methane must be the first intermediate in the biosynthesis of uroporphyrinogen III. Both uroporphyrinogens must proceed through different pathways after the first porphobilinogen units became bound to the deaminase.

References

Bullock, E. (1965), *Nature (London) 205*, 70.
Conford, P. (1964), *Biochem. J. 91*, 64.
Dalton, J., and Dougherty, R. C. (1969), *Nature (London) 223*, 1151.
Eriksen, L. (1958), *Scand. J. Clin. Lab. Invest. 10*, 319.
Falk, J. E. (1964), *Porphyrins Metalloporphyrins*, 193, 197.
Frydman, R. B., and Frydman, B. (1970), *Arch. Biochem. Biophys. 136*, 193.
Frydman, B., Reil, S., Despuy, M. E., and Rapoport, H. (1969), *J. Amer. Chem. Soc. 91*, 2738.
Frydman, B., Reil, S., Valasinas, A., Frydman, R. B., and Rapoport, H. (1971), *J. Amer. Chem. Soc. 93*, 2738.
Granick, S., and Levere, R. D. (1964), *Progr. Hematol. 4*, 1.
Lascelles, J. (1964), Tetrapyrrole Biosynthesis and Its Regulation, New York, N. Y., W. A. Benjamin, p 47.
Llambias, E. B. C., and Battle, A. M. del C. (1971), *Biochem. J. 121*, 327.
Margoliash, E. (1961), *Annu. Rev. Biochem. 30*, 549.
Mathewson, J. H., and Corwin, A. H. (1961), *J. Amer. Chem. Soc. 83*, 135.
Pluscec, J., and Bogorad, L. (1970), *Biochemistry 9*, 4736.
Porra, R. J., and Falk, J. E. (1964), *Biochem. J. 90*, 69.
Radmer, R., and Bogorad, L. (1972), *Biochemistry 11*, 904.
Stevens, E., and Frydman, B. (1968), *Biochim. Biophys. Acta 151*, 429.
Stevens, E., Frydman, R. B., and Frydman, B. (1968), *Biochim. Biophys. Acta 158*, 496.

Glycoproteins from the Surface of Metaphase Cells†

Mary Catherine Glick and Clayton A. Buck*

ABSTRACT: Several criteria related to carbohydrates of the cell surface were examined in baby hamster kidney fibroblasts, BHK_{21}/C_{13}, arrested in metaphase with vinblastin sulfate. Analysis by gel filtration of the glycopeptides from the surface of metaphase arrest cells revealed a pattern of fucose-containing glycopeptides similar to that seen previously for rapidly growing or virus-transformed cells. Surface glycopeptides from cells treated with vinblastin sulfate but not in metaphase have a distribution similar to surface glycopeptides derived from BHK_{21}/C_{13} cells in exponential growth. The results suggest that the glycopeptides expressed on the cell surfaces during mitosis are similar to those permanently expressed after viral transformation. The metaphase cells show a 43% decrease in the total sialic acid content when compared to cells treated with vinblastin sulfate but not in metaphase. Cells arrested by thymidine have a sialic acid content more similar to the cells in exponential growth, indicating that the fluctuations in silalic acid content of vinblastin treated cells are not merely the result of cessation of growth. In addition, a threefold increase was observed in the amount of radioactive fucose incorporated into metaphase-arrest cells when compared with vinblastin sulfate treated cells which had not yet entered metaphase. The activities of seven glycosidases in the metaphase cells show little or no deviation from the cells treated with vinblastin sulfate but not in metaphase or as previously shown from the cells in exponential growth.

Examination of the carbohydrate content of a synchronous population of KB cells has shown that the individual monosaccharides of the cell are markedly reduced as the cell population divides (Glick *et al.*, 1971). When the molar ratios of the monosaccharides are related to sialic acid throughout the cell cycle the fluctuations of the carbohydrates appear in an orderly fashion. For example, when the cell population is dividing, the molar ratio of sialic acid to fucose is 1; when the population is not dividing the molar ratio is 2.

Changes from the normal distribution of glycopeptides derived from cell surfaces have been described after transformation by RNA or DNA viruses (Buck *et al.*, 1970, 1971a). These changes are associated partially with the state of growth of the cells, that is, whether the cells are growing rapidly or have formed a confluent monolayer (Buck *et al.*, 1971b).

In order to more precisely localize the changes in the mitotic event of the cell cycle, baby hamster kidney fibroblasts (BHK_{21}/C_{13}) were arrested in metaphase with vinblastin sulfate. Cells in metaphase change to a round morphology and come off the monolayer making it possible to isolate a population which is 80–90% in metaphase. Furthermore, the cells which are not in metaphase remain attached to the monolayer and serve as control cells which have been treated with vinblastin. These two populations of cells from the same flask are similar with the exception that one population is arrested in metaphase. These two populations were compared with BHK_{21}/C_{13} fibroblasts which were growing exponentially. Three parameters concerning the carbohydrates of the cell surface were examined: (1) the distribution of glycopeptides of the cell surface, (2) the sialic acid content of the cells, and (3) the activities of a number of glycosidases.

