appear that hydrostatic pressure itself is at least not the only cause of the increased oxygen affinity, if indeed it is a cause it all. Hyperbaric gases themselves seem to interfere with functions of the hemoglobin molecule to an extent that depends on their concentration and on their chemical nature.

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References and Notes

1. Oxygen dissociation curves were determined with the system directly under pressure in a 1-cm optical pressure cell of 2.7-ml total volume, attached to a dual-wavelength spectrophotometer [L. A. Kiesow, J. W. Bless, D. P. Nelson, J. B. Shelton, *Clin. Chim. Acta* **41**, 123 (1972)]. Since this method involves, at least for the purpose of titration, only a single, liquid phase, it could be adapted easily in pressure applications. The method utilizes the biochemical generation of O_2 from an H_2O_2 titrant solution by catalase which is added to a dilute suspension of blood in Krebs-Ringer phosphate solution, *p*H 7.1, at 37°C, with 200 mg of glucose added per 100 ml. Before being transferred into the pressure cell, the blood suspension is deoxygenated with the respective inert gas. After pressurization, oxygen is then generated in the stirred blood suspension by the stepwise addition of small volumes (about 3 μl) of approximately 0.02*M* H_2O_2 from a pressurized micrometer syringe that is also part of the optical pressure cell. During this stepwise generation of O_2 from H_2O_2, the change in the difference of optical absorbance of the blood suspension at 439 and 448 nm is constantly recorded by the dual-wavelength spectrophotometer. For each H_2O_2 addition, and therefore for each O_2 generation, an equilibrium absorbance value is thus obtained which represents the concentration of oxyhemoglobin formed in the red blood cells at this particular oxygen partial pressure. Although the system contains both a gas and a liquid phase, it can be considered as a single phase for the distribution of the generated oxygen. This is possible since the liquid phase is separated from the gas phase by approximately 70 mm of a liquid-filled capillary tubing. This tubing allows only the diffusion of gases, and its content does not participate in the constant mixing process and in the distribution of the generated oxygen for which it forms a diffusion barrier. Further details of this adaptation of the method for determining O_2 dissociation curves in pressure applications are being prepared for publication by the authors.
2. Reversible changes of tertiary or quaternary protein structures could occur with hyperbaric gases and could involve the intracellular hemoglobin molecule. However, these changes are also considered to be encompassed by the term reversible chemical equilibria.
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5. The 2,3-DPG content of fresh, normal human RBC's has been determined as 4.8 ± 0.3 m*M* [L. A. Kiesow and J. W. Bless, *Anal. Biochem.* **51**, 91 (1973)].
6. Experiments by F. Johnson and F. Schlegel [*J. Cell. Comp. Physiol.* **31**, 421 (1948)] have shown that the oxygenation equilibria of dilute hemoglobin solutions are not affected by hydrostatic pressure per se up to 680 atm.
7. The effect of hydrostatic pressure alone could not be studied, since the available equipment requires the remote presence of a gaseous phase. This phase absorbs by compression the slight increase in liquid volume which results from the introduction of the oxygen-generating titrant into the pressure cell.

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SV40 Virus Transformation of Mouse 3T3 Cells Does Not Specifically Enhance Sugar Transport

Abstract. *The apparent enhancement of 2-deoxy-D-glucose uptake by mouse 3T3 cells accompanying transformation by SV40 virus is not due primarily to an effect on the transport process but to enhanced phosphorylation of the sugar by intracellular kinases. Moreover, the effect is not specifically a function of the presence of the viral genome, but is a reflection of the overall growth rate and physiological state of the cell.*

