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# Effect of anti-C5a antibody on blood–lung and blood–brain barrier in rabbits after decompression

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Hjelde A, Brubakk AO, Bergh K, Videm V, Ustad A-L. Effect of anti-C5a antibody on blood–lung and blood–brain barrier in rabbits after decompression. Undersea Hyper Med 1999; 26(4):249–256.—The complement activation product C5a may be an important mediator of tissue injury after decompression stress. This study investigated whether the administration of anti-C5a antibody may reduce changes after decompression in the lung and in the brain. Two groups of rabbits were used; one receiving anti-C5a monoclonal antibody ($n = 7$) and the other receiving a sham antibody as control ($n = 7$) before pressure exposure. Five rabbits (4 in the anti-C5a group and 1 in the control group) died during the 2-h observation period postdive due to massive bubbling. Polymorphonuclear leukocyte (PMN) infiltration of lung tissue and pulmonary edema was observed, but this accumulation was unaffected by anti-C5a pretreatment. However, a significant positive correlation was observed between PMN accumulation and survival time postdive. Brain-specific gravity was lower for the group treated with anti-C5a antibody compared to the control group. Further, it was lower for those rabbits that died early compared to the ones that survived the 2-h period. This study was unable to prove a protective effect on the blood–brain and blood–lung barrier by injecting anti-C5a antibody. A possible beneficial effect of anti-C5a antibody may be masked by the mechanical damage caused by the gas bubbles.

*anti-C5a, decompression, vascular bubbles, brain edema, neutrophils, lung*

Vascular gas bubbles may occur during most decompressions. The surface of the bubbles acts as a foreign substance and is capable of activating alternative complement pathways in vitro (1–3). Bergh et al. (2) showed that the degree of activation was related to the amount of gas bubbles introduced.

Activation of the complement cascade results in the generation of several fragments important as mediators of the acute inflammatory response. In particular, the anaphylaytoxin C5a exerts a chemotactic and aggregating effect on polymorphonuclear leukocytes (PMN). C5a has a specific receptor on human PMN (4,5) and binding results in increased neutrophil adherence to the endothelium. C5a-initiated leukocyte activation leads to release of free oxygen radicals and other mediators which may cause endothelial injury (6,7), increased vascular permeability (8,9), and subsequent edema (10).

Several authors have described the role of complement-stimulated neutrophils in the development of acute lung injury (6–8,11–13). Pulmonary sequestration of activated neutrophils is well documented (6–8,14,15). The marked attenuation of lung injury in C5-deficient mice, exposed to high oxygen concentrations, indicates a requirement for C5 in the development of pulmonary edema in response to hyperoxia (16). Further evidence that C5a-induced effects on lung injury are mediated by neutrophils comes from studies in neutrophil-depleted animals, which developed less extensive acute lung injury when complement was activated (7,14). Monkeys pretreated with anti-C5a antibodies showed a decreased mortality to *Escherichia coli* infusion compared with control animals (17,18). These data suggest that both an intact complement system and the presence of neutrophils are required for producing endothelial damage.

Under normal conditions, cerebral blood vessels, unlike peripheral blood vessels, are impermeable to circulating proteins. Previous studies have demonstrated that neutrophils accumulate in rat brain as part of the acute inflammatory response to traumatic brain injury (19,20). Schoettle et al. (19) found a significant correlation between neutrophil (PMN) accumulation and the development of cerebral edema. Oxygen-derived free radicals, when infused intracerebrally into rats, induced changes in the permeability of the blood–brain barrier (BBB), cellular injury, and edema (21). Pretreatment with a soluble complement receptor type 1 (sCR1) reduced

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neutrophil accumulation in the brain, suggesting that complement is involved in the acute inflammatory response to traumatic brain injury in rats (22).

An increase in BBB and blood-lung barrier (BLB) permeability may also arise from a mechanical injury of the endothelial cells directly from the gas bubbles. This may occur in addition to an inflammatory response indirectly via the bubbles. Gas bubbles can cause changes in barrier permeability even in the absence of clinical manifestations of decompression sickness (23). Breakdown of the BBB and BLB may allow proteins and other substances to move into the extravascular brain tissue, with subsequent formation of edema. Previous studies have demonstrated that dysbaric exposure increased BBB (23,24) and BLB (23) permeability, and this permeability was increased in animals with intravascular gas bubbles (23). Others have also demonstrated that this BBB may be broken by introduction of gas bubbles into the brain circulation (25,26).

