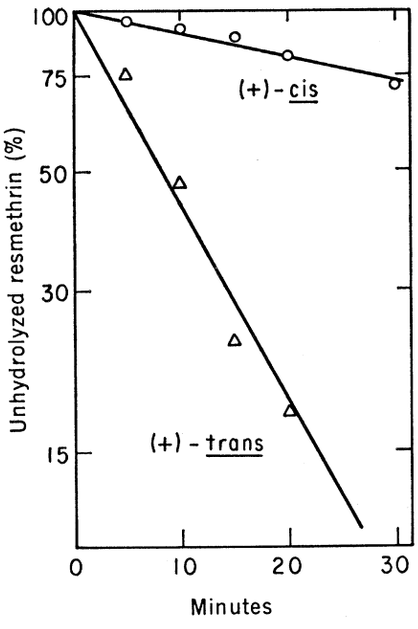

Fig. 1. Specificity of esterases for hydrolysis of (+)-*trans*-resmethrin (bioresmethrin) and (+)-*cis*-resmethrin. Acetone powder preparations of mouse hepatic microsomes were used at a concentration of 17.8 mg of protein per reaction.

tributyl phosphorotrithioate (DEF) at a dose of 50 mg/kg. In mice DEF increases the toxicity of malathion by inhibiting the malathion-detoxifying esterases (*6*); DEF also increases the acute toxicity to mice of compounds **2** and **4** by 3- to 5-fold and of compound **3** by more than 188-fold, but it does not alter the toxicity of bioresmethrin. When hepatic microsomes from mice given preliminary treatment with DEF are assayed without NADPH fortification, their activity relative to microsomes from untreated controls is inhibited by 95 percent for bioresmethrin hydrolysis and by 86 percent for hydrolysis of the (+)-*trans*-ethano derivative. The increased toxicity of the ethano derivative to the treated mice may result from inhibition of detoxifying esterases, but the toxicity of bioresmethrin is not increased even with more complete esterase inhibition; therefore, some other factor or factors must also contribute to the low mammalian toxicity of bioresmethrin.

Possibly, a portion of the detoxifying action involves oxidase attack in addition to esterase cleavage occurring in different degrees on bioresmethrin and the ethano derivative. Neither piperonyl butoxide nor sesamex, known inhibitors of the microsomal oxidases (*7*), alters the susceptibility of mice to poisoning by bioresmethrin or the ethano derivative when the synergists are administered intraperitoneally at 150 to 170 mg/kg 30 minutes prior to the pyrethroid. This finding indicates that oxidation of these pyrethroids is either not important in their detoxification or that it is not the only detoxifying mechanism. These alternative hypotheses were examined by enzyme studies.

When fresh microsomes from untreated mice are used, the rate of metabolism of bioresmethrin is increased by 1.3-fold on addition of NADPH but that of the ethano analog is not affected. It appears that the cyclopentylidenemethyl moiety of the ethano derivative is not as readily oxidized as the *trans*-methyl group of the isobutenyl moiety; therefore, the hepatic microsomes of normal mice metabolize the ethanochrysanthemate almost entirely by hydrolysis rather than by the combined esterase and oxidase action occurring with bioresmethrin. When fresh microsomes from DEF-treated mice are used and NADPH is added, the rate of bioresmethrin metabolism is 4.7-times greater than that of the ethano derivative. Thus, it is probable that in DEF-treated mice the enzymes in the fresh microsomes remaining active in bioresmethrin metabolism are largely oxidases while with the ethano derivative they are largely esterases. Therefore, the low mammalian toxicity of bioresmethrin appears to result from the combined attack of esterases and oxidases that lead to rapid detoxification even when the activity of one or the other of the two enzyme systems is inhibited.

The esterase hydrolyzing bioresmethrin are not restricted to liver microsomes. They are also found in other subcellular fractions of mouse liver, and other mouse tissues including the brain, and in certain insect homogenates (*8*).

An understanding of the enzymatic mechanisms for pyrethroid detoxification is important for the continuing use of these extremely effective insecticide chemicals and for the development of even more potent and selective materials. The naturally occurring form of chrysanthemic acid, the (+)-*trans* isomer, combined with suitable primary alcohols yields highly insecticidal esters that are of low mammalian toxicity (*1*) in part because of the ease of hydrolysis of these pyrethroids by mammalian liver esterases.

