AD-A148 889 THE EFFECT OF INERT GASES ON ION TRANSPORT ACROSS THE ERYTHROCYTE MEMBRANE(U) NEW MEXICO UNIV ALBUQUERQUE SCHOOL OF MEDICINE W R GALEY 18 DEC 84 1/1

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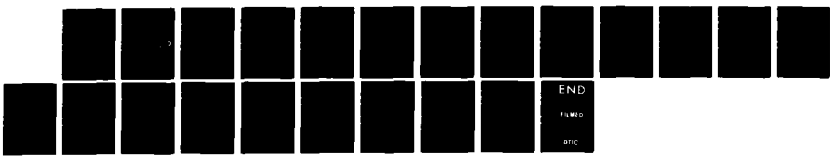

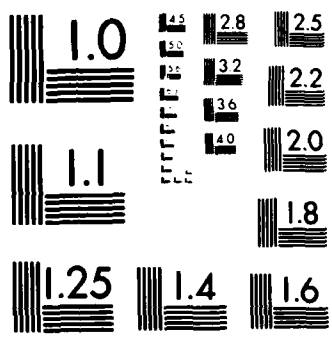

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Under this contract we have made several major important observations which bear directly on our proposed goals.

First, we have shown that hyperbaric argon stimulates active transport of sodium and potassium in the human erythrocyte. These increases in active uptake of potassium and active extrusion of sodium from the cell are related to the partial pressure of the ENG.

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Second, we have shown that moderate hydrostatic pressures (as exerted through helium, an ENNG) depress active transport in these same cells. This depression is seen under hyperbaric pressures of argon-helium mixtures to exert an effect proportional to the pressure exerted.

These first two observations indicate first, that there are effects of an elemental narcotic gas (ENG) on ion transport across cell membranes and second, that these effects are antagonized by hydrostatic pressure. This antagonism then parallels the observed actions of pressure to antagonize (reverse) inert-gas-narcosis. It should be remembered that these effects are being seen at pressures which correspond to those encountered in physically possibly submarine descents.

The third major observation is that nitrogen, a less potent ENG than argon, increases active transport across cell membranes but to a lesser degree. This shows again the parallel between the behavioral actions of hyperbaric ENGs and their effects on cell membrane ion transport.

The fourth observation is that although active transport is stimulated by ENGs and inhibited by pressure, the ability of Na^{-K}, ATPase (the transport enzyme) of cell membranes to hydrolize ATP is not affected by these conditions. This suggests that the effects of pressure and ENGs may be on the stochiomestry or efficiency of the pump rather than on its activity per se.

Fifth, our studies have indicated that hydrostatic pressure and ENNG (Helium) exerted gas pressure are analagous in that they both inhibit potassium uptake by erythrocytes and synaptosomes.

We have also shown that the effects of pressure and hyperbaric ENG (argon) are the same on the uptake of ^{42}K by rat brain synaptosomes and human red cells. This supports the use of the human red cells as a model of the much more difficult to work with, nervous tissue.

In summary we have shown that elemental narcotic gases and pressure have effects on cell membrane transport consistent with their antagonistic actions in vivo.

Final Technical Report on The Effect of Inert Gases on Ion Transport Across the Erythrocyte Membrane

Contract No. N00014-76-C-0813

Work Unit No. NR 201-120

Principal Investigator: William R. Galey, Ph.D.
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Report Date: December 18, 1984

Statement of Research Goals

Although numerous elemental gases and organic compounds act as general anesthetic agents, very few if any of the mechanisms by which these agents act are known at the cellular or subcellular level. Through studies of the effects of elemental narcotic gases (ENGs) and elemental non-narcotic gases (ENNGs) on cellular membrane structure and function have attempted to elucidate the molecular basis of elemental gas narcosis. Particular emphasis has been placed on defining the effects of these gases on cell membrane transport of the cations Na^+ and K^+ since they are intimately involved in the cellular excitability process known to be perturbed by narcotic agents.

In order to elicit the anesthetic effects of most elemental narcotic gases it is necessary to present the gases at partial pressures exceeding one atmosphere. This requirement presents an interesting situation since not only is pressure required to elicit the anesthetic perturbations of ENGs it is well established that pressure antagonizes anesthesia and narcosis. Furthermore, pressure alone can create in man and animals a hyperexcitability state known as HPNS. We have consequently used pressure as a tool to study the mechanisms of elemental gas narcosis and its reversal and investigate how these mechanisms relate to the etiology of HPNS.

Summary of the Major Accomplishments

Under this contract we have made several major important observations which bear directly on our proposed goals.

First, we have shown that hyperbaric argon stimulates active transport of sodium and potassium in the human erythrocyte. These increases in active uptake of potassium and active extrusion of sodium from the cell are related to the partial pressure of the ENG.

Second, we have shown that moderate hydrostatic pressures (as exerted through helium, an ENNG) depress active transport in these same cells. This depression is seen under hyperbaric pressures of argon-helium mixtures to exert an effect proportional to the pressure exerted.

These first two observations indicate first, that there are effects of an elemental narcotic gas (ENG) on ion transport across cell membranes and second, that these effects are antagonized by hydrostatic pressure. This antagonism then parallels the observed actions of pressure to antagonize (reverse) inert-gas-narcosis. It should be remembered that these effects are being seen at pressures which correspond to those encountered in physically possible submarine descents.

The third major observation is that nitrogen, a less potent ENG than argon, increases active transport across cell membranes

but to a lesser degree. This shows again the parallel between the behavioral actions of hyperbaric ENGs and their effects on cell membrane ion transport.

The fourth observation is that although active transport is stimulated by ENGs and inhibited by pressure, the ability of Na-K, ATPase (the transport enzyme) of cell membranes to hydrolize ATP is not effected by these conditions. This suggests that the effects of pressure and ENGs may be on the stochiometry or efficiency of the pump rather than on its activity per se.

Fifth, our studies have indicated that hydrostatic pressure and ENNG (Helium) exerted gas pressure are analagous in that they both inhibit potassium uptake by erythrocytes and synaptosomes.

We have also shown that the effects of pressure and hyperbaric ENG (argon) are the same on the uptake of ^{42}K by rat brain synaptosomes and human red cells. This supports the use of the human red cells as a model of the much more difficult to work with, nervous tissue.