† From the Department of Therapeutic Research, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, and the Division of Biology, Kansas State University, Manhattan, Kansas 66506. *Received July 12, 1972.* This work was supported by U. S. Public Health Service Grants 5 P01 A107005-06, CA 12426-01, and FR7036, and American Cancer Society Grant No. PRA-68.

* Address correspondence to this author at Kansas State University.

Methods and Materials

Cell Culture. Baby hamster kidney fibroblasts (BHK_{21}/C_{13}) and BHK_{21}/C_{13} cells transformed by the Bryan strain of Rous sarcoma virus (C_{13}/B_4) were from early passaged stocks and no experiments were performed on cells after the 12th passage in our laboratories. The cells were examined for *Mycoplasma* at routine time intervals and were negative. Culturing of the cells in roller bottles has been described (Buck *et al.*, 1970). Unless otherwise stated, the cells were counted in a Coulter Counter. The size distribution of the cells was determined by the automatic plotter.

Cells in Metaphase. BHK_{21}/C_{13} cells were grown as described (Buck *et al.*, 1970) with the exception that 24 hr prior to harvesting 1.2×10^{-5} mg of vinblastin sulfate (Velban, Eli Lilly and Co., Indianapolis, Ind.) was added per milliliter of medium. At the time of harvest, the cells were gently shaken off the monolayer and handled separately from the cells remaining adhered to the culture bottle. The metaphase cells were chilled, centrifuged at 600*g* for 5 min, and washed three times with 10 ml of 0.16 M NaCl at 5°. The cells were counted in a hemocytometer and represented approximately 20–30% of the total cells from the culture. Examination under the phase contrast microscope showed that more than 85% of the cells had the nuclear characteristics of metaphase.

Velban Treated Cells Not in Metaphase. The cells adhering to the glass were washed and removed from the monolayer by treatment with trypsin as described (Buck *et al.*, 1970). The exception was that in experiments where carbohydrate analyses were performed, no soybean trypsin inhibitor was utilized to stop the action of the trypsin.

Cells Arrested with Thymidine. BHK_{21}/C_{13} cells (2.5×10^7 cells) were arrested by the addition of 7 mM thymidine to the roller bottles 24 hr before harvesting (Glick *et al.*, 1971). No growth of the cells was observed after the addition of this concentration of thymidine although the cells were still viable as shown by their release from the arrested state. Some growth was observed using smaller amounts of thymidine.

Preparation of Trypsinates. The material removed from the cell surface with trypsin will be referred to as "trypsinate" and was processed as described previously (Buck *et al.*, 1970). To obtain similar material from the mitotic cells, the cells harvested in metaphase were treated with trypsin for 5 min at 37°. Soybean trypsin inhibitor was added and the cells were chilled and centrifuged at 600*g*. The supernatant material and the cells were then processed as described (Buck *et al.*, 1970).

Preparation of Surface Membranes. The washed cells, in metaphase or treated with Velban but not in metaphase, were suspended in 0.16 M NaCl to a concentration of 5×10^7 cells/ml. Surface membranes were prepared from these cells by the zinc ion procedure (Warren and Glick, 1969).

Distribution of Glycopeptides. The procedures for examining the distribution of glycopeptides from the cell surface have been described (Buck *et al.*, 1970). The exception was that in the experiments reported here, the radioactive compounds were added 24 hr before harvesting, at the time of the addition of Velban. Pronase digestion and gel filtration on Sephadex G-50 of membranes and trypsinates were as described previously (Buck *et al.*, 1970), as was processing for scintillation counting (Buck *et al.*, 1971b).

Assays for Glycosidases. The cells arrested in metaphase, the cells treated with Velban but not in metaphase, and cells which were growing exponentially were harvested and washed as described above. Each of these groups of cells ($3–6 \times 10^7$ cells) was suspended in 1 ml of 0.1% Triton × 100 (Bosmann and Bernacki, 1970) and partially broken by approximately 50 strokes in a pointed tight-fitting glass homogenizer. Aliquots were removed for protein determination. These homogenates were examined for glycosidase activities which were determined as nanomoles of the appropriate substrate cleaved per hour per milligram of protein at 37°. *p*-Nitrophenyl derivatives of the appropriate monosaccharides served as substrates. The amount of *p*-nitrophenol released was related to a standard curve of authentic *p*-nitrophenol. Each experiment consisted of three linear points for the individual enzymes. Acid phosphatase was determined in the cell extracts in a similar manner using *p*-nitrophenyl phosphate as the substrate.

