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Blood-brain and blood-lung barrier alteration by dysbaric exposure

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Chryssanthou, C., M. Springer, and S. Lipschitz. 1977. Blood-brain and blood-lung barrier alteration by dysbaric exposure. Undersea Biomed. Res. 4(2): 117–129.—Failure of certain circulating substances to penetrate specific organs led to the concept of blood-organ barriers. Such barriers can be altered by various physical or chemical means. This report concerns modification of the blood-brain barrier (BBB) and blood-lung barrier (BLB) by dysbaric exposure. Trypan blue was intravenously administered to 19 experimental rabbits (subjected to compression-decompression) and to 11 controls (kept at ambient pressure). Gross and microscopic examination and measurements of dye extracted from tissues revealed greater dye penetration into lung and brain of the experimental animals. Dye concentration in brain was 12.10 μg/g tissue in experimental and 2.93 μg in control animals; in lungs it was 935 μg and 434 μg, respectively ($0.01 > P$ 0.001). Increased permeability of BBB and BLB was associated with intravascular bubbles. The mechanism of BBB and BLB alteration may involve chemical agents activated by gas-blood interface or vascular injury produced by bubbles. These observations could have pathogenetic implications in decompression sickness and may suggest new methods for facilitating penetration of therapeutic agents into the brain.

blood-organ barriers
trypan blue
rabbits
permeability
compression-decompression
intravascular bubbles
decompression sickness
pathogenesis

After Goldman (1913) demonstrated the peculiar impermeability of the brain to trypan blue at the turn of the century, the concept of barriers was advanced to explain the observation that certain circulating substances fail to penetrate specific organs or tissues. The presence of the so-called blood-brain barrier (BBB) has been long established. The relative impermeability of other organs or tissues to certain drugs, vital dyes, colloids and other substances led to the postulation of barriers which separate blood from the fetal side of the placenta, the testis, the interior of the eye, etc. The existence of a blood-lung barrier (BLB) was proposed to account for the failure of this organ to stain by intravenously administered chlorophyllin, trypan blue, and tetrazolium salts (Chryssanthou and Antopol 1961; Chryssanthou and Antopol 1963).

The permeability of blood-organ barriers can be altered by various chemical and physical means. Venoms, allergic agents, bacterial products, bile salts, artificially induced seizures, X-irradiation and gas embolization have been reported to alter the BBB (Bouton 1940;

Bjerner, Broman, and Swensson 1944; Broman and Lindberg-Broman 1945; Broman 1949; Clemente and Holst 1954; Eckman, King, and Brunson 1958; Johansson 1975) and bradykinin and bacterial endotoxins were shown to increase permeability of the BLB (Chryssanthou and Antopol 1961; Chryssanthou and Antopol 1963).

The present study is, to our knowledge, the first report concerning alterations of the BBB and BLB induced by exposure to dysbaric conditions.

MATERIALS

Albino female rabbits weighing 3–4 kg were employed. They were housed in metal cages in animal rooms with controlled temperature (65°–68° F) and relative humidity (50%) and were fed Purina Rabbit Chow and water ad libitum. The animals were kept under these conditions for a stabilization period of at least two weeks before they were used.

A hyperbaric chamber (Bethlehem Corporation, Model 1835 HP) with controlled temperature and relative humidity was utilized. The chamber was pressurized with air (dry air cylinders, Matheson Company, Inc.). Solutions of 1000 u/ml sodium heparin (Upjohn Company) and of 2% trypan blue (K and K Laboratories, Inc.) in sterile normal saline were prepared for intravenous injections. The extractant (for trypan blue extraction from tissues or blood) consisted of four volumes of 95% ethanol and one volume of 17% benzalkonium chloride (Zephiran, City Chemical Corp.)