In summary we have shown that elemental narcotic gases and pressure have effects on cell membrane transport consistent with their antagonistic actions in vivo.

Red Cell Studies

Our studies on red cells have involved measuring unidirectional fluxes of sodium and/or potassium across the red cell membrane while these cells were subjected to atmospheric pressures ranging from 1 ATA to 90 ATA. To measure these fluxes we have determined the kinetics of loss or gain of the tracer isotopes ^{24}Na and ^{42}K.

In initial studies we determined the fluxes of Na and K across the red cell membranes in a ATA air, 68 ATA Helium, and 68 ATA Argon. Since helium has been shown to have little or no anesthetic effect at pressures even above 68 ATA, we have assumed pressures of helium to represent pure hydrostatic pressure. On the other hand hyperbaric pressures of argon represent not only the hydrostatic pressure exerted by the gas, but also the equivalent partial pressure of an elemental gas that is known to be very narcotic (an ENG). Finally fluxes at 1 ATA of air serve as a baseline to which one can compare fluxes obtained in the hyperbaric environments.

These studies have shown that the passive fluxes of Na and K (i.e., Na influx and K efflux and ouabain inhibited Na efflux and K influx) are unaffected by hydrostatic pressures of a hyperbaric ENG as represented by argon. Figure 1 depicts the effects of 68 ATA of pressure for an ENG on active transport across the red blood cell membrane. As is apparent, hydrostatic pressure significantly depresses the active influx of K and active efflux of Na from the red blood cell when compared to the transport

occurring at 1 ATA of air. Conversely the same pressure of an ENG, argon, significantly increases active transport when compared to the same pressure of the non-anesthetizing ENNG, helium.

By treating the red cells with the drug ouabain which is known to be a specific poison of the Na and K active transport pump we have shown that the effects of the ENG and hydrostatic pressures are indeed limited to the active fluxes of Na and K since the observed changes are blocked by 10^{-4} M concentrations of the drug. Thus, it appears that the effect of hydrostatic pressure is to inhibit active transport while the effect of a narcotic gas is to increase active transport.

Having established these phenomena we were anxious to determine whether pressure of an ENNG or an ENG acted directly through stimulation or inhibition of the Na, K, ATPase within the RBC membrane. Consequently, we prepared ghosts of red blood cells and compared the ouabain inhibitable Na, K, ATPase activity of these ghosts under: 1) 68 ATA of (helium) hydrostatic presure, 2) 68 ATA of argon and, 3) 1 ATA of air. As is seen in Figure 2 there are no statistically discernable differences in the activity of this enzyme under these three experimental conditions. Consequently we have concluded that either the efficiency rather than the absolute activity of the Na, K pump is modified by hydrostatic pressure and hyperbaric ENGs or that the broken cell ghost Na, K, ATPase preparation fails to mimic the conditions seen by the whole cells under these conditions.

Having established the existence of the perturbation of active transport at 68 ATA we deemed it necessary to investigate the phenomena at other relevant pressures. Figure 3 presents the results of those studies on active K influx. In Part A of this figure we see plotted the change in active transport caused by increasing hyperbaric pressures of argon as compared to equivalent pressures of helium. Part B of Figure 3 graphs the effect of pressure alone on active transport. It appears that the stimulation of active transport by hyperbaric argon is increased by increasing pressure between 1 and 86 ATA. On the other hand, we see that there is little or no effect of hydrostatic pressure on active transport until pressures above 34 ATA are reached. The depression of active transport by hydrostatic pressure then seems to plateau at between 68 and 88 ATA. These data then show that there appear to be two independent pressure related phenomena operating to perturb the transport of ions across the red cell membrane which has been subjected to the influence of hyperbaric pressures of an ENG such as argon. The first of these perturbants is that of the hydrostatic pressure alone while a second opposing process depends on the narcotic properties of the gas.

To investigate the apparent antagonism between these two processes we have studied the effects of different partial pressures of argon at constant total pressure. The results of these studies are seen in Figure 4 which relates the active influx of K^+ to different partial pressures of argon at three total

hydrostatic pressures. The differences between the partial pressure of argon and the total pressure is made up by helium. For example, data points for line B of Figure 4 represent the influx of K^+ at 68 ATA of total pressure with partial pressures of argon being 0, 34 and 68 ATA while data points in line C represent fluxes at the same partial pressures of argon but at a total hydrostatic pressure of 88 ATA. It is clear from this graph that at all three total pressures increasing partial pressures of argon stimulate the active transport by the red cell membrane. Furthermore, we see that the rates of these increases with pressure (slopes of the three lines) are similar even though they have different x intercepts. This indicates that the phenomenon responsible for the increased transport is the same at all hydrostatic pressures studied and that the effect of the ENG, argon is dependent upon its partial pressure alone. The decreasing x intercepts with increasing total hydrostatic pressure show that pressure diminishes or antagonizes the effects of the ENG.

In Figure 5 I have plotted data which demonstrates the effects of the ENG argon on the phenomenon created by hydrostatic pressure. The lower line labeled "Helium only" describes the effect previously mentioned, namely that increasing hydrostatic pressures decrease active transport in the red blood cell. The upper line of Figure 5 shows the effect of a given partial pressure of an ENG on this decrease. A statistical analysis of the best lines through the two data sets (Helium and 34 ATA argon) show that there is no difference between their slopes. The parallel nature of the two lines shows that the effect of argon is constant throughout the pressure range studied. The fact that the 34 ATA Argon line lines above the hydrostatic pressure line shows that argon's effect is antagonistic to pressure alone and tends to increase active transport. We have also found, as is shown in Table 1, that N_2 increases active transport of K^+ across the human erythrocyte membrane. It should be noted that higher pressures of N_2 are required to produce an effect still somewhat smaller than that observed with Ar. This is evidence that the phenomena we have studied is associated with the mechanisms of elemental gas narcosis since nitrogen is a significantly less narcotic gas than argon and requires higher partial pressures to initiate narcosis as well as changes in active transport. The second observation of interest resulting from these studies is that N_2 at 117 ATA significantly increases passive K^+ efflux from red cells. On the other hand, He at 117 ATA inhibits active transport, but has no effect on passive transport. Thus, it appears that hyperbaric N_2 does increase passive K^+ flux and that the effect is not attributable to pressure.

