Effect of Blood pH and CO_2 Tension on Performance of the Heart-Lung Preparation. (32566)

N. S. Nejad and Eric Ogden
(With the technical assistance of Patricia Corsaut)
Naval Aerospace Medical Center, Pensacola, Fla., and Environmental Biology Division, Ames Research Center, NASA, Moffett Field, Calif.

Jerusalem and Starling(1) as early as 1910 reported that major changes in CO_2 in either direction elicit cardiac dilation in an isolated heart. Since then it has been reported that while the heart in intact animals is highly tolerant to severe hypercapnia, it is very sensitive to high pCO_2 when it is isolated(2-5). Boniface and Brown(4) with the aid of a Cushny myocardiograph measured the effect of carbon dioxide on the contractile force of a representative segment of the right ventricle *in situ*. They observed a pronounced cardiac dilation when the animal was subjected to 30% CO_2. Nahas and Cavert(3) reported acute myocardial failure in the heart-lung preparation exposed to CO_2 of 10% or above.

The present study examines some effects of moderate elevation of inspired CO_2 (0-10%) on cardiac performance, as evaluated by stroke work (SW) and left atrial pressure (LAP), in the heart-lung preparation.

Methods. Starling(6) heart-lung preparations (HLP) made from 27 mongrel dogs (9-11 kg) were ventilated with a pump connected to a spirometer filled with a gas mixture of 40% O_2, 0-10% CO_2, and the balance nitrogen. The preparations were supported by a continuous infusion of 5% glucose (10 mg/min), and insulin (0.008 unit/min). Expired CO_2 and O_2 were monitored continuously with a Beckman Model LB-1 gas analyzer and a Model E2 oxygen analyzer, respectively.

Statham pressure transducers (PR23 and P23Dd) recorded pressures from the left atrial appendage and the aortic end of the left subclavian artery. A Shipley-Wilson flowmeter(7) was connected in the arterial flowline across the arterial resistance clamp, and the pulmonary flow was recorded with a pulsed field electromagnetic flowmeter. A portion of the systemic flow was shunted through a modular cuvette for continuous measurements of pO_2, pCO_2, and pH of the arterial blood. Because of the uncertainty of the accuracy at very low readings of pCO_2, all readings of 10 mm Hg or less were considered to be in the same category. The details of the experimental design, instrumentation, and calibrations are described elsewhere(8).

The recorded changes of CO_2 tension in the blood, however, did not reach a steady state for about 10 minutes because of the slow response time of the instrument. The arterial pCO_2 was then allowed to remain constant for a period of 15 to 25 minutes before the inspired CO_2 concentration was changed again. Measurements were made throughout the experiment at one-minute intervals.

The performance of the heart was evaluated by the relationship between SW and mean LAP. The mean left atrial pressure was considered as an index of the filling pressure; the stroke work was considered as an index of performance independent of heart rate.

Results. Fig. 1 shows the progressive decrease in the heart rate as arterial pCO_2 increased in 18 separate experiments in response to the changes in inspired CO_2. In these experiments no attempt was made to maintain the blood pH constant. The data clearly indicate an inverse relationship between the heart rate and pCO_2.

Fig. 2 shows the progressive decrease in the heart rate as arterial pH is lowered in 6 HLP. In these experiments arterial pCO_2 was kept constant at or below 10 mm Hg. The pH was changed with infusion of 0.5 N HCl at the rate of 1.5 cc/min. It is clearly evident that there was an approximately linear relationship between the pH of the blood over the range 7.10 to 7.98 and the slowing of the heart rate. It is probable that the decrease in the heart rate with a rise in arterial pCO_2 shown in Fig. 1 was largely due to pH changes.

Fig. 3 presents data from a single preparation typical of 8 experiments showing the effect of inhalation of CO_2. Work curves are