Early comparative studies of normal and tumor cells revealed that tumor cells exhibited increased anaerobic and aerobic glycolysis (*1*). More recent studies of animal cells transformed by various tumor viruses have shown that profound changes in the nature of the cell surface occur during malignant transformation; these changes result in loss of contact inhibition (*2*), appearance of specific antigens (*3*), differences in agglutinability by certain plant lectins (*4*), and differences in cell membrane glycoproteins and glycolipids (*5*). Within the last 2 years, a number of reports have appeared which may indicate that enhanced utilization of glucose by transformed cells is also related to changes in the cell membrane—striking increases in the rate of sugar transport have been reported in cells transformed by a number of tumor viruses. Changes in the rate of sugar uptake have been reported in mouse (*6*, *7*) and rat (*8*) cells transformed by murine sarcoma viruses, in chick embryo cells (*9*) transformed by Rous sarcoma virus, in baby hamster kidney cells (*10*) transformed by polyoma virus, and in mouse cells (3T3) transformed by SV40 virus (*10*).

A number of these studies were carried out with metabolizable sugars such as glucose, galactose, glucosamine, or mannose (*6*, *8*, *9*) and did not separate sugar transport from the total process of sugar uptake and metabolism. Other experiments carried out with the nonmetabolizable analog 2-deoxy-D-glucose (2-dOG) (*7–11*) are more reliable, since

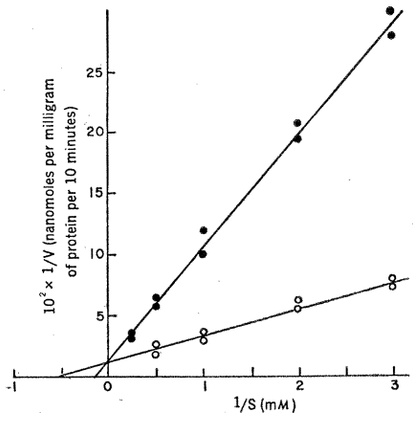

Fig. 1. Lineweaver-Burk plot of the inhibition of 2-deoxy-D-glucose uptake by D-glucose. Cover slips with adherent SV-3T3 cells were rinsed four times with glucose-free Hanks solution at 37°C and incubated with uniformly labeled 2-deoxy-D-[^{14}C]glucose (0.5 μc/μmole) (International Chemical and Nuclear Corp.) at the concentrations indicated for 10 minutes at 37°C, in the absence (○) and presence (●) of 7 m*M* D-glucose. Cover slips were then washed through four successive changes of ice-cold glucose-free Hanks solution, drained, and dropped into vials containing 15 ml of Bray's scintillation fluid (*18*) and 0.5 ml of distilled H_2O for counting. $1/V$, Reciprocal velocity; $1/S$, reciprocal substrate concentation.

Table 1. Characteristics of cell lines used. Cover slips with adherent cells were incubated in growth medium containing 200 μc of [^{3}H]thymidine ([^{3}H]TdR) (New England Nuclear) for 45 minutes, washed four times in ice-cold glucose-free Hanks solution, and placed in petri dishes containing ice-cold 10 percent trichloroacetic acid (TCA) for 1 hour. The cover slips were then washed three times in cold 10 percent TCA, three times in 95 percent ethanol, and dried; the TCA-precipitable radioactivity was determined by liquid scintillation counting as described in the legend of Fig. 1. Incorporation of [^{3}H]thymidine into acid-precipitable material was measured for duplicate cultures grown on cover slips; protein determinations were performed in quadruplicate on parallel cover slip–grown cultures.

Cell type	Virus genome	Density-dependent growth	Incorporation of [^{3}H]TdR (count/min)	Protein (mg)	[^{3}H]TdR (count/min per milligram of protein)
3T3	Absent	Yes	2,000	0.079	25,430
3T6	Absent	No	9,030	.111	81,300
SV-3T3	Present	No	14,070	.118	119,200
SV-3T3-FL	Present	Yes	4,670	.104	44,900

this sugar is not incorporated into cellular material. However, it can be phosphorylated to form 2-deoxy-D-glucose-6-phosphate (2-dOG-P) by the action of animal cell kinases (*12*). This can lead to misinterpretation, since phosphorylation of the sugar yields an anionic product that will not exchange with extracellular 2-dOG; an intracellular trapping is provided thereby, which would drive the equilibrium toward the phosphorylated product. Thus, an increase in the level or activity of intracellular hexokinase (E.C. 2.7.1.1) or glucokinase (E.C. 2.7.1.2) could bring about an apparent increase in both rate and final amount of accumulated 2-dOG, if only total intracellular radioactivity is measured. Therefore, we measured the intracellular pools of both the free ^{14}C-labeled 2-dOG and phosphorylated ^{14}C-labeled 2-dOG-P sugar in each of four lines of mouse cells.