Other experiments investigated whether anoxia <u>per se</u> was responsible for some of the observed effects created by the elemental gases we have studied. These experiments are important since, although red cell metabolism does not require O_2, it is possible that the elemental gases, N_2, Ar, and/or He may interact with hemoglobin within the cell to affect changes in metabolism

and, therefore, ATP supplies available for driving active ion transport. This is extremely relevant since the environment in which our studies are conducted is one of Ar, Ar-He mixtures or N_2 with very little or no O_2 being present. The data from experiments we have conducted on cells oxygenated at 1 ATA of O_2 and 1 ATA N_2 (the red cells being completely deoxygenated) show that there is no significant effect of deoxygenation on ion transport (see Table 2).

Hence, we have established from these studies that between pressures of 0 and 117 ATA:

1. Hyperbaric pressures of argon stimulate active transport of Na and K in the human red cell.

2. Hydrostatic pressures as applied by hyperbaric pressures of helium depress active transport in red cells.

3. Neither hyperbaric pressures of Helium nor Argon affect passive transport of Na or K across the red cell membrane

4. The effects of hyperbaric Argon and hydrostatic pressure are inhibitable by ouabain.

5. Neither hydrostatic pressure nor hyperbaric argon affects the activity of Na, K, ATPase of broken red cell membranes.

6. The effects of hydrostatic pressures are antagonistic to those of an ENG (argon) at hyperbaric pressures.

7. Nitrogen, a ENG which is less potent than Ar behaves similarly to Ar in the perturbation of ion transport but requires greater pressure than Ar to bring about quantitatively similar changes.

8. The effects of the ENGs are not due to anoxia of the cells being studied.

Synaptosome Studies:

A great deal of effort has been expended in the development of a system for he study of nerve synaptosomes prepared from rat brain. the efforts have been to:

1. Modify pressure chambers so as to allow us to conduct studies on small volumes of synaptosomes.

2. Develop an efficient, reproducible and rapid method for separating synaptosomes from their incubation medium an absolute requirement for conducting transport studies).

3. Develop the most efficient methodology for studying ^{24}Na and ^{42}K transport across the synaptosome membrane.

4. Develop techniques for the characterization and testing of the osmotic integrity of synaptosomes.

5. Characterize the electrolyte and water accessible pools and spaces in the synaptosomes and determine whether the synaptosomes are capable of active transport.

6. Conduct studies on the transport of ^{24}Na and ^{42}K across the synaptosome membrane under 1 ATA of air and hyperbaric pressures of Ar and He respectively.

The development of an efficient, reproducible and rapid method for separating synaptosomes from their incubation medium is required in order that the synaptosomes may be removed from the radioisotopically labeled incubation medium. Subsequently, the amount of ^{42}K or ^{24}Na which has accumulated in or extruded from the synaptosomes can then be determined. The technique we have developed for obtaining the medium free synaptosomes utilizes a very high speed table top centrifuge and successive washings of the synaptosomal pellet.

Studies of synaptosomal ion permeabilities have presented several unique challenges. It has become apparent that rat brain derived synaptosomes cannot withstand repeated compression and decompression necessary for repetitive sampling using our present pressure apparatus. The consequence of this increased fragility was a vastly increased error in the determination of synaptosomal membrane fluxes since repeated compression--decompression caused lysis of some synaptosomes, leading to decreased vesicle numbers and apparent decreases in ionic uptake. To alleviate difficulties we initiated several changes in our experimental protocol. First we utilize the isotope uptake to the amount of protein in each sample. Since the protein is a direct measure of the number of unlysed synaptosomes (lysed membranes do not pellet upon centrifugation at the speeds and spin times used in sample preparation) dividing each sample radioctivity by the amount of protein in each sample unities the amount of isotope uptake to the number of synaptosomes present in the sample. A second change was made in the procedure to eliminate repeated compression and decompression of synaptosomes. Isotope (^{42}K) is added to one of these suspensions and pressurized with Helium, Argon or hydrostatic pressure. After a given period of time, for instance two minutes, has elapsed the synaptosomal suspension is decompressed and a sample taken in duplicate. Subsequently an identical amount of isotope is added to another synaptosomal suspension and pressurized for a different period of time, perhaps four minutes, depressurized and a sample taken. Utilizing this procedure four time periods for isotope flux are obtained. The protein concentrations are then determined for each sample and the time-course for the isotope uptake during the various time periods is plotted. In this way the synaptosomes were pressurized and depressurized only once, resulting in much less synaptosomal damage and lysis. Furthermore, since synaptosomal uptake of

potassium is so much more rapid than that of erythrocytes (coming to equilibrium in a matter of minutes rather than hours), we were able to take data points closer together while eliminating the time required to bring the synaptosomal suspension to pressur and then to depressurize for sampling. Since the kinetics for synaptosomal ion transport are so rapid if repetitive sampling was used the time for compression and decompression would become a sizable fraction of the time-course of the process.

The data obtained in the manner described above was then subjected to a mathematical processing analagous to the Eadie-Hofstee analyses used in enzyme kinetics. This analysis is used in our studies to determine the equilibrium point for isotopic fluxes and prepare the data for subsequent mathematical determination of the rate of isotope uptake.

The data were analyzed to determine permeability coefficients of the synaptosomal membranes to the ion being studied. Our studies have shown that ^{42}K permeability of synaptosomes is increased by 69 ATA of argon and decreased by 69 ATA of the ENNG, Helium. Representative experiments showing these results are seen in Figures 6 and 7. As can be seen in Figure 6, the rate of isotope uptake determined from the slope of the line is greater for synaptosomes under 69 ATA of Argon than for an identical suspension of synaptosomes at 1 atmosphere of air. Conversely synaptosomes under 69 ATA of Helium (Fig. 7) show a slower rate of ^{42}K uptakes than control synaptosomes. Our finding that hyperbaric argon stimulates uptake of potassium by synaptosomes while pressure alone inhibits that uptake is very satisfying since it verifies the effects of pressure and elemental narcotic gases that we have previously observed in human red cells and lends strong support to the notion that the red cell, (which is much easier to work with), can be used to model the effects of pressure and narcotic agents on membrane properties of nervous tissue.