METHODS

The animals were numbered, weighed, and randomly divided into an experimental (subjected to compression-decompression) and a control group (kept at ambient pressure). Both control and experimental animals received an intravenous injection (marginal ear vein) of trypan blue solution (4 ml/kg). The experimental animals were injected within 4 min after decompression to sea level. They were then observed for clinical manifestations of decompression sickness until they died or were killed. The animals which died within 75 min after decompression were not included in these studies. Those which survived for more than 90 min postdecompression were killed by intravenous administration of sodium nembutal. Control animals were killed at intervals corresponding to those of the experimental animals. Just prior to killing, or when death appeared imminent in the experimental group, all animals received an intravenous injection of heparin solution (1 ml/kg) to maintain liquidity of the blood and permit perfusion of tissues. Prior to heparin administration a blood sample was obtained for dye concentration determination. Immediately after death the animals were autopsied and the degree of gross staining of the lungs and brain recorded. The animals were also inspected for the presence of gas bubbles in tissues or in blood vessels. The lungs were perfused with normal saline. Representative portions of lung and brain were fixed in formalin for histologic processing. Tissue sections (5-μm thick) stained with hematoxylin-eosin, light eosin, and unstained preparations were subjected to light and phase microscopy. The extent of trypan blue penetration into tissues was graded. The remaining lung and brain tissue was frozen for dye extraction at a later time. The data were statistically evaluated (Student's t-test).

Hyperbaric exposure

The experimental animals were subjected, one at a time, to 90 psig (202.5 fsw) air pressure for 12 min and then decompressed to sea level in 23 min. Figure 1 shows the dive profile

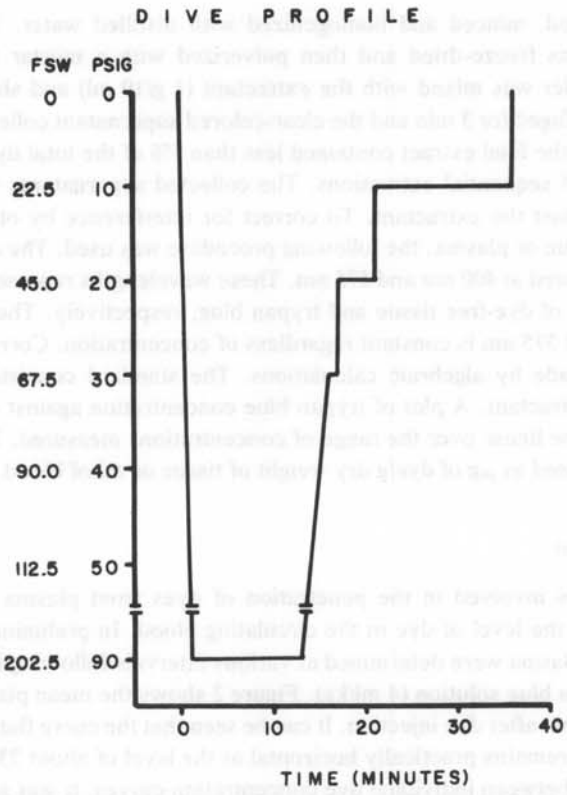

Fig. 1. Compression-decompression schedule used for experimental group of rabbits.

employed. This exposure produced decompression sickness with paraplegia in one third of the animals.

Gross and microscopic grading of tissue staining

The degree of tissue staining was graded grossly from 1+ to 4+ according to the intensity and extent of staining and microscopically from 1+ to 3+ on the basis of frequency of fields revealing the presence of dye in tissue and intensity of staining. The grading was done blindly by two observers.

Perfusion of lungs

The lungs of both control and experimental animals were perfused with normal saline (under 25 mmHg) by means of a catheter inserted into the pulmonary artery. Perfusion continued until the outflow of the perfusate was clear.

Determination of dye concentration

Trypan blue was extracted from tissues or from plasma samples by a method modified from Caster (Caster, Simon, and Armstrong 1953; Caster, Simon, and Armstrong 1954). Frozen

tissues were thawed, minced and homogenized with distilled water. The homogenate (or plasma sample) was freeze-dried and then pulverized with a mortar and pestle to a fine powder. The powder was mixed with the extractant (1 g/10 ml) and shaken for 1 min. The mixture was centrifuged for 3 min and the clear-colored supernatant collected. This procedure was repeated until the final extract contained less than 5% of the total dye extracted. Usually this required 4 to 5 sequential extractions. The collected supernatants were read in a spectrophotometer against the extractant. To correct for interference by other colored material extracted from tissue or plasma, the following procedure was used. The optical density of the extracts was measured at 400 nm and 575 nm. These wavelengths represent the maxima in the absorbance curves of dye-free tissue and trypan blue, respectively. The ratio of the absorbance at 400 nm and 575 nm is constant regardless of concentration. Correction for interfering substances was made by algebraic calculations. The standard consisted of the dye alone dissolved in the extractant. A plot of trypan blue concentration against optical density readings was found to be linear over the range of concentrations measured. The concentration of the dye was expressed as μg of dye/g dry weight of tissue or ml of blood.

