*Journal of Bioenergetics and Biomembranes, Vol. 18, No. 6, 1986*

# Transplasmalemma Electron Transport Is Changed in Simian Virus 40 Transformed Liver Cells

I. L. Sun,[1] P. Navas,[1] F. L. Crane,[1,4] J. Y. Chou,[2] and H. Löw[3]

*Received February 24, 1986*

## Abstract

Transplasma membrane electron transport activity by fetal rat liver cells (RLA209-15) infected with a temperature-sensitive strain of SV40 has been measured with cells grown at the restrictive temperature (40°C) and permissive temperature (33°C). The transformed cells grown at 33°C had only one-half the rate of external ferricyanide reduction as the nontransformed cells held at 40°C. Both the $K_m$ and $V_{max}$ for ferricyanide reduction were changed in the transformed state. The change in $V_{max}$ can be based on a decrease of NADH in the transformed cells. The change in rate with ferricyanide does not depend on change in surface charge. Reduction of external ferricyanide was accompanied by release of protons from the cells. The ratio of protons released to ferricyanide reduced was higher in the transformed cells than in the nontransformed cells. Since the transplasma membrane electron transport has been shown to stimulate cell growth under limiting serum, the changes in the plasma membrane electron transport and proton release in transformed cells may relate to modification of growth control.

**Key Words:** Plasma membrane; pyridine nucleotide oxidation; temperature sensitive SV40; liver cells; transmembrane electron transport; cell transformation; enzyme kinetics.

## Introduction

A transplasma membrane redox system, found in many types of cells, has been related to the control of cell growth. Activation of transplasma membrane electron transport with ferricyanide stimulates the growth of melanoma cells in serum-deficient media (Ellem and Kay, 1983) and promotes HeLa cell proliferation in the absence of fetal calf serum or other

[1]Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907.
[2]Human Genetics Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20205.
[3]Endocrinology Department, Karolinska Institute, S10401 Stockholm, Sweden.
[4]To whom correspondence should be addressed.



0145-479X/86/1200-0471$05.00/0 

growth factors (Sun *et al.*, 1984a). A series of impermeable oxidants with redox potentials down to −125 mV have similar growth promoting effects (Sun *et al.*, 1984b). All of these oxidants are reduced by the transplasma membrane electron transport system. Oxidants which are not reduced by the transmembrane electron transport do not stimulate growth (Sun *et al.*, 1984b). Growth-promoting hormones, such as insulin, stimulate growth in the absence of serum and also stimulate transmembrane redox activities (Sun *et al.*, 1984a). Diferric transferrin, which is an essential growth factor for many cells, can act as an electron acceptor for the plasma membrane redox system (Crane *et al.*, 1985a).

Redox effects on growth are not unexpected, since animal cells which have nonfunctional mitochondria still require oxygen for growth (Scheffer *et al.*, 1981). Antineoplastic drugs (Sun and Crane, 1981, 1984a, b, c) which inhibit cell growth also inhibit the transplasma membrane redox system. There is also some evidence that virus-transformed or tumor cells have lower transmembrane redox activities than nontransformed cells. These cells include Simian Virus 40 transformed *RLA209-15* liver cells (Sun *et al.*, 1983), SV40-transformed *RPNA209-1* pineal cells (I. L. Sun, unpublished), 3T3 cells (Crane *et al.*, 1985b), Esb (*LS178Y-ES* and *EbLS178Y-E*) cells (Cherry *et al.*, 1981), and hepatoma McA-RH7777 cells (Crane *et al.*, 1985b). A lower NADH ferricyanide reductase activity in purified plasma membrane from transformed 3T3 cells has been reported (Sheinin and Onodera, 1972). The transmembrane redox activity in transformed cells is also more sensitive to inhibition by antitumor drugs (Sun *et al.*, 1983).

To understand the regulation of transmembrane redox systems in cancer cells it is necessary to have a valid control system in which to study the normal redox function. Cultured rat fetal liver cells (*RLA209-15*) that retain differentiated hepatic functions have been established by transforming normal liver cells with *tsA* mutant of SV40 that is temperature sensitive in the gene required for the maintenance of transformation (Chou and Schlegel-Haueter, 1981). The cell line expresses the transformed phenotype at the permissive temperature (33°C) but mimics the normal nontransformed hepatocytes at the restrictive temperature (40°C). This cell line provides us a favorable model for the study of the nature of transmembrane redox modification under reversible conditions of malignant transformation.

Numerous studies have shown that pyridine nucleotide pools are significantly altered in proliferating tissues in comparison to those of nonproliferating tissues (Jedeikin and Weinhouse, 1955; Caiger *et al.*, 1962; Nemeth and Dickerman, 1960; Briggs, 1960; Burch and Von Dippe, 1964; Clark *et al.*, 1966; Ferris and Clark, 1972). We have previously observed a decrease in NAD(H) levels of rat liver following administration of a carcinogen (2-acetylaminofluorene) (Sun *et al.*, 1985). Furthermore, changes in pyridine

nucleotide levels as a function of growth in normal and transformed 3T3 cells have been reported (Jacobson and Jacobson, 1976). NADH or NADPH may increase (Schwartz *et al.*, 1974; Warburg, 1977) or decrease (Jedeikin and Weinhouse, 1955) as a result of transformation. Although the significance of the relationship between NAD(H) levels and cell proliferation is not clear, their possible involvement in growth control are of great interest. The NADH level can also control the $V_{max}$ for the transplasma membrane dehydrogenase involved in redox stimulation of growth. Therefore the level of NAD and NADP and their redox states have been measured as a function of growth in RLA209-15 (40°C) cells which exhibit density-dependent inhibition of growth and RLA209-15 (33°C) cells which have lost this property.