Reports and Publications eminating from this Project:

1. W.R. Galey and P.S. Van Nice. Alteration of electrolyte transport by an anesthetic noble gas. Physiologist 18: 428, 1975.

2. P.S. Van Nice and W.R. Galey. The opposing effects of argon and helium on active transport of potassium in the human erythrocyte. Physiologist 20: 97, 1977.

3. P.S. Van Nice and W.R. Galey. The effects of pressure and argon on the activity of human erythrocyte Na^+, K^+ -ATPase. Fed. Proc. 579, 1977.

4. E.I. Seibel and W.R. Galey. A rapid separation of synaptosomal preparations by centrifugation. Physiologist, 21, 107, 1978.

5. L.R. Maez and W.R. Galey. Pools and spaces of rat brain synaptosomes. Proc. V MBS Symp. 7: 36, 1979.

6. P.S. Van Nice and W.R. Galey. Actions of hyperbaric elemental gases on electrolyte transport in the human erythrocyte. Am. J. Appl. Physiol. 49: 204-210, 1980.

7. W.R. Galey. Effects of hyperbaric and elemental narcotic gases on cellular membrane ion transport. 2nd International Res. Confer.: Molecular Mechanisms of Anesthesia 33, 1979.

8. W.R. Galey and P.S. Van Nice. The effects of hyperbaric and elemental narcotic gases on cellular membrane transport. In Progress in Anesthesiology: Molecular Mechanisms of Anesthesia, Volume II Editor B.R. Fink. Raven Press Publishers, New York, 411, 1980.

9. W.R. Galey, P.S. Van Nice and C.V. Beato. Hydrostatic Pressure: Its effects on cellular membrane ion transport. Abstr. 7th Symposium on Underwater Physiology (1980).

10. P.S. Van Nice and W.R. Galey: The actions of hyperbaric nitrogen on erythrocyte membrane K^+ transport. Submitted for publication. Biochem. Biophys. Acta 419, 1983.

11. W.R. Galey and E.I. Seibel-Ross. An efficient method for separating cellular vesicles (synaptosomes) from their maturation medium. Biochem. Biophs. Meth. In Press (1984)

12. E.I. Seibel. The effects of pressure and hyperbaric inert gases on ion transport across rat brain synaptosomes. Univ. of New Mexico Doctoral Dissertation, 1981.

Personnel involved in this project

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Robert Smith, B.S.

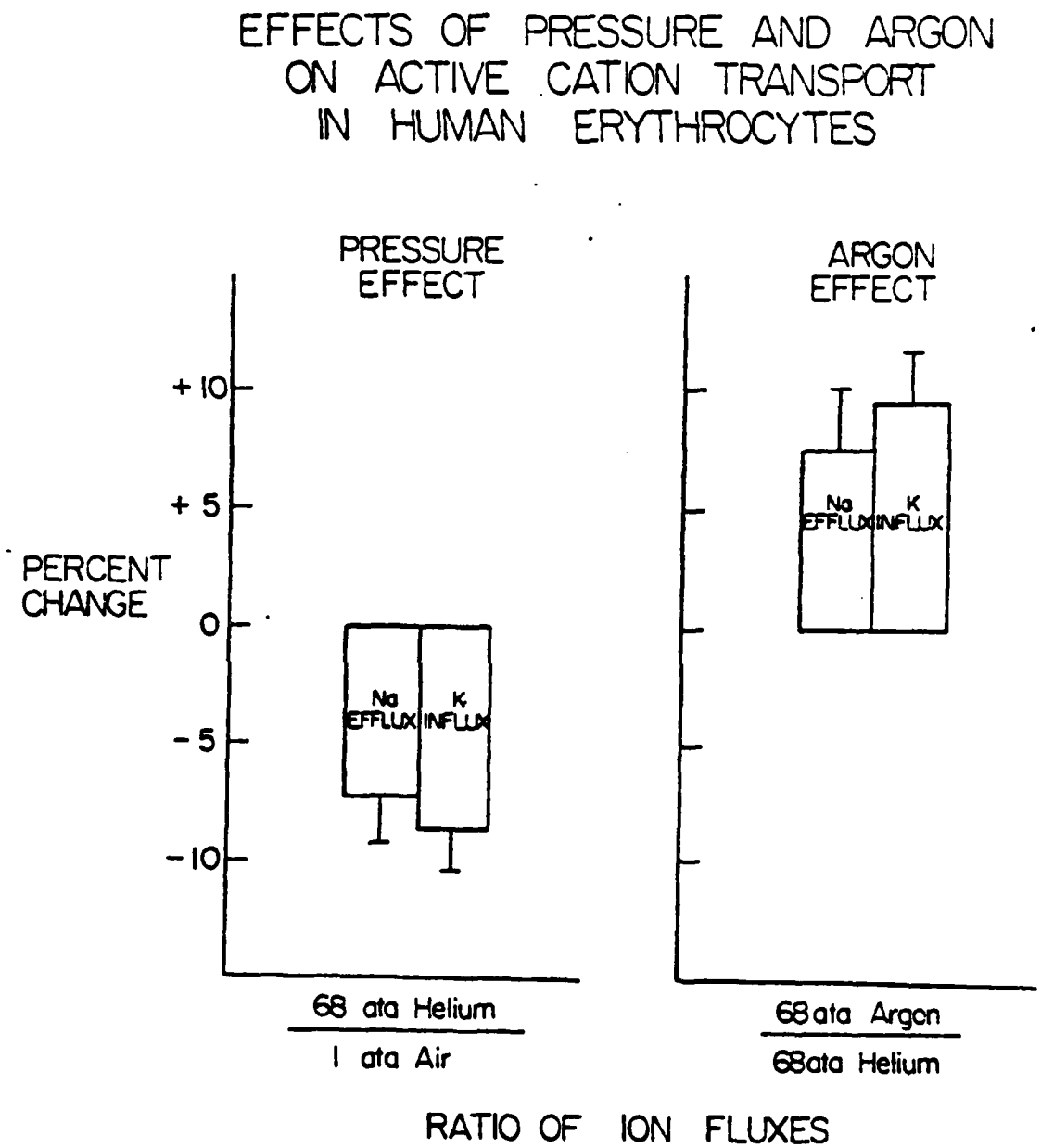


Figure 1. Opposing Effects of 68 Atmospheres of Argon and 68 Atmospheres of Helium on Active Transport in the Human Erythrocyte.