The purpose of the present study was to investigate whether pretreatment with anti-C5a antibody will attenuate the extent of complement-induced PMN sequestration and thus reduce pulmonary inflammation and cerebral edema in rabbits after decompression.

## MATERIALS AND METHODS

Fourteen chinchilla rabbits of both sexes, locally bred, weighing 2.76 ± 0.05 kg were used in this study. The animals were bred and housed at the Animal Care Facilities of the University Hospital, Trondheim, and were regularly examined by a veterinarian. No signs of illness were detected during this period. The experiments were performed in accordance with national legislation and approved by the Norwegian Council for Animal Experimentation. Using a randomized, blinded protocol, the rabbits were divided into two groups. Thirty minutes before pressure exposure, one group ($n$ = 7) was pretreated with 12.6 mg anti-C5a Mab 4B1C11 (anti-C5a group) whereas 12.6 mg of a sham antibody (Mab 3A3D10) was given to another group ($n$ = 7, control group), The antibody was infused intravenously through the marginal ear vein as a bolus over 1 min, immediately after the first blood sampling period.

*Anesthesia*: The rabbits were tranquillized with an intramuscular injection of midazolam 5 mg (Dormicum, F. Hoffmann-La Roche AG, Basel, Switzerland) and fluanisone 7 mg + fentanyl 0.22 mg (Hypnorm, Janssen-Cilag Ltd, Saunderton, Buckinghamshire, England). Following this preanesthetic treatment the rabbits were transported to the operating room where they were placed on a thermostatically controlled heating blanket. Body temperature was monitored rectally and maintained at 39° ± 0.5°C. Within 45 min, the rabbits received an additional intramuscular injection of midazolam (2.5 mg) and fentanyl (0.11 mg)/fluanisone (3.5 mg). The depth of anesthesia was assessed by leg-withdrawal reflexes and blood gas determinations. Anesthesia was maintained postdive by giving additional doses of fentanyl/fluanisone (diluted 1:10 with water) intravenously, 1 ml $\cdot$ kg$^{-1}$ $\cdot$ h$^{-1}$, when necessary. This supplementation protocol was based on pilot studies (data not shown).

*Blood sampling and preparation*: Blood samples were collected from the central ear artery before the dive (predive) and after the dive; after a 1-h observation (*postdive 1h*) and a 2-h observation time (*postdive 2h*), respectively. Samples (1 ml) for hematologic analysis were obtained in EDTA tubes (Beckton Dickinson, Meylan Cedex, France) and kept at room temperature until analyzed on a hematology analyzer (Technicon H*1, Technicon Instruments Corporation, Tarrytown, NY) for Hgb, leukocyte (WBC), and differential counts. One half milliliter of blood was drawn into heparin-wetted syringes for arterial blood gas analysis ($Po_2$, $Pco_2$, pH) and were measured on a blood gas analyzer (ABL 330, Radiometer, Copenhagen). The data were automatically corrected for body temperature of the rabbit. The measurements were made within 2 min of sampling. Samples (1.5 ml) for analysis of C5a-desArg were drawn into EDTA (final concentration 13 mmol $\cdot$ liter$^{-1}$) tubes and immediately placed on ice. The plasma was separated by centrifuging at 600 $g$ for 10 min within 30 min and was stored at −80°C until analyzed.

*Dive profile*: Approximately 45 min after premedication, the spontaneously breathing rabbits were placed one at a time in a 300-liter pressure chamber. They were compressed to 500 kPa on air at a rate of 100 kPa $\cdot$ min$^{-1}$ and stayed at this level for 20 min. They were decompressed to the surface at a rate of 200 kPa $\cdot$ min$^{-1}$.