## Experimental

### *Growth of Cells and Culture Conditions*

*RLA209-15* fetal liver cells were cultured in α-modified minimal essential medium (α MEM, from Irvine Scientific Company, with arginine, supplemented with 0.4 mM ornithine), 100 μg/ml streptomycin, and 100 u./ml penicillin plus 4% fetal bovine serum and gassed with 5% $CO_2$ and 95% air as described previously (Chou and Schlegel-Haueter, 1981). After 3 days of culture at 33°C, some cells were cultured for 2 more days at 40°C to suppress transformation and cause a return to normal phenotype. The rest of cells remain at 33°C to maintain the transformed phenotype. Cells were then harvested in the same media and washed in salts–Tris–EDTA buffer (140 mM NaCl, 2.5 mM KCl, 0.6 mM $Na_2HPO_4$, 25 mM Trizma base, and 0.05 mM EDTA, pH 7.4) (TD buffer). The pellet was resuspended in the same buffer to a final concentration of 0.1 gram wet weight per ml (gww/ml). As controls, we used the McA-RH7777 hepatoma and primary rat fetal hepatocyte cells, which were cultured at 33°C in the same media.

### *Assay of Ferricyanide Reduction by Cells*

The rate of ferricyanide reduction was measured at 37°C with cells (1–10 mg) suspended in 2.8 ml TD buffer with ferricyanide added to 0.2–0.4 mM. Ferricyanide reduction was measured in an Aminco DW2a spectrophotometer in the dual-wavelength mode at 420 nm minus 500 nm. The cuvette was stirred with a magnetic stirrer. Extinction coefficient for ferricyanide is $1\ mM^{-1}\ cm^{-1}$. There was an initial fast rate of ferricyanide reduction by cells for 1–2 min followed by a steady slower rate which continues for 10 min or longer (Sun *et al.*, 1983, 1984a, c; Clark *et al.*, 1981).

### *Study of Kinetics of Enzymic Reactions*

The affinity of the surface site for the oxidant, ferricyanide, was determined. Reductase activity was assayed with different concentrations of ferricyanide and the results plotted in a double reciprocal Lineweaver–Burk plot to determine $K_m$ and $V_{max}$ (Dixon and Webb, 1979).

### *Assay of Proton Extrusion*

Ferricyanide-induced proton generation was measured in a 2-ml cuvette and an Orion 701 pH meter and a Corning combination electrode. Cells were suspended in Krebs–Henseleit solution without phosphate or bicarbonate (low buffer) or in a sucrose salts solution (10 ml KCl, 10 mM NaCl, 10 mM $CaCl_2$, 0.1 M sucrose, and 5% TD Tris buffer) to a final concentration of 0.005 gww/ml. The sample was stirred continuously and bubbled with air to remove $CO_2$. After the pH came to an equilibrium, ferricyanide (0.1–0.5 mM) was added. The proton release was measured by the change in pH from 7.4 to 7.3. The rate was measured after a rapid initial decline following ferricyanide addition so it corresponds to the slow rate of ferricyanide reduction. Buffer capacity was calibrated with 0.01 N HCL.

### *Assay for Pyridine Nucleotide Pool*

The pyridine nucleotide pool was determrined by extraction of NAD or NADP or NADPH from cells with perchloric acid and alkali, respectively, as described previously (Jacobson and Jacobson, 1976). The extracted pyridine nucleotide was then quantitated using a cycling assay involving alcohol dehydrogenase and glucose-6-phosphate dehydrogenase (Matsumara and Miyachi, 1980).

## Results

### *Transplasma Membrane Ferricyanide Reduction by RLA209-15 Fetal Liver Cells*

*RLA209-15* cells, exhibiting a transformed phenotype (33°C), have a slower rate of external ferricyanide reduction than their counterparts exhibiting a nontransformed phenotype (40°C) (Fig. 1). Both transformed and nontransformed cells showed an initial rapid phase of ferricyanide reduction which lasts for approximately 2 min and a slow steady-state phase which proceeded for 30 min or longer. A similar pattern of ferricyanide reduction has been reported with rat liver cells (Clark *et al.*, 1981) and HeLa cells (Sun *et al.*, 1984c). The difference in ferricyanide reduction rate between transformed and nontransformed *RLA209-15* cells held for cells at the log phase of growth and in the stationary phase. Cells with transformed phenotype