Dye levels in plasma

Since diffusion is involved in the penetration of dyes from plasma to the tissues, it is important to know the level of dye in the circulating blood. In preliminary experiments dye concentrations in plasma were determined at various intervals following intravenous administration of 2% trypan blue solution (4 ml/kg). Figure 2 shows the mean plasma levels of trypan blue from 5 to 180 min after dye injection. It can be seen that the curve flattens 75 min after dye administration and remains practically horizontal at the level of about 230 μg/ml. Since there was little variation between individual dye concentration curves, it was assumed that animals which died or were killed at least 75 min after dye injection had approximately equal concentrations of circulating dye. This was confirmed in several experimental and control animals by determining dye concentration in blood samples obtained just prior to death.

RESULTS

Clinical manifestations

Seven of 21 animals subjected to dysbaric conditions developed decompression sickness. The disease was manifested by paralysis of the hind legs, twitching, and severe respiratory distress with panting and gasping. Two of the animals which suffered decompression sickness died a few minutes after decompression and consequently were excluded from the study.

Gross and microscopic examination

Lungs

On gross examination the lungs of all experimental animals appeared stained. In 53% of the animals the staining was intense, with a patchy or diffuse distribution. In the control group the lungs were stained in 82% of the animals, but the staining was usually focal and weak. Only one control animal (9%) exhibited intense staining. Figure 3 shows the gross appearance of experimental and control lungs. Microscopic examination of unstained preparations of exper-

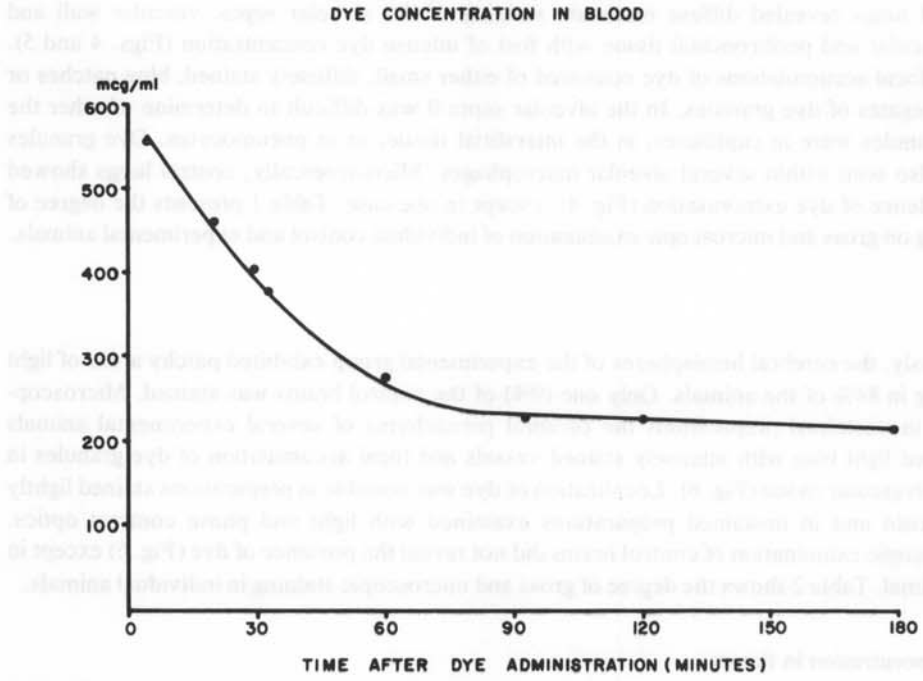

Fig. 2. Mean blood levels of trypan blue at various intervals after intravenous injection of dye.

