Changes in Blood and Plasma Volumes in Dogs During Decompression Sickness

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Plasma and red cell volumes were measured using isotope labelling in 12 dogs given various types of decompression from 220 foot air dives. A 25- to 30-min dive produced a limb bend, and a 35- to 40-min dive produced a spinal cord lesion (paresis). A short dive to 70-90 ft following the 220-ft dive was used to prevent severe chokes and cardiopulmonary collapse. Four dogs making safe dives (no decompression sickness) and four dogs with limb bends showed no change in blood, RBC, or plasma volumes, while four dogs with paretic dives showed loss of plasma and elevated hematocrit. The I^{125} albumin was less reliable than the Cr^{51}-RBC label in demonstrating the plasma volume shifts. Plasma loss is a significant component of severe decompression sickness and should be considered in severe clinical cases.

HYPOVOLEMIA and hemoconcentration occur in severe decompression sickness (DCS). Cockett[6] described a reduction of plasma volume in dogs after decompression from an air dive to 165 ft for 1 hr. He measured plasma volume with radioiodinated albumin 30 min and 5 hrs after the dive and attributed the post-decompression reduction to a shift of plasma from the intravascular to the extravascular space. Hemoconcentration measured by a rising hematocrit has been described in a few human cases of DCS and is thought to be a sign of severe disease.[2,4,5] Haymaker[12] noted in severe cases of DCS which came to autopsy that large amounts of plasma were sequestered in the lungs, producing pulmonary edema. Since the relationship of plasma volume to severity of DCS is not clear, we studied this relationship in dogs given different types of DCS. The time-course of blood, plasma, and red cell volume changes were followed after dives within the accepted limits of safety; after dives which caused limb-bends, a relatively minor form of DCS; and after dives which caused paresis, a severe form of DCS. Since the integrity of the plasma space is affected by increased capillary permeability, measurements of the intravascular space with radioiodinated albumin may be misleading. Erythrocytes do not leak from the vascular space when capillary permeability increases; and when labelled with Cr^{51}, they provide a detectable indicator which resides only in the intravascular space. Thus red blood cell volume can be measured directly and with an appropriate hematocrit value, the total intravascular volume can be calculated. Albumin labelled with I^{125} and autologous red cells labelled with Cr^{51} can be injected together so that albumin and red cell spaces can be measured simultaneously.

MATERIALS AND METHODS

Conditioned mongrel dogs, weighing between 15 and 20 kg, were studied not less than 3 weeks after a splenectomy had been performed. The study group comprised 12 dogs—four were used as controls, four sustained limb-bends without evidence of paresis, and four sustained a neurological injury from DCS. All animals were studied awake* with a short percutaneous indwelling plastic cannula in an extremity vein. The animals were given a double dive; the first a 220-ft dive for 25 to 40-mins followed by direct ascent to the surface at 60 ft/min. Afer a short surface interval, a second dive to 70-80 ft was made. This second exposure was used to prevent cardiorespiratory collapse. The animals were observed carefully during a slow return to the surface, the rate of ascent being determined by the animal's behavior and observable evidence of a lesion.

All animals had blood samples drawn periodically at 100 to 140 mins prior to the dive. These were used to establish a normal disappearance curve for each indica-

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The animals used in this study were handled in accordance with the provisions of public law 89-44 as amended by public law 91-579, the Animal Welfare Act of 1970, and the principles outlined in the Guide for the Care and use of Laboratory Animals; U.S. Dept. of Health Education and Welfare Publication No. (NIH) 72-73.

The opinions contained herein are the private ones of the authors and are not to be construed as reflecting the views of the Navy Dept.