*Bubble detection*: All rabbits were monitored for gas bubbles postdive using a 5-MHz transducer connected to an ultrasonic scanner (Vingmed 750,Vingmed A/S Horten, Norway). The amount of bubbles was evaluated using a grading system from 0 to 5. Grade 0 denoted no bubbles, 1 was occasional bubbles, 2 was at least one bubble every fourth heart cycle, 3 was at least one bubble every heart cycle, 4 was continuous bubbling, and 5 was massive bubbling. This scoring system is approximately exponential compared to the number of bubbles in the right ventricle (27), and the bubble grade was converted to bubbles per square centimeter using this table (27).

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*Observation period*: After a 2-h observation period at the surface, the animals were given a lethal dose of potassium chloride intravenously, under anesthesia. The lungs were immediately removed for histologic study, while the brains were excised for subsequent specific gravity measurements with respect to development of brain edema. The brains were directly submerged in kerosene to avoid any evaporation from the surfaces.

*Lung histology*: Selected samples from the right and left upper and lower lung from all rabbits were fixed in a solution consisting of 70% ethanol, 4% formaldehyde, and 5% acetic acid. A total of four samples per lung per animal were taken. The next day the specimens were transferred to 80% ethanol before dehydration and embedding in paraffin for histopathology by standard techniques. Sections were cut at 5 µm, stained with hematoxylin-eosin-safran (H&E&S). The accumulation of PMN was estimated by an investigator who was blinded to the treatment given to the rabbits. Four fields from each lung section were examined at ×400 in a Nikon YS2-H light microscope. This microscope was equipped with an eye-piece containing a 10 × 10 reticle grid (0.5 × 0.5 mm), thus providing a counting device. The field area was determined by the number of grid points falling on the tissue. By counting the total number of PMN in that field divided by the number of lung tissue grid points, PMN was calculated as a number of PMN per unit lung tissue. Each rabbit was represented by a mean value of 8 data points from the left and right lung, respectively. The results are expressed as mean number of PMN per unit lung tissue. One unit lung tissue equals 0.25 $mm^2$.

*Determination of the wet-dry weight*: Dry weight of the lung tissue was determined from a small section, less than 1 g, from the right lung. The tissue was weighed (wet weight), incubated at 120°C for 7 days, and then weighed again (dry weight). Percent lung water content was calculated as (wet weight – dry weight)/wet weight*100 and was used to estimate the severity of pulmonary edema.

*Preparation of anti-C5a and control monoclonal antibodies*: The production of murine neutralizing anti-rabbit C5a Mab has been described in detail elsewhere (28). Mab 4B1C11 (IgG1 class) was produced in tissue culture to minimize the contamination of endotoxin and microbes often observed when ascitic fluid production is employed. The hybridoma cell line was cultivated in miniPERM Bioreactor (Heraeus Instruments, Hanau, Germany), and the culture medium was extensively dialyzed against endotoxin-free isotonic saline and frozen in aliquots which were thawed immediately before use. The capacity of the IgG given (12.6 mg) will be sufficient to bind every C5a molecule generated in plasma in vivo. As control antibody, we used an IgG1 Mab (Mab 3A3D10) raised in our laboratory against a psoriasis-associated dermal antigen, cultivated and prepared similarly and administered at equivalent amounts.

*Quantification of C5a*: The quantification of rabbit C5a-desArg in EDTA plasma was performed by a C5a-specific sandwich enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies, described in detail by Bergh and Iversen elsewhere (28). In the text, C5a will be used synonymously with its plasma analogue C5a-desArg.

*Specific gravity measurements*: To evaluate changes in the permeability of the BBB, a gravimetric method was employed. Specific gravity (density) of selected brain tissue samples was determined using a brombenzene/kerosene graduated column, in principle as described by others (29,30). A linear density gradient was established in a 250-ml graduated glass cylinder. The column was made by slowly adding the lighter mixture (1.030 $g \cdot ml^{-1}$) of brombenzene (50 ml) and kerosene (100 ml) to the denser mixture (1.065 $g \cdot ml^{-1}$) of brombenzene (57.6 ml) and kerosene (92.4 ml) into a graded cylinder to form a continuos gradient with densities running from 1.030 $g \cdot ml^{-1}$ at the top to 1.065 $g \cdot ml^{-1}$ at the bottom. The benzene-kerosene gradient column was calibrated immediately after each brain-specific gravity determination, using $K_2SO_4$ solutions of eight different concentrations, with specific gravity ranging from 35.9 to 73.9 $g \cdot liter^{-1}$, corresponding to specific gravities of 1.0283 and 1.0572 $g \cdot ml^{-1}$ (31). While immersed in kerosene, the brain was sectioned into in 5 coronal blocks (2–3 mm). From two of these blocks tissue samples of approximately 3–5 mg were dissected at different white (corpus callosum and basal white area) and gray matter (medial and lateral cortex) from each hemisphere. The samples were carefully transferred on the top of the brombenzene/kerosene column and allowed to sink (equilibrate) for 2 min before their final positions were recorded. Specific gravity (density, g/ml) was estimated graphically by interpolation from the position of salt solutions of known specific gravity. Each rabbit was represented by a mean value of 16 data points of white (corpus callosum) and gray matter (cortical), respectively. The intraindividual variation was less than 0.2%.