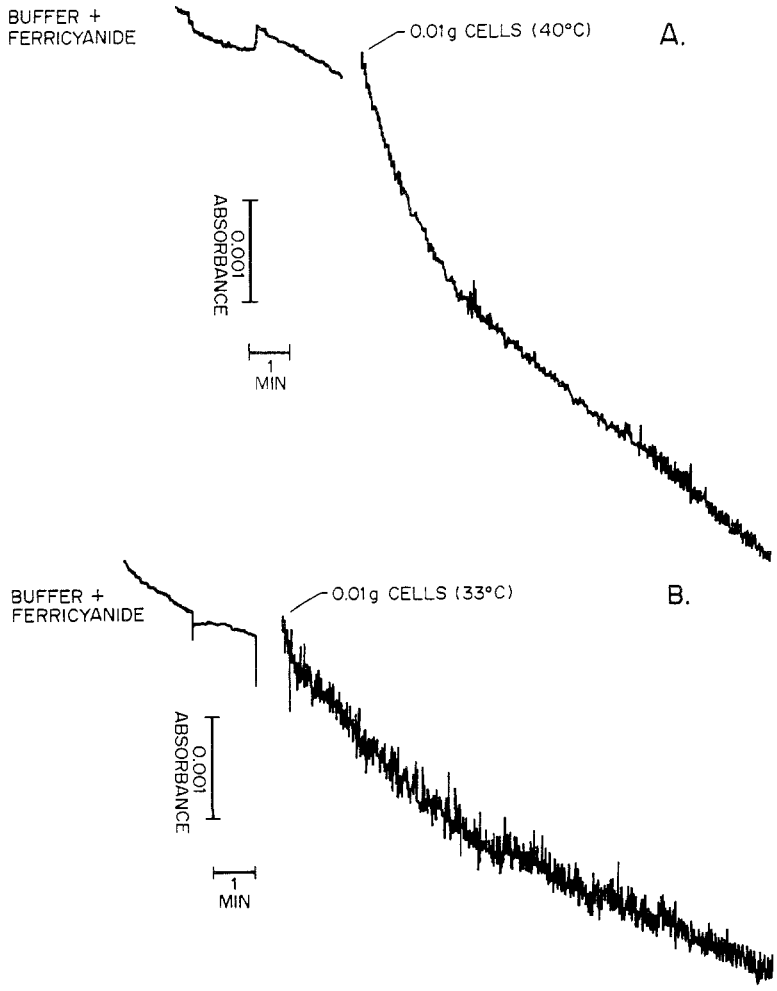


**Fig. 1.** Spectrophotometer tracings showing ferricyanide reduction by transformed (33°C) and nontransformed (40°C) RLA209-15 rat fetal liver cells. Assays in salts–Tris–EDTA buffer (TD) with dual beam measuring changes in absorbance at 420–500 nm are run at 3.7° C.

gave less than one-half the rate found with cells of nontransformed phenotype (Table I). The same relationship is seen in SV40-transformed 3T3 cells (Löw, Grebing, Crane, unpublished) in which the rate is only half of that of normal 3T3 cells. A similar relation is seen by comparison of normal liver cells and hepatoma in which hepatoma cells have a rate of ferricyanide reduction 60% less in the slow phase than isolated fetal liver cells (Table I). We have shown previously that FSb mouse lymphoma cells have a very low rate of ferricyanide reduction (0.01 $\mu$mol min$^{-1}$ gww$^{-1}$) (Cherry *et al.*, 1981).

**Table I.** Rates of Transplasma Membrane Ferricyanide Reduction by RLA20915 Fetal Liver Cells Grown at 33 and 40°C[a]

| Cell type | Growth conditions | Rate (nmol $min^{-1}$ $gww^{-1}$) |
|---|---|---|
| RLA209-15 | 40°C (nontransformed phenotype) | 320 |
| RLA209-15 | 33°C (transformed phenotype) | 100 |
| Rat liver (perfused) | Normal | 280 |
| Primary fetal liver | Normal | 610 |
| Rat liver McA-RH7777 | Hepatoma | 210 |

[a]Ferricyanide reduction rates are the steady rate after the first 2 min of initial fast rate. Perfused rat liver data is based on Clark *et al.* (1981). All assays run at 37°C.

### *Kinetics of Enzymatic Reactions*

Affinity of the surface site for ferricyanide was determined by assay of reduction with different concentrations of ferricyanide and plotting the results in a double reciprocal Lineweaver–Burk plot to determine $K_m$ and $V_{max}$. The $K_m$ for ferricyanide reduction in both the fast and slow phase was much higher for cells grown at 33°C then for cells grown at 40°C (Figs 2A, 2B). Cells with nontransformed phenotype had a higher $V_{max}$ in both slow phase and fast phase.

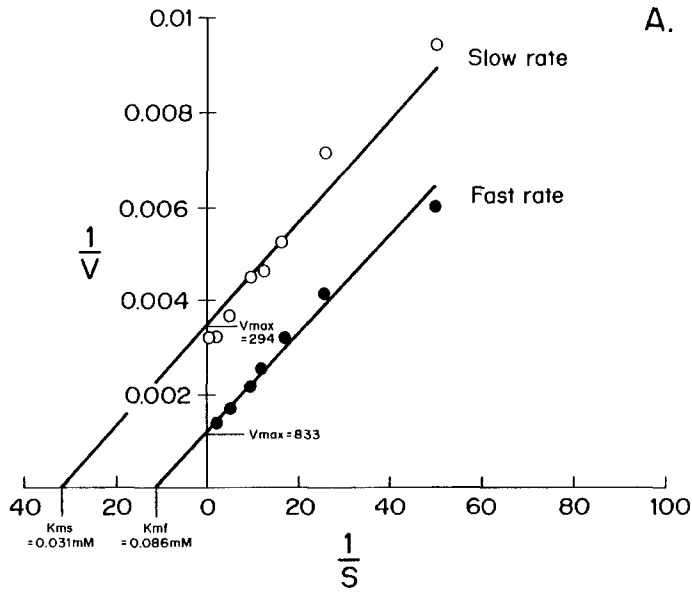


**Fig. 2A.** Lineweaver–Burk plot of relation between ferricyanide concentration and the fast and slow rate of ferrricyanide reduction by nontransformed (40°C) rat fetal liver cells RLA209-15 assay in TD buffer.