*Awake animals were necessary in this study because of the difficulty in obtaining a given response to a specific hyperbaric exposure. The individual variation among dogs precluded use of a fixed schedule to produce the desired response, and only by observation of behavioral changes could we decide when to partially recompress the dog to prevent severe DCS.

tor. After this initial period, those animals used for controls were placed in a 3 x 7 ft hyperbaric chamber and held at the surface for 5 mins while the chamber was vented to produce the noise equivalent to a dive. After 120 mins of further sampling, a dive of 220 ft for 5 mins was performed. Observation through the chamber ports showed no evidence of abnormal behavior or any activity suggesting discomfort of the animal. After a 4 min decompression, blood samples were again drawn periodically for measurement of isotope concentration. No adverse affects were noted from this dive profile, and the animals evidenced no discomfort upon return to the surface. The four animals in the limb-bend series were studied in the same manner as controls except that the first dive was a safe dive to 220 ft for 5 mins, followed by a dive to 220 ft for 20 to 25 mins with decompression to the surface over 4 mins (Fig. 1). They were recompressed to 70-90 ft after a 3-min surface interval during which no clinical signs of DCS were evident. After a stay of about 2 mins at depth, the ascent to the surface usually lasted 15 to 20 mins and was controlled by careful observation of the animal to preserve the limb-bend and avoid more serious forms of decompression sickness.

Upon arrival at the surface, these animals exhibited impaired motion of one limb. Limb-bends were manifested by an animal's reluctance to use a limb accompanied by an intact placing reflex, a normoactive deep tendon reflex and no demonstrable weakness in that limb. Aside from a limping gait, these dogs showed no evidence of distress. Four animals were given a dive (Fig. 2) designed to produce spinal cord paralysis. Again, the paretic dive was preceded by a safe dive control of 220 ft for 5 mins. The animals were then dived to 220 ft for 40 mins, brought directly to the surface at 60 ft/min, observed until early signs of decompression developed, then returned to 70 ft. Cardiopulmonary difficulties were controlled by recompression to 70 ft and slow return to the surface. Upon surfacing, the animals exhibited rapid respiration, lethargy, and signs of neurological damage such as loss of panniculus reflex, paralysis of one or more extremities, hypoactive or hyperactive deep tendon reflexes, and extensor rigidity of one or more limbs. Blood samples were drawn up to 50 mins after completion of the dive and the dogs were then sacrificed with a large dose of barbiturate anesthesia.

Isotope Counting Procedure: Standard Cr^{51} labelling[1] of the dog's own red cells was done on the morning of the experiment. For each sample, 1.5 ml of blood were drawn and then 1.0 ml was added to 4 ml of distilled water in a tube designed for use in an automatic 2-channel gamma counter (Nuclear-Chicago Corp.). When all samples, blanks and standards were prepared, they were read in the gamma counter for I^{125} and Cr^{51} activity. The count data were punched onto paper tape for computer analysis. Samples for hematocrit using a micro-determination were also drawn from each 1.5 ml sample.

Mathematical Analysis: Red cell and plasma volumes were calculated by extrapolating the concentration-time curves for Cr^{51} and I^{125} respectively backward to zero time. Volume is found by the formula $V = I \div C$ where V is the calculated volume, I the amount of isotope injected and C, the concentration at zero time. The backward extrapolation was performed by computer using a second order polynomial as described below.

Since we were interested in the time course of the plasma and blood volume changes, we developed a method similar to the technique of Gibson and Evans[9] for calculating blood volume at each data point after injection of the isotopes. The initial 100- to 140-min control period in each dog was used to determine the curve of isotope concentrations *vs* time, the normal disappearance curve. To determine the best-fit curve for the control data, a second order curve was fitted to the log-concentrations *vs* time curve. This curve in the control dogs gave reasonable predictions of the time course of isotope concentration up to 400 mins after injection of the label. The log-concentration curve is not linear, indicating that the decay curve of isotope concentration is not a mono-exponential. Since we were interested in predicting the