*Pilot study*: A pilot study, including four rabbits, was performed to assess the accumulation of PMN in the lung in anesthetic rabbits, nonexposed to hyperbaric situations. Two rabbits (group A) were killed immediately with a lethal dose of potassium chloride following the preanesthetic treatment, while two rabbits (group B) followed a

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similar observation protocol as the rabbits in the experimental group before they were killed. The lungs from both groups were removed for histologic study.

*Statistical analysis*: Due to the small number of animals, we used non-parametric methods. The data were subjected to analysis using Mann–Whitney U and Wilcoxon signed rank test for unpaired and paired data as appropriate. To test any significance of correlation between parameters of interest, Spearman's rank correlation was used. The results were expressed as mean and standard deviation. A $P$ value less than 0.05 was considered significant.

## RESULTS

Intravascular bubbles in the rabbits detected upon decompression varied from no bubbles to massive bubbling. The median bubble grade, 3, was similar for both groups. The mean bubble count was $3.7 \pm 5.5$ bubbles $\cdot$ $cm^{-2}$ (range 0–15) in the anti-C5a group and $2.2 \pm 2.6$ bubbles $\cdot$ $cm^{-2}$ (range 0–5) in the control group ($P$ = 0.740). Four rabbits, two in each group, had no detectable bubbles. Five rabbits (4 in the anti-C5a group and 1 in the control group) died postdive after $29.2 \pm 22.2$ min during the observation period due to massive bubbling (mean 6.1 $\pm$ 5.3 bubbles $\cdot$ $cm^{-2}$). Nine rabbits (3 in the anti-C5a group and 6 in the control group) survived the 120-min observation period (mean $1.2 \pm 2.1$ bubbles $\cdot$ $cm^{-2}$). The difference in bubble count between the surviving rabbits and the rabbits that died early was significant ($P$ = 0.022).

Peripheral blood leukocytes (WBC), neutrophil granulocytes (PMN), lymphocytes (LY), and trombocytes (PLT) are shown in Table 1. We have no data *postdive 1h* from the five rabbits that died before the observation time ended. There was a significant 39% decrease (range 21–71%, $n$ = 14) in the total leukocyte count *predive* to *postdive 1h* ($P$ = 0.001). The observed decrease was due to a significant 62% reduction (range 48–76%) in the number of lymphocytes in this period ($P$ = 0.001), and the lymphocyte count remained low during the whole observation period. From *predive* to *postdive 2h*, however, a significant 80% increase (range −20 to 215%, $n$ = 9) in the number of neutrophil granulocytes was observed in the rabbits ($P$ = 0.011). There was no significant difference between the anti-C5a group and the control group for WBC ($P$ = 0.565), PMN ($P$ = 0.406), or LY ($P$ = 0.250). An 18% significant reduction was found in the number of platelets (range +2 to 68%, $n$ = 14) from *predive* to *postdive 1h* ($P$ = 0.006). This reduction was more pronounced in rabbits with a higher bubble grade, except for one rabbit that had no changes. This rabbit, however (bubble grade 4), experienced a 48% decrease from *predive* to *postdive 2h*. Rabbits with no bubbles had minimal changes in the number of platelets during the observation period.

Mean C5a values *predive* and *postdive 1h* for the anti-C5a group were $91.6 \pm 12.6$ and $18.0 \pm 2.9$ ng $