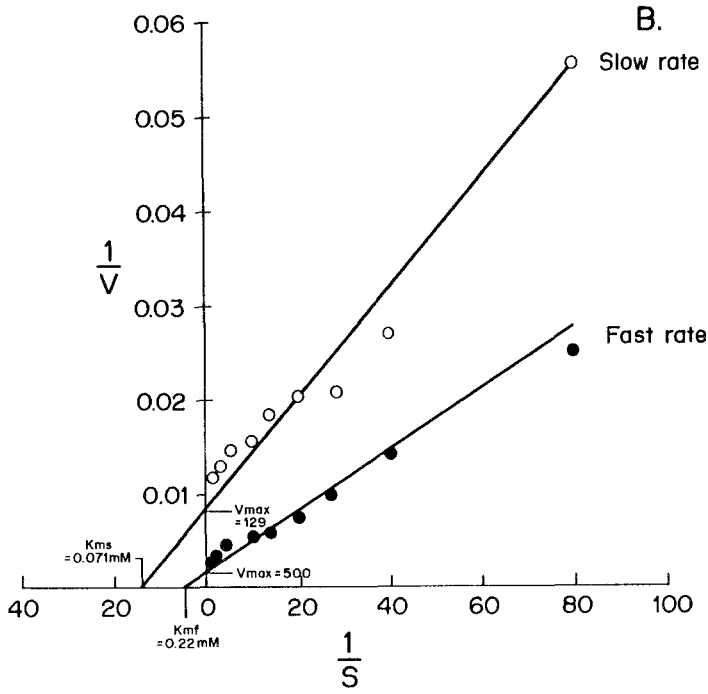


**Fig. 2B.** Lineweaver–Burk plot of relation between ferricyanide concentration and the fast and slow rate of ferricyanide reduction by transformed (33°C) rat fetal liver cells RLA209-15 assay in TD buffer.

### *Effect of Ionic Strength on Ferricyanide Reduction Rate*

The transformation of cells is known to involve changes in glycolipids and glycoproteins at the cell surface which may modify the negative surface potential (Hakamori, 1975). Since ferricyanide has a negative charge, the change in activity could be based on a change in affinity for the cell surface with change in surface charge after transformation. An increase of ionic strength in the media will counter the surface charge on the membrane by electrostatic screening. If the lower rate of ferricyanide anion reduction by the transformed cells is due to an increased negative charge on these cells, then higher ionic strength should decrease the difference in activity between the transformed and nontransformed cells (Pethig *et al.*, 1984). As shown in Table II, the difference in ferricyanide reduction between the two cell types was observed at both low and high salt concentrations. Furthermore, the activity with both transformed and nontransformed cells was lower in the low salt media than in 150 mM sodium chloride, so the surface charge was not the primary control on the ferricyanide reduction rate.

*Pyridine Nucleotide Concentration in RLA209-15 during Growth*

We have determined the pyridine nucleotide concentration in *RLA209-15* liver cells during growth both at the permissive temperature (33°C) and at the nonpermissive temperature (40°C). In 40°C cultures, NAD concentration decreased after 2 days growth and NADH concentration increased proportionally (Fig. 3A). In 33°C cultures, there was a decrease of NADH concentration reflected by an increase of NAD (Fig. 3B). Accumulation of the reduced form of NAD in the nontransformed phenotype was correlated to the abundance of the oxidized form in the transformed phenotype.

To study if the difference between the two phenotypes was a consequence of the interconversion between reduced and oxidized forms of pyridine nucleotides, the pyridine nucleotide pool was measured in cells

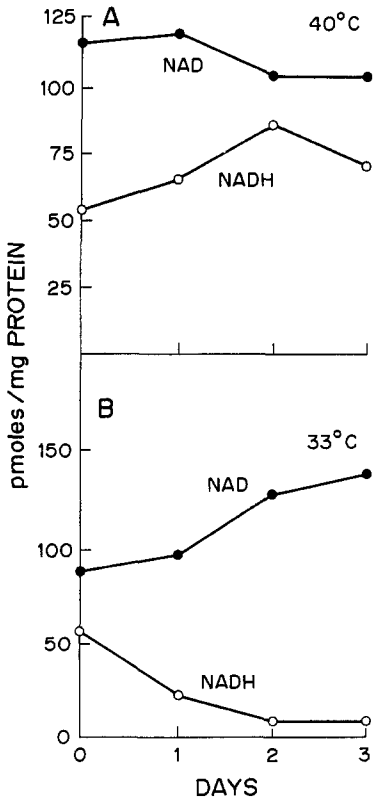


**Fig. 3.** Changes in diphosphopyridine nucleotide redox state in transformed (33°C) and nontransformed (40°C) rat fetal liver cells after the temperature shift. Two sets of cultures were plated at 33°C and grown for 2 days. After two days one set was shifted to 40°C while the other was maintained at 33°C. Pyridine nucleotide concentrations are shown for the day the change was made and three subsequent days. Medium was changed every 24 hr.

