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THE EFFECTS OF ACUTE HYPOXIA ON THE BODY
FLUIDS OF GALLUS DOMESTICUS

by

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INTRODUCTION

The effects of hypoxia have been known since Paul Bert's (1) experiments on animals and man revealed an increase in the oxygen carrying capacity of the blood apparently due to an erythrocytic polycythemia. Dallwig et. al. (8) evoked a similar increase in the total number of circulating red blood cells in dogs and rabbits with gas mixtures ranging between 10% and 13% oxygen under normobaric conditions. More recent works (9, 11, 14, 16) have shown that this increase in erythrocyte concentration may be due initially to a hemoconcentration brought about by a reduction in plasma volume. Fryers (10), working with different groups of rats, reported a significant reduction in their plasma volumes after acclimatization to 4,500 m (15,000 ft.) for periods ranging from 3-100 days. Hannon et. al. (11) showed a similar reduction in humans at 4,230 m (14,100 ft.) after only a few hours of exposure. Similar findings also have been reported in humans by Pugh (14), Surks et. al. (21) and Sanchez et. al. (16) who feel that this hemoconcentration is a compensatory mechanism to enable a great number of red blood cells to be oxygenated per unit volume of blood.

On the other hand, Hurtado et. al. (12) made observations on six humans at 4,540 m (14,900 ft.) and found a decrease in the plasma volume in four individuals and an increase in two after a period of two hours. Observations on five of the subjects two days later revealed a decrease in plasma volume in two and an increase in three, one 13.7% above normal.

Dill et. al. (9) also found varying results in the plasma volumes of humans while working at 3,000 m (9,900 ft.). These apparently conflicting data suggest that in mammals fluids may be gained or lost from the plasma depending on the degree and duration of the hypoxic exposure.

Previous studies on the effects of acute hypoxia on body fluids (1, 8, 9, 10, 11, 12, 14, 16, 21) have dealt with mammals or with exposures of several days duration at oxygen concentrations of 10% to 14%. Virtually no information is available on the effects of oxygen concentrations of less than 10% to 14% on body fluids in nonmammalian species such as the domestic fowl.

The study reported herein initially was undertaken to demonstrate whether acute exposure of domestic fowl to a simulated altitude to 3,750 m (12,500 ft.) -- an altitude at which they are known to reside (2, 5, 18) -- would produce a hemoconcentration in this species. After 12 hours of this exposure -- approximately 13% oxygen at normobaric conditions -- no changes were observed in the plasma volume, packed cell volume or plasma protein concentration. However, at an oxygen concentration of 8% (approximately 25,000 ft. or 7,930 m) for a period of two hours, hemodilution rather than hemoconcentration was observed. Since this effect was the opposite of what was expected based on previous data (2, 5, 18) the study was changed to measure both total body water and plasma volume changes resulting from acute hypoxia. The results are reported here.

METHODS AND MATERIALS

Animals. Adult, male, Single Comb White Leghorns (SCWL), 10-20 months of age, with an average body mass of 2.5 kg. were used in these experiments. The animals were on a 12L:12D photoperiod, in an animal room, in standard laying cages for a minimum of seven days prior to experimentation. All trials were performed under similar environmental conditions and at the same hours of the day, to minimize differences due to circadian rhythms. Blood and plasma determinations were performed in samples drawn from a cutaneous ulnar vein cannula. Cannulation surgery was done 24 hours prior to the experiment under a local anesthetic (Procaine hydrochloride*).

Chamber. The exposure chamber was fabricated by covering a wooden framework enclosure with heavy plastic (Fig. 1). After placing the animal into this chamber, the inlet air was diluted with tanked nitrogen to produce an ambient of 8% oxygen atmosphere, usually within 20 minutes. The hypoxic stress was induced by exposing the animal to this condition for two hours in each case. The ventilation rate of the chamber was adjusted to give approximately two air changes per hour. The concentration of oxygen was monitored with an Oxygen Analyzer (Beckman, model-E2) on gas exiting the chamber through a T-tube attached to the exhaust port. Carbon dioxide concentration was monitored similarly with a Medical Gas Analyzer (Beckman, model LB-1), and never rose above 0.2%.