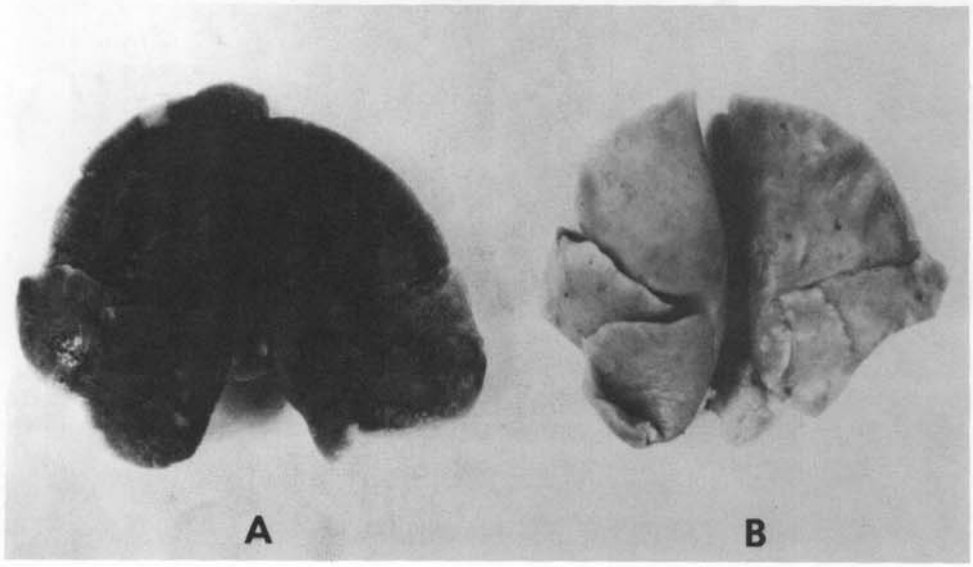

Fig. 3. Lung from rabbit subjected to compression-decompression (A) showing diffuse and intense staining. In contrast, corresponding control lung (B) appears practically unstained. (Experimental and control animals in this figure and in following microphotographs have been injected with same amount of dye and killed after same interval following dye injection.)

imental lungs revealed diffuse moderate staining of the alveolar septa, vascular wall and perivascular and peribronchial tissue with foci of intense dye concentration (Figs. 4 and 5). These focal accumulations of dye consisted of either small, diffusely stained, blue patches or of aggregates of dye granules. In the alveolar septa it was difficult to determine whether the dye granules were in capillaries, in the interstitial tissue, or in pneumocytes. Dye granules were also seen within several alveolar macrophages. Microscopically, control lungs showed no evidence of dye extravasation (Fig. 4). except in one case. Table 1 presents the degree of staining on gross and microscopic examination of individual control and experimental animals.

Brain

Grossly, the cerebral hemispheres of the experimental group exhibited patchy areas of light staining in 84% of the animals. Only one (9%) of the control brains was stained. Microscopically, in unstained preparations the cerebral parenchyma of several experimental animals appeared light blue with intensely stained vessels and focal accumulation of dye granules in the perivascular tissue (Fig. 6). Localization of dye was possible in preparations stained lightly with eosin and in unstained preparations examined with light and phase contrast optics. Microscopic examination of control brains did not reveal the presence of dye (Fig. 6) except in one animal. Table 2 shows the degree of gross and microscopic staining in individual animals.

Dye concentration in tissues

The mean dye concentrations in the lungs and brain of controls and of animals subjected to dysbaric conditions are shown in Table 3. The differences in the mean value between control