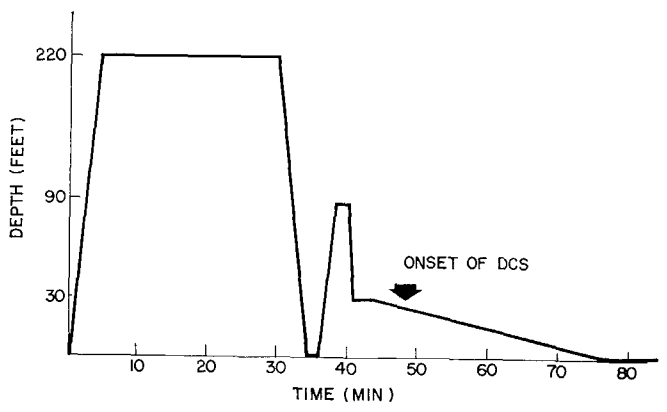


Fig. 1. Dive profile which produced limb bend in four dogs. Second dive to 90 ft is used to prevent severe decompression sickness. Slow ascent to surface is done while observing dog in chamber to preserve the limb bend.

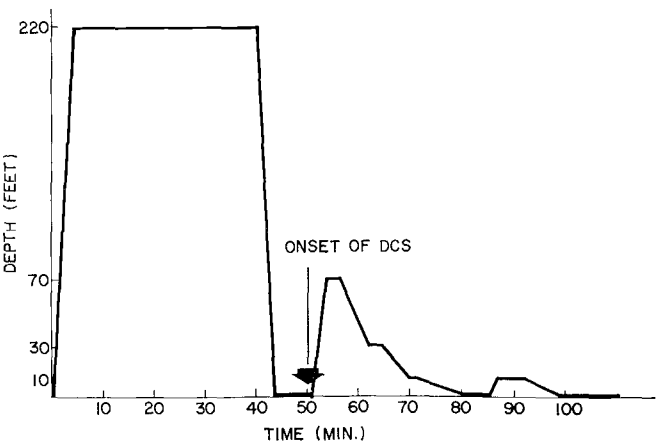


Fig. 2. Dive profile which produced a paretic lesion in four dogs. Second dive to 70 ft prevents severe DCS and slow ascent is used to preserve the lesion.

curve up to 400 mins, and not in a specific analysis of the component exponentials making up the concentration curve, we sought a mathematical model which fit the data, rather than one which predicted the compartmental components of the curve. For our purposes, a second-order curve fit well in almost all cases. Swan *et al.*[26] discussed the problems with this method and developed a technique for eliminating some of the errors of the method. Our use of a nonlinear regression curve and the technique of least squares fitting of the data are other means of reducing the error in this method. In a few dogs, the second order curve had an inflection point, usually at 200 to 250 mins, where the predicted concentrations began to rise. This was corrected by converting the predicted second-order curve to first order beginning one sample before the inflection, and continuing the predicted curve as a mono-exponential. The technique worked well in those animals where it was used. The presence of an inflection point was detected by the analysis program which printed a message that the first order option was being used for the tail of the curve. In addition to predicting the concentration curves, the program corrected the 2-channel isotope data for channel crosstalk using single isotope reference solutions which were counted before the blood samples in each experiment. At each sample point, a comparison was made between the actual and predicted isotope concentrations. The ratio of predicted to actual concentration times the initial volumes gave the red cell plasma volumes at each point. From these data, and the hematocrit, a comparison of blood, plasma and red cell volumes for each indicator could be obtained, as well as a total blood volume which was the sum of measured plasma and red cell volumes. The computer program also provided curves of each volume, the actual and calculated isotope concentrations and hematocrit along with numerical data.

For each condition (control, limb-bend, paresis) data from the four animals were combined to find the mean and standard error of sample points for the curves of blood, plasma and red cell volumes and hematocrit.

In one animal not included in the control series, 220 ml of whole blood were withdrawn on the morning of the

	CR^{51} - RBC			I^{125} - ALBUMIN		
	BLOOD VOL	PLASMA VOL	RBC VOL	BLOOD VOL	PLASMA VOL	RBC VOL
CONTROL	1833	1230	592	1817	1227	591
+ 200 ML BLOOD	2030	1334	696	2082	1368	714
Δ V	207	104	104	265	141	123

Fig. 3. Results of one experiment used to evaluate the indirect volume measurement method used for the DCS experiments. Two blood labels were used: Cr^{51} tagged RBC and I^{125} labelled albumin. There were 200 ml augologous whole blood injected. ΔV represents measured addition using both labels.