Chemical Analyses and Other Procedures. Sialic acid and protein analyses were performed as described (Glick *et al.*, 1970). The procedures for determination of sialic acid were reduced fivefold, making it possible to determine the sialic acid content of 5×10^5 cells. Fucose was determined by gas–liquid chromatography of the alditol acetate (Lehnhardt and Winzler, 1968) as adapted for analysis of whole cells (Glick *et al.*, 1970). Details for the scintillation counting of radioactive material in the whole cells have been described (Buck *et al.*, 1971b). Agglutination by Concanavalin A (Calbiochem) was performed as described (Inbar and Sachs, 1969) with 250 μg of Concanavalin A per 1×10^7 cells.

Isotopes and Chemicals. [1-^{14}C]L-Fucose (50.8 mCi/mmol) and [H]L-fucose, generally labeled, (4.3 mCi/mmol) were obtained from New England Nuclear Corp., Boston, Mass. *p*-Nitrophenyl phosphate, disodium, and the following *p*-nitrophenyl glycosides were obtained from Sigma Chemical Co., St. Louis, Mo.: α-D-mannopyranoside; α- and β-D-glucopyranosides ; α- and β-galactopyranosides; α- and β-L-fucosides; α,β-*N*-acetyl-D-galactosaminide; and β-D-*N*-acetyl-D-glucosaminide. All other chemicals have been referred to previously (Buck *et al.*, 1970, 1971b; Glick *et al.*, 1971).

Results

Glycopeptides from the Trypsinates of Cells Arrested in Metaphase. BHK_{21}/C_{13} cells were arrested in metaphase with Velban in the presence of [^{14}C]- or [^{3}H]L-fucose. The material removed from the surface of these cells by trypsin (trypsinate) was further digested with pronase. Figure 1 shows the profiles which resulted when the fucose-containing glycopeptides obtained by these procedures were chromatographed on Sephadex G-50. Figure 1a compares directly the trypsinates from cells arrested in metaphase with those from Velban treated cells which were not in metaphase. The profiles shown in Figures 1b and c give a comparison of the fucose-containing glycopeptides from either the surface of the metaphase arrest (Figure 1b) or the Velban treated cells (Figure 1c) with those obtained from virus-transformed cells (C_{13}/B_4). The surface material from the metaphase-arrest cells shows a clear shoulder of rapidly migrating glycopeptides corresponding to the first major peak of glycopeptides from the C_{13}/B_4 cells (Figure 1a and b). In contrast, this well-resolved shoulder is not present in the trypsinate from BHK_{21}/C_{13} cells from the same culture but not having reached metaphase (Figure 1a and c). Note that in both Figure 1b and c the first two major peaks from the C_{13}/B_4 trypsinates were clearly resolved. The significances of variable amounts of radioactivity in fractions 50–70 are not clear. The profile