**Table II.** Effect of Salt Concentration on the Rate of Ferricyanide Reduction by Transformed (33°C) and Nontransformed (40°C) Rat Liver Cells (RLA209-15)[a]

| Assay media | Ferricyanide reduction (nmol $min^{-1}$ $gww^{-1}$) | | Ratio 40°/33° |
|---|---|---|---|
| | 40° | 33° | |
| 250 mM Sucrose | 218 | 98 | 2.2 |
| 150 mM Sodium chloride | 236 | 108 | 2.2 |

[a]Slow rate after 2 min shown, all with 2.5 mM Tris Cl buffer, pH 7.4. Assay at 37°C.

growing for 2 days at 40°C and then shifted down to 33°C for an additional 2 days. The change in pyridine nucleotide in *RLA209-15* cells grown at 33 and 40°C was reversible as was the change in phenotype. When cells grown for 2 days at 40°C were shifted back to 33°C, NAD concentration was increased and NADH was decreased (Fig. 4A). In contrast, the NADP/NADPH concentrations show a parallel relation without an apparent interchange

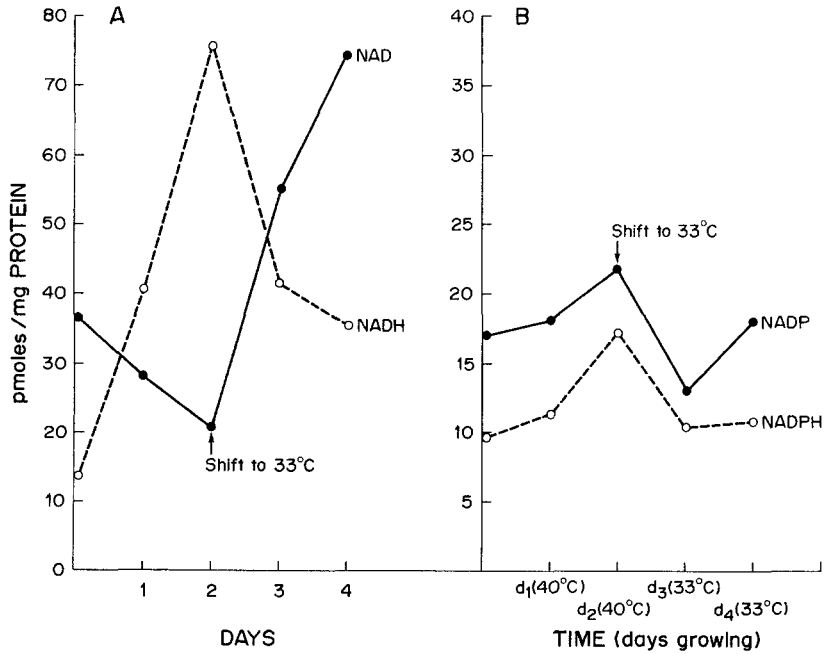


**Fig. 4.** Response of pyridine nucleotide pools in RLA209-15 cells to a shift from 40 to 33°C and subsequent growth for 4 days. Cultures were plated at 33°C with a small amount of initial inoculum, so the cells were maintained in the log phase throughout the experiment. After 2 days growth at 33°C, cultures were shifted from 33 to 40°C. After 2 days grown at 40°C, cultures were then shifted back to 33°C. Extraction of pyridine nucleotides was performed each day during the experiment.