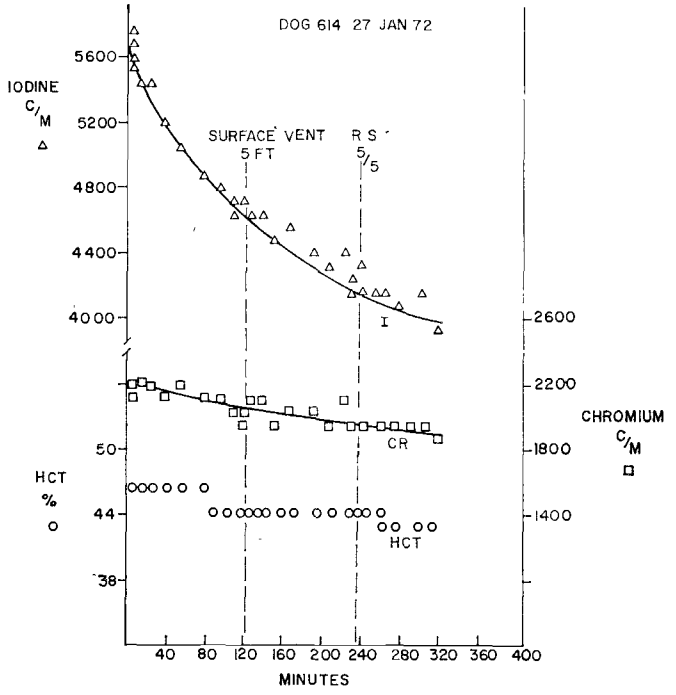


Fig. 4. Hematocrit and isotope decay curves for Cr^{51} and I^{125} in a control study. Solid lines represent best-fit curve to the 0-120 min samples. Open triangles are actual I^{125} concentrations in counts/minute-ml, open squares are actual Cr^{51} concentration, open circles are hematocrit values measured from the same blood samples.

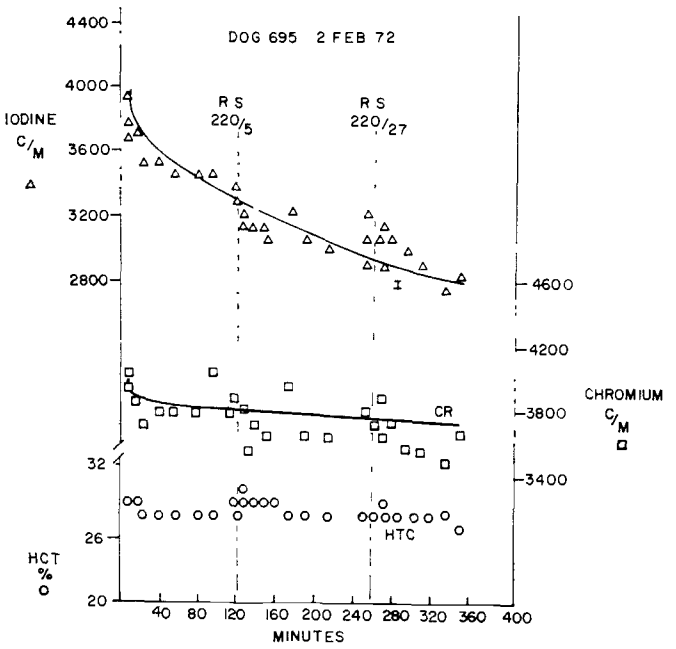
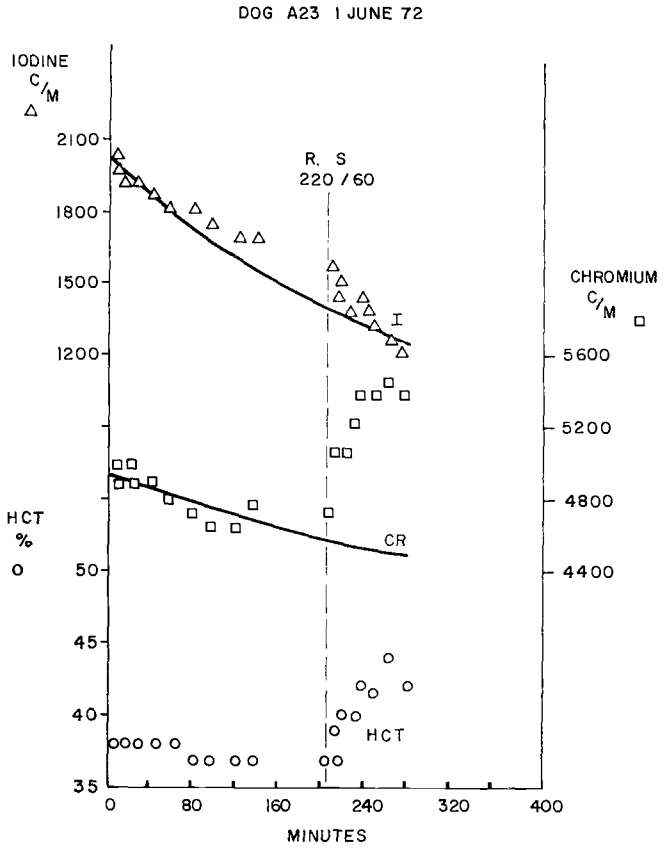