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Oxygen Dissociation in Human Erythrocytes: Its Response to Hyperbaric Environments

Abstract. *Hyperbaric nitrogen at a pressure of 100 atmospheres increases significantly the affinity of erythrocytic hemoglobin for oxygen. This results in oxygen dissociation curves which are shifted to lower than normal oxygen partial pressures. The pressure effect is completely reversible and can therefore be observed only during exposure of the blood cells to the hyperbaric gas.*

The ability of red blood cells (RBC's) to bind and to release oxygen is probably best described by their oxygen dissociation curves. If such curves are determined in freshly collected human RBC's while they are exposed to an elevated nitrogen pressure, an increase in the affinity of erythrocytic hemo-

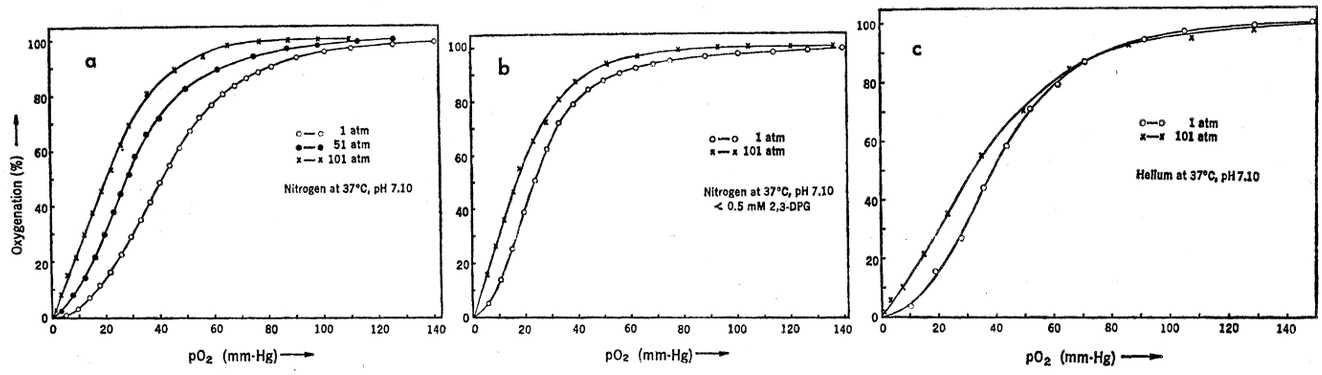

Fig. 1. Effects of hyperbaric gases on the oxygen dissociation of red blood cells. Blood samples are diluted by Krebs-Ringer phosphate solution with glucose added (*p*H 7.1), then deoxygenated and transferred into an optical pressure cell for determination of the oxygen dissociation curve under gas pressure at 37°C. (a) Oxygen dissociation curves of fresh human RBC's at 1, 51, and 101 atm of nitrogen. (b) Oxygen dissociation curves of stored human RBC's with 2,3-DPG concentrations below 0.5 m*M* at 1 and 101 atm of nitrogen. (c) The effect of 1 and 101 atm of helium on the oxygen dissociation curve of fresh human RBC's; this shows the reduced pressure effect of helium compared with nitrogen.

globin for oxygen can be observed (Fig. 1a). Only recently have such measurements become feasible due to the development of a new method for the determination of oxygen dissociation curves of RBC suspensions by spectrophotometric titration (*1*). When assayed with the system under pressure, oxygen dissociation curves are observed which reflect a marked change in oxygen affinity—that is, a higher affinity of hemoglobin for oxygen—compared to curves obtained with the same blood sample and method at ambient pressure. In fact, the increase in oxygen affinity of the RBC hemoglobin, when measured at 100 atm of nitrogen and 37°C (Fig. 1a), is so pronounced that the resulting oxygen dissociation curve is somewhat similar in position and shape to the hyperbolic curve obtained with a solution of myoglobin or monomeric hemoglobin at ambient pressure under otherwise identical conditions. As is to be expected, an intermediate nitrogen pressure of 50 atm also produces a shift of the oxygen dissociation curve to lower oxygen partial pressures. Yet, even under these conditions, the observed increase in hemoglobin-oxygen affinity, although quantitatively smaller, remains significant (Fig. 1a). From additional data, not shown in Fig. 1a, it is evident that a linear increase in the nitrogen partial pressure results in a nonlinear increase of hemoglobin-oxygen affinity. In fact, this affinity seems to approach a maximum value which cannot be far from that observed at 100 atm of nitrogen and 37°C. Although strongly suggested by the experimental data, this hypothesis could not be tested directly, since the available equipment was limited to a maximum pressure of 100 atm.

