in chick embryo cells when glucose-containing Earle's medium was used.

When VS virus was grown in various cell lines and overlaid with medium containing 10 mM DOG there was a considerable reduction in viral yields (Table 1) in both glucose- and pyruvate-containing media. However, in Vero cells the same level of DOG reduced the yield of virus only 49% in glucose-containing medium compared to 99% with medium containing pyruvate. These results suggest that the inhibitory effect of DOG on viral yields is dependent upon the type of host-cell infected. It was evident that the viral yields from all control infected cultures, except H. Ep #2, incubated in glucose-

Table 1. The effect of 2-deoxy-D-glucose on the yield of VS virus in various cell lines.*

Cell line	G-MEM-10FCS			P-MEM-10FCS		
	Control	DOG-treated	% Inhibition	Control	DOG-treated	% Inhibition
BHK-21	1.1×10^7	3.4×10^5	96.9	2.0×10^6	5.7×10^4	97.2
H. Ep#2	1.9×10^7	6.9×10^5	96.4	1.5×10^7	6.4×10^4	99.6
L-929	1.2×10^7	2.5×10^5	97.9	1.1×10^6	2.8×10^4	97.5
PK-15	1.5×10^7	5.5×10^5	96.4	6.8×10^6	2.0×10^4	99.7
Vero	9.5×10^6	2.8×10^6	49.0	6.0×10^5	2.2×10^3	99.6

*Forty-eight hour old cultures of BHK-21, H. Ep #2, PK-15, L-929 and Vero cells were incubated with VS virus for 60 minutes, washed and exposed to 10 mM DOG in pyruvate- or glucose-containing media. Control infected cultures were incubated in pyruvate- or glucose-containing media without DOG. At 24 hours postinfection viral yields were determined.

containing medium were about one log higher than those in medium with pyruvate as the energy source.

The Effect of Preincubation of 2-Deoxy-D-Glucose on the Capacity of Vero Cells to Inhibit VS Multiplication

To test the possibility that DOG might act at the cell membrane to prevent cell-virus interaction, Vero cell cultures were preincubated with pyruvate-containing medium in the presence of 0, 5, 25 and 50 mM DOG for 24 hours at 37°C before virus was added. It can be seen in Table 2 that final yields of virus were similar even in the presence of 50 mM DOG.

Test for Contact Activity of 2-Deoxy-D-Glucose on VS Virus

An experiment was done to determine the effect of treating clarified virus with 50 mM DOG as described in Materials and Methods. The viral yields of non-treated controls compared favourably with those treated with the compound (Table 3). Therefore, DOG does not inactivate the virus by direct contact.

Table 2. The effect of preincubation of Vero cultures in pyruvate medium containing DOG prior to infection.*

DOG (mM)	Virus yield (PFU/ml)
0	8.5×10^7
5	8.8×10^7
25	8.7×10^7
50	7.9×10^7

*Vero cultures were preincubated in pyruvate-containing medium in the presence of DOG in various concentrations for 24 hours. The cultures were washed, infected with VS virus, overlaid with P-MEM-10FCS and 24 hours later assayed for infectivity.

Table 3. The effect of contact activity of 2-deoxy-D-glucose on VS virus.*

DOG (mM)	Virus yield (PFU/ml)
0	8.4×10^7
50	8.5×10^7

*Clarified virus was incubated in 50 mM DOG for 1.5 hours at room temperature.

The Effect of Early Addition of 2-Deoxy-D-Glucose on the Yield
of Virus

To establish when the presence of DOG was required to exert its inhibiting effect, 43 hour old Vero cells were inoculated with VS virus in the absence and presence of 5 and 25 mM DOG in pyruvate-containing medium. At 1 hour postinfection monolayers were washed and recovered with pyruvate medium containing the appropriate concentration of 0, 5 or 25 mM DOG for the duration of the test period. One hour postinfection monolayers inoculated with virus in the absence of DOG were treated similarly. Twenty-four hours later all cultures were frozen and titrated.

The results in Table 4 show that the addition of DOG 1 hour postinfection had a greater inhibiting effect on virus production than DOG added at the time of infection.

Dose Response Relationships

The influence of DOG on VS virus replication in Vero and

Table 4. The effect of addition of 2-deoxy-D-glucose to Vero cells at time of virus infection and one hour postinfection.

DOG (mM)	DOG added at time of infection		DOG added at one hour postinfection	
	Virus yields (PFU/ml)	% Inhibition	Virus yields (PFU/ml)	% Inhibition
5	4.5×10^7	64.0	2.0×10^7	84.0
25	8.0×10^5	99.3	1.8×10^5	99.9

Control cultures infected with VS virus in the absence of DOG had a titer of approximately 1.2×10^8 PFU/ml.

L-929 cells was tested over a range of concentrations from 5 to 50 mM in medium containing glucose or pyruvate as the energy source. As shown in Fig. 1, the yield of infectious virus was progressively affected as the concentration of DOG was increased when infected Vero cells were incubated in medium with glucose or pyruvate. Furthermore, the magnitude of inhibition by DOG was considerably greater in pyruvate-containing medium.

In L-929 cultures no difference in susceptibility to DOG in the presence of medium containing glucose or pyruvate was demonstrated (Fig. 2). But reduced viral yields were obtained as the concentration of antimetabolite increased.

The Effect of the Addition of 2-Deoxy-D-Glucose during the Viral Growth Cycle

Growth curves of VS virus in Vero cells were performed with glucose- or pyruvate-containing medium. At various intervals after inoculation, DOG was added to give a final

Fig. 1. Relationship between dose of 2-deoxy-D-glucose and viral yields in Vero cells with pyruvate or glucose as the energy source. Forty-eight hour old Vero cells were infected with VS virus, washed and overlaid with glucose- or pyruvate-containing media having various concentrations of DOG as described in Materials and Methods. Control cultures were incubated in medium lacking in DOG. Symbols: (●) pyruvate-containing medium; (○) glucose-containing medium.