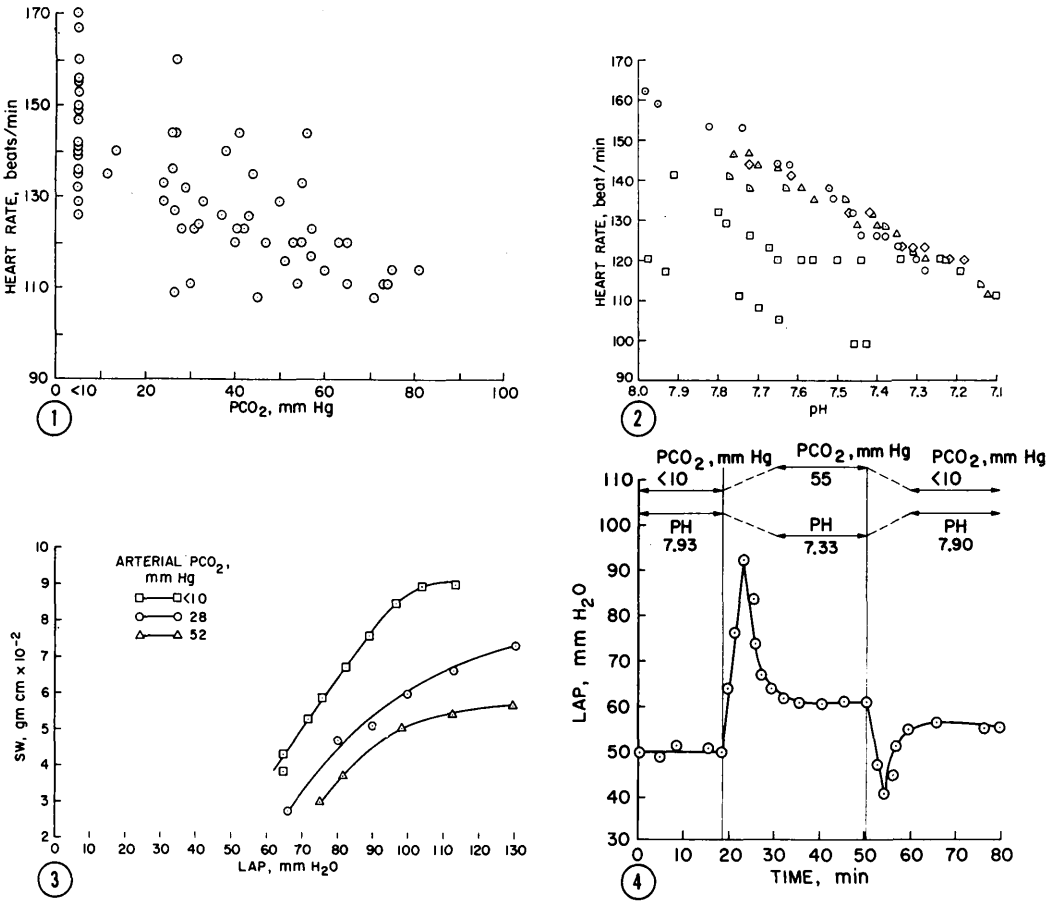

FIG. 1. Effect of arterial pCO_2 on heart rate. These data are from 18 experiments. Each dog is represented by 2-3 points.

FIG. 2. Effect of arterial pH on heart rate. Arterial pCO_2 was kept constant at or below 10 mm Hg. The pH was changed with infusion of 0.5 N HCl, 1.5 cc/min. The change of the heart rate at various arterial pH levels is shown in 6 separate heart-lung preparations. Each symbol represents a different experiment.

FIG. 3. Effect of inhalation of CO_2 on cardiac work curve. Three work curves from the same preparation at constant aortic pressure. SW = Stroke work. LAP = Left atrial pressure.

FIG. 4. Effect of varying arterial pCO_2 on left atrial pressure at constant stroke work. The inspired gas was changed at the 2 vertical lines. Arterial pCO_2 and pH are indicated. One representative experiment.

shown at arterial pCO_2 values of below 10, 28, and 52 mm Hg. The performance of the heart was evaluated by a comparison of these work curves. Left atrial pressures are plotted against left ventricular stroke work (*i.e.*, pulmonary flow times the mean aortic pressure/heart rate). The workload was varied by changing the flow and keeping the arterial pressure constant. These graphs show that the work curve was depressed with an increase in arterial CO_2 tension. The optimum work curve in a heart-lung preparation appeared to be at nearly zero arterial pCO_2.

The effect of arterial pCO_2 on LAP at constant stroke work was studied, and data from a single preparation typical of 5 such experiments are shown in Fig. 4. In this preparation the flow was maintained at about 500 cc/min at a mean arterial pressure of 80 mm Hg. This graph shows that with increase of pCO_2 from below 10 mm Hg to 55 mm Hg, there was an immediate rise in LAP from 50 to 92 mm H_2O, followed by a drop

to a new stable level at 60 mm H_2O. When the arterial CO_2 tension was subsequently returned to nearly zero, a rebound in LAP (to 40 mm H_2O) was noted before stabilizing at about the previous level. These responses were essentially the same for all 5 experiments.