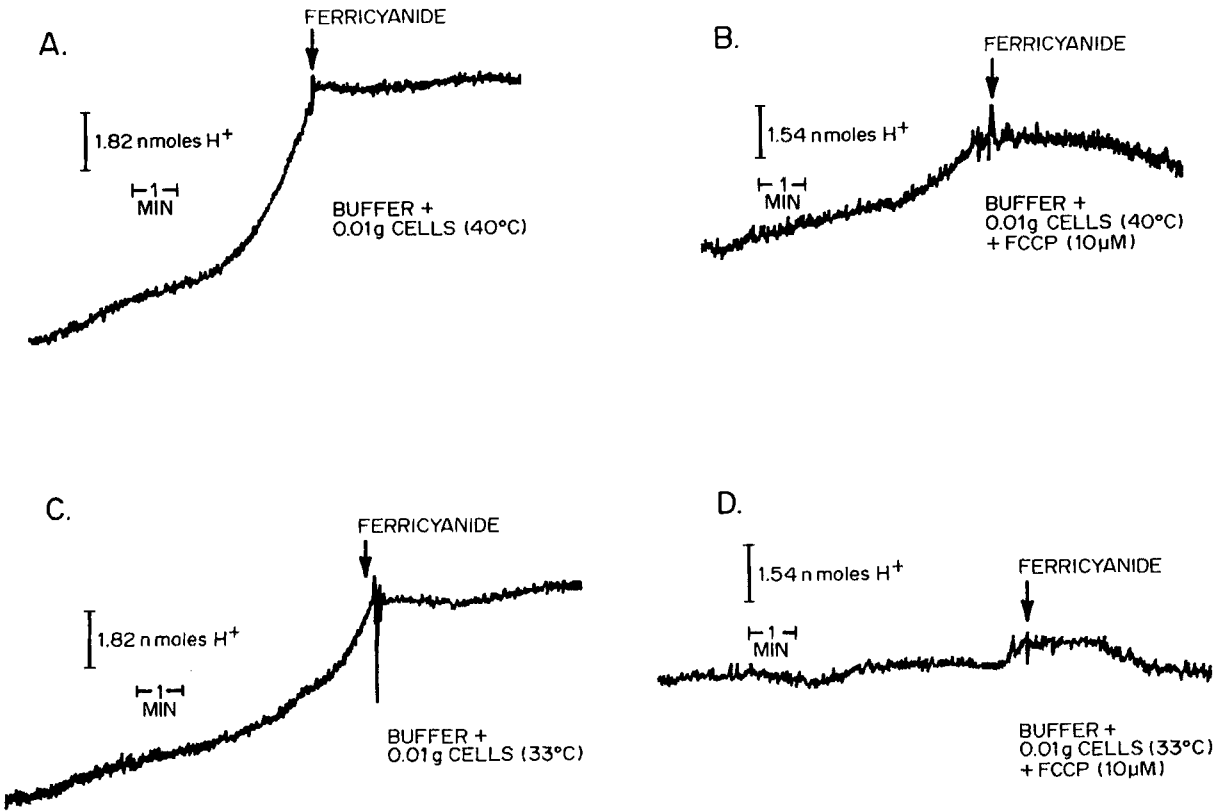


**Fig. 5.** Tracing of pH changes in the presence of RLA209-15 cells with added ferricyanide. (A) 40°C cells; (B) 40°C cells with FCCP (10 $\mu$M) added before ferricyanide; (C) 33°C cells; (D) 33°C cells with FCCP (10 $\mu$M). Procedures described in Experimental section.

**Table III.** Ferricyanide-Induced Proton Release and the Slow Rate of Ferricyanide Reduction by RLA209-15 Cells Grown at 33 and 40°C[a]

| Cell temperature | Rate of proton release (nmol $H^+$ $min^{-1}$ $gww^{-1}$) | Rate of ferricyanide reduction (nmol $min^{-1}$ $gww^{-1}$) | $\frac{H^+}{e}$ |
|---|---|---|---|
| 33°C (transformed phenotype) | 552 | 36 | 15.3 |
| 40°C (nontransformed phenotype) | 588 | 88 | 6.68 |

[a]The ferricyanide reduction rate in the buffer used for the determination of pH change is lower than the rate shown in Table I because of the lower ionic strength. The rate of proton release shown is the increased proton release when ferricyanide is added to the cells. The rates are measured 3 min after adding ferricyanide so they are the slow-phase reaction.

between oxidized and reduced forms (Fig. 4B). Thus the state of transformation was reflected in a change in the NAD/NADH ratio.

### *Ferricyanide-Induced Proton Release in RLA209-15*

When ferricyanide was added to the cell suspension in a sucrose salt solution, the pH of the suspension medium decreased, showing proton release from cells (Fig. 5). If the transplasma membrane electron transport activity was also measured in the sucrose salt solution, a higher ratio of proton release/electron transport was observed in 33°C cultures than that of 40°C cultures (Table III). The modification of this ratio may explain the loss of control in growth in transformed cells.

## Discussion

Ferricyanide can act as an artificial electron acceptor for the transmembrane redox system which is present in all cells that have been studied (Crane *et al.*, 1985b). The stimulation of cell growth by ferricyanide observed by Ellem and Kay (1983) and by this laboratory (Sun *et al.*, 1984a, b, c) shows that low levels of ferricyanide support sufficient electron transport to stimulate growth in the absence of serum factors. Higher levels of ferricyanide, however, may increase transmembrane electron transport to a level which inhibits growth by causing excess oxidation of reducing agents in the cytoplasm.

The basis for redox stimulation of growth is not known, but the release of protons from the cell which accompanies the redox activity may be related to the control of cell division. It has been shown in several cell types that an increase of cytoplasmic pH (alkalinization) is associated with cell division

(Gerson *et al.*, 1982; Moolenaar *et al.*, 1983; Frelin *et al.*, 1983). Ferricyanide induced proton release across the HeLa cell membrane which can increase the pH of the cytoplasm (H. Löw and F. L. Crane, unpublished). By this action the transmembrane reductase would help make cytoplasm more alkaline and thus induce mitosis.

In this study, we have demonstrated that normal and transformed cells differ greatly in their ability to effect the reduction of the exogenous oxidant, ferricyanide. Both the $K_m$ and $V_{max}$ for ferricyanide are changed. The transmembrane proton movement which accompanies electron transport retains more activity in the transformed cells in relation to electron transport rate so that the $H^+/e^-$ ratio increases in transformed cells.