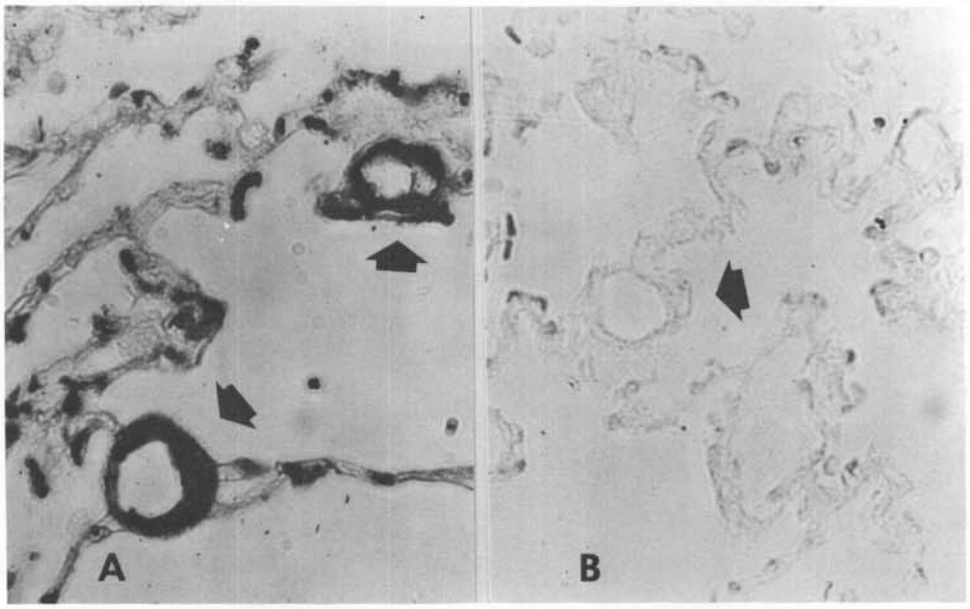

Fig. 4. Lung from a rabbit subjected to compression-decompression (A) exhibiting diffuse moderate staining of alveolar septa with focal concentrations of dye and intensely stained vascular walls (arrows). Compare with corresponding control (B) showing absence of dye from alveolar and vascular wall (arrow). (Unstained preparation, original magnification × 256.)

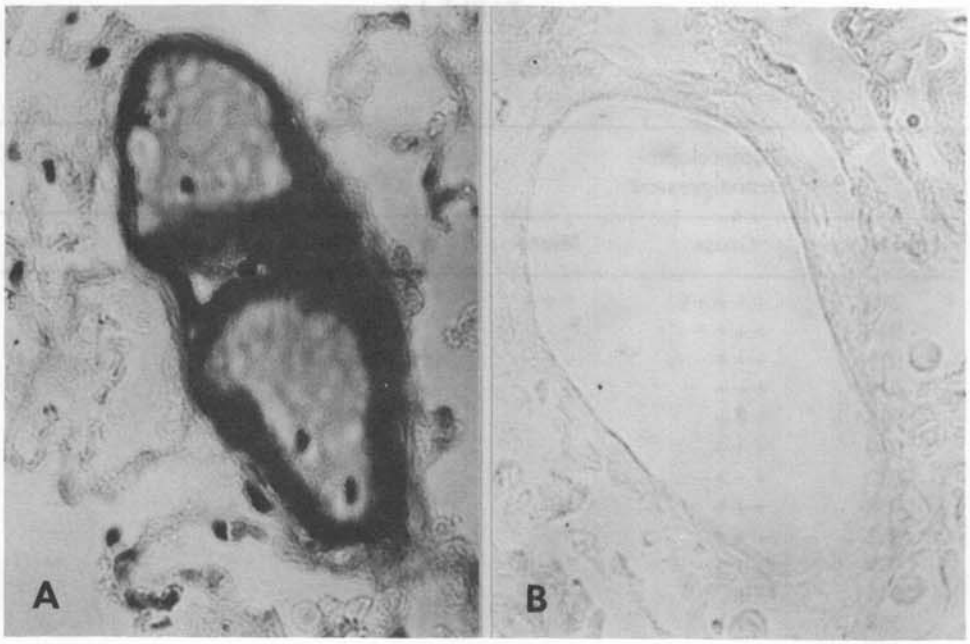

Fig. 5. Deeply stained vascular wall in lung of experimental animal (A) stands in contrast to the unstained vessel in corresponding control lung (B). (Unstained preparation, original magnification × 160.)