Fig. 5 presents data from a single preparation typical of 7 experiments on 7 separate dogs. Both graphs, here, show the effect of inhalation of CO_2 (4%, 6%, 8%) on stroke work at constant LAP (65 mm H_2O). On the left hand graph, the work was computed from the pulmonary flow and the mean aortic pressure; these represent the total myocardial work. On the right hand graph, the systemic flow was used to calculate the stroke work which here represents the effective work of the left ventricle, omitting the work involved in maintaining the coronary flow. Both graphs show a stepwise decline in stroke work with a stepwise increase of pCO_2 from <10 to 75 mm Hg. Recovery in performance occurred when the pCO_2 was brought back to <10.

Table I presents data obtained from 7 experiments in which the left atrial pressure at constant stroke work was recorded when the arterial pCO_2 and pH were separately changed. Fig. 6 is a detailed presentation of Experiment 1 in the series in Table I. In Table I only the maximum changes of pH and corresponding LAP are shown; whereas, in Fig. 6 the entire experiment is plotted. These experiments were carried out in an attempt to separate the direct effect of pCO_2 and pH on the performance of the heart. After the baseline period of the experiment (Fig. 6) the blood pH was lowered to a value of 7.25 by increasing the arterial pCO_2 to 55 mm Hg by ventilating 8% CO_2, and this in turn depressed the performance of the heart, as indicated by a rise of left atrial pressure from 50 to 117 mm H_2O. After completion of the initial overshoot, further recovery in the heart perfomance was brought about by raising the pH of the blood with continuous infusion of 0.5 M $NaHCO_3$ at the rate of 1.5 cc/min. Throughout this period the pCO_2 was kept at about 55 mm Hg. In the next part of this experiment the arterial pCO_2 was maintained at below 10 mm Hg, while the blood pH was

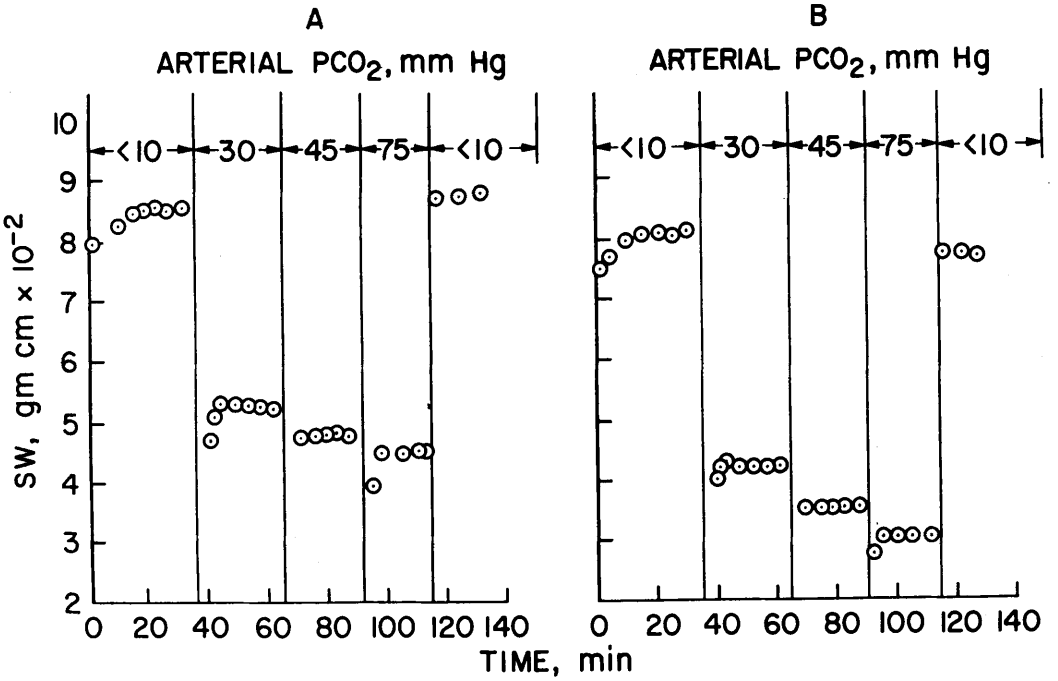

FIG. 5. Effect of arterial pCO_2 changes on stroke work at constant left atrial pressure (65 mm H_2O). The values shown here were taken after the recorded arterial pCO_2 was maintained at the levels indicated. One representative experiment. (A). Stroke work calculated from pulmonary flow measurements. (B). Stroke work calculated from aortic flow measurements.