Fig. 5. I^{125} and Cr^{51} kinetics and hematocrit in a dog with limb bend. Solid line is best-fit curve from 120 min control period. Control dive of 220 ft for 5 min is indicated by vertical dashed line. Experimental dive of 220 ft was for 27 mins.

Fig. 6. I^{125} and Cr^{51} kinetics and hematocrit in a dog with a spinal cord lesion from decompression sickness. A single dive to 220 ft for 60 min was performed (vertical dashed line). Note rise in hematocrit and Cr^{51} concentration after dive.



experiment. Of this, 20 ml were used for labelling red cells with Cr^{51} the remaining 200 ml was heparinized for later injection. After 2 hrs, the isotope indicator was injected and samples were taken at intervals for 140 mins. At 140 mins, the 200 ml was re-infused into the dog and sampling was continued for another 100 mins. The data were subjected to analysis as described above and accurately indicated the reinfusion of the previously withdrawn blood. Fig. 3 summarizes the data from this experiment.

RESULTS

Fig. 4 shows the pattern of isotope concentration *vs* time for one of the control animals. The rate of decay for Cr^{51}, though more rapid than in man, was slower than that of I^{125} and consistent in all the dogs studied. The mathematical model predicted the tail of the chromium curve accurately in the control series so the rapid decline in Cr^{51} label did not appear to alter the experimental data.

Fig.5 shows isotope concentration curves for an animal from the limb-bend group. This dog performed two