Our second objective was to determine whether the apparent increased rate of sugar uptake in transformed cells is specifically mediated by the presence of a transforming virus genome. We used four lines of mouse embryo fibroblasts whose designations, growth characteristics, and virus-carrier state are outlined in Table 1. The SV-3T3-FL cells are a so-called flat revertant mutant of SV40 virus–transformed 3T3 cells that have regained the property of density-dependent growth inhibition while retaining the SV40 genome in an integrated state (*13*). All experiments were carried out with cells grown to confluency on cover slips (*14*). To determine the extent of density-dependent growth inhibition in each experiment, incorporation of [^{3}H]thymidine into acid-precipitable material was determined as a reflection of DNA synthetic activity. The data presented in Table 1 are typical.

The Lineweaver-Burk plots in Fig. 1 establish that D-glucose inhibits the uptake of 2-dOG by SV-3T3 cells in a simple competitive manner, indicating that these sugars are transported by a common stereospecific carrier. Moreover, the apparent inhibitor constant (K_i) for glucose inhibition calculated from these data is 2.3 m*M* (*15*), a value close to the apparent Michaelis constant (K_m) for 2-dOG uptake of 2 m*M*. Thus, both sugars appear to have in addition a nearly equal affinity for the transport system. In agreement with the results of Isselbacher (*11*), we found that 3T3 cells had the same apparent K_m for 2-dOG transport as SV-3T3 cells, but the latter exhibited a higher apparent maximum velocity.

The rate of appearance of total intracellular 2-dOG is greater in the cell lines that do not show density-dependent growth (SV-3T3 and 3T6) (see Fig. 2). Total intracellular 2-dOG in the SV-3T3-FL cells is significantly less than in either SV-3T3 or 3T6, and only slightly more than that in 3T3. Thus, it appears that the rate of total uptake of 2-dOG is not specifically a function of the presence of the SV40 viral genome, but rather is related to the growth rate, as measured by incorporation of [^{3}H]thymidine (Table 1).