TABLE I. Effect of Arterial pCO_2 and pH on Left Arterial Pressure at Constant Work.

Exp No.	Treatment: Ventilation, % CO_2*	Treatment: Infusion†	Arterial blood: pCO_2, mm Hg	Arterial blood: pH	Left atrial pressure,‡ mm H_2O
1	0	—	<10	7.95	50
	8	—	55	7.25	117
	8	$NaHCO_3$	"	7.60	40
	0	—	<10	8.00	"
	"	HCl	"	7.20	105
	"	$NaHCO_3$	"	7.70	46
2	0	—	<10	8.00	60
	8	—	50	7.25	190
	"	$NaHCO_3$	"	8.00	45
	0	—	<10	"	"
	"	HCl	"	7.28	120
	"	$NaHCO_3$	"	8.00	77
3	0	—	<10	8.00	47
	8	—	55	7.28	117
	"	$NaHCO_3$	"	7.95	40
4	0	—	<10	7.90	45
	"	HCl	10	7.00	85
	"	$NaHCO_3$	"	7.72	33
5	0	—	<10	7.90	53
	"	HCl	10	7.05	165
	"	$NaHCO_3$	"	7.80	30
6	0	—	10	7.90	90
	"	HCl	"	7.25	143
	"	$NaHCO_3$	"	7.90	67
7	0	—	<10	8.00	60
	"	HCl	"	7.43	210
	"	$NaHCO_3$	"	7.86	57

* All gas mixtures contain 40% O_2, zero or 8% CO_2, balance N_2.

† .5 N HCl infusion, 1.5 cc/min; .5 M $NaHCO_3$ infusion, 1.5 cc/min.

‡ Note: In this table LAP varies with pH in every case but varies with arterial CO_2 tension only when pH changes.

lowered by continuous infusion of 0.5 N HCl at the rate of 1.5 cc/min. The left atrial pressure rose from 40 to 105 mm H_2O and was then lowered to 46 mm H_2O by continuous infusion of $NaHCO_3$.

Fig. 7 presents data obtained from a single preparation typical of 4 experiments (Nos. 4-7) shown in Table I. In these experiments the arterial pCO_2 was kept at or below 10 mm Hg. The changes in blood pH were made with infusion of 0.5 N HCl or 0.5 M $NaHCO_3$ at a rate of 1.5 cc/min. At constant stroke work the left atrial pressure was elevated to 85 mm H_2O as the blood pH was lowered to a final value of 7.0. The left atrial pressure was subsequently lowered to 33 mm H_2O with infusion of $NaHCO_3$.

Table I and Fig. 6 and 7 indicate that the performance changes produced by inhalation of CO_2 are more closely related to the consequent changes of blood pH than to the direct effect of blood CO_2 tension.

Discussion. The decrease in heart rate produced by a change in arterial CO_2 tension appears to be due to change in pH. By evaluating the performance on a stroke work basis, we are avoiding the chronotropic effects of temperature. In any case, the heart rate changes observed in the present study were not sufficient to account for a negative inotropic effect shown here.

There was little difference between the behavior of stroke work calculated with and without coronary flow (Fig. 5). This suggests that any effect arterial pCO_2 or pH may have on the coronary flow is negligible for present considerations. Increasing the composition of CO_2 in the inspired air produced progressive deterioration in cardiac performance as the arterial pCO_2 rose from near zero to 75 mm Hg. This depression in the heart performance is reversible.

Our observations are in sharp contrast to those of Jerusalem and Starling(1) who found that there is an optimum tension of CO_2 in the blood at which the heart performs at its maximum. In the present experiments after changes in inspired CO_2 in either direction the above mentioned inotropic effects appeared dramatically in a very marked degree. This is considered an overshoot since within 5-10 minutes the produced inotropic changes diminished and the heart assumed a performance characteristic of the new equilibrium level.

Studies presented here on the negative inotropic effect of CO_2 inhalation have attempted to disassociate the effects of changes in blood pCO_2 or pH. It appears that the performance of the heart regularly increases with increasing pH and diminishes with falling pH regardless of the arterial CO_2 tension (Table I). When the pH is maintained constant either in the neighborhood of pH 8.0 or pH 7.0, CO_2 tension is clearly without inotropic effect.