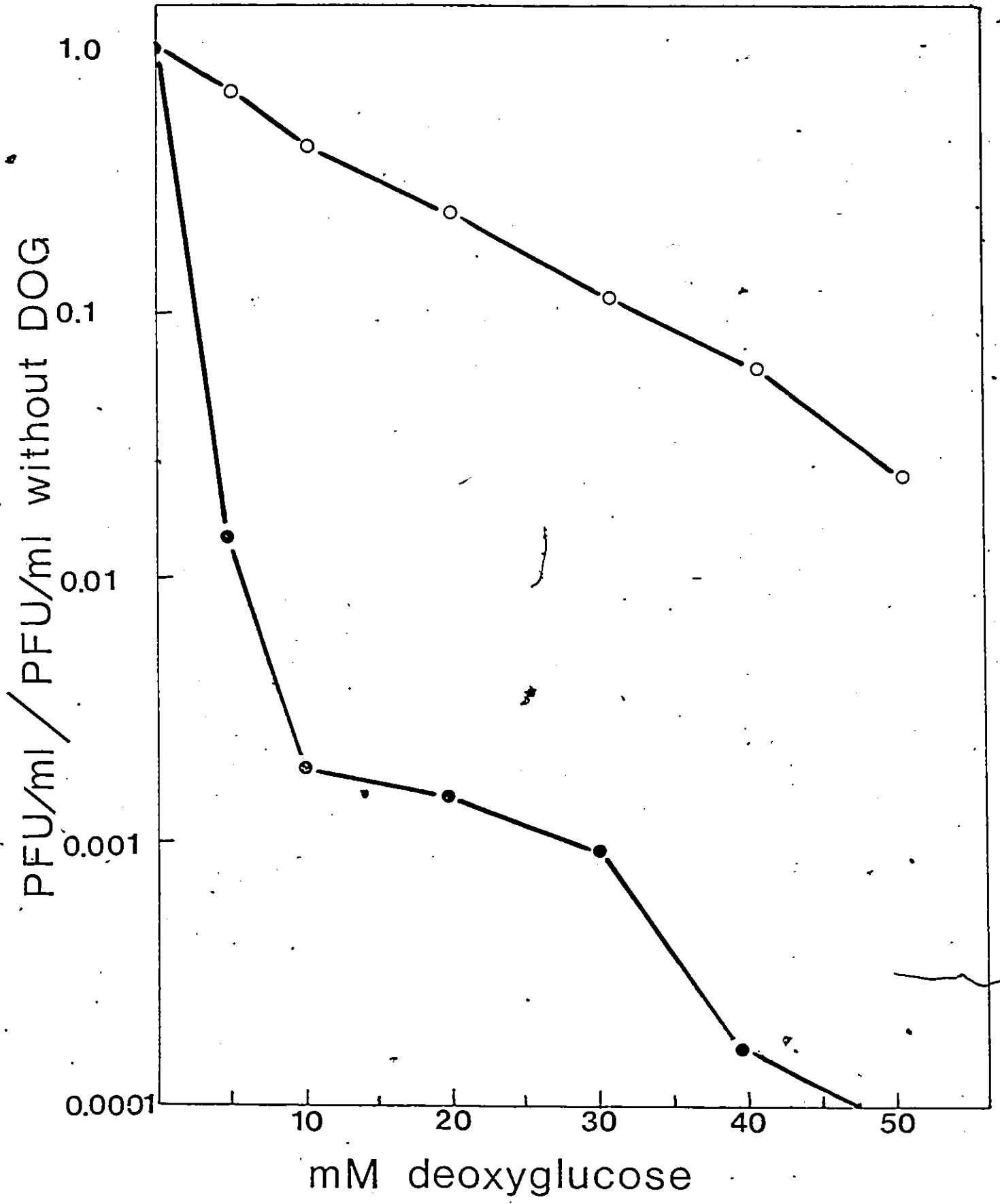
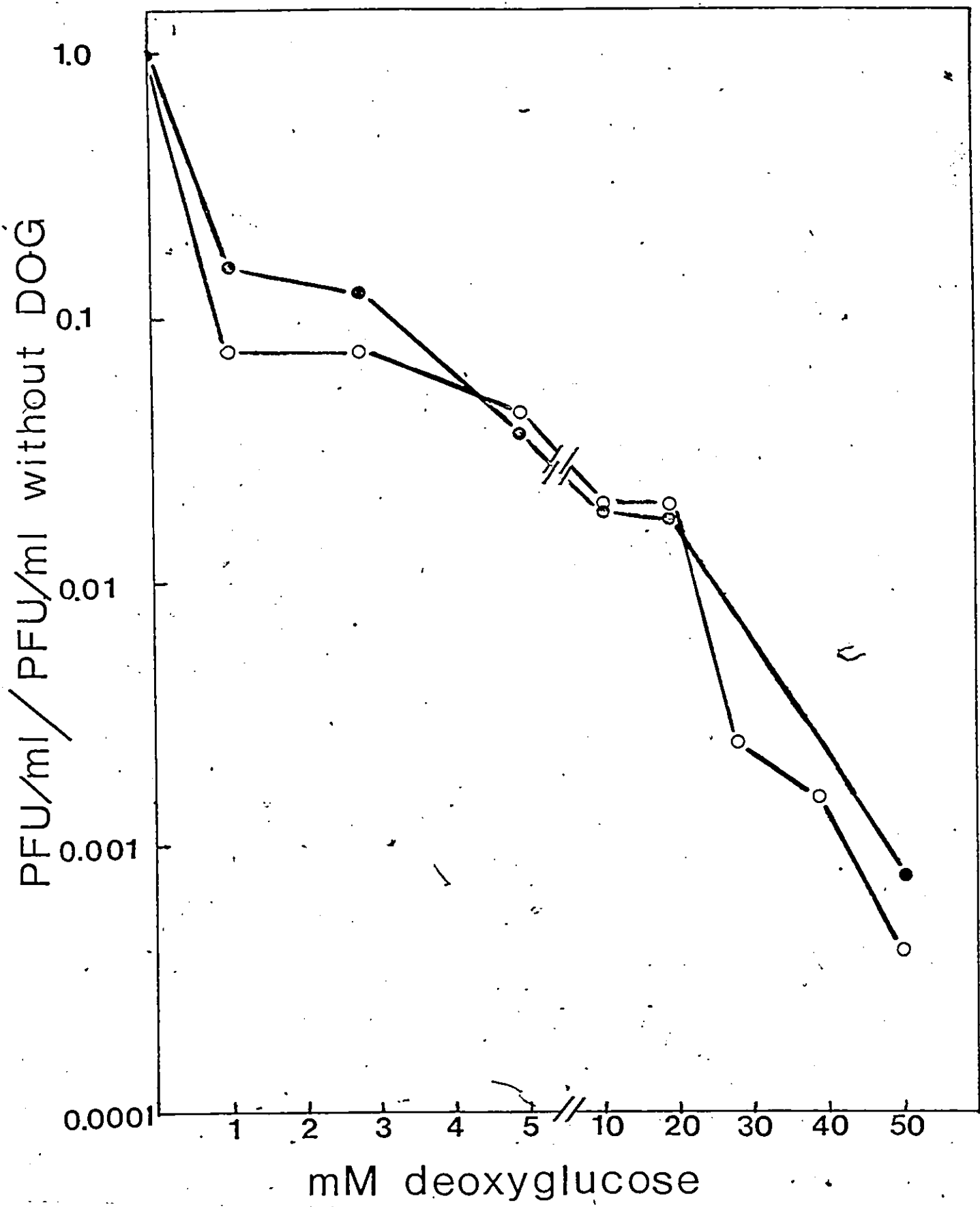


Fig. 2. Relationship between dose of 2-deoxy-D-glucose and viral yields in L-929 cells with pyruvate or glucose as the energy source. Experimental design as for Fig. 1.

Symbols: (●) pyruvate-containing medium; (○) glucose-containing medium.



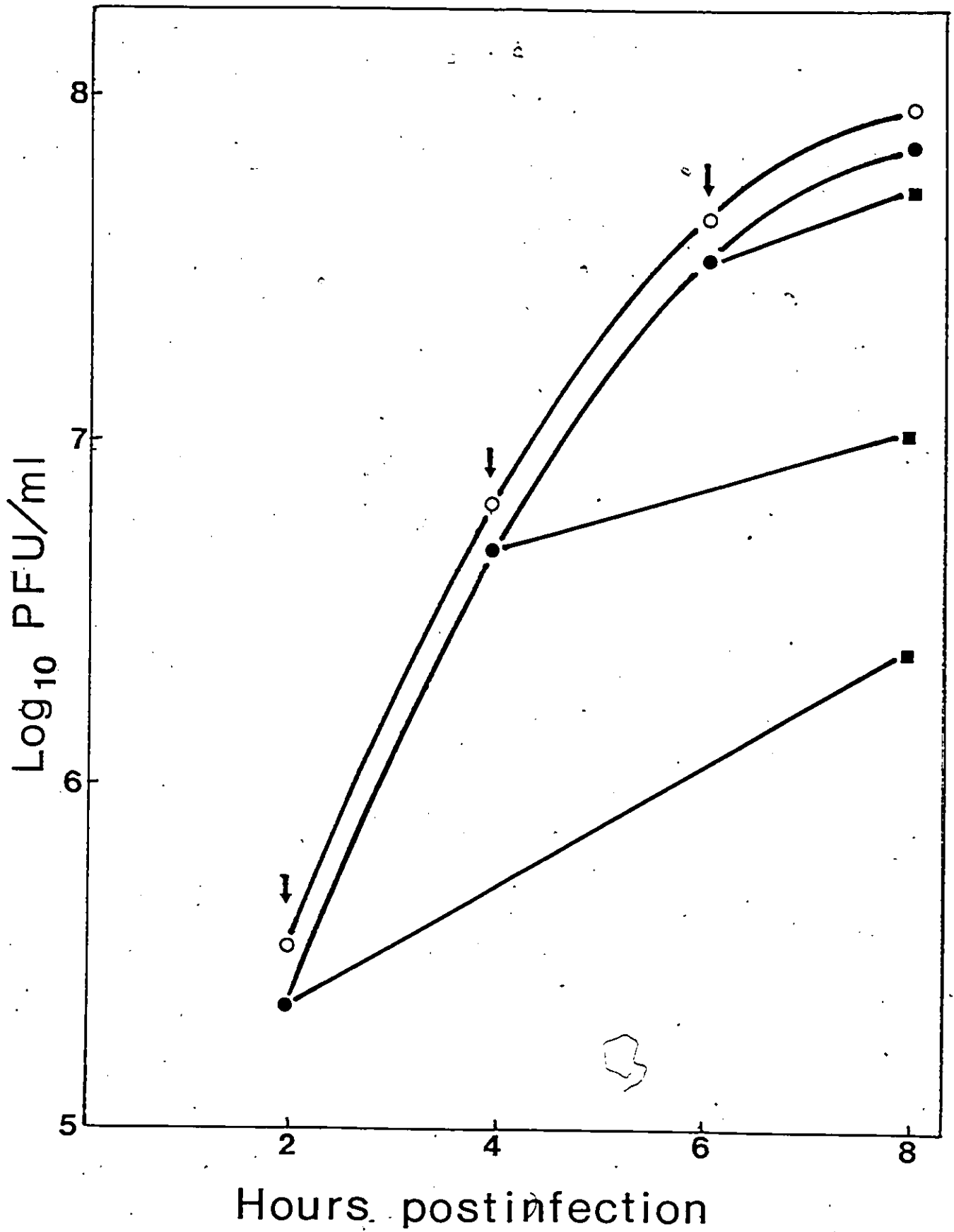
concentration of 5 mM and final yields of virus from cultures were titrated after incubation for 8 hours. In addition, other infected cultures were harvested at intervals and titrated to establish control growth curves.