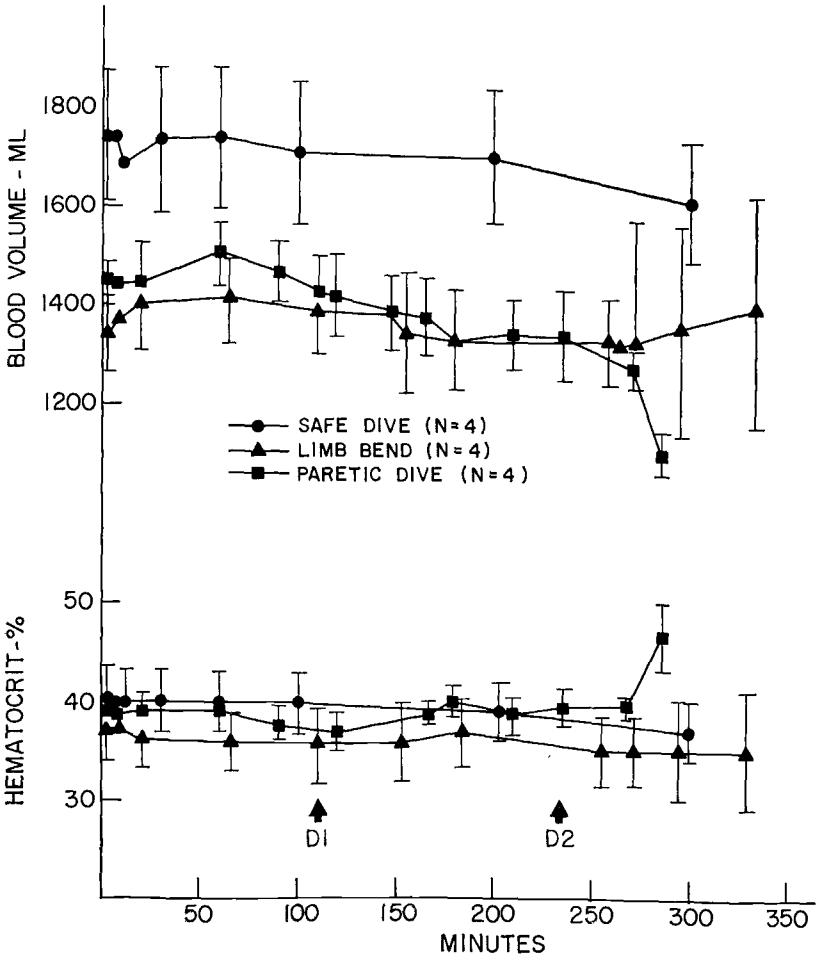


Fig. 7. Composite blood volume and hematocrit data for all experiments. Each point shows mean ± standard error. Note fall in blood volume, rise in hematocrit in the paretic series. No significant changes are noted in safe or limb-bend dives. D1— control dive, D2— experimental dive.

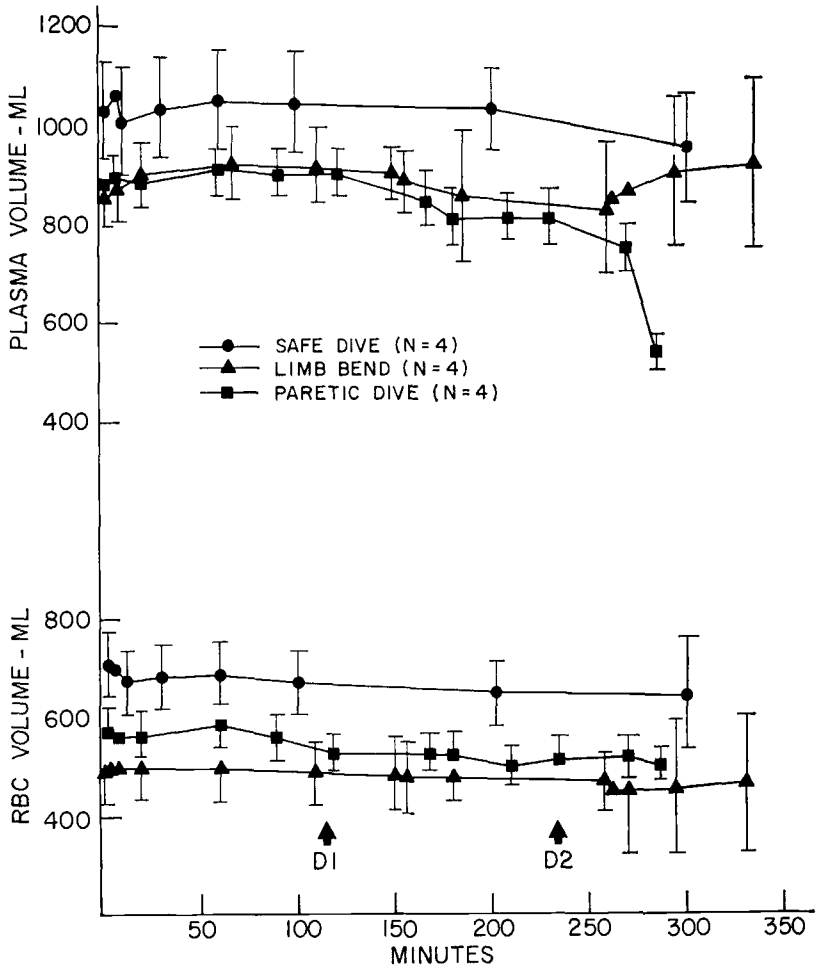


Fig. 8. Composite plasma and RBC volume data for all experiments calculated from Cr^{51} kinetics. Plasma volume falls only after the paretic dive. (D2). RBC volume remains unchanged in all experiments.

separate dives. The first dive was the safe dive control. No sustained changes in blood, plasma or red cell volumes were found after either control or limb-bend-producing dives.