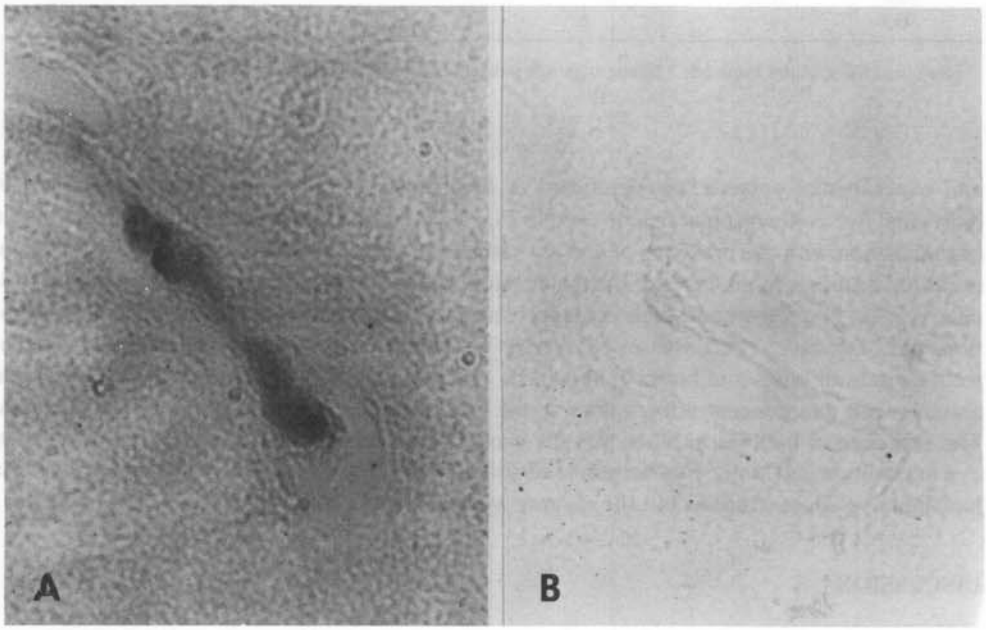

Fig. 6. White matter from cerebrum of an animal subjected to compression-decompression (A) exhibiting diffuse moderate staining of parenchyma and intensely stained vessel. Corresponding control (B) showing absence of dye in parenchyma and vessel. (Unstained preparation, original magnification × 256.)

TABLE 1

Degree of staining in lung

Compressed - Decompressed			Controls		
Animal No.	Gross	Micro	Animal No.	Gross	Micro
299	++++§	+++	962	+++	−
946	++++	*	300	++	+
939	+++	++	941	++	−
929	+++	+	943	++	−
935	+++	+	947	++	*
940	+++	+	986	++	−
932	+++	*	292	+	−
938	+++	*	942	+	−
952	+++	*	944	+	−
979	+++	*	287	−	−
945	++	+++	291	−	−
298	++	++			
937	++	++			
934	++	+			
936	++	+			
954	++	+			
981	++	+			
989	++	*			
933	+	+			

§See text for grading method; * tissue was not subjected to microscopic examination.

and experimental animals are significant at high levels of confidence. The distribution of individual dye concentration can be seen in Fig. 7. The same figure correlates the level of dye concentration with the presence of grossly visible intravascular gas bubbles and with decompression sickness. In all cases of decompression sickness there were grossly visible intravascular gas bubbles. However, their presence was not always associated with clinical manifestations of the disease. There was some overlap between control and experimental dye concentrations in both lungs and brain. It should be noted, however, that none of the experimental animals with dye concentration values in the control range exhibited intravascular bubbles. The presence of bubbles in blood vessels was always associated with high concentration of dye in the brain and lungs. Furthermore, all animals which manifested decompression sickness had high dye concentrations but the reverse was not always true.

DISCUSSION

Permeability of blood-organ barriers is not an "all-or-none" phenomenon. Most substances, including vital dyes, penetrate these barriers, though sometimes slightly or slowly. Therefore, barrier permeability to a substance should be quantitated and preferably expressed in terms of

TABLE 2

Degree of staining in brain

Compressed - Decompressed			Controls		
Animal No.	Gross	Micro	Animal No.	Gross	Micro
951	+++§	++	941	+	+
940	++	++	287	–	–
945	++	++	289	–	–
298	++	+	292	–	–
937	++	+	300	–	–
938	++	+	942	–	–
939	++	–	943	–	–
946	++	–	944	–	–
929	+	+	947	–	–
935	+	+	962	–	–
952	+	+	989	–	–
981	+	+			
933	+	–			
934	+	–			
989	+	–			
299	+	*			
954	–	+			
979	–	–			
932	–	*			

§See text for grading method; *tissue was not subjected to microscopic examination.

TABLE 3

Concentration of dye in tissue

Tissue	Compressed-Decompressed	Control	Significance
Lung	935 ± 128*	434 ± 66	$0.001 < P < 0.01$
Brain	12.1 ± 1.95	2.95 ± 0.63	$0.001 < P < 0.01$

Values are mcg/g dry tissue; *mean ± SEM; significant by Student's *t*-test for small samples of unpaired observations.