The results are plotted in Fig. 5. The maximal inhibiting effect of DOG on the final yield of virus was greatest when it was added in the early portion of the viral development cycle. Irrespective of the time of addition of DOG, some inhibition in viral yield was evident. This lack of immediate shut-down of virus multiplication most likely reflects the time necessary for the uptake and activation of DOG during which infectious virus is still produced.

Reversal of the Inhibitory Activity of 2-Deoxy-D-Glucose on the Multiplication of VS Virus with Glucose or Mannose

Since the inhibitory activity of DOG on the multiplication of VS virus in cell cultures was readily reversed by glucose, the reversal activities of glucose and mannose were compared.

Fig. 3. Effect of 2-deoxy-D-glucose added at different times after infection on the multiplication of VS virus. Cells were infected with VS virus and incubated in glucose- or pyruvate-containing media. DOG (5 mM) was added at the indicated times (arrows) after infection. At 8 hours postinfection cell-free (■) virus was determined. Virus grown in glucose- (○) or pyruvate- (●) containing medium was also determined at the times indicated on the abscissa.



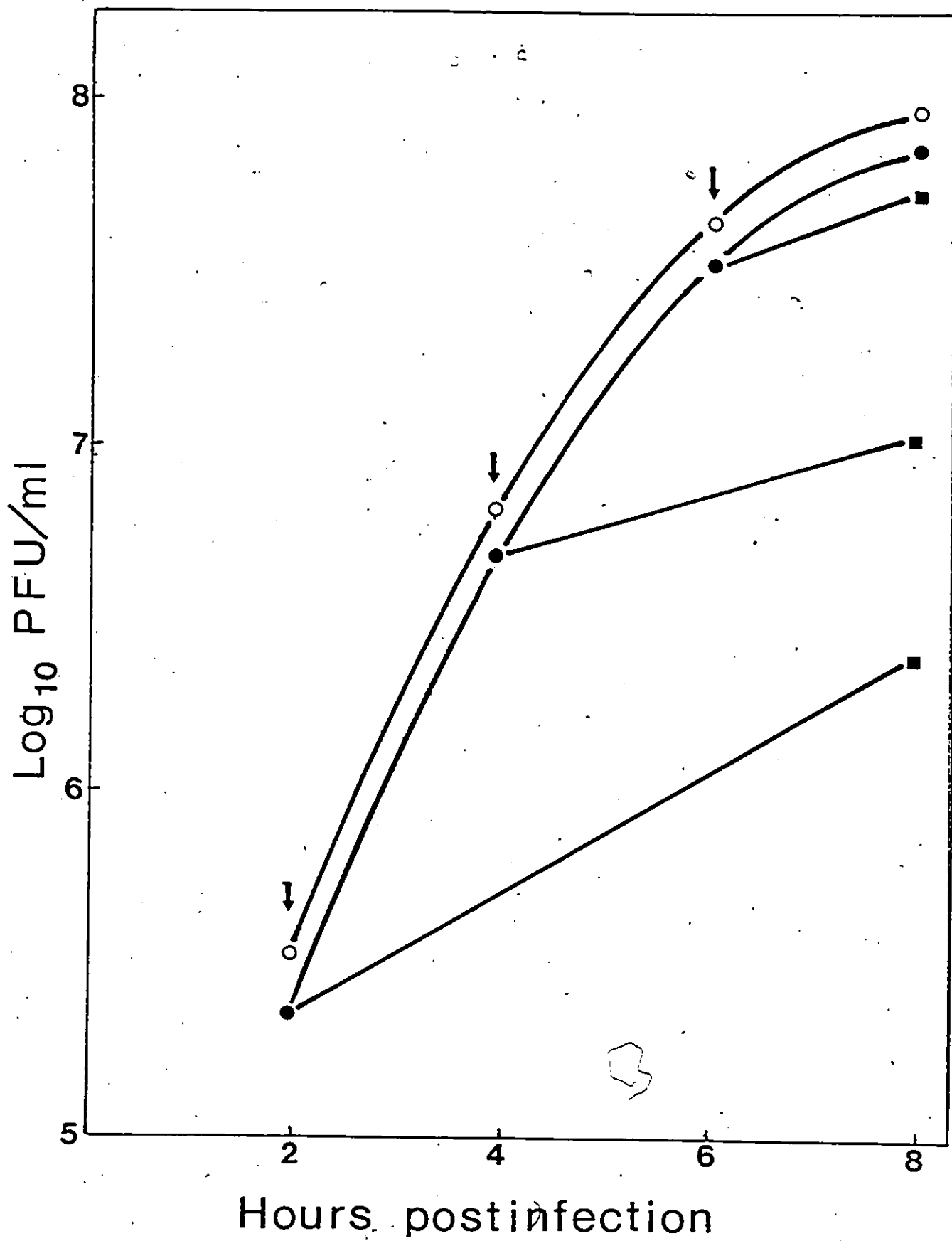
In Fig. 4 it is seen that glucose and mannose were effective in reversing the inhibitory effect of DOG on virus multiplication in Vero cells, and that mannose was somewhat more active than glucose. Also shown is the immediate rise in titer of infectious virus after addition of sugars at a level of 10 mM. This apparent lack of a lag period to overcome the inhibitory effect of DOG suggests that early events during viral multiplication proceed even in the presence of the antimetabolite.

Effect of 2-Deoxy-D-Glucose on RNA Synthesis of VS Virus

Synthesis of VS viral RNA in vitro has been studied by inhibiting the production of cellular RNA by the addition of 5 mcg/ml Actinomycin D (Appendix C) and pulsing cultures for 1 hour with 10 μ Ci 3 H-Uridine at various intervals postinfection after which the cell layers were processed as described in Materials and Methods.

RNA production by viral particles in the presence of 5

Fig. 4. Comparison of glucose and mannose for reversal of the inhibiting activity of 2-deoxy-D-glucose on the multiplication of VS virus. Vero cells were infected with VS virus and after infection the cells were incubated in pyruvate- or glucose-containing media. The cultures grown in pyruvate-containing medium received 5 mM DOG. To some of the latter cultures glucose or mannose were added to a final concentration of 10 mM 4 hours after infection (see arrow). The infectivity was assayed at times as indicated on the abscissa. Symbols: (O) glucose-containing medium, no DOG; (●) pyruvate-containing medium, 5 mM DOG; (▲) same as (●) but glucose was added 4 hours post-infection; (△) same as (●) but mannose was added 4 hours post-infection.



mM DOG was approximately 50% of that by VS virus in the absence of DOG (Fig. 5) and decreased by 4 hours postinfection to the background level.

SDS-Polyacrylamide Gel Electrophoresis of VS Viral Particles

a) Molecular weight determination of viral polypeptides


Electrophoresis of viral band III from a 5, 20, 30, 45% sucrose gradient and molecular weight protein standard combination containing Ribonuclease A, Chymotrypsinogen A, Ovalbumin and Aldolase, was carried out as described in Materials and Methods.

The densitometric tracings obtained are diagrammed in Fig. 6.

Migration distances and molecular weight calculations (Tables 5 and 6, and Fig. 7) yielded the designations of L, G, N, NS and M to the peaks obtained by electrophoresis. The nomenclature of the viral polypeptides was taken from a proposal by Wagner et al., (1972).

Fig. 5. Effect of 2-deoxy-D-glucose on RNA synthesis of VS virus. General conditions of infection were given in Materials and Methods. At zero time cultures were infected with VS virus or were mock-infected. Cross-hatched bars represent the production of RNA by VS virus in the absence of DOG. Solid bars represent production of RNA by VS virus in the presence of 5 mM DOG, and open bars represent production of RNA in mock-infected cells incubated in the presence of 5 mM DOG and 3 mcg/ml Actinomycin-D.