A representative curve of isotope concentration for a dog from the paretic series is shown in Fig. 6. This animal had no control dive. He was studied in the large chamber complex at the Naval Medical Research Institute where both the animal and the investigative team could dive together. Because of the additional problems of locking the investigators into the chamber after the 200 ft dive, the control dive was eliminated from the protocol. After the dive there was a change in plasma and blood volumes, and a rise in hematocrit and isotope concentration following the onset of DCS. Signs of neurological impairment were established by examination of the dogs before and after the onset of DCS.

All animals in this series sustained neurological deficits and, in addition, had respiratory difficulty. Death often occurred 35 to 40 mins after surfacing.

The average data for all dogs in each series are shown in Figs. 7 and 8. These curves are derived from the chromium concentration data and show the changes in red cell and plasma volume that occurred after the onset of paresis. This change in plasma volume is not found in controls or in the limb-bend series and in no instance was there a change in red cell volume. Plasma volume

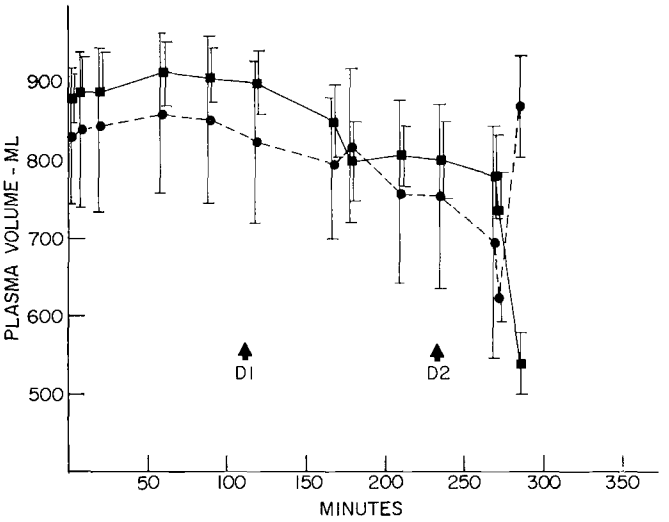


Fig. 9. Comparison of plasma volume changes in four paretic dives measured with two blood labels. Solid squares represent volume derived from Cr^{51}—RBC label and hematocrit, circles represent plasma volume measured with I^{125} albumin label. A marked rise in plasma volume is noted in albumin curve after second dive (D2) while the RBC curve shows a reduction in plasma volume. D1 is a control dive.

measured with I^{125} labelled albumin showed a smaller change during paretic decompression sickness. Indeed the plasma volume showed a rise when measured with I^{125}. (Fig. 9). This might be expected because the al-

bumin space may actually enlarge as capillary permeability is increased causing albumin to leak at an accelerated rate into the interstitial space. Hematocrit and whole blood volume curves for the three groups are shown in Fig. 7. The hematocrit in the control and limb-bend groups showed no change while a marked rise was found in all animals in the paretic group. We have previously observed similar hematocrit changes during severe DCS in other animals who developed paresis after similar dive profiles. Whole blood volume changes are due to changes in plasma volume in the paretic group.

DISCUSSION

It is likely that limb-bends are the mildest form of a progression of abnormalities which culminate in hypotension, plasma loss, pulmonary edema, and death. The quantity of gas released from tissues and the magnitude of the hemodynamic and hematologic reaction to the gas emboli may determine where, in a spectrum of symptoms or severity, the individual diver with DCS will be. In this study, we have been able to correlate the severity of the clinical lesion of DCS with blood and plasma volume changes, and have corroborated the data of others that plasma loss occurs in DCS.