Moreover, separation of the intracellular pool into free 2-dOG and 2-

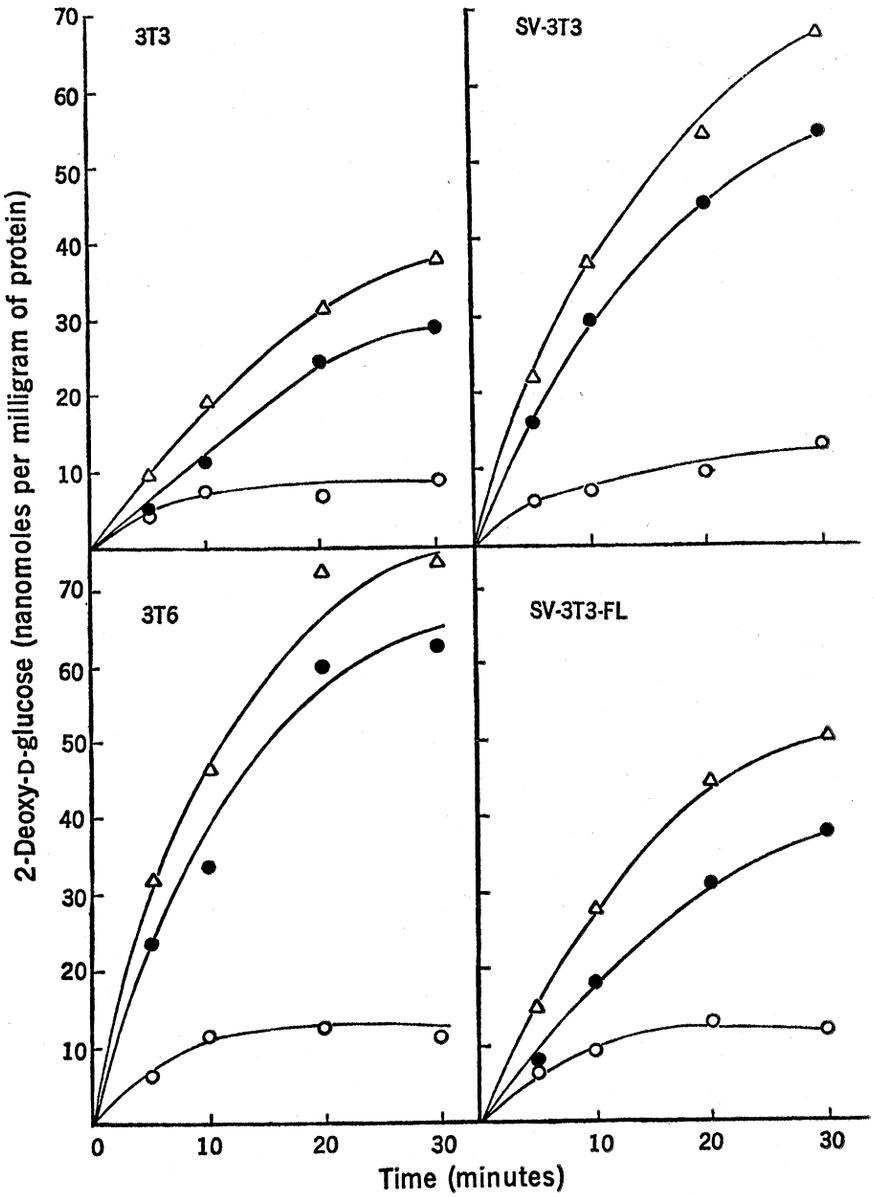

Fig. 2. Uptake and phosphorylation of 2-deoxy-D-glucose. Cover slips with adherent cells were washed as described in the legend of Fig. 1, incubated in 2 m*M*, uniformly labeled 2-deoxy-D-[^{14}C]glucose (0.5 μc/μmole) at 37°C, and removed at appropriate time intervals. After being rinsed four times in ice-cold glucose-free Hanks solution, cover slips were immediately immersed in 1 ml of boiling water for 1 minute to extract intracellular sugar pools. Free 2-deoxy-D-glucose and 2-deoxy-D-glucose phosphate were separated by chromatographing 0.5-ml samples of extract on columns (4 by 0.6 cm) of Bio-Rad AG1-x2 anion exchange resin (100 to 200 mesh) in the formate form (*19*). Free sugars were eluted with five 0.5-ml portions of distilled water; sugar phosphates were subsequently eluted with seven 0.5-ml portions of 0.5*M* ammonium formate in 0.2*M* formic acid. The eluted materials were collected directly in Bray's scintillation fluid (*18*) for radioassay (△, total; ○, free 2-dOG; and ●, 2-dOG-P).

dOG-P components reveals that both the rate of uptake and final steady-state concentration of free 2-dOG are essentially the same in all four cell types. The increased total intracellular 2-dOG in SV-3T3 and 3T6 is accounted for entirely by increased amounts of the phosphorylated sugar (2-dOG-P) in these density-independent cell types.

The final steady-state concentration of free 2-dOG in all cases was approximately 11 nmole per milligram of cell protein. If one assumes that protein accounts for 60 percent of the total dry weight of the cells, that the cell is 80 percent water, and that all the cell water is available to external solute, this would represent an intracellular concentration of 1.7 m*M* 2-dOG, a value very close to the external concentration.