7



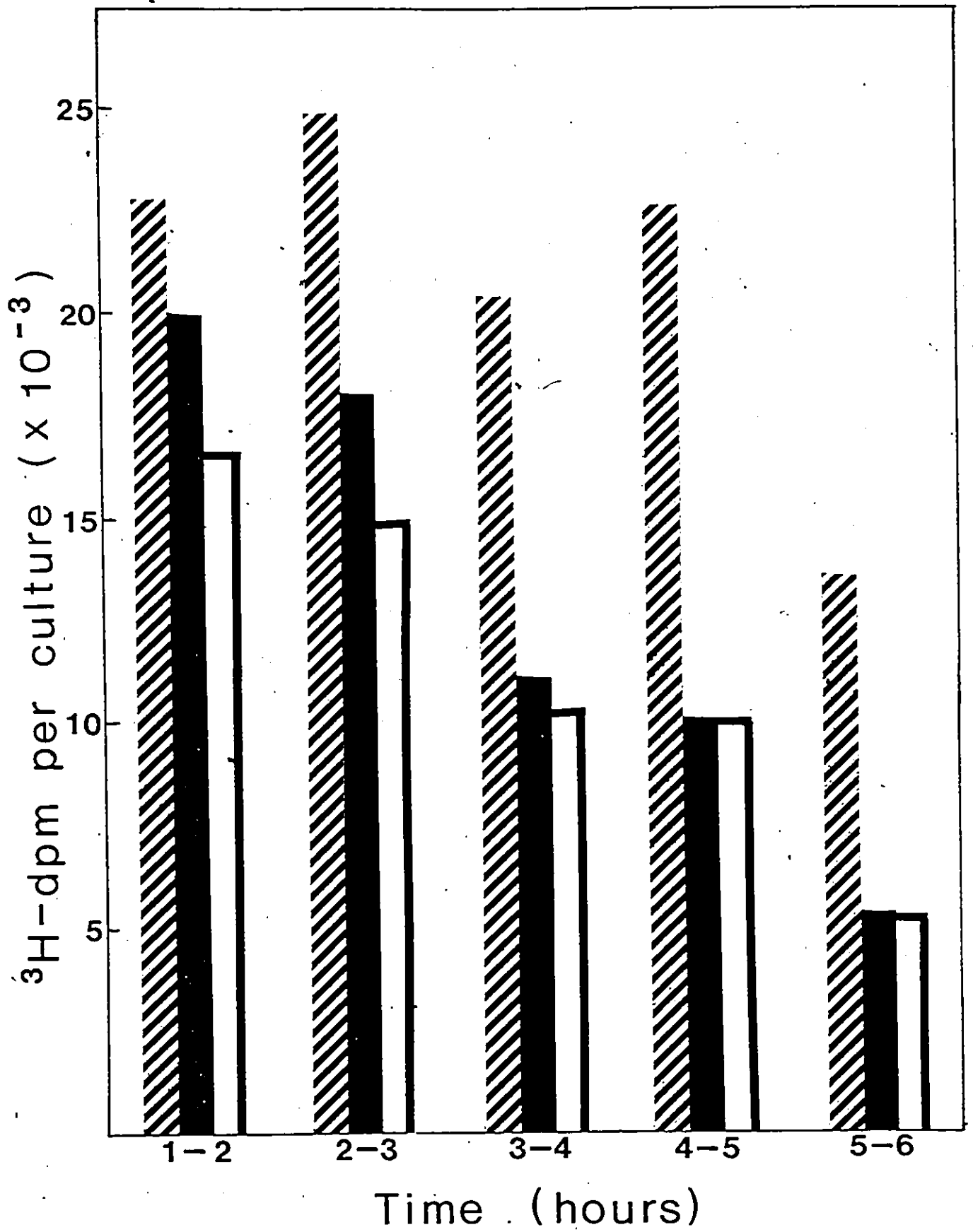


Fig. 6. Densitometric tracings of VS viral band III (A)
and the molecular weight standard proteins (B).

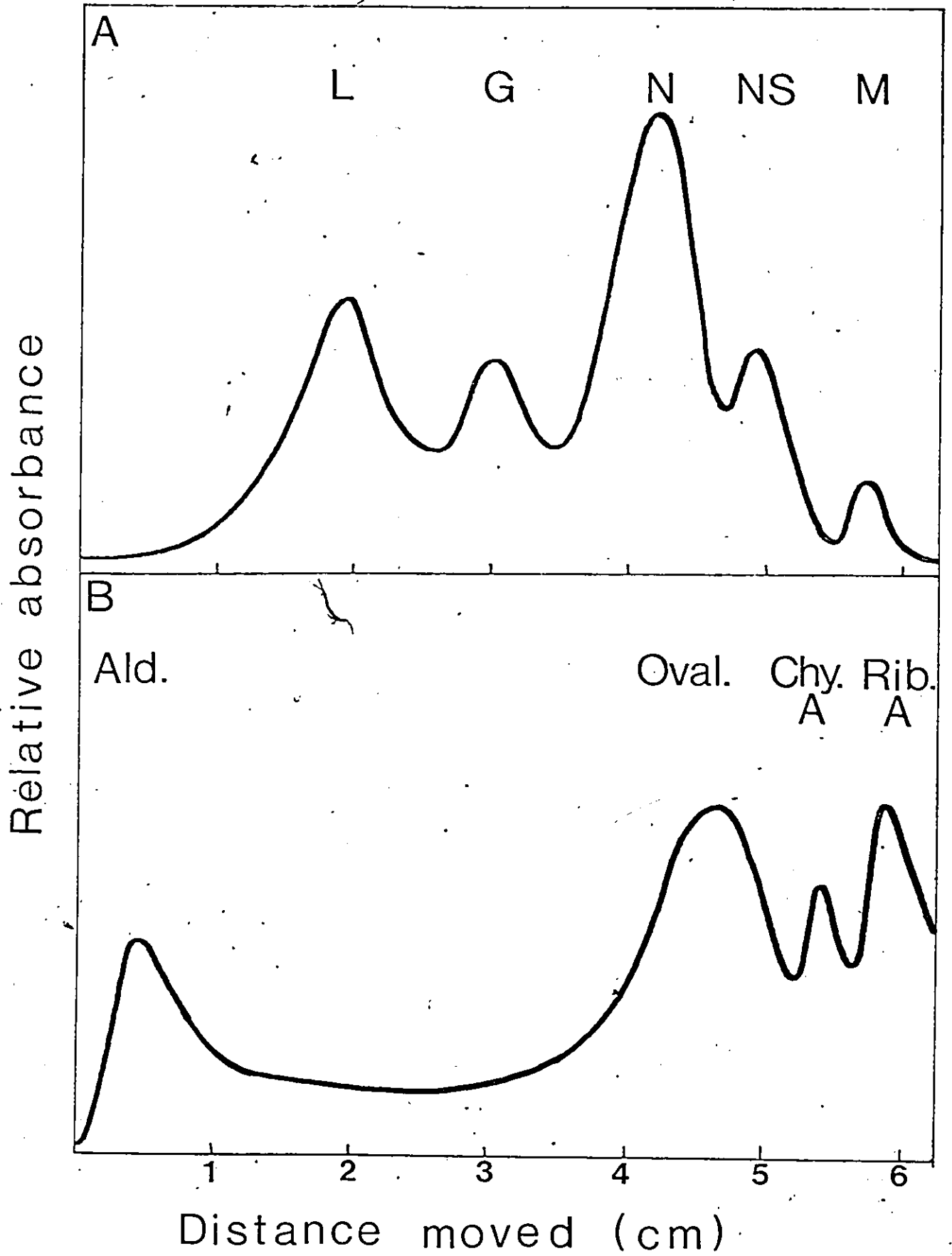


Table 5. Determination of mobility of the molecular weight standard proteins on SDS-polyacrylamide gel electrophoresis.