Post-dive bubbles in the hearts of divers without clinical evidence of DCS have been established.[8] This finding suggests that DCS is a disease which is dependent on bubble dose. In our limb-bend animals we noted no change in blood or plasma values, hematocrit was stable, and the animals showed no significant systemic response to the inert gas load which they experienced. This relatively minor type of DCS gave way to bends with CNS injury, evidence of pulmonary dysfunction and finally circulatory collapse. Only with this severe DCS syndrome, was there a loss of plasma from the vascular space, reduced blood volume and hemoconcentration. The large inert gas load in this case apparently was enough to cause normal defenses against vascular embolism to become overwhelmed. Thus the amount of free gas in blood and tissues determines the degree of severity of DCS not only by producing a greater degree of obstruction, but also by secondary effects, such as the blood and plasma volume changes described herein.

The exact cause of these secondary changes remains speculative but effects of obstruction by bubble emboli as well as activation of vasoactive compounds, platelets, or clotting factors, should be considered.

The primary treatment for DCS is recompression followed by a slow decompression. In many instances, this is sufficient and no other therapy is needed for the diver. However, as high as 20% of cases in some series[18,29] may not respond to initial recompression or will have recurrences or some permanent residual injury. We know at present, however, that a series of systemic reactions occur which may not respond to recompression alone.[16,17,20,21,28] When hemoconcentration occurs in severe DCS, there has been injury to capillary endothelium which may not immediately reverse with recompression. Plasma leakage from the vascular to interstitial spaces with subsequent loss of blood volume, rising hematocrit, and a tendency for blood to sludge[13] may not respond to recompression alone.

Plasma leakage into the interstitial spaces should become manifest as edema. In our dogs, evidence of pulmonary edema was noted terminally in all the paretic animals. Haymaker[12] noted pulmonary edema in *post mortem* examination of divers and aviators who died from severe DCS and Dahlgren & Josephson[7] found evidence of pulmonary edema in dogs that had been given acute pulmonary air embolism by intravenous injection. Thus the lungs appear to be at least one of the target organs where capillary injury occurs. Khan *et al.*[15] found evidence of bubble-induced bronchospasm in dogs given intravenous air. Using antagonists, they concluded that serotonin and possibly other amines were responsible for these changes. From this study and others[14] the lung appears to be the target organ for venous air emboli. Since the lungs are probably the first organ subjected to the gas embolism of DCS[10,25,27] it is reasonable to expect that pulmonary vascular alterations would occur.

The mechanism whereby bubbles cause injury to capillary endothelium is presently unclear. Although direct evidence is lacking, a number of mechanisms have been suggested. A most important area of consideration is the activation of blood constituents by the gas-blood interface. When platelet aggregation occurs at the bubble surface *in vivo*[19,24] the subsequent platelet release reaction produces serotonin and other active compounds which can alter capillary permeability. We have shown[11] that Hageman factor (Factor 12) activation occurs when bubbles are present in blood *in vitro*. Reactions subsequent to platelet aggregation or Factor 12 activation cause serotonin release, kinin and complement activation, and activation of the intrinsic clotting mechanism.[22,23] Many of the compounds released when these various systems undergo activation are known to produce capillary endothelial damage as well as vaso-constriction or dilation, and broncho-constriction. Any of these responses can cause increased capillary permeability and loss of plasma from the vascular space.

Because of the possibility of other effects besides the obstruction of lymphatics and small blood vessels produced by the bubble, therapy for DCS which consists only of recompression may not always be adequate. Indeed, cases occur where divers improve only temporarily on recompression therapy, then show deterioration while still at treatment depth in a chamber. Since, in severe cases, hypovolemia, hypotension, and pulmonary edema may be present, therapy directed at these abnormalities may be necessary as an adjunct to recompression.

Thus, a comprehensive treatment plan which includes not only recompression but also administration of agents designed to block the various systems which cause injury to tissue and blood vessels, should be considered in the treatment of decompression sickness.

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