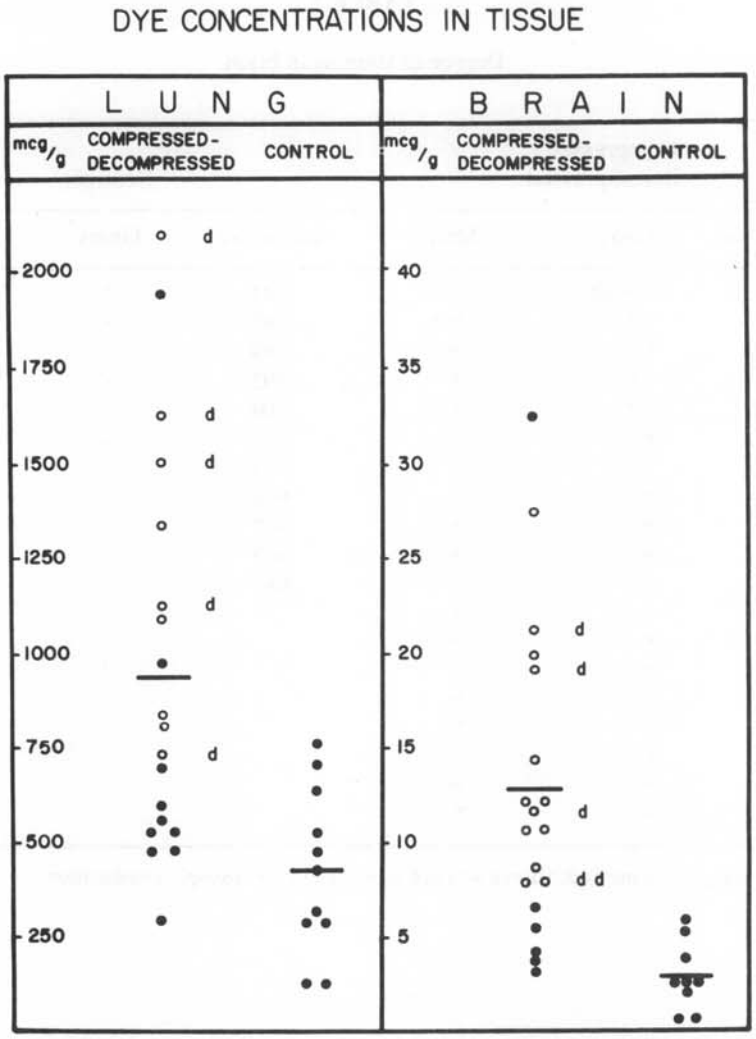

Fig. 7. Dye concentrations in lungs and brain of experimental and control animals. Note that in experimental animals with intravascular gas bubbles (○) dye concentrations are higher than in animals with bubble-free vessels (●). Horizontal bars represent mean values and (d) indicates animals which suffered decompression sickness.

rate of penetration. Comparison of barrier permeability to a circulating dye under different conditions is meaningful only when concentrations of the dye in tissue and plasma are determined and temporal parameters evaluated.

In the present study transgression of trypan blue through the BBB and BLB was quantitated in both control and experimental animals at a time when all animals had approximately equal plasma levels of trypan blue.

The results of gross and microscopic examination and dye concentration determinations show that a significantly greater amount of dye penetrated the barriers in experimental animals

than in controls. This indicates that dysbaric exposure increased the BBB and BLB permeability to trypan blue.

Bouton (1940) observed as early as 1940 that the brain appears grossly stained following air embolization and Broman (1949) showed disturbances in cerebrovascular permeability resulting from either air or fat embolism. It is not certain whether in our experiments barrier alterations were caused by gas or other emboli. There was, however, a definite correlation between grossly visible intravascular gas bubbles and increased dye concentrations in lung and brain. All animals which manifested decompression sickness exhibited increased barrier permeability but alteration of barriers was not always associated with decompression sickness. These observations suggest that gas bubbles can cause changes in barrier permeability even in the absence of clinical manifestations of decompression sickness.