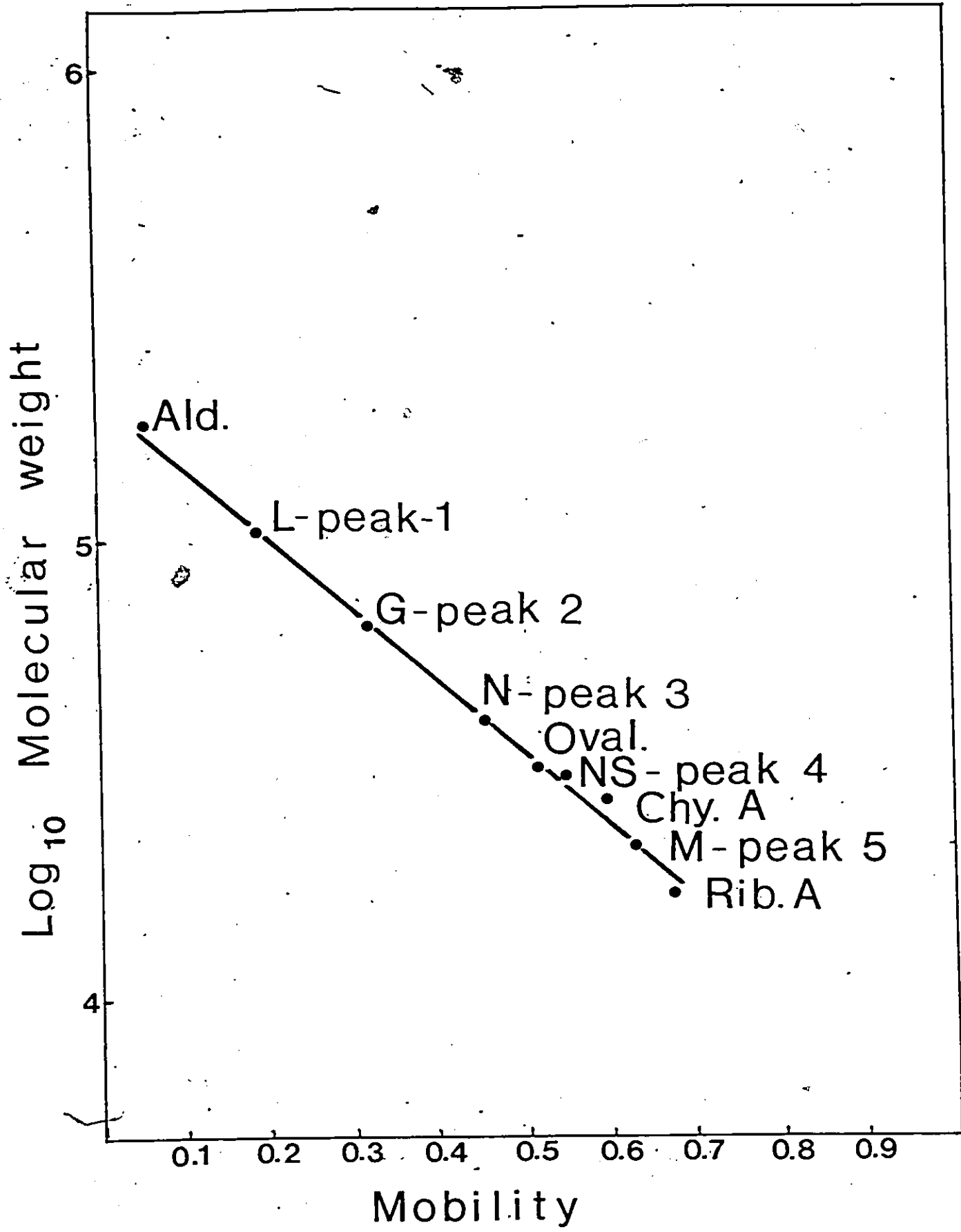
Molecular weight standard protein	Migration of band from top of gel (cm)	<u>Migration of band</u> Migration of marker dye (bromphenol blue)	Molecular weight (daltons)
Aldolase	0.6	0.07	15.8×10^4
Ovalbumin	4.8	0.53	4.5×10^4
Chymo- trypsinogen A	5.4	0.60	2.5×10^4
Ribonuclease A	6.0	0.67	1.37×10^4

Table 6. Determination of mobility of VS viral proteins on SDS-polyacrylamide gel electrophoresis and approximate molecular weight estimations.

Viral peak	Migration of band from top of gel (cm)	Migration of band Migration of marker dye (bromphenol blue)	Molecular weight (daltons) estimated	Molecular weight (daltons) reported*
1	2.0	0.22	108,000	190,000
2	3.6	0.40	66,100	69,000
3	4.2	0.47	42,700	50,000
4	4.8	0.53	31,700	40,000-45,000
5	5.7	0.63	21,400	29,000

*Wagner et al., (1972).

Fig. 7. Determination of approximate molecular weights of VS viral polypeptides according to a standard molecular weight protein curve.



The L protein, having a molecular weight of approximately 190,000 is associated with the VS virion nucleocapsid and intracellular nucleocapsids.

The G protein (M. W. ca 69,000) is the only identifiable VS virion protein which is glycosylated. There is evidence that migration in gels is possibly related to the degree of glycosylation and can differ for glycoproteins extracted from intracellular components and released VS virions. These glycoproteins are identified as the only components of the spikes protruding from the viral envelope and can be removed relatively easily with proteolytic enzymes and non-ionic detergents (Cartwright et al., 1969). The G protein is primarily associated with the plasma membrane of VS virus-infected cells. It is the major antigenic determinant of the VS virus and is responsible for neutralization of infectivity by antibodies and for type specificity of the virus.

A structural protein of the viral nucleocapsid with a

molecular weight of approximately 50,000 is referred to as the N protein. It is tightly bound to virion RNA.

The NS protein with a molecular weight of 40,000 to 45,000 is a minor component of the VS viral nucleocapsid core. It is found in association with proteins L and N as well as viral RNA.

An integral component of the VS viral membrane is the M protein (M. W. ca 29,000). It is a matrix protein serving to bind the ribonucleocapsid to the viral envelope.

b) Synthesis of VS viral proteins in the presence of 2-deoxy-D-glucose

The polyacrylamide gel patterns shown in Fig. 8 and 9 demonstrate that under conditions inhibiting viral multiplication by approximately 97% the synthesis of all viral proteins is inhibited.

In polyacrylamide gel electrophoretic patterns of VS virus-infected Vero cells, labelled with amino acids and treated with 5 mM dose of DOG, it can be seen that all

Fig. 8. Polyacrylamide gel electrophoresis of VS virus-infected Vero cells labelled with ^3H -amino acids and treated with 2-deoxy-D-glucose. Cells were infected with VS virus and incubated in glucose (A) or pyruvate (B and C) containing media having 3 mcg/ml Actinomycin D. The cultures received no (A and B) or 5 mM DOG (C). Two hours postinfection a mixture of equal amounts of ^3H -labelled tyrosine and leucine (total of 20 μCi per culture) was added. The cells were processed 8 hours postinfection.