Oxygen dissociation curves of RBC suspensions before and after compression to 100 atm of nitrogen for 20 minutes were also compared. This comparison shows that the change in hemoglobin-oxygen affinity occurs only under pressure and that the effect is fully reversible on decompression. This would suggest that the phenomenon stems from the response of chemical equilibria to pressure changes and not from irreversible cellular damage (*2*). These equilibrium changes are then probably responsible for the observed pressure dependency of the oxygen affinity. Indeed, examination by scanning electron microscopy of RBC's exposed to and fixed under 100 atm of nitrogen does not reveal any gross changes in the structure of these cells, nor do they show any hemolysis during the exposure to hyperbaric gas.

Red blood cells are known to undergo a significant modification of their oxygen affinity during storage. Therefore, the effects of pressure on human erythrocytes obtained from recently outdated acid citrate dextrose blood units were examined. As shown in Fig. 1b, the effect of 100 atm of nitrogen on the oxygen affinity of the hemoglobin of stored RBC's is greatly reduced compared to that observed with fresh cell preparations. The oxygen dissociation curve obtained with stored RBC's at atmospheric pressure shows the well-known shift to lower values of the partial pressure of oxygen. This shift has been attributed to the decrease in the 2,3-diphosphoglycerate (2,3-DPG) content of RBC's which occurs during storage (*3*). It has been shown that the binding of 2,3-DPG to hemoglobin decreases its affinity for oxygen and shifts the binding curve to higher pO_2 values (*4*). Conversely, a decrease in the 2,3-DPG content of RBC's shifts the binding curve to lower pO_2 values. We determined the concentration of 2,3-DPG in the aged RBC's to be less than one-tenth of that in fresh cells (*5*). It is, therefore, reasonable to assume that the observed reduction of the effect of pressure on the oxygen affinity of hemoglobin in aged RBC's may be mediated by 2,3-DPG. In other words, the oxygenation equilibrium responds to nitrogen pressure only if 2,3-DPG is available for binding by hemoglobin, or inversely, an oxygen affinity already increased due to a lack of 2,3-DPG shows a proportionately reduced additional increase due to the hyperbaric gas (*6*).

There are at least three different ways by which hyperbaric nitrogen could cause the observed effects on the oxygen dissociation in erythrocytes. The effect could be the result of the hydrostatic pressure (*7*) associated with the use of hyperbaric nitrogen; it could be the result of molecular nitrogen gas itself; or it could be the result of a trace contaminant in the nitrogen gas, although it was of the highest purity available. Figure 1c shows that hyperbaric helium also produces shifts in the RBC oxygen dissociation curve to lower oxygen partial pressures. However, the increase in the affinity of hemoglobin for oxygen caused by 100 atm of helium gas is quantitatively smaller than the increase produced by an identical pressure of nitrogen. Therefore, it would

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.

30 October 1972; revised 1 January 1973

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

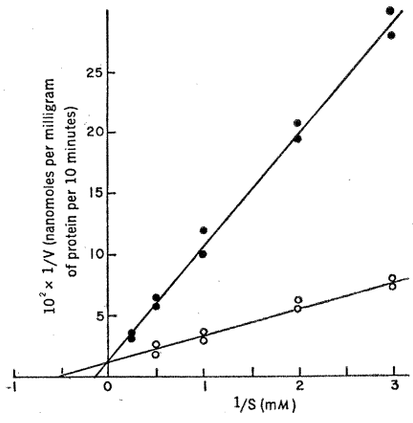

Fig. 1. Lineweaver-Burk plot of the inhibition of 2-deoxy-D-glucose uptake by D-glucose. Cover slips with adherent SV-3T3 cells were rinsed four times with glucose-free Hanks solution at 37°C and incubated with uniformly labeled 2-deoxy-D-[^{14}C]glucose (0.5 μc/μmole) (International Chemical and Nuclear 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