Recently it was reported that acid pH in both *in vitro*(9) and *in vivo*(10) experiments inhibits norepinephrine-induced lipolysis; this would reduce the ability to mobilize fat stored

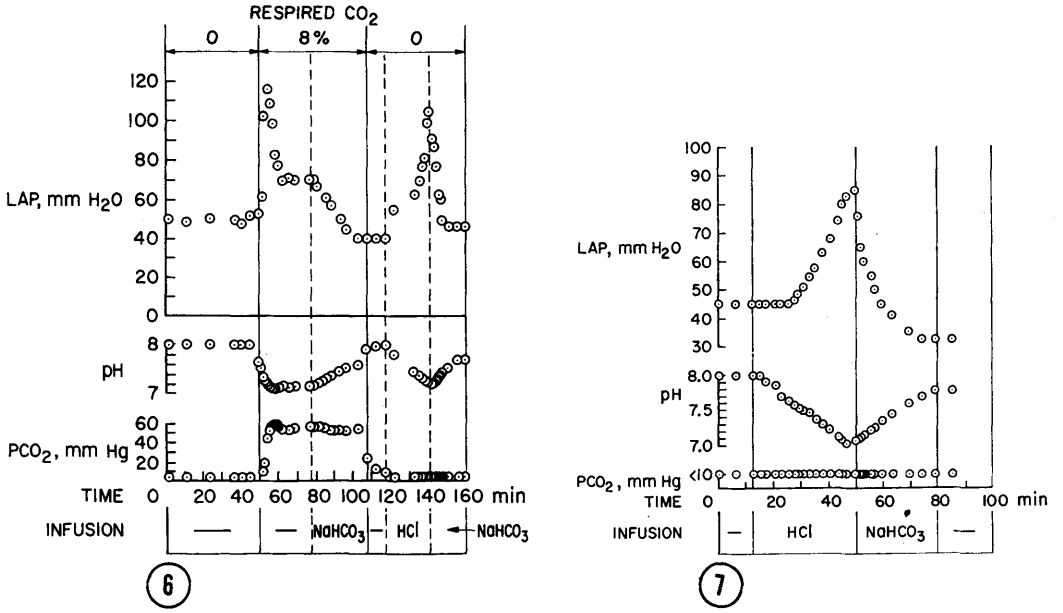

FIG. 6. Effect of arterial pH and pCO_2 on left atrial pressure-stroke work constant. Respiratory CO_2 concentration shown on top was changed from zero to 8% and back to zero while the arterial blood was infused with base or acid at the rate of 1.5 cc/min. See text.

FIG. 7. Relationship of arterial blood pH and LAP. Stroke work constant. Respiratory CO_2 was kept at zero. Blood pH decreased by infusion of 0.5 N HCl, 1.5 cc/min, and then increased by infusion of 0.5 M $NaHCO_3$, 1.5 cc/min.

within and around the heart and lungs and thus limit metabolism. This phenomenon may well explain the negative inotropic effect observed in our experiments at low pH.

The immediate partial adjustment to hypercapnic depression and the rebound phenomenon upon termination of carbon dioxide stress may be accounted for by one or both of the following explanations:

a. Release of endogenous catecholamines while the heart was under CO_2 stress and the persistence of the action of these compounds even after the carbon dioxide stress was removed.

b. A state of ionic disequilibrium due to pH changes across the cell membrane. There is evidence that the force of contraction is a function of the rate of repolarization (K^+ exit) of the membrane(11). The passage of potassium across the cell membrane may well be facilitated by pH changes(12).

Summary. When the performance of heart-lung preparations was evaluated by the relationship between stroke work and left atrial pressure, a change of the CO_2 content of the inspired air from zero to 10% caused a progressive decrease in performance. The use of HCl or $NaHCO_3$ allowed for changing the pH and pCO_2 of the arterial blood separately. Arterial blood pH rather than blood pCO_2 appeared to be the decisive factor in mediating this change. Whenever a change of inspired air composition was made in either direction, the new performance level was preceded by a marked overshoot. A fall in arterial pH was accompanied by a slowing of the heart rate.