Measurements. Plasma volumes were determined by a modification of the Burton et. al. (4) technique using human

*Curts Laboratories, Kansas City

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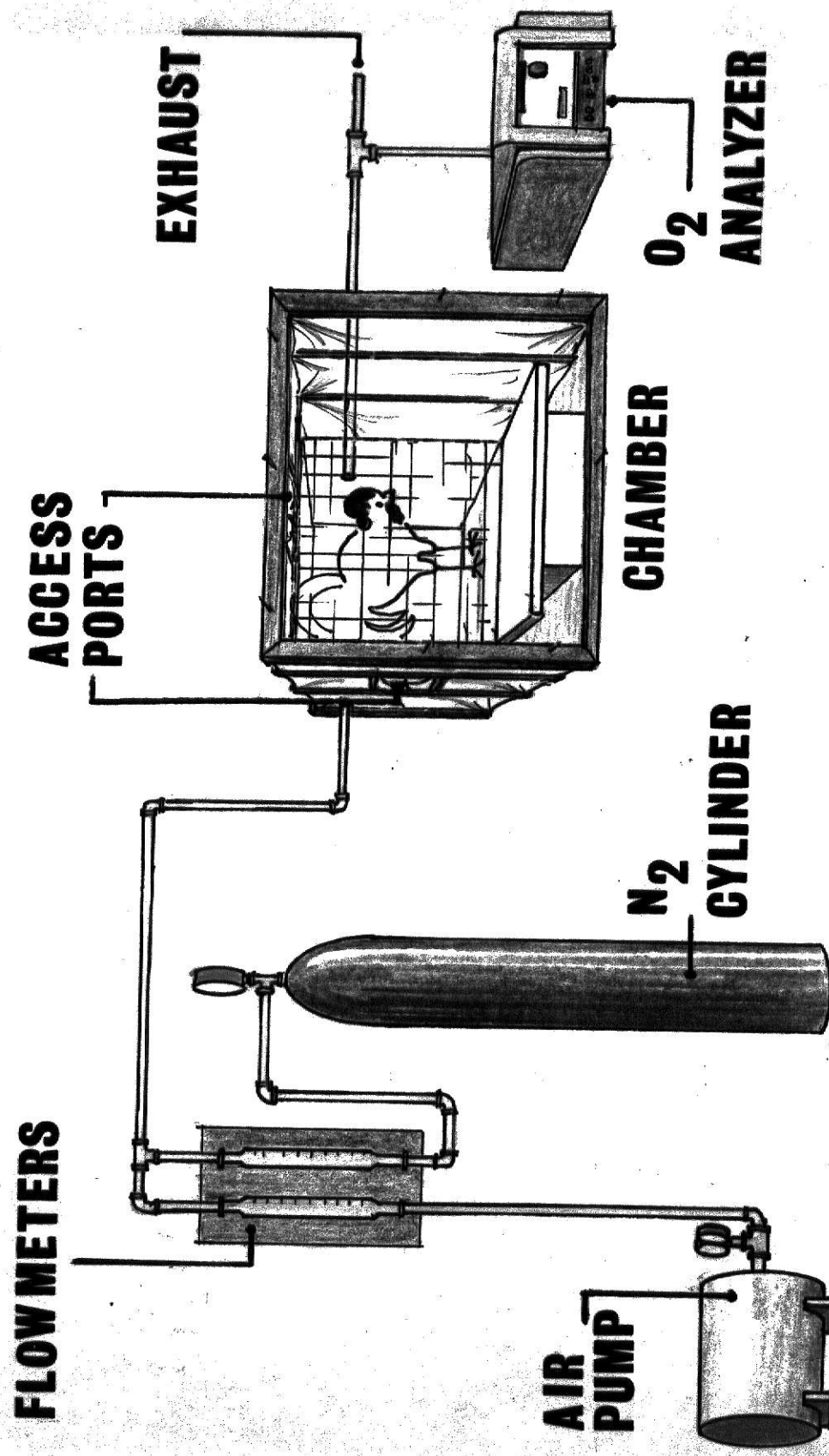


Fig. I Schematic of apparatus used to induce hypoxic (8% oxygen) and normoxic (21% oxygen) conditions. The carbon dioxide analyzer (Beckman, model LB-1) was used periodically at the exhaust port to monitor the CO₂ levels.

radio-iodinated (I^{125}) human serum albumin (RISA) (Appendix I). This procedure was carried out immediately prior to and at the end of the two hour experiment while the bird was still hypoxic. A two hour time limit was used because preliminary studies showed that the chickens would convulse and die if kept at 8% for longer durations.