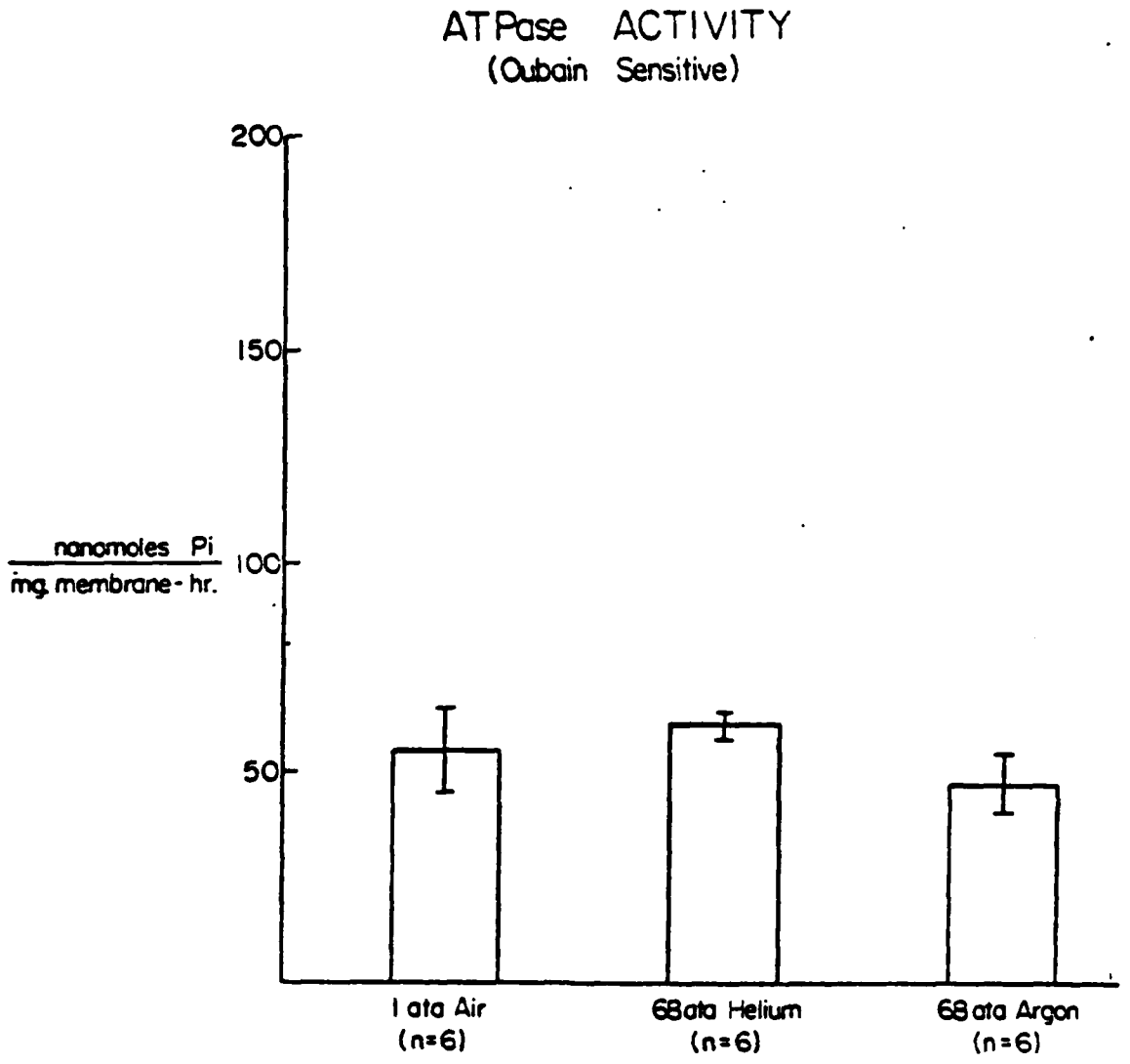


Figure 2. The Effects of 68 Atmospheres of Argon and Helium on the Activity of Ouabain-Sensitive ATPase (80 mM Sodium, 30 mM Potassium).