Since mammalian cells other than kidney and intestinal epithelial cells transport sugars by a facilitated diffusion process that yields a final equilibrium distribution of substrate and does not involve concentrative uptake against a gradient (*16*), we take the rate of appearance of the unaltered free 2-dOG molecule in the intracellular pool to be a measure of the transport process. Thus we suggest that the increased total uptake of 2-dOG measured in SV-3T3 and 3T6 cells is not a result of enhanced transport of the sugar, but a consequence of increased phosphorylation by intracellular kinases subsequent to transport. This effect is not specifically a function of SV40 virus transformation, since it is absent in the flat revertant (SV-3T3-FL) that retains the viral genome, and it is present in 3T6 cells that contain no DNA tumor virus. We postulate that the initial phosphorylation of sugar may be one of the series of metabolic reactions that is modulated in accord with growth rate, which has been referred to as the "pleiotypic response" (*17*) and which may become perturbed in a number of ways, including virus transformation.

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15. Apparent K_i was calculated from the formula

$$K_i = \frac{I}{\frac{K_p}{K_m} - 1}$$

where I is the concentration of inhibitor, and K_p and K_m are values read graphically as the intersection of the lines with the abscissa in the presence and absence of inhibitor, respectively.
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20. We thank Dr. R. Pollack for cultures of SV-3T3 and SV-3T3-FL, Dr. H. Green for cultures of 3T3 and 3T6 cells, and M. McCloud and M. A. Brustalon for technical assistance. Supported by NSF grant GB 31543, Damon Runyon Memorial grant DRG-1191, NIH grant AI-10096, and the University of Connecticut Research Foundation. C.C. is the recipient of a research career development award from the National Institute of Allergy and Infectious Diseases.

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Putamen: Activity of Single Units during Slow and Rapid Arm Movements

Abstract. *The activity of putamen neurons was studied in a monkey during the performance of both slow and rapid arm movements. More than half of all movement-related units discharged preferentially in relation to slow movements and less than 10 percent in relation to rapid movements. These findings indicate that at least a portion of the basal ganglia (the putamen) is primarily involved in the control of slow movements and are consistent with the hypothesis of Kornhuber that the primary motor function of the basal ganglia is to generate slow ("ramp") rather than rapid ("ballistic") movements.*

Clinicopathologic studies in man indicate that the basal ganglia play an important role in the control of movement and posture (*1*). Kornhuber (*2*) has recently interpreted clinical and experimental evidence to indicate that the primary motor function of the basal ganglia is to generate slow ("ramp") movements, whereas the function of the cerebellum is to preprogram and initiate rapid ("ballistic") movements. If this view is correct, one might predict that the activity of basal ganglia neurons would be preferentially related to slow rather than rapid movements. To test this hypothesis, the activity of neurons in the putamen (a prominent portion of the basal ganglia) was recorded in a monkey during the performance of a motor task involving both slow and rapid arm movements.

For these studies a monkey (*Macaca mulatta*) was trained to reach out and grasp a lever and to execute slow and rapid (pushing and pulling) arm movements between two zones 1 cm wide and 5 cm apart. To begin the task, the animal had to move the lever into the starting zone and hold there for an unpredictable period of 2 to 6 seconds. A white lamp indicated to the monkey that the lever was within the correct zone. Then, when a green lamp came on, the animal had to make a slow (ramp) movement into the opposite zone. The movement time between the zones had to be greater than 0.7 second but less than 1.0 second. After executing a slow movement, the animal held again for 2 to 6 seconds within the opposite zone. Now, when a red lamp came on, the monkey had to move the lever as rapidly as possible (ballistic movement) back to the original starting zone.

The ballistic movements were arrested by impact against mechanical stops 1 cm beyond each zone. A juice reward was delivered after each rapid movement if the movement time between the zones was less than 140 msec. After the rapid movement, the animal again positioned the lever within the starting zone and the sequence of slow and rapid movements was repeated. By changing the starting zone, it was possible to have the animal make both slow and rapid movements in each direction,