A modification of the tritiated water procedure of Chapman and Black (7) was used to determine the total body water pool. The animals were injected with 250 μ Ci of tritiated water via a cannula in the cutaneous ulnar vein. A 2.0 ml. blood sample was taken two hours post-injection. Preliminary data showed that two hours was ample time to allow for the tritiated water (T_2O) to equilibrate with the body fluids. The animal was immediately reinjected with the same amount of T_2O and a similar sample taken after two hours of hypoxic (8%) or normoxic (21%) conditions. The blood samples were immediately frozen until all had been collected. These samples were lyophilized to complete dryness and the water collected in a cold trap (dry ice and acetone). A 1.0 ml. portion of the water was added to 5.0 ml. of dioxane based liquid scintillation solution (3) and counted in a deep well liquid scintillation counter (Packard, Tri-Carb) for ten minutes. A standard, prepared by diluting a portion of the solution used for injection (1:1250), was counted simultaneously with the samples. Counting efficiency was determined for each sample volume by counting similar volumes of the

standard. All sample volumes were expressed as $\text{m}\mu\text{Ci } ^3\text{H/ml. H}_2\text{O}$. Total body water was determined by the standard method as outlined by Chapman and Black (7).

Packed cell volumes (PCV) were determined by the micro-capillary hematocrit method. Total plasma proteins were determined with a hand protometer (National Instrument Company). Erythrocyte fragility was measured utilizing the technique of Wintrobe (23) using different saline concentrations.

RESULTS

The data of experiment #1, summarized in table 1, show that all animals experienced a non-significant body weight loss, although this was greater in the hypoxic than in the normoxic birds. Moreover, there were no significant differences between the pre- and post-treatment body water pool sizes in either the normoxic or hypoxic groups. The magnitude of water loss in hypoxic animals was approximately equivalent to the body weight loss.

In experiment #2, the plasma volumes in all hypoxic animals increased significantly ($P < 0.05$) above their pre-treatment values (table 2). Further, the controls displayed a slight, but nonsignificant, increase in their plasma volumes that may be due to inherent error in the technique as there was no change in the PCV's or plasma proteins indicative of hemodilution. Zipf (24) has shown that the overall probable error for the RISA technique is less than plus or minus 5%. The percentage increase in the control group reported here amounts to +5.8%. When this value (+5.8%) was subtracted from the total percentage increase for the hypoxic birds (+16.8%), the resultant increase above normal in plasma volume due to hypoxia was +11.0%. This represents a net increase in vascular volume, or hemodilution, apparently at the expense of another pool (e.g., the intracellular volume). Since body weight changes in control and hypoxic birds are of the same magnitude as those in table 1, the total body water loss was about the same in both experiments.

Table 1. Body weight and total body water changes in male, domestic fowl under hypoxic (8% oxygen) and normoxic (21% oxygen) conditions.

CONTROL--21% OXYGEN ¹			
SAMPLE ²	BODY WEIGHT (gm)	TOTAL BODY WATER (ml)	TOTAL BODY WATER (% body mass)
Initial	2528 ± 77.3	1694 ± 25.	67.28 ± 1.80
Final	2508 ± 77.	1697 ± 70.	67.71 ± 2.79
± Δ% ⁴	-0.7	+0.1	+0.6
EXPERIMENTAL--8% OXYGEN			
Initial	2352 ± 110.	1603 ± 57.	66.68 ± 1.26
Final	2301 ± 112.	1558 ± 89.	67.71 ± 1.54
± Δ%	-2.1	-2.8	+1.5

¹11 Animals in control group; 8 in experimental.

²Initial sample at 0 time; final after 2 hours.

³Mean ± standard error.

⁴± Δ% = (Final value - Initial value/Initial value) X 100.