1. Jerusalem, E., Starling, E. H., J. Physiol., 1910, v40, 279.

2. Brown, E. B., Jr., Miller, F., Am. J. Physiol., 1952, v170, 550.

3. Nahas, G. G., Cavert, H. M., ibid., 1957, v190, 483.

4. Boniface, K. J., Brown, J. M., ibid., 1953, v172, 752.

5. Manley, E. S., Jr., Nash, C. B., Woodbury, R. A., ibid., 1964, v207, 634.

6. Knowlton, F. P., Starling, E. H., J. Physiol., 1912, v44, 206.

7. Shipley, R. E., Wilson, C., Proc. Soc. Exp. Biol. & Med., 1951, v78, 724.

8. Nejad, N. S., Ogden, E., ibid., 1967, v126, 762.

9. Triner, L., Nahas, G. G., Science, 1965, v150, 1725.

10. Nahas, G. G., Poyart, C., Am. J. Physiol., 1967, v212, 765.

11. Burgen, A. S. V., Terroux, K. G., J. Physiol., 1953b, v120, 449.

12. Fenn, W. O., Cobb, D. M., Am. J. Physiol., 1935, v112, 41.

Received July 25, 1967. P.S.E.B.M., 1967, v126.

In vivo Effect of Dehydroepiandrosterone on Red Blood Cells Glucose-6-Phosphate Dehydrogenase. (32567)

A. LOPEZ-S.* AND W. A. KREHL

Clinical Research Center, Department of Medicine, University of Iowa, Iowa City

The biological mechanism of action of steroid hormones still remains unknown. Reports from the literature suggest that they may exert their regulatory effect by an effect on certain enzymatic functions. That specific enzymes are influenced by certain steroids has been reported by McKerns(1) and others (2-3). With regard to dehydroepiandrosterone (DHA) it has been shown by different investigators that this steroid hormone is a potent *in vitro* inhibitor of the enzyme glucose-6-phosphate dehydrogenase. This information is summarized in Table I.

Such a metabolic inhibition *in vivo* has interesting implications, particularly if the inhibition of glucose-6-phosphate dehydrogenase resulted in a decreased availability of TPNH, which is an important co-factor in the synthesis of fatty acids.

Materials and methods. The present study was designed to test the *in vivo* effect of dehydroepiandrosterone on the enzyme glucose-6-phosphate dehydrogenase of red blood cells. For this, two similar experiments were carried out. In the first study, 100 mg of pure DHA (dehydroisoandrosterone, Sigma Chemical Co.) were administered for 3 consecutive days to a healthy adult male engaged in normal professional activities. Red blood cell glucose-6-phosphate dehydrogenase activity was measured every other day for one week, before the administration of DHA (control periods), during the 3 days of administration, and thereafter; each time the determination of G-6-PD was done in triplicate. The urinary excretion of 17 ketosteroids and DHA was also measured daily during the same period of time, in duplicate. A similar experiment was repeated 9 months later on the same subject but using a more accurate method of measuring dehydroepiandrosterone; a total of 500 mg of DHA in 4 consecutive days was administered in this second experiment.

Glucose-6-phosphate dehydrogenase was determined using the method of Kornberg and Horecker(4) by measuring the increase of TPNH absorbance at 340 millimicrons per unit of time in a medium consisting of enzyme (r.b.c.) substrate (glucose-6-phosphate), buffer (triethanolamine) and TPN (as a co-factor). All these reagents were obtained from C. F. Boehinger and Soehne, GMBH, Mannheim, Germany.

Dehydroepiandrosterone: In the first experiment urinary DHA was hydrolyzed by the method of Burstein and Lieberman(5) and then separated by column chromatography by the method of Fotherby(6). The isolated DHA was then measured colorimetrically by a modification of the Petenkofer reaction(7) described by Inagaki(8). In the second experiment, the DHA, after hydrolysis,

TABLE I. *In vitro* Effect of Dehydroepiandrosterone on Glucose-6-Phosphate Dehydrogenase.

System	Concentration	% Inhibition	Ref.
Adrenal cortex (cows and rats)	5×10^{-5}M	80	(15)
" "	1×10^{-6}M	20	(15)
Human erythrocytes	4×10^{-5}M	80	(12)
" "	1×10^{-6}M	28	(12)
" "	4×10^{-7}M	18	(12)
Rat adrenals	4×10^{-5}M	90	(12)
" "	1×10^{-6}M	45	(12)
" "	4×10^{-7}M	21	(12)
Lactating guinea pigs (mammary gland)	3×10^{-4}M		(16)
Rat adrenal	5×10^{-5}M	80	(17)

* Present Address: Dept. of Medicine, Louisiana State Univ. School of Med., New Orleans.