The exact mechanism by which dysbaric exposure alters barrier permeability is obscure. Hypoxia caused by emoblization or other circulatory disturbances is not a likely pathogenetic factor since it has been shown that anoxic states of several hours' duration have no deleterious effects on cerebrovascular permeability to trypan blue (Broman 1949). In our experiments anoxia, if present, could not have lasted for more than one hour. Furthermore, changes of the BBB have been reported within a few seconds following intracarotid injection of air, oxygen, or carbon dioxide (Johansson 1975). One could also discount possible effects of vasodilation or changes in blood pH and osmotic pressure since even extreme variations in these parameters failed to alter barrier permeability (Broman 1949). It seems more likely that gas bubbles cause direct mechanical injury of blood vessels or that vascular permeability is altered by the action of chemical agents released or activated in the course of reactions initiated by blood-bubble interface activity (Chryssanthou 1973; Hallenbeck, Bove, and Elliot 1973; Chryssanthou 1974b). Smooth muscle activating factor (SMAF), which was shown to be activated by gas bubbles (Chryssanthou 1974a), increases vascular permeability (Chryssanthou, Teichner, Goldstein, Kalberer, and Antopol 1970), and bradykinin, which has been implicated in decompression sickness and could be activated by gas bubbles (Chryssanthou, Teichner, Goldstein, and Antopol 1973; Hallenbeck et al. 1973; Chryssanthou 1974b), has been reported to alter the BLB (Chryssanthou and Antopol 1963). It is also conceivable that protein denaturation at the blood-bubble interface may result in release of protein-bound dye, thus increasing plasma concentration of free dye.

All these postulated mechanisms implicate gas bubbles. It is still possible that the observed correlation between intravascular gas bubbles and barrier alteration has no pathogenetic significance and that changes in permeability are caused by other factors related to compression or decompression.

In discussing mechanisms of barrier alteration, one should have the anatomical and functional characteristics of the barriers in mind. The BBB is generally believed to be related to the tight interendothelial junctions and the virtual absence of pinocytotic vesicles from brain capillaries (Oldendorf 1974). The nature of the BLB, on the other hand, is still unclear. It is therefore possible that alterations of the BBB and BLB involve different mechanisms.

Changes in the permeability of BBB and BLB may have pathogenetic implications in decompression sickness. It has already been emphasized that all animals which suffered decompression sickness exhibited increase in barrier permeability. Metabolites, released or activated humoral agents, and other plasma components which normally do not penetrate the BBB and BLB may, under dysbaric conditions, gain access to the brain or lung and cause pathologic changes. Parenthetically, it can be noted that the observed dysbaric alterations of the BBB may suggest new methods for administering chemotherapeutic agents, antibiotics or neuroactive drugs which under normal conditions do not penetrate the BBB.

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Chryssanthou, C., M. Springer, and S. Lipschitz. 1977. Altération de la barrière hémato-encéphalique et hématopulmonaire par l'exposition dysbarique. Undersea Biomed. Res. 4(2): 117–129.—La constatation que certaines des substances circulantes ne réussissent pas à pénétrer dans certaines organes est à l'origine du concept des barrières hémato-organiques. Ces barrières se laissent altérer par des moyens physiques ou chimiques. Nous rapportons la modification de la barrière hématoencéphalique et de la barrière hématopulmonaire par l'exposition dysbarique. Des injections intravéneuses de bleu trypan ont été administrées à 19 lapins "expérimentaux" (qui ont subi la compression-décompression) et à 11 animaux témoins, qu'on a gardés à la pression ambiante. Les examens anatomiques et microscopiques, ainsi que la détermination du taux de colorant extrait des tissus, ont mis en évidence une plus grande pénétration du colorant dans les poumons et les cerveaux des animaux expérimentaux. La concentration cérébrale en était 12,10 μg/g tissu chez les expérimentaux, et 2,93 μg/g chez les témoins; la concentration pulmonaire en était 935 μg/g et 434 μg/g, respectivement ($0{,}01 > P$ 0,001). La perméabilité augmentée des barrières hémato-encéphaliques et pulmonaires est associée aux bulles intravasculaires. Le mécanisme de l'altération des barrières peut impliquer des agents chimiques activés par l'interface gaz-sang ou des lesions vasculaires dues aux bulles. Ces observations peuvent contribuer à l'élucidation de la pathogénie de la maladie de décompression, et suggérer de nouvelles méthodes pour faciliter la pénétration des agents thérapeutiques dans le cerveau.

barrières hemato-organiques
bleu trypan
lapins
perméabilité
compression-décompression
bulles intravasculaires
maladie de décompression
pathogénie

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