Table 2. Body weight and plasma volume changes in male, domestic fowl under hypoxic (8% oxygen) and normoxic (21% oxygen) conditions.

CONTROL -- 21% OXYGEN ¹				
SAMPLE ²	BODY WEIGHT (gm)	P ³	PLASMA VOLUME (ml)	P PLASMA VOLUME (% body weight)
Initial	2444 ± 83. ⁴	N.S.	118 ± 6.	4.8 ± 0.1
Final	2423 ± 82.		125 ± 7.	5.1 ± 0.2
+ Δ% ⁵	-0.8		+5.9	+6.2
EXPERIMENTAL--8% OXYGEN				
Initial	2345 ± 107.	N.S.	101 ± 4.	4.3 ± 0.1
Final	2294 ± 105.		118 ± 6.	5.2 ± 0.3
+ Δ%	-2.1		+16.8	+19.8

¹11 animals in control group; 8 in experimental.

²Initial sample at 0 time; final after 2 hours.

³Statistical probability - Student's T-test.

⁴Mean ± standard error.

⁵+ Δ% = (Final value - Initial value/Initial value) X 100.

Another consistent finding was the significant decrease in packed cell volume and plasma protein values (table 3) for hypoxic compared to control birds. These changes in the hypoxic animals -- a 8.3% decrease in PCV and 10.0% decrease in plasma protein concentration -- also indicate a hemodilution effect.

There was no change in the osmotic fragility of the erythrocytes due to hypoxia. All cells in both control and hypoxic animals began to hemolyze at 0.40% and were completely hemolyzed by 0.30% sodium chloride. Similar erythrocyte fragility data have been reported for avian red blood cells by Soliman and Amrousi (19) and for human red cells by Stickney et. al. (20).

Visual observations made on the birds while breathing 8% oxygen revealed that they appeared to be in a very weakened and lethargic state. In many instances they assumed a prone position during the entire trial. For a few animals the oxygen concentration was apparently too low since they convulsed and died. One hundred percent mortality was observed when the concentration was kept below 8% for any length of time (i.e., more than one hour). Moreover, it was found that the birds did not begin hyperventilating until the oxygen concentration was at or below 12%, supporting the work of Ray and Fedde (15).

Although the increase in the plasma volume of +11.0%

Table 3. Packed cell volume and plasma protein changes in male, domestic fowl under hypoxic (8% oxygen) and normoxic (21% oxygen) conditions

CONTROL--21% OXYGEN¹

SAMPLE ²	PACKED CELL VOLUME (%)	P ³	PLASMA PROTEINS (gm/100 ml)	P
Initial	42.7 ± 0.6 ⁴	N.S.	6.9 ± 0.1	N.S.
Final	42.6 ± 0.6		6.9 ± 0.1	
+ Δ% ⁵	-0.2		-0.4	
EXPERIMENTAL--8% OXYGEN				

¹18 animals in control group; 13 in experimental. Data are from animals used in both experiments #1 and #2.

²Initial sample at 0 times; final after 2 hours

³Statistical probability - Student's T-test.

⁴Mean ± standard error

⁵± Δ% = (Final value - Initial value/Initial value) X 100.

due to hypoxia (table 2) exceeds the percentage change of PCV of -8.3% (table 3) there is no significant change in the total red blood cell volume before and after hypoxia (table 4). Thus, a detectable erythrocytic polycythemia did not occur during the two hour trial. Apparently the difference between the plasma volume change and the PCV change is due to an inherent technique error for it has been shown (18) that SCWL are highly efficient in increasing their packed cell volumes while exposed, chronically, to an altitude of 3,700 m (12,500 ft.).

Table 4. Red blood cell volume changes in male, domestic fowl under hypoxic (8% oxygen) and normoxic (21% oxygen) conditions.

CONTROL--21% OXYGEN ¹			p ⁴
SAMPLE ²	RED BLOOD CELL VOLUME ³ (ml)		
Initial	84 ± 11.5	N.S.	
Final	88 ± 13.		
+ Δ% ⁶	+0.04		
EXPERIMENTAL--8% OXYGEN			
Initial	76 ± 12.	N.S.	
Final	78 ± 15.		
+ Δ%	+0.02		

¹11 Animals in control group; 8 in experimental.