All plasma membranes which have been prepared show NADH-ferricyanide reductase activity (Crane *et al.*, 1979; Goldenberg, 1982), but the major problem in the study of transmembrane redox systems is to identify the electron donor. NADH appears to be the electron donor in yeast cells and carrot cells (Chalmers *et al.*, 1984; Craig and Crane, 1981; Crane *et al.*, 1982), but the electron donor remains unknown in perfused liver (Clark *et al.*, 1982). Erythrocyte plasma membrane can reduce ferricyanide using NADH but not NADPH as an electron donor (Grebing *et al.*, 1984). External ferricyanide causes a rapid decline in cytoplasmic NADPH and glutathione, as well as NADH (Arese *et al.*, 1972) in erythrocytes.

In our results we show a higher ferricyanide reduction rate and higher level of NADH in nontransformed cells (40°C) than in transformed cells (33°C). It has been demonstrated that the levels of NAD(H) (Jedeikin and Weinhouse, 1955; Sun *et al.*, 1985; Jacobson *et al.*, 1980; Rankin *et al.*, 1980) and NADH ferricyanide reductase activity (Sun *et al.*, 1985; Miyake *et al.*, 1974) are decreased after transformation. It is possible that the decrease in NADH in cells grown at 33°C may result in a decrease in $V_{max}$ for transmembrane ferricyanide reduction. The change in apparent $K_m$ for ferricyanide at the cell surface suggests a modification of the enzyme itself or some closely associated surface feature. Since increased ionic strength increases the rate of ferricyanide reduction but does not change the relative activity for transformed compared to nontransformed cells, a simple increase in negative surface potential on the transformed cells does not account for the activity change.

We have previously shown that the ferricyanide reduction by transformed cells is more sensitive to inhibition by adriamycin than the ferricyanide reduction by nontransformed cells (Sun *et al.*, 1983). The change in activity of the enzyme can be an expression of a change in the properties of the enzyme proteins or a change in some controlling protein in the membrane. The differential inhibition by adriamycin indicates a more basic change than change in substrate levels.

Results presented here show that the NADP(H) pools run in a similar pattern and in a similar range in *RLA209-15* grown at 33 and 40°C. Study of pyridine nucleotide levels in normal and transformed 3T3 cells also indicates that NADP(H) levels are much less density dependent in comparison to NAD(H) (Jacobson and Jacobson, 1976). NAD(H), therefore, may play a more important role in the control of animal cell division. Although the mechanisms that regulate the levels of NAD(H) within cells are not well understood, the levels appear to be significantly higher in nontransformed cells than in transformed cells. The lowering of the NADH pool and the increase of the oxidized form, NAD, is a unique pattern found in the transformed phenotype. It is possible that such an alteration is necessary for the processes involved in density-dependent inhibition of growth and that transformed cells contain a defect in the mechanism that normally elevates NADH. A high value of the $NAD^+$/NADH ratio has also been reported in some tumors (Jedeikin and Weinhouse, 1955).

The regulation of NAD(H) biosynthesis is potentially complex, since several alternative pathways have been described in mammalian cells (Jacobson and Jacobson, 1976). Regulation may be achieved by modulation of the activity of enzymes, such as nicotinamide phosphoribosyl transferase and/or NAD pyrophosphorylase in biosynthetic pathways, or degradative enzymes, such as NAD glycohydrolase. Further studies on these factors may aid in the elucidation of the mechanisms that regulate NAD(H) biosynthesis in transformed and nontransformed cells. The activity of the transplasma membrane dehydrogenase may contribute to control of the redox state of the pyridine nucleotide pool. The decrease in activity which is observed in transformed cells may reflect the decline in NADH within the cell so that the NADH concentration becomes rate limiting for the dehydrogenase.

## Acknowledgment

This work was supported by grants from the National Science Foundation PCM8314603 and an NIH Career Award to FLC GMK6, 21839 and NIH P01CA36761 to D. J. Morré. We wish to thank Carin Grebing for advice on pyridine nucleotide determinations.

## References

Arese, P., Bosia, A., and Pescamona, G. P. (1972). In *VI Intern. Symposium uber Structur und Function der Erythrozyten.* (Rapport, S., and Jung, F., eds.), Akademie-Verlag, Berlin, pp. 91–101.

Briggs, M. H. (1960). *Nature (London)* **187**, 249–250.

Burch, H. B., and Von Dippe, P. (1964). *J. Biol. Chem.* **239**, 1898–1899.

Caiger, P. R., Morton, K., Filsell, O. H., and Jarrett, J. G. (1962). *Biochem. J.* **85**, 351–359.