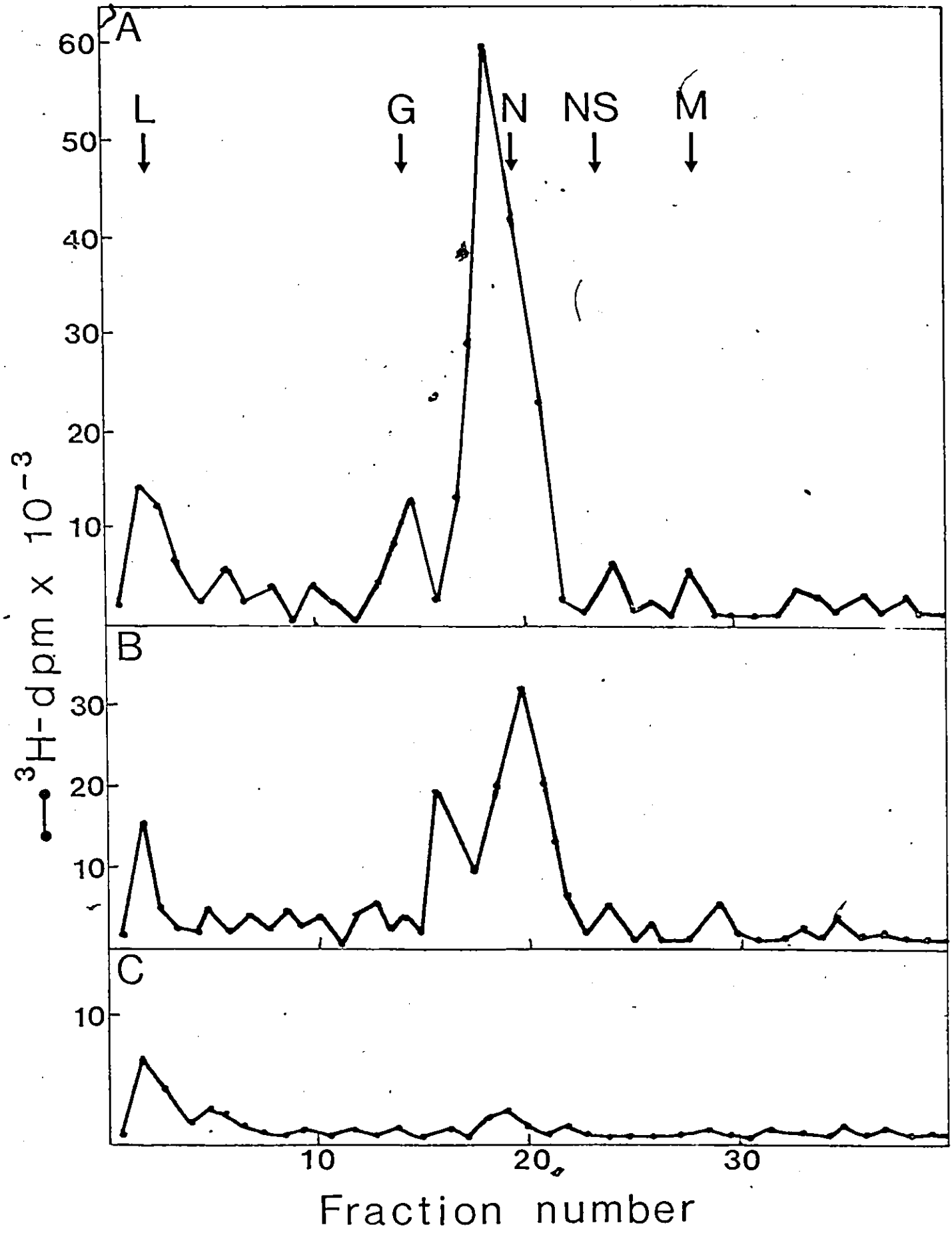
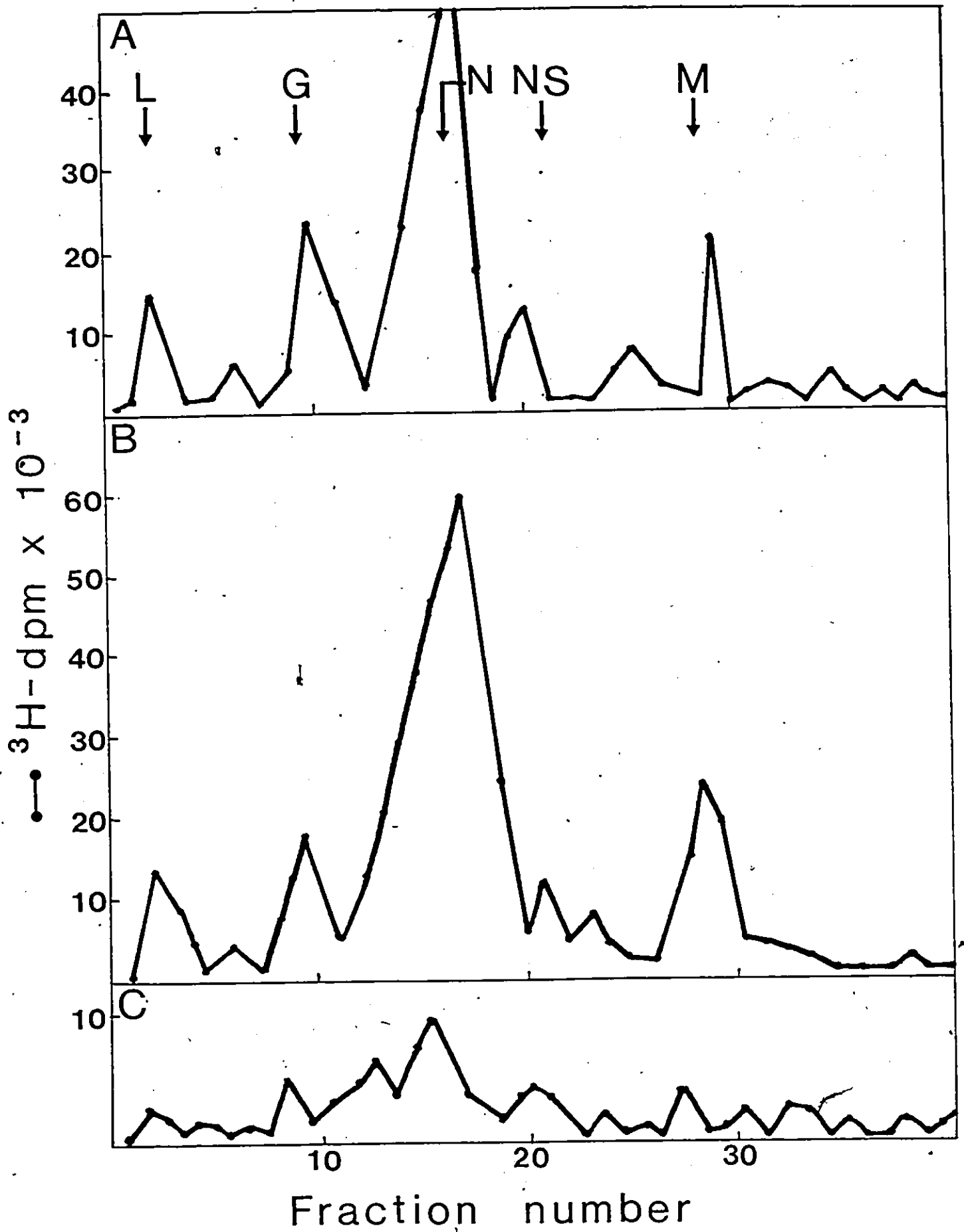


Fig. 9. Polyacrylamide gel electrophoresis of VS virus labelled with ^3H -amino acids and treated with 5 mM 2-deoxy-D-glucose. Cells were infected with VS virus and incubated in glucose (A) or pyruvate (B and C) containing media. Virus was isolated from cultures containing no (A and B) or 5 mM DOG (C). Two hours postinfection a mixture of equal amounts of ^3H -labelled tyrosine and leucine was added (total of $20\ \mu\text{Ci}$ per culture). Virus was purified after 5 cycles of freezing and thawing 8 hours postinfection as described in Materials and Methods.



polypeptide production is greatly inhibited (Fig. 8C). However, the peaks, although much reduced in size, occur in the same position as in untreated virus-infected cells (Fig. 8A and B).

In polyacrylamide gel electrophoresis of purified virus it is evident that the addition of DOG to a level of 5 mM to the overlay medium resulted in viral particles containing aberrant proteins. There appears to be a shift in the mobility of the G protein. In fact, two peaks are evident where the single G peak usually occurs. Perhaps one peak is unglycosylated G protein (the faster moving peak) and the other could be G protein that incorporated DOG instead of glucose therefore resulting in a slower moving aberrant polypeptide. Scholtissek et al., (1974) reported the occurrence of false glycoprotein with a faster than normal mobility in VS virus isolated from infected cells treated with low doses of DOG. It is possible that higher doses of DOG result in a double peak as that occurring in Fig. 9C.

DISCUSSION

The inhibition of multiplication of VS virus by 2-deoxy-D-glucose (DOG) has been studied in Vero cells using medium containing pyruvate as the energy source. It was suggested earlier (Kaluza et al., 1973) that pyruvate, while acting as an energy source, does not interfere with the uptake of DOG. In Vero cells the inhibitory effect of DOG was less apparent in glucose-containing medium than in pyruvate-containing medium. In other cell lines this difference was not as great. Apparently the inhibitory effect of DOG is mediated through a virus-cell-antimetabolite interaction and is not a direct effect on either the cell membrane or virus. Under the conditions used the early steps of virus multiplication such as adsorption and penetration occur undisturbed since, after counteracting the block at later times after infection by the addition of either glucose or mannose, infectious particles were produced without any lag. Virus multiplication was inhibited at any

time during the growth cycle by the antimetabolite however shut-down is not immediate. Uptake and activation of the antimetabolite is necessary during which time the production of infectious virus is possible. In contrast to influenza (Klenk et al., 1972) and Semliki Forest viruses (Kaluza et al., 1973) DOG interferes not only with the production of glycoproteins, but with the synthesis of all proteins of VS virus. Viral RNA synthesis was reduced and therefore fewer viral proteins (as seen by polyacrylamide gel electrophoresis) and fewer infectious viral particles were produced compared with nontreated controls. Protein and RNA synthesis of non-infected cells were not significantly impaired under these conditions (Scholtissek et al., 1975). The electrophoretic mobility of glycoproteins of VS virus was changed by incubating infected cells with DOG. Two peaks were evident in place of the single peak representing the G protein. DOG therefore must have a specific effect on synthesis and/or

processing of viral glycoproteins. This observation is comparable to the effect of DOG on influenza, Semliki Forest and herpes viruses multiplication (Kaluza et al., 1973; Klenk et al., 1972 and Courtney et al., 1973). As in the latter systems the aberrant synthesis of the glycoproteins of VS virus adds to the dramatic effect on the production of infectious viral particles. Perhaps DOG interferes with the processing of viral glycoproteins resulting in the reduction of glycosylated proteins as well as being incorporated itself producing aberrant glycoproteins. VS-viral nucleocapsids may become enveloped with the altered glycoprotein component, or with the proper glycoprotein, thereby producing non-infectious or infectious particles. In order for the antimetabolite to be incorporated into viral glycoproteins it must be assumed that it is activated via nucleoside diphosphate derivatives like other sugars. In opposition to Kipnis and Cori (1959), who stated that DOG is not metabolized past the 2-deoxy-D-glucose-6-phosphate stage,

Schmidt et al., (1974), using hydrolytic and chromatographic techniques and specific enzymatic degradation, have found and characterized the following activated intermediates of DOG: the 1-phosphate derivative, the 1,6-diphosphate derivative, UDP-2-deoxy-D-glucose, GDP-2-deoxy-D-glucose and 2-deoxy-D-glucuronic acid 6-phosphate. It is therefore possible for DOG to be incorporated into viral membranes resulting in the reduction of viral yields.