²Initial sample at 0 times; final after 2 hours.

³Red blood cell volume equals Total Blood Volume -
Plasma Vol. Total Blood Volume = $\frac{\text{Plasma Volume}}{1-PCV}$

¹11 Animals in control group; 8 in experimental.

²Initial sample at 0 times; final after 2 hours.

³Red blood cell volume equals Total Blood Volume - Plasma Vol. Total Blood Volume = $\frac{\text{Plasma Volume}}{1-PCV}$

⁴Statistical probability - Student's T-test.

⁵Mean ± standard error.

⁶+ Δ% = Final value - Initial Value/Initial value) X 100.

DISCUSSION

It is commonly accepted (9, 11, 14, 16, 17) that hemoconcentration is a phenomenon usually seen following exposure to hypoxia and acts as a compensatory mechanism to increase the oxygen carrying capacity of the blood. A hemodilution would decrease the efficiency of this process and also decrease the oxygen supply to many vital parts of the body (e.g., the brain). The results reported here indicate that a chicken breathing 8% oxygen is unable to compensate for this severe oxygen deprivation. Butler (6) has shown that the chicken, unlike many mammals, displays a severe bradycardia with marked decrease in arterial diastolic pressure suggesting a lowered peripheral resistance at an inspired oxygen concentration of 8%. His data also suggest that cardiovascular collapse may be due to a loss of autonomic control. Thus, a decreased blood hydrostatic pressure could change the equilibrium at the level of the capillaries with a resultant net influx of fluids from the tissues. This also is supported by work of Kadono and Besch (13) who reported a decrease in mean systemic blood pressure of approximately 20% in SCWL following exposure to a 12% oxygen environment.

Preliminary studies performed in this laboratory demonstrate that hemodilution in anesthetized domestic fowl may occur within minutes after exposure to acute hypoxia (8% oxygen) but return to normal pretreatment values after the

hypoxic stimulus has been removed. Male, SCWL were unidirectionally ventilated (15) in series, at 550 ml/minute with two gas mixtures, 8% and 100% oxygen. A venous PCV and plasma protein concentration were taken while the animal was spontaneously breathing room air and again after a five minute exposure to 8% oxygen. The animal was then immediately ventilated with 100% oxygen for ten minutes at a similar flow rate. In all cases the PCV and plasma protein concentration decreased while the animal was ventilated with the 8% oxygen. After being ventilated with the 100% oxygen the PCV and plasma protein concentrations returned to their normal pretreatment values (table 5). These data indicate that a hemodilution occurs rather quickly (about six minutes) following exposure to 8% oxygen. The rapidity of this response is similar to the one reported for diastolic blood pressure (6) during acute hypoxia and is further evidence of a relationship between decreased peripheral resistance and hemodilution.

Although the hypoxic animals lost a slightly greater percentage of their total body weight than the controls during the experiment (table 1) they did not lose a significant amount of body water. That weight reduction may have been the result of water loss from the respiratory tract as a result of hyperventilation. Surks et. al. (21) studying the body composition of soldiers at 4,250 m (14,100 ft.) found body weight reductions but no evidence of a negative water balance. Hannon et. al. (11) using the deuterium oxide technique, found no

Table 5. PCV and plasma protein changes in male, anesthetized SCWL unidirectionally ventilated at 550 ml/minute with various gas mixtures.

TIME (min.)	% OXYGEN	PCV (%)	P ¹	PLASMA PROTEINS (gm/100 ml)	P
T ₀ ²	21 ³	49.1 ± 0.6 ⁴		6.1 ± 0.2	
T ₀ + 6	8	45.1 ± 1.2	<0.05	5.5 ± 0.1	<0.05
T ₀ + 15	100	48.9 ± 1.2	N.S.	6.1 ± 0.2	<0.05

¹Statistical probability - Student's T-test.

²T₀ = time 0

³⁴ determinations were performed for each time and oxygen concentration.

⁴Mean ± standard error.

change in the body water compartment of humans at an altitude of 4,250 m (14,100 ft.).