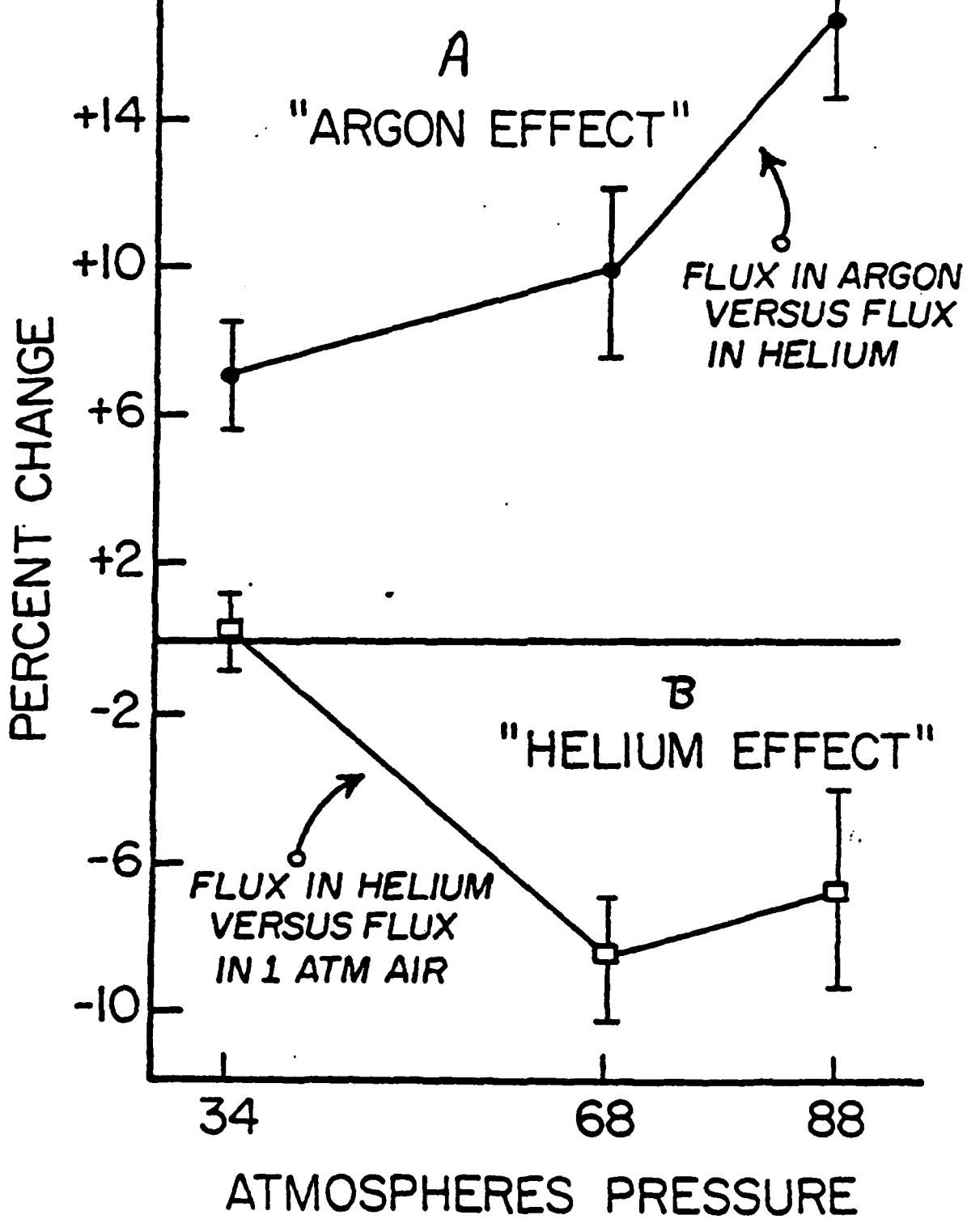


Figure 3. The Opposing Effects of Argon and Helium on Potassium Influx.

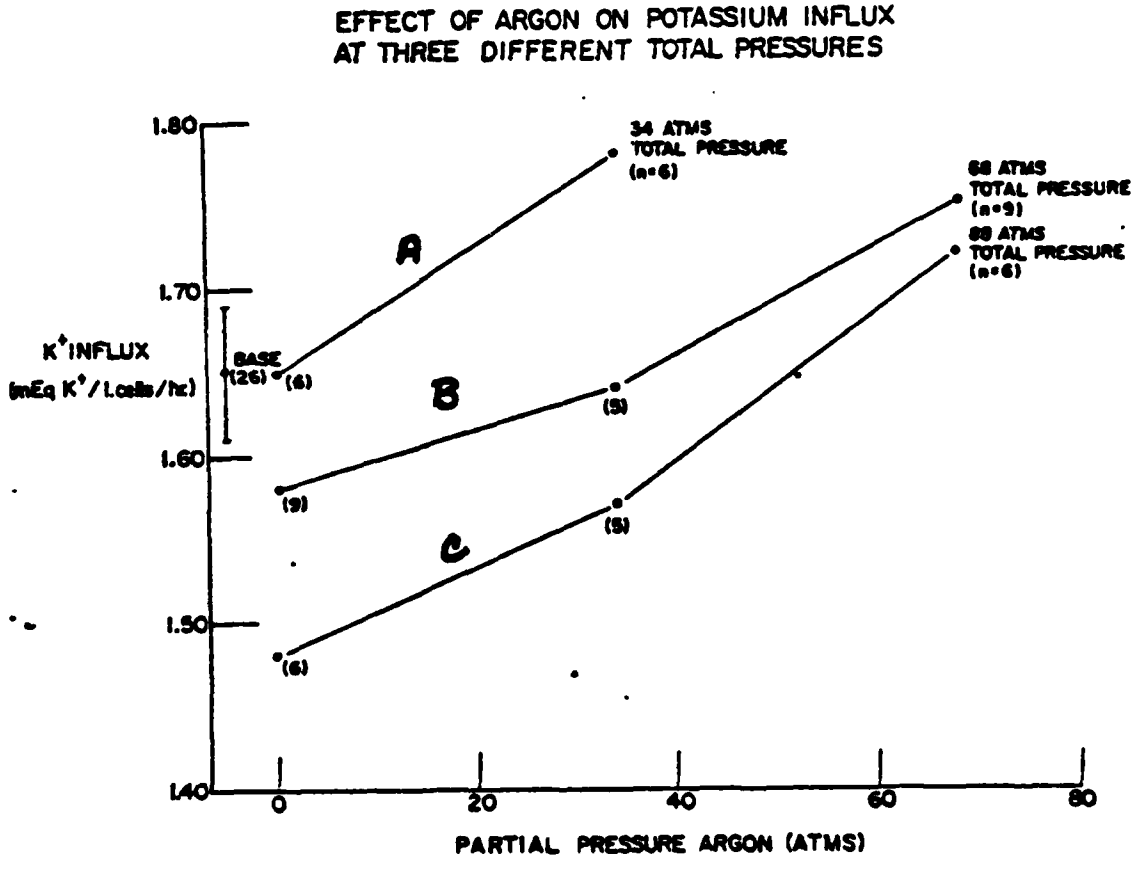


Figure 4. Effects of Argon on Potassium Influx at Three Different Constant Pressures.