SUMMARY

DOG had a great inhibitory effect on VS viral multiplication in Vero cells when pyruvate was used as an energy source. The effect of the antimetabolite in glucose-containing medium was not as great. Addition of DOG caused a decrease in viral yields at any time during the growth cycle of VS virus.

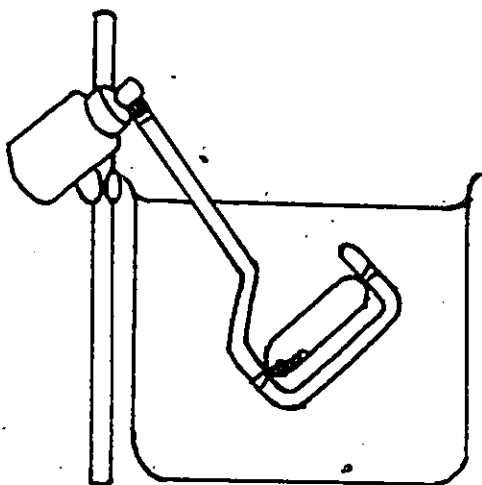
Reversal of the effect was possible by the addition of either glucose or mannose. RNA synthesis was greatly reduced. This resulted in reduced viral protein peaks in polyacrylamide gel electrophoresis. In isolated VS virions grown in Vero cells in medium containing both pyruvate and DOG two peaks were found in place of the single G glycoprotein. DOG therefore may be acting on the multiplication of VS virus first by causing a decrease in RNA synthesis and therefore a decrease in viral protein production as well as to have an effect on the synthesis and/or processing of viral glycoproteins. DOG may be causing the production of aberrant glycoproteins which are incorporated

into the viral membrane resulting in non-infectious particles.

APPENDIX A

Dialysis of Fetal Calf Serum

Dialysis of heat-inactivated fetal calf serum was performed by placing 100 ml of the serum in a dialysis bag along with 15 or 20 2 mm glass beads. The bag was then rotated for 3 hours in cold 0.9% NaCl in a set-up as diagrammed below:

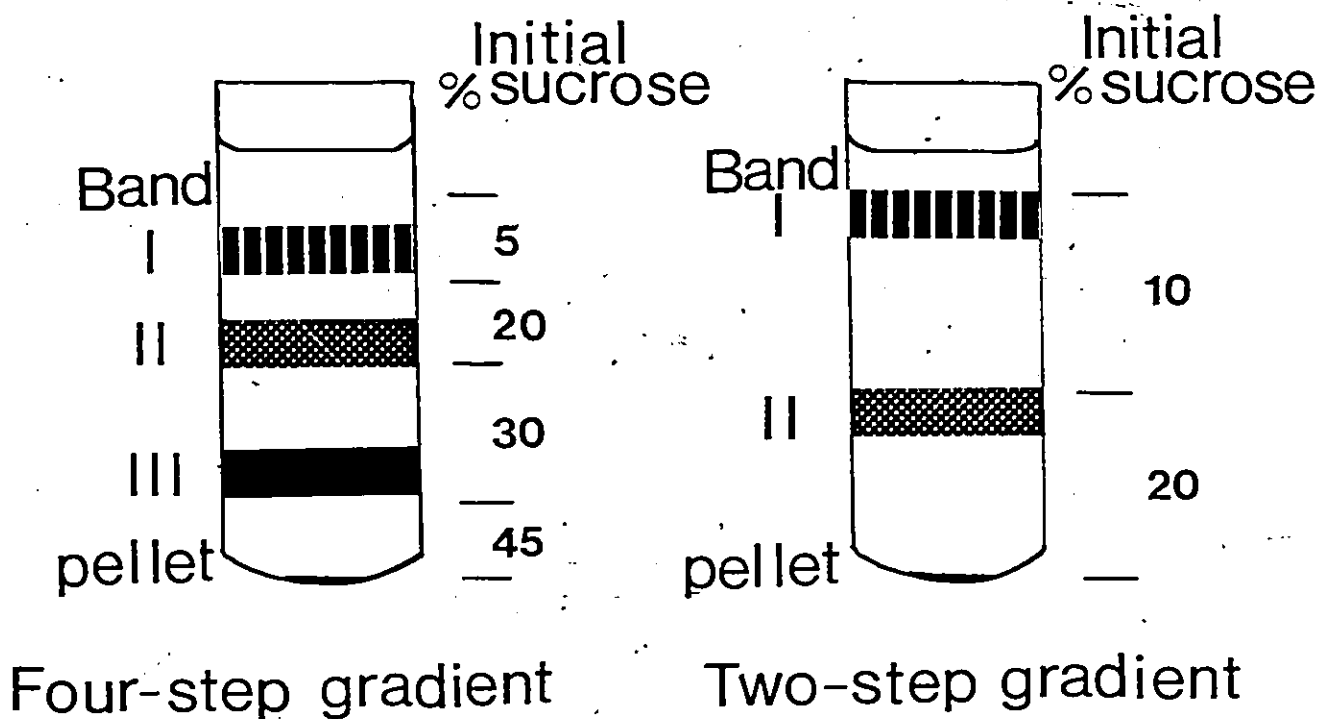


Before dialysis serum contained 3.225 mg/ml glucose as determined enzymatically using Glucostat prepared reagents. After dialysis 0.494 mg/ml glucose remained. Therefore, an 85% decrease in glucose content of the serum was achieved by a 3 hour dialysis.

APPENDIX B

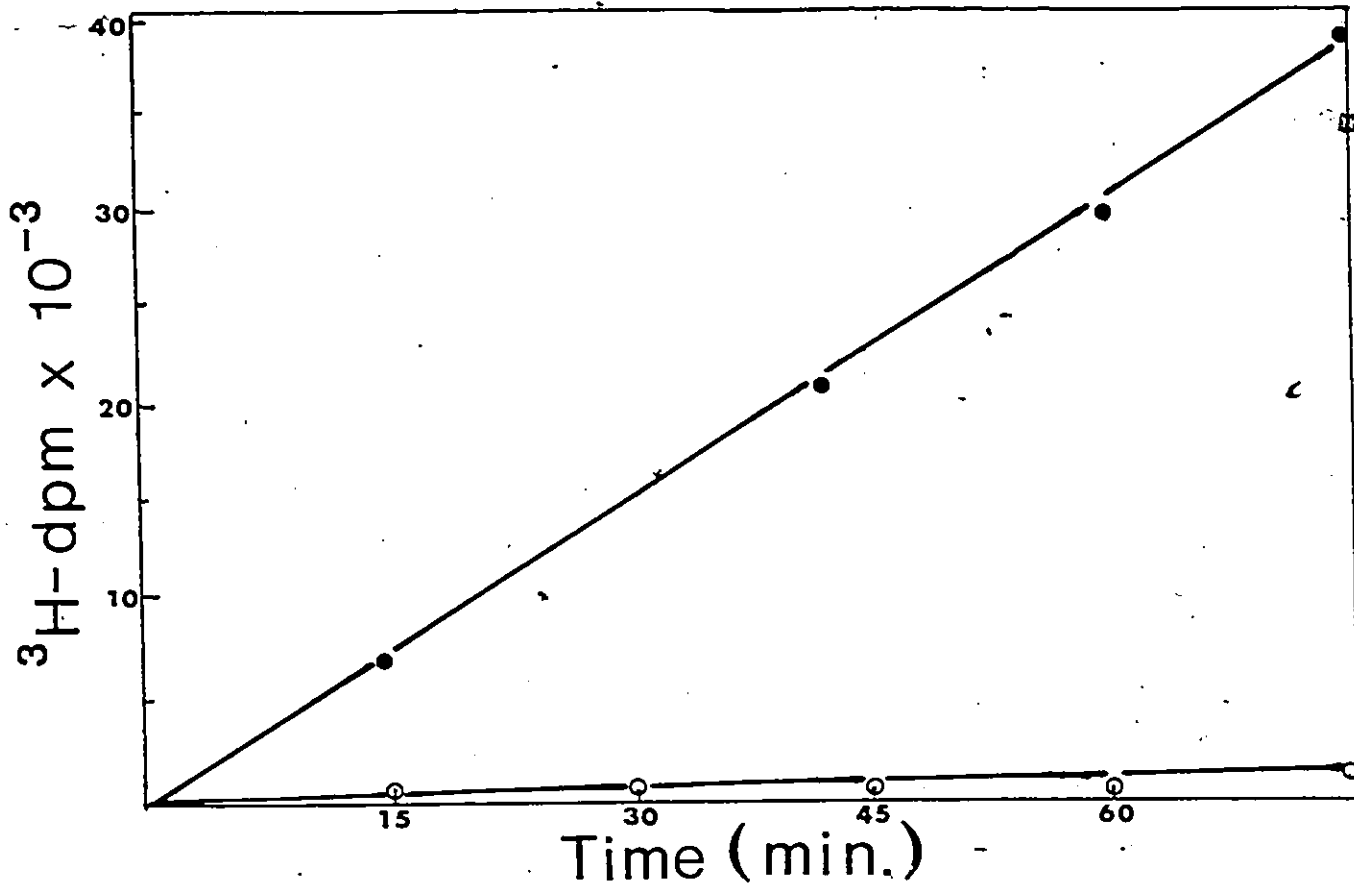
Preparation of Discontinuous Sucrose Gradients