The slight difference between the percentage changes for plasma proteins (-10.0%, table 3) and plasma volume (+11.0%, table 2) suggests that there may be some loss of protein from the vascular compartment perhaps as a result of an increased capillary permeability due to hypoxia. Siggard-Anderson et. al. (17) have shown that there is no increase in vascular permeability in humans as a result of acute hypoxia. VanLiere and Stickney (22) report that the capillary permeability to fluid and protein does not increase until the venous PO_2 falls below 10-12 torr in humans. Thus, it seems that the loss of protein from the blood plasma probably cannot account for this discrepancy and that hemodilution is due to the increase in plasma volume.

Based on the red cell fragility data obtained in this study as well as that reported elsewhere (19), it is not likely that any part of the decreased PCV was due to hemolysis.

The general conclusion is that domestic fowl exposed to 8% oxygen, under the conditions described in this study, will experience a net increase in their plasma volume while total body water will remain the same. The sources of this fluid must be either a) intracellular water or b) interstitial water. A study of these pool changes during acute hypoxia in domestic fowl may be worthy of further investigation.

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APPENDIX I

To determine the plasma volume the indicator dilution technique was used. The indicator was radio-iodinated (^{125}I) human serum albumin (RISA) obtained from Abbott Laboratories.

The labeled albumin was supplied in 110 ml. vials containing 10 μCi $^{125}\text{I}/\text{ml}$. A 5 ml. aliquot of the original sample was diluted in a 10 ml. volumetric flask to contain 5 μCi $^{125}\text{I}/\text{ml}$. The animals were injected with 0.5 ml. of this solution via a cannula in their cutaneous ulnar vein. Three post injection samples of 0.3-0.5 ml. were taken at 3, 5, and 10 minutes. The samples were limited to 0.3-0.5 ml. in order to avoid the problem of hemodilution as reported by Sturkie (1965). Previous work by Sturkie (1965) has shown that the mixing time of dye in vascular systems of chickens is approximately three minutes. These data and that reported by Burton et. al. (1967) suggest that three minutes is ample time for the complete mixing of RISA. The whole blood samples were spun down and a 0.1 ml. portion of the plasma collected. The plasma was diluted to a volume of 1.0 ml. and counted. A standard (std.) was made by diluting a 0.5 ml. portion of the injectable solution to 100 ml. in a volumetric flask. A 0.1 ml. aliquot of the standard was counted simultaneously with the plasma samples in order to determine the counting efficiency for this system. The efficiency factor was used to convert net counts

per minute to $\mu\text{Ci } ^{125}\text{I/ml.}$ of plasma.

It was found that the labeled albumin left the vascular compartment at a slow rate. This is seen in the straight line relationship of a semilogarithmic plot of $\mu\text{Ci } ^{125}\text{I/ml.}$ of plasma vs. time (Fig. 1), which indicates that the concentration of the labeled albumin changes exponentially with time. Since the slope of the regression line was close to zero for the first 30 minutes the mean of the counts per minute of the three plasma samples was used to calculate the plasma volume. The following relationship was used:

$$\text{Plasma Volume} = \frac{\mu\text{Ci } ^{125}\text{I injected}}{\mu\text{Ci } ^{125}\text{I/ml. plasma}}$$