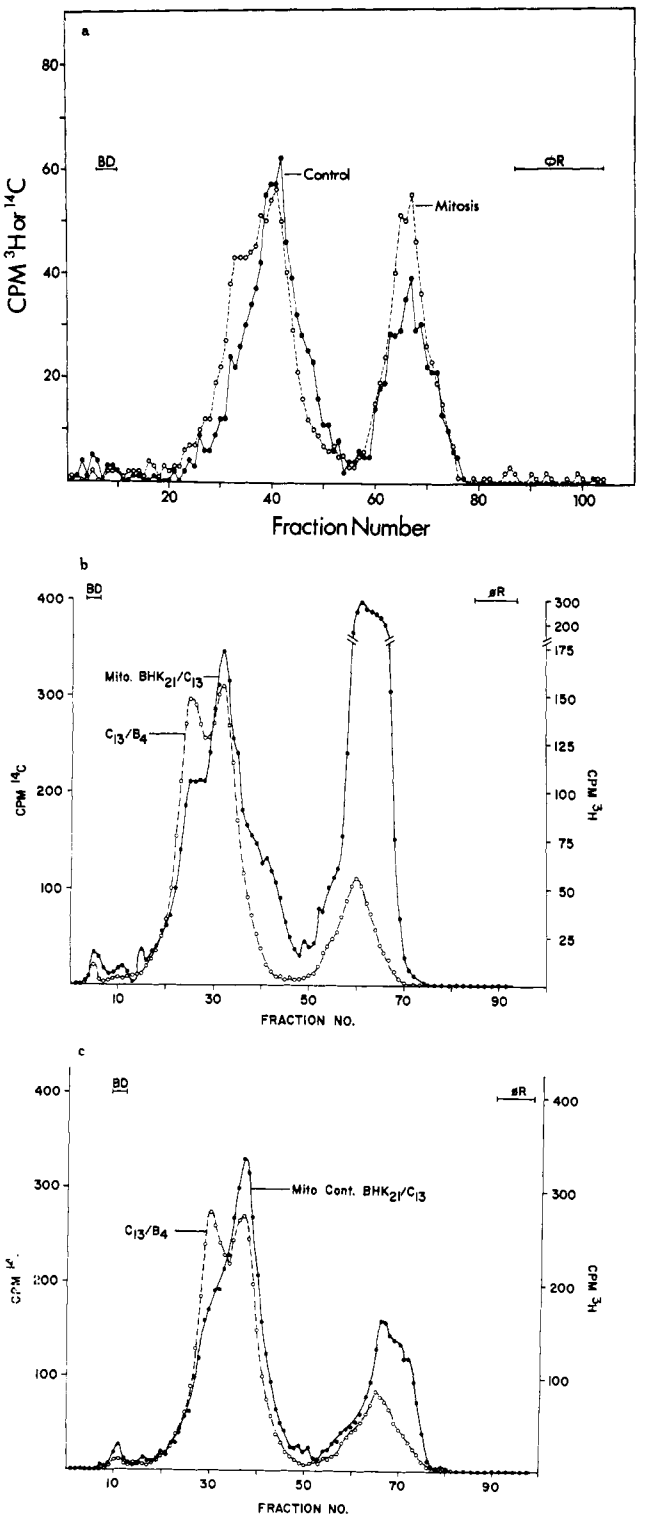

FIGURE 1: Cochromatography on Sephadex G-50 of pronase-digested trypsinates from BHK_{21}/C_{13} cells treated with Velban. Velban was added (1.2×10^{-5} mg/ml of medium) and the cells cultured in the presence of 100 μCi of [^{3}H]L-fucose (●) or 10 μCi of [^{14}C]L-fucose (○) for 24 hr. All details are described in the text. (a) Trypsinate of cells in metaphase (mitosis) pronase digested with trypsinate of cells treated with Velban but not in metaphase (control). (b) Trypsinate from cells harvested in metaphase (mitosis) pronase digested with the trypsinate of C_{13}/B_4 cells (C_{13}/B_4). (c) Trypsinate from cells from the same culture treated with Velban but not in metaphase (control) pronase digested with trypsinate from C_{13}/B_4 cells (C_{13}/B_4). Blue Dextran and Phenol Red were included as high and low molecular weight markers: BD, fraction in which Blue Dextran was eluted; ϕR, fractions in which Phenol Red was eluted.

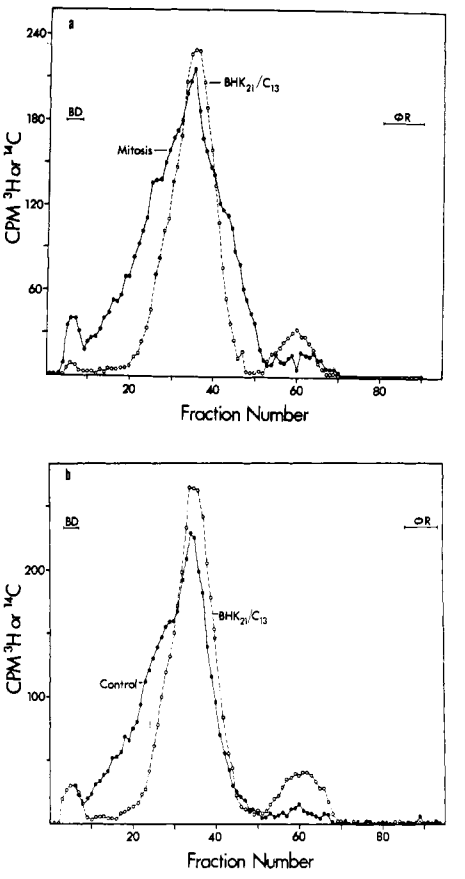

FIGURE 2: Cochromatography on Sephadex G-50 of pronase-digested surface membranes isolated from BHK_{21}/C_{13} cells treated with Velban and the trypsinate from BHK_{21}/C_{13} cells growing exponentially. The cells were grown in the presence of 100 μCi of [^{3}H]-L-fucose (●) or 10 μCi of [^{14}C]L-fucose (○) for 24 hr. All details are described in the text. From the same culture (a) one population of cells was harvested in metaphase (mitosis) and (b) the remaining cells harvested after treatment with Velban but which were not in metaphase (control). Blue Dextran and Phenol Red were included as high and low molecular weight markers: BD, fractions in which Blue Dextran was eluted; ϕR, fractions in which Phenol Red was eluted.

of the glycopeptides from the surface of the Velban treated cells which were not in metaphase (Figure 1c) was similar to that obtained from cells under normal conditions of growth (Figure 2).