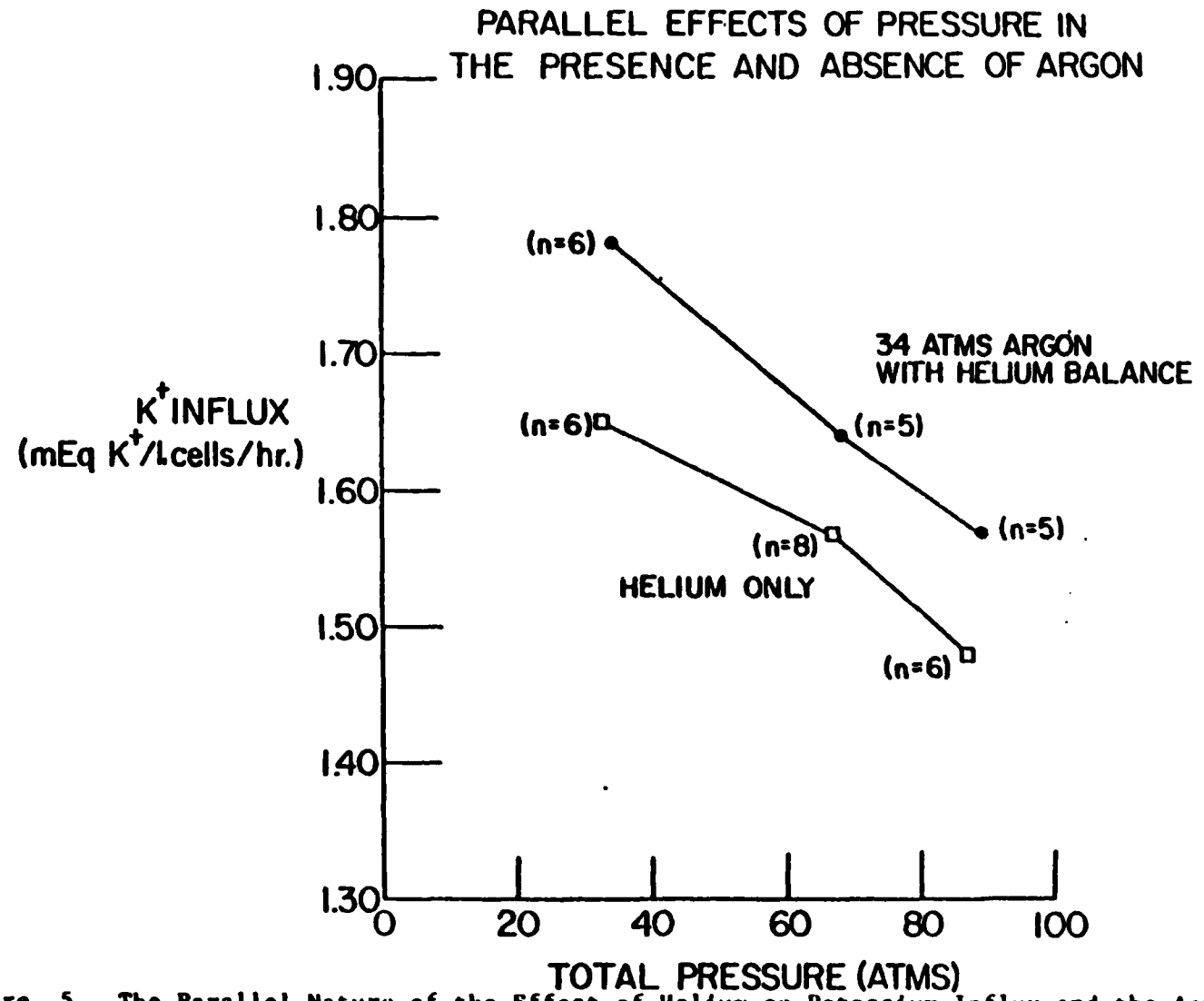


Figure 5. The Parallel Nature of the Effect of Helium on Potassium Influx and the Antagonism by Helium of the Effect of 34 Atmospheres of Argon on Potassium Influx. Slopes of least squares fits of above data points are not different ($P>.95$)

TABLE I

EFFECTS OF PRESSURE AND NITROGEN

ON ION TRANSPORT ACROSS THE RED CELL MEMBRANE

CONDITION	FLUXES (mEq Electrolyte/Liter cells · hr)	
	K INFLUX ± SD (active)	K EFFLUX ± SD (passive)
1 ATA Air	1.54 ± 0.18	2.15 ± 0.24
117 ATA Helium	[A]1.43 ± 0.24	1.96 ± 0.50
117 ATA Nitrogen	[B]1.51 ± 0.27	[A,B]2.57 ± 0.48

[A]Flux is significantly different from Flux in 1 ATA Air, $P < 0.05$

[B]Flux is significantly different from Flux in 117 ATA Helium, $P < 0.05$

TABLE 2

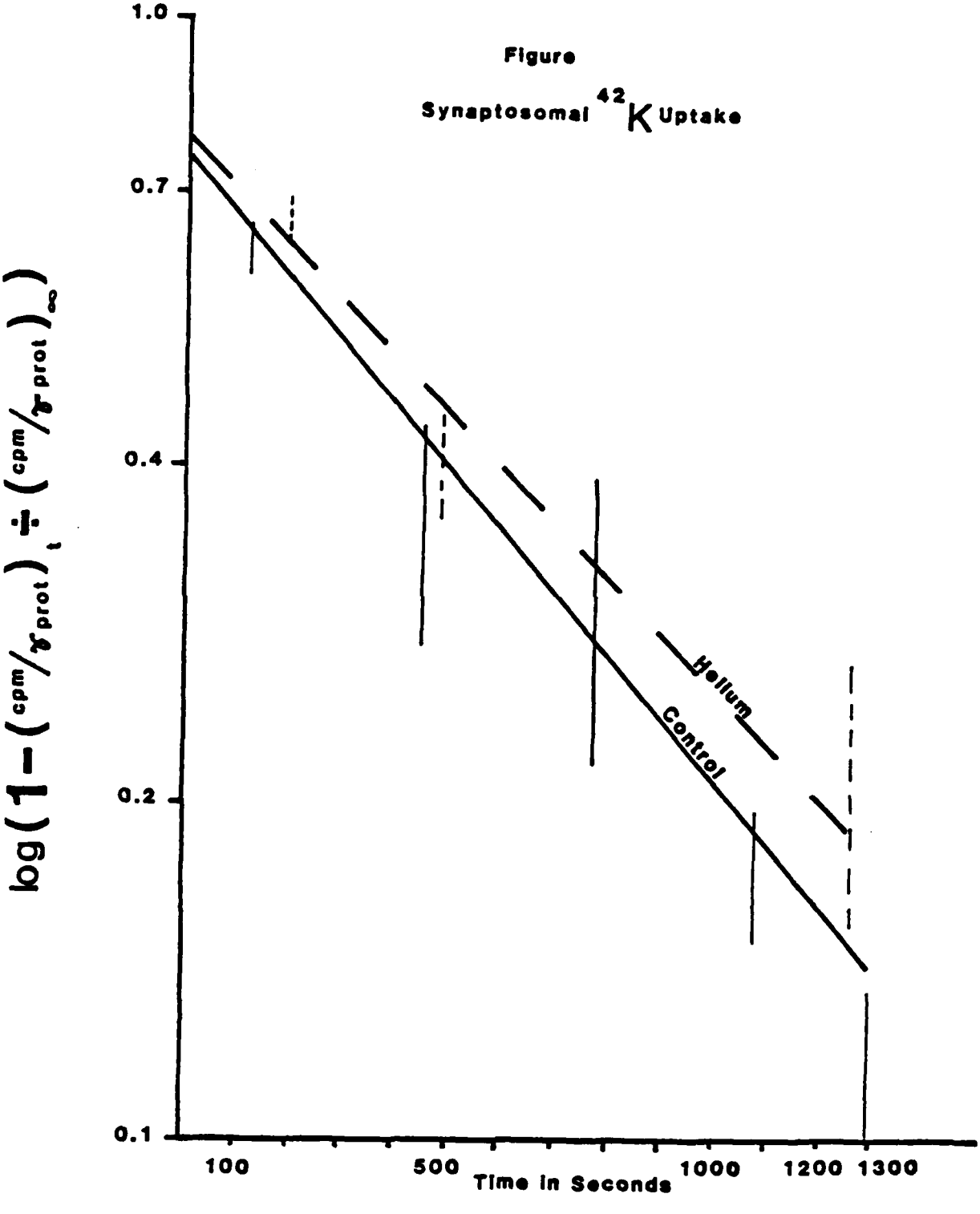
DIFFERENCES BETWEEN POTASSIUM INFLUX RATES OF OXYGENATED RED CELLS AND DEOXYGENATED RED CELLS (SIXTEEN TRIALS)