Chalmers, J. D. C., Coleman, J. O. D., and Walton, N. J. (1984). *Plant Cell Rep.* **3**, 243–246.
Cherry, J. M., MacKellar, W., Morré, D. J., Crane, F. L., Jacobson, L. B., and Schirrmacher, V. (1981). *Biochim. Biophys. Acta* **634**, 11–18.
Chou, J. Y., and Schlegel-Haueter, S. E. (1981). *J. Cell Biol.* **89**, 216–222.
Clark, J. B., Greenhaum, A. L., and McLean, P. (1966). *Biochem. J.* **98**, 546–556.
Clark, M. G., Partick, W. J., Patten, G. S., Crane, F. L., Löw, H., and Grebing, C. (1981). *Biochem. J.* **200**, 565–572.
Clark, M. G., Partick, E. J., and Crane, F. L. (1982). *Biochem. J.* **204**, 795–801.
Craig, T. A., and Crane, F. L. (1981). *Proc. Indiana Acad. Sci.* **90**, 150–155.
Crane, F. L., Goldenberg, H., Morré, D. J., and Löw, H. (1979). Dehydrogenases of the Plasma Membrane, in *Subcellular Biochemistry*, Vol. 6 (Roodyn, D. B., ed.), Plenum, New York, pp. 345–399.
Crane, F. L., Roberts, H., Linnane, A. W., and Löw, H. (1982). *J. Bioenerg. Biomembr.* **14**, 191–205.
Crane, F. L., Sun, I. L., Morré, D. J., and Löw, H. (1985a). *J. Cell Biol.* **101**, 483a.
Crane, F. L., Sun, I. L., Clark, M. G., Grebing, C., and Löw, H. (1985b). *Biochim. Biophys. Acta* **811**, 233–264.
Dixon, M., and Webb, E. C. (1979). Enzyme Kinetics. *Enzymes*, 3rd edn., Academic Press, New York, pp. 386–391.
Ellem, K. A. O., and Kay, G. F. (1983). *Biochem. Biophys. Res. Communs.* **112**, 185–190.
Ferris, G. M., and Clark, J. B. (1972). *Biochem. J.* **128**, 869–877.
Frelin, C., Vigne, P., and Lazounski, M. (1983). *J. Biol. Chem.* **258**, 6272–6276.
Gerson, D. F., Kiefer, H., and Eufe, W. (1982). *Science* **216**, 1009–1010.
Grebing, C., Crane, F. L., Löw, H., and Hall, K. (1984). *J. Bioenerg. Biomembr.* **16**, 517–532.
Goldenberg, H. (1982). *Biochim. Biophys. Acta* **694**, 203–223.
Hakamori, S. I. (1975). *Biochim. Biophys. Acta* **417**, 55–89.
Jacobson, E. L., and Jacobson, M. K. (1976). *Arch. Biochem. Biophys.* **175**, 627–634.
Jacobson, M. K., Levi, V., Juarez-Salinas, H., Barton, R. A., and Jacobson, E. L. (1980). *Cancer Res.* **40**, 1797–1802.
Jedeikin, E. L., and Weinhouse, S. (1955). *J. Biol. Chem.* **213**, 271–280.
Matsumara, M., and Miyachi, S. (1980). *Methods Enzymol.* **69**, 465–470.
Miyake, Y., Gaylor, J. L., and Morris, H. P. (1974). *J. Biol. Chem.* **249**, 1980–1987.
Moolenaar, W. H., Tsien, R. Y., Van der Saag, P. T., and De Loat, S. W. (1983). *Nature (London)* **304**, 645–648.
Nemeth, A. M., and Dickerman, H. (1960). *J. Biol. Chem.* **235**, 1761–1764.
Pethig, R., Gascoyne, P. R. C., McLaughlin, J. A., and Szent-Györgyi, A. (1984). *Proc. Nat. Acad. Sci. USA* **81**, 2088–2091.
Rankin, P. W., Jacobson, M. K., Mitchell, J. R., and Busbee, D. B. (1980). *Cencer Res.* **40**, 1803–1807.
Scheffer, I. E., Defrancisco, L., Ditta, K., Soderberg, G., Breen, A. M., and Mascarello, T. (1981). In *Biochemical and Clinical Aspects of Coenzyme Q*, Vol. 3 (Fokers, K., and Yamamura, K., eds.), Elsevier, Amsterdam, pp. 245–255.
Schwartz, J. P., Passonneau, J. V., Johnson, G. S., and Pastan, I. (1974). *J. Biol. Chem.* **249**, 4138–4143.
Scheinin, R., and Onodera, K. (1972). *Biochim. Biophys. Acta* **272**, 49–63.
Sun, I. L., and Crane, F. L. (1981). *Biochem. Biophys. Res. Commun.* **101**, 299–306.
Sun, I. L., and Crane, F. L. (1984a). *Biochem. Int.* **9**, 299–306.
Sun, I. L., and Crane, F. L. (1984b). *Biochem. Pharmacol.* **34**, 617–622.
Sun, I. L., and Crane, F. L. (1984c). *Proc. Indiana Acad. Sci.* **93**, 267–274.
Sun, I. L., Crane, F. L., Chou, J. Y., Löw, H., and Grebing, C. (1983). *Biochem. Biophys. Res. Commun.* **116**, 210–216.
Sun, I. L., Crane, F. L., Grebing, C., and Löw, H. (1984a). *Exp. Cell Res.* **156**, 528–536.
Sun, I. L., Crane, F. L., Löw, H., and Grebing, C. (1984b). *Biochem. Biophys. Res. Commun.* **125**, 649–654.

Sun, I. L., Crane, F. L., Grebing, C., and Löw, H. (1984c). *J. Bioenerg. Biomembr.* **16**, 583–595.
Sun, I., MacKellar, W., Crane, F. L., Barr, R., Elliot, W. L., Varnold, R. L., Heinstein, P. F., and Mooré, D. J. (1985). *Cancer Res.* **45**, 157–163.
Warburg, G. (1977). *N. Engl. J. Med.* **296**, 486–493, 541–551.