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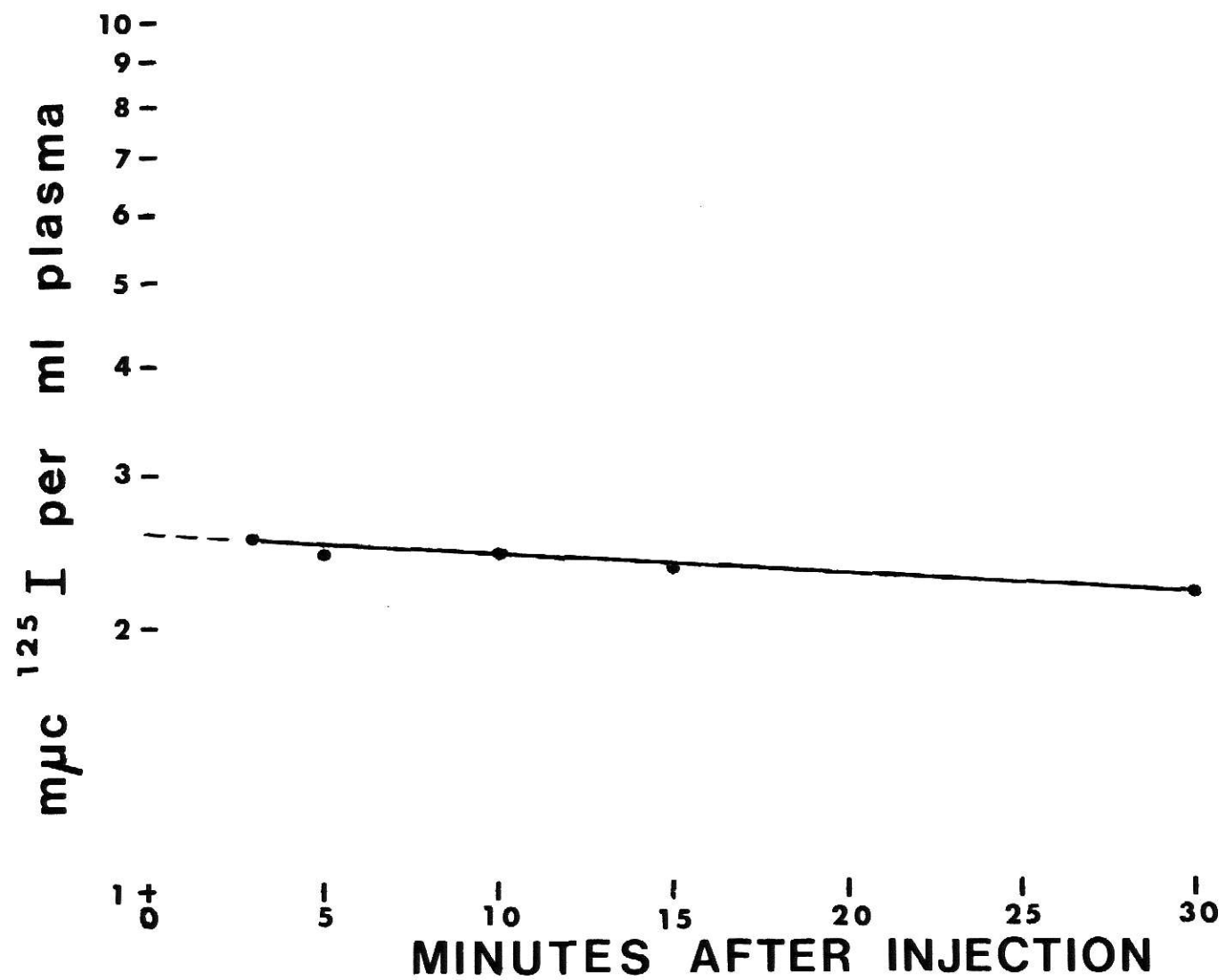


Figure 1. Semilogarithmic plot of specific activity of radio-iodinated serum albumin (RISA).

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ABSTRACT

Unanesthetized, adult, male Single Comb White Leghorns (SCWL) chickens, averaging 2.5 kg. in weight were acutely exposed to hypoxia (8% oxygen) in an environmental chamber. The air in the chamber was diluted with tanked nitrogen to obtain the desired oxygen concentration (8%). Fluid shifts were monitored by utilizing tritiated water (total body water) and radio-iodinated (^{125}I) human serum albumin (plasma volume). Total body water remained unchanged while there was a definite increase of +11% plasma volume ($P < 0.05$) after a two hour exposure to 8% oxygen. Another consistent finding was a significant decrease ($P < 0.05$) in both packed cell volume (-8.3%) and plasma protein concentration (-10.0%) as a result of hypoxia. Thus, an increase in plasma volume with a concomitant decrease in packed cell volume and plasma protein levels while total body water remained the same, indicates that there was a hemodilution at the expense of some other body fluid pool (i.e., intracellular fluid or interstitial fluid). Preliminary studies for this research on anesthetized SCWL, unidirectionally ventilated with 8% oxygen demonstrated that this hemodilution occurred within six minutes after exposure. Also, the increase in plasma volume returned to normal pretreatment values in a similar amount of time after the removal of the hypoxic gas.