Glycopeptides from Surface Membranes of Cells Arrested in Metaphase. Surface membranes were prepared from cells arrested in metaphase with Velban and cells treated with Velban but not in metaphase harvested from the same vessels. The cells were made radioactive by growth in the presence of [^{3}H]L-fucose. Figure 2 shows the profile obtained by chromatography on Sephadex G-50 of the fucose-containing glycopeptides of the surface membranes from these cells. As an internal marker, the membranes were compared with material removed from the surface of randomly growing BHK_{21}/C_{13} cells by trypsin. Prior to chromatography the fractions to be compared were mixed and digested with pronase. The elution pattern of the glycopeptides from surface membranes of metaphase-arrest cells (Figure 2a) shows the presence of faster eluting material, similar to that seen in the material removed from the surface of these cells with trypsin (compare Figures 1a and 2a). The surface membranes from the cells treated with Velban but not in metaphase also

TABLE I: Sialic Acid and Protein Content of BHK_{21}/C_{13} Cells in Various States of Growth.

Conditions of Cells at Time of Harvest[a]	Sialic Acid[b] μmol $\times$ 10^{-9}/Cell	Sialic Acid[b] nmol/mg of Cell Protein	Protein[b] mg $\times$ 10^{-7}/Cell
Metaphase	4.0 ± 1.0	8.9 ± 2.2	4.8 ± 0.5
Velban treated but not in metaphase	6.4 ± 0.9	15.6 ± 3.8	4.1 ± 0.8
Thymidine arrest	3.6 ± 0.5	13.9 ± 0.7	2.7 ± 0.2
Exponential growth	2.7 ± 0.4	12.7 ± 2.7	2.2 ± 0.3

[a] BHK_{21}/C_{13} cells were cultured for 24 hr under the usual conditions (exponential growth) or treatment with 7 mM thymidine (thymidine arrest) or 1.2×10^{-5} mg of Velban/ml of medium. The latter cells, treated with Velban, were harvested as two separate populations, those which could be shaken off the monolayer (metaphase) and those remaining adhered to the culture flask which were subsequently removed with trypsin (Velban treated but not in metaphase). All details and chemical procedures are described in the text. [b] The numbers represent the mean and standard deviations for five separate experiments performed in duplicate with the exception of the thymidine arrested cells which represent three separate experiments performed in duplicate.

TABLE II: Differences between Metaphase and Control Cells.

Condition of Cells at Time of Harvest[a]	per mg of Cell Protein: Total Fucose Content,[b] nmol	per mg of Cell Protein: [^{3}H]L-Fucose Incorporated[c] cpm $\times$ 10^4	Agglutination with Concanavalin A
Metaphase	3.6	3.0	Positive[d]
Velban treated but not in metaphase	ND[e]	1.0	Negative

[a] See Table I. [b] Average values of two separate experiments performed in duplicate. [c] Average values of three separate experiments performed in duplicate. [d] At least 70% of the cells were agglutinated into clumps of 5 or more cells. [e] Not determined.

had fucose-containing glycopeptides which elute more rapidly from Sephadex G-50 (Figure 2b). This was in contrast to the distribution of the glycopeptides found in the trypsinates from these cells in which little rapidly migrating material was visible (compare Figures 1c and 2b).

Sialic Acid Content of Whole Cells in Various Stages of Growth. The sialic acid content of BHK_{21}/C_{13} cells in metaphase was compared with the sialic acid content of cells (1) treated with Velban but not in metaphase, (2) grown exponentially, and (3) arrested with thymidine. The values reported for all of the cells (Table I) with the exception of the metaphase-arrest cells included the sialic acid content of the material removed from the cell surface with trypsin, since this comprises part of the outer surface of the cell. In all cases this value amounted to 25–35% of the total cell content of sialic acid.

The protein content of the cells in the various stages of growth as well as the size determination indicated that the cells treated with Velban were considerably larger than the cells which were growing exponentially (Table I). The thymidine arrested cells were not as large as the Velban treated cells. Therefore, when the sialic acid contents of the whole cells were expressed on a per milligram of cell protein basis (Table I) the sialic acid content of the metaphase cells was less than that of the other cells. The metaphase cells contained 43% less sialic acid than the Velban treated cells, not in metaphase, and 30% less sialic acid than cells in exponential growth. The thymidine arrested cells contained an amount of sialic acid more similar to those in exponential growth.

Other Differences between Metaphase and Control Cells. The amount of [^{3}H]L-fucose incorporated by the metaphase cells was three times more than that incorporated by the control cells (Table II). The total fucose content of the metaphase cells was 3.6 nmol/mg of protein (Table II). In these experiments, the molar ratio of fucose to sialic acid was 0.40.

A difference was also noted between these two cell populations in the agglutination reaction with Concanavalin A (Table II). The cells in metaphase agglutinated while the Velban treated cells, not in metaphase, showed no agglutination. BHK_{21}/C_{13} cells, growing exponentially, showed no agglutination under similar conditions. The agglutinated cells were examined in a hemocytometer and more than 70% of the cells were in clumps of five or more cells.

Comparison of Glycosidase Activities of Metaphase and Control Cells. In order to determine if there was a specific degradation of the oligosaccharide portion of the glycoproteins during mitosis, Triton homogenates of cells in metaphase (mitosis) and cells from the same culture treated with Velban but not in metaphase (control) were examined for glycosidase activities.

The glycosidase activities can be expressed in two groups, enzymes with high activity levels (Figure 3a) and those with lower activities (Figure 3b). The activities of the enzymes given in Figure 3a are approximately ten times more than those in Figure 3b. When the activities were expressed as nanomoles of substrate cleaved per hour per milligram of mitotic or control cell protein, β-*N*-acetyl-D-glucosaminidase (350 and 330 nmol), β-D-galactosidase (378 and 300 nmol), and α,β-*N*-acetyl-D-galactosaminidase (120 and 140 nmol) were in former group. The latter group comprised α-D-mannosidase (42 and 69 nmol), β-D-glucosidase (23 and 44 nmol), α-L-fucosidase (37 and 40 nmol), α-D-galactosidase (22 and 17 nmol), β-L-fucosidase (12 and 9 nmol), and α-D-glucosidase (9 and 8 nmol). The activities of acid phosphatase (583 and 679 nmol) are also given in Figure 4a. As a point of reference, α-D-mannosidase was included in both figures.

Only small differences were found between the glycosidase activities of the mitotic and control cells under the conditions used. Two exceptions which showed statistically significant differences within each experiment were α-D-mannosidase and β-D-glucosidase. Both of these enzymes from the mitotic cells showed decreased activities (53 and 61%, respectively) when compared to these activities in the control cells (Figure 3b).

Mitotic and control cells contained approximately the same amounts of protein, 4.8×10^{-7} and 4.1×10^{-7} mg/cell,

respectively (Table I). Because of this, the enzyme activities remained similar when expressed on a per cell basis.

Discussion

These studies were initiated to further examine the role of glycoproteins of the cell membrane in mitosis. When cells are treated with Velban, a population of cells can be isolated which are 80–90% in metaphase. This affords the opportunity to examine cells arrested in a discrete phase of the cell cycle.

The results presented here suggest that the fucose-containing glycopeptides which are expressed on the cell surface during mitosis are similar to those which are permanently expressed after virus transformation (Buck *et al.*, 1970, 1971a) or transiently expressed during very rapid growth of nontransformed BHK_{21}/C_{13} cells (Buck *et al.*, 1971b). Small amounts of the more rapidly migrating glycopeptides were obtained from the surface membranes of both metaphase-arrest and control cells even under conditions where these glycopeptides were not present in the trypsinates from these same cells. In other studies, analyses of the carbohydrates of these glycoprotides suggest that the apparent increase in molecular weight does not represent merely the addition of fucose to existing glycoproteins, but the addition of other monosaccharides as well (Glick, 1971).

The fact that the BHK_{21}/C_{13} fibroblasts in mitosis were agglutinated by Concanavalin A (Table II) is another indication of a similarity with virus transformed cells. The cells treated with Velban but not in metaphase do not agglutinate. Concanavalin A is a carbohydrate binding protein (Goldstein *et al.*, 1965) and has been described by Inbar and Sachs (1969) to agglutinate transformed cells to a greater extent than nontransformed cells. Fox *et al.*, (1971) obtained a result similar to that shown here using wheat germ agglutinin conjugated with fluorescin isothiocyanate. They found that the tagged wheat germ agglutinin was bound to 3T3 cells during mitosis but not in other phases of the cell cycle.

Bosmann and Bernacki (1970) have examined the activities of *N*-acetyl-D-glucosaminidase, β-D-galactosidase, *N*-acetyl-D-galactosaminidase, and acid phosphatase in L5178 cells synchronized with excess thymidine and Colcemid. They found these enzymes increased in a continuous linear pattern starting with the lowest level at the end of mitosis. Our experiments show little difference in the activities of these glycosidases from BHK_{21}/C_{13} cells in metaphase or cells treated with Velban but not in metaphase (Figure 3). Both of these cell populations show glycosidase activities similar to BHK_{21}/C_{13} cells which were growing exponentially (Glick, 1971). There is no dramatic change in the activities of any of the carbohydrate degrading enzymes during or immediately preceding metaphase. However, a 50–60% decrease in the activities of α-D-mannosidase and β-D-glucosidase was consistently found in the metaphase cells. No attempts were made to vary the conditions of assaying these enzymes although the presence of certain ions altered the glycosidase activities of red blood cell stroma (Bosmann, 1971). The difference between our results and those of Bosmann and Bernacki (1970) may reflect differences between the cell lines or the methods for obtaining cells in mitosis. The activities of fucosidase and sialidase will be the subject of further studies.

A comparison of the cells in metaphase with those treated with Velban but not in metaphase shows that the amount

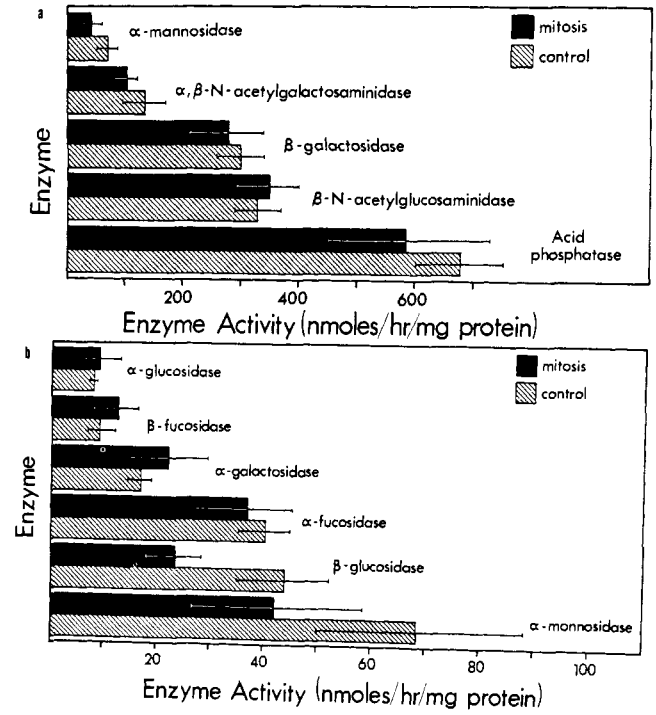

FIGURE 3: Glycosidase activities of cells in metaphase. BHK_{21}/C_{13} cells arrested in metaphase with Velban (mitosis; solid) or treated with Velban but not in metaphase (control; hatched) were homogenized in 0.1% Triton X100, and assayed for glycosidase and acid phosphatase activities. Each assay mixture consisted of 0.3 μmol of the appropriate substrate with the exception of *p*-nitrophenol α-D-mannopyranoside of which 1.2 μmol was used. The enzyme homogenates in 0.1% Triton X100 and 0.6 ml of 0.05 M sodium citrate buffer, pH 4.5, made a final volume of 0.9 ml. The mixtures were incubated at 37° for 1 hr and the reactions were terminated with 0.6 ml of 0.2 M potassium borate, pH 9.8. The released *p*-nitrophenol was measured at 420 mμ. Authentic *p*-nitrophenol was used as a standard. Substrates were as stated in the text. The amounts of *p*-nitrophenol released were expressed as nmoles per hr per mg of protein: (a) the enzymes which cleaved greater than 100 nmol per hr per mg of protein and (b) less than 100 nmol per hr per mg of protein. Each experiment consisted of two-three linear points for the individual enzymes. The mean and standard deviations of three separate experiments are given.

of sialic acid found in the metaphase cells was decreased by 43% when compared to the Velban treated cells not in metaphase (Table I). These results are similar to those obtained previously which showed specific decreases in the monosaccharides during the hours of mitosis in a synchronized population of KB cells (Glick *et al.*, 1971). Sialic acid increased before the population began to divide and decreased after the onset of mitosis. The threefold increase in the incorporation of radioactive fucose (Table II) by the metaphase cells could precede the sudden return after division to premitotic levels of the total fucose content of the synchronous population (Glick *et al.*, 1971). It is quite possible that the "control" cells represent a population of cells about to enter metaphase. This is reflected not only by the increased amount of sialic acid but also by the increased size distribution and protein content when compared to cells which are growing exponentially.

A role for carbohydrates in cell division has been suggested by several kinds of experiments. Cells have been known to stop dividing when exposed to specific monosaccharides such as fucose (Cox and Gesner, 1968) or to plant lectins

such as Concanavalin A (Burger and Noonan, 1970; Shoham *et al.*, 1970). Lymphocytes are stimulated to divide in the presence of certain lectins (see review, Ling, 1968). The cell surface receptor sites for several of these lectins have been characterized as glycoproteins (Kornfeld *et al.*, 1971). Presumably these mechanisms involve carbohydrates at the cell surface, but exactly how such phenomena can take place has yet to be elucidated. The data reported here further emphasize the possible role of surface carbohydrates in cellular processes.

Acknowledgments

We wish to thank Dr. Ian Macpherson, Imperial Cancer Fund, London for supplying early passage BHK_{21}/C_{13} cells, Dr. Leonard Hayflick, Stanford University, for examining our cultures for *Mycoplasma*, Mr. Anthony Neuberger, Cambridge University, Cambridge, for statistical analyses, and Mrs. Pamela Kaplan, this Department, for performing the enzyme assays. The technical assistance of Mrs. Suzanne Redmond, Mrs, Sigrid Skau, and Mrs. Florence Massey is fully acknowledged.

References

Bosmann, H. B. (1971), *J. Membrane Biol. 4*, 113.
Bosmann, H. B., and Bernacki, R. J. (1970), *Expt. Cell Res. 61*, 379.
Buck, C. A., Glick, M. C., and Warren, L. (1970), *Biochemistry 9*, 4567.
Buck, C. A., Glick, M. C., and Warren, L. (1971a), *Science 172*, 169.
Buck, C. A., Glick, M. C., and Warren, L. (1971b), *Biochemistry 10*, 2176.
Burger, M. M., and Noonan, K. D. (1970), *Nature (London) 228*, 512.
Cox, R. P., and Gesner, B. M. (1968), *Cancer Res. 28*, 1162.
Fox, T. O., Sheppard, J. R., and Burger, M. M. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 244.
Glick, M. C. (1971), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 30*, No. 3, 1044.
Glick, M. C., Comstock, C. A., and Warren, L. (1970), *Biochem. Biophys. Acta 219*, 290.
Glick, M. C., Gerner, E. W., and Warren, L. (1971), *J. Cell Physiol. 77*, 1.
Goldstein, I. J., Hollerman, C. E., and Smith, E. E. (1965), *Biochemistry 4*, 876.
Inbar, M., and Sachs, L. (1969), *Proc. Nat. Acad. Sci. U. S. 63*, 1418.
Kornfeld, S., Rogers, J., and Gregory, W. (1971), *J. Biol. Chem. 246*, 6581.
Lehnhardt, W. F., and Winzler, R. J. (1968), *J. Chromatogr. 34*, 471.
Ling, N. R. (1968), Lymphocyte Stimulation, Amsterdam, North Holland Publishing Co.
Shoham, J., Inbar, M., and Sachs, L. (1970), *Nature (London) 227*, 1244.
Warren, L., and Glick, M. C. (1969), *in* Fundamental Techniques in Virology, Habel, K., and Salzman, N. P., Ed., New York, N. Y., Academic Press, p 66.

Amino Acid Sequences of Mouse 2.5S Nerve Growth Factor. I. Isolation and Characterization of the Soluble Tryptic and Chymotryptic Peptides†

Ruth Hogue Angeletti,‡ Delio Mercanti,‡ and Ralph A. Bradshaw*,§

ABSTRACT: The determination of the amino acid sequence of nerve growth factor (NGF), prepared as the 2.5S derivative from mouse submandibular glands, has been initiated. Tryptic digestion of *S*-carboxymethyl- and *S*-aminoethyl-NGF, followed by purification and characterization of the resulting peptides, was performed. These peptides accounted for 87 residues in the NGF molecule but contained only 5 of the 6 half-cystinyl residues and were missing the amino-terminal fragment. Digestion of the *S*-carboxymethyl derivative with chymotrypsin yielded 19 peptides covering 108 unique residues. By combined Edman degradation and carboxypeptidase A and B digestion, 96 of the 108 residues, accounted for by peptides from the three separate digests were placed in sequence.

Nerve growth factor (NGF)[1] is a protein which controls the growth of sympathetic nerve cells during development and throughout adult life (Angeletti *et al.*, 1968; Levi-Montalcini *et al.*, 1972). The biological effects accompanying these processes have been examined extensively and reported in detail (Levi-Montalcini and Angeletti, 1968). However, in

† From the Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri 63110, and from the Department of Biology, Washington University, St. Louis, Missouri 63130. *Received August 14, 1972.* This work was supported by research grants from the National Institutes of Health AM-13362 and NS-03777 and a Health Science Advancement award to Washington University (5 SO4FR06115).

* To whom to address correspondence at the Department of Biological Chemistry, Washington University School of Medicine.

‡ Present address: Laboratorio di Biologia Cellulare, CNR, Rome, Italy.

§ Research Career Development awardee of the National Institutes of Health (AM-23968).

[1] Abbreviations used are: NGF, nerve growth factor; SCM-, *S*-carboxymethyl; SAE-, *S*-aminoethyl; T-, tryptic peptides; TPCK-, (1-tosylamido-2-phenyl)ethyl chloromethyl ketone; MTH-, methylthiohydantoin; dansyl-, 5-dimethylaminonaphthosulfonic acid; ST-, SAE-NGF tryptic peptides; C-, chymotryptic peptides.