POTASSIUM INFLUX (mM K/l. cells/hr.)		
Donor	Deoxygenated	Oxygenated*
D.S.	1.31	1.27
	1.76	2.09
N.P.	1.25	1.38
M.P.	1.77	1.68
	1.90	1.83
J.S.	1.43	1.57
	1.56	1.55
R.A.	1.25	1.34
M.S.	1.32	1.43
S.P.	1.13	1.25
D.P.	1.39	1.54
J.M.	1.90	1.92
A.M.	1.35	1.33
B.S.	1.61	1.61
L.S.	1.92	1.80
R.V.	1.76	2.09

Significance level by Student's t-test for paired values, 2-tailed.
*not significantly different from deoxygenated ($P>.05$)

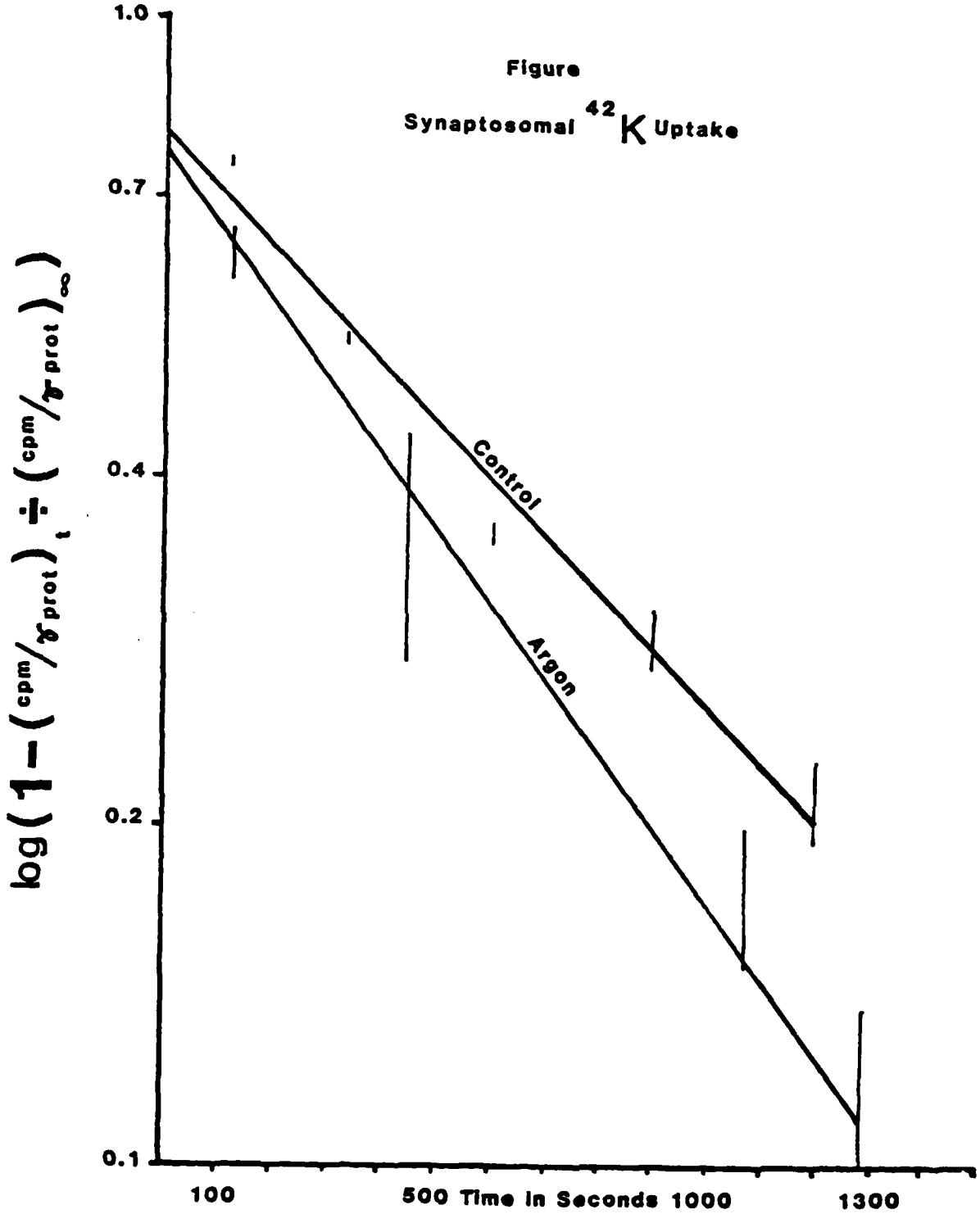
Figure

Synaptosomal ^{42}K Uptake



Figure

Synaptosomal ^{42}K Uptake



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