Discontinuous gradient columns were prepared by floating layers of 2.0, 2.0, 4.0 and 2.0 ml of 5, 20, 30 and 45% (4-step gradient) or 5.0 and 5.0 ml of 10 and 20% (2-step gradient) sucrose solutions in 9/16 x 3 1/2 inch nitrocellulose centrifuge tubes. Ribonuclease-free grade sucrose was dissolved in phosphate-buffered saline, pH 7.4. Before use the layered solutions were allowed to stand 3 hours at 4°C.



APPENDIX C

Inhibition of Production of Cellular RNA by Actinomycin D



Forty-eight hour old Vero cells were overlaid with P-MEM-10FCS containing 3 mcg/ml Actinomycin D (○). Control cultures received P-MEM-10FCS lacking Actinomycin D (●). At zero time all cultures were given 4 μCi of ^3H -Uridine. At indicated times after addition of label cells were processed as described in Materials and Methods. 3 mcg/ml Actinomycin D was found to greatly inhibit cellular RNA production.

REFERENCES

- BALL, H. A., A. H. WICK and C. SANDERS. 1957. Influence of glucose antimetabolites on the Walker tumour. *Cancer Res.* 17: 235.
- BARBAN, S and H. O. SCHULZE. 1961. The effects of 2-deoxyglucose on the growth and metabolism of cultured human cells. *J. Biol. Chem.* 236: 1887.
- COURTNEY, R. J., S. M. STEINER and M. BENYESH-MELNICK. 1973. Effects of 2-deoxy-D-glucose on herpes simplex virus replication. *Virology* 52: 447.
- DANIELS, J. B., M. D. EATON and M. E. PERRY. 1952. Effect of glucose on growth of influenza virus in deembryonated eggs and tissue cultures. *J. Immunol.* 69: 321.
- EAGLE, H. 1959. Amino acid metabolism in mammalian cell cultures. *Science* 130: 432.
- KALUZA, G, C. SCHOLTISSEK and R. ROTT. 1972. Inhibition of the multiplication of enveloped RNA-viruses by glucosamine and 2-deoxy-D-glucose. *J. gen. Virol.* 14: 251.
- KALUZA, G, M. F. G. SCHMIDT and C. SCHOLTISSEK. 1973. Effect of 2-deoxy-D-glucose on the multiplication of Semliki Forest virus and the reversal of the block by mannose. *Virology* 54: 179.

- KILBOURNE, E. D. 1959. Inhibition of influenza virus multiplication with a glucose antimetabolite (2-deoxy-D-glucose). *Nature (London)* 183: 271.
- KIPNIS, D. M. and C. F. CORI. 1959. Studies of tissue permeability. V. The penetration and phosphorylation of 2-deoxyglucose in the rat diaphragm. *J. Biol. Chem.* 234: 171.
- KLENK, H.-D., C. SCHOLTISSEK and R. ROTT. 1972. Inhibition of glycoprotein biosynthesis of influenza virus by D-glucosamine and 2-deoxy-D-glucose. *Virology* 49: 723.
- LANDAU, B. R., J. LASZLO, J. STENGLE and D. BURK. 1958. Certain metabolic and pharmacologic effects in cancer patients given infusions of 2-deoxy-D-glucose. *J. Nat. Cancer Inst.* 21: 485.
- LASZLO, J., B. LANDAU, K. WIGHT and D. BURK. 1958. The effect of glucose analogues on the metabolism of human leukemic cells. *J. Nat. Cancer Inst.* 21: 475.
- LETNANSKY, K. 1964. The influence of 2-deoxy-D-glucose on the nucleotide content of Ehrlich ascited carcinoma cells. *Biochim. Biophys. Acta.* 87: 1.

- LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL.
1951. Protein measurement with the Folin phenol reagent.
J. Biol. Chem. 193: 265.
- LUDWIG, H. and R. ROTT. 1975. Effect of 2-deoxy-D-glucose on
herpesvirus-induced inhibition of cellular DNA synthesis.
J. Virol. 16: 217.
- MAIZEL, J. V., Jr. 1966. Acrylamide-gel electrophorograms by
mechanical fractionation: radioactive adenovirus proteins.
Science 151: 988...
- MAIZEL, J. V., Jr., D. F. SUMMERS and M. D. SCHARFF. 1970.
SDS-acrylamide gel electrophoresis and its application to
the proteins of poliovirus- and adenovirus-infected
human cells. J. Cell. Physiol. 76: 275.
- MAIZEL, J. V., Jr. 1971. Methods in Virology V. Chapter 5, page
179. Academic Press, Inc., New York, New York.
- PLAGEMANN, P. G. W. 1973. Deoxyglucose transport by uninfected
murine sarcoma virus-transformed, and murine leukemia
virus-infected mouse cells. J. Cell. Physiol. 83: 421.
- RENNER, E. D., P. G. W. PLAGEMANN and R. W. BERNLOHR. 1972.
Permeation of glucose by simple and facilitated diffusion
by Novikoff rat hepatoma cells in suspension culture and
relationship to glucose metabolism. J. Biol. Chem. 18:
5765.

- SABINA, L. R. and T. W. MUNRO. 1969. Effects of trypsin on replication of parainfluenza-3 virus in Hep. #2 cell cultures. Can. J. Microbiol. 15: 577.
- SCHLOEMER, R.H. and R. R. WAGNER. 1974. Sialoglycoprotein of vesicular stomatitis virus: role of the neuraminic acid in infection. J. Virol. 14: 270.
- SCHMIDT, M. F. G., R. T. SCHWARZ and C. SCHOLTISSEK. 1974. Nucleoside-diphosphate derivatives of 2-deoxy-D-glucose in animal cells. Eur. J. Biochem. 48: 237.
- SCHOLTISSEK, C. 1971. Detection of an unstable RNA in chick fibroblasts after reduction of the UTP pool by glucosamine. Eur. J. Biochem. 24: 358.
- SCHOLTISSEK, C., R. ROTT, G. HAU and G. KALUZA. 1974. Inhibition of the multiplication of vesicular stomatitis and Newcastle disease virus by 2-deoxy-D-glucose. J. Virol. 13: 1186.
- SCHOLTISSEK, C., R. ROTT and H.-D. KLENK. 1975. Two different mechanisms of the inhibition of the multiplication of enveloped viruses by glucosamine. Virology 63: 191.

- SHAPIRO, A. L. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. *Biochem. Biophys. Res. Comm.* 28; 815.
- SPEAR, P. G. and B. ROIZMAN. 1972. Proteins specified by herpes simplex virus. V. Purification and structural proteins of the herpesvirion. *J. Virol.* 9;143.
- WAGNER, R. R., R. M. SNYDER and S. YAMAZAKI. 1970. Proteins of vesicular stomatitis virus; kinetics and cellular sites of synthesis. *J. Virol.* 5; 548.
- WAGNER, R. R., L. PREVEC, F. BROWN, D. F. SUMMERS, F. SOBOL and R. MacLEOD. 1972. Classification of rhabdovirus proteins: a proposal. *J. Virol.* 10; 1228.
- WAGNER, R. R. 1976. Third international colloquium on rhabdoviruses. *ASM News* 42; 616.
- WEBER, K. and M. OSBORN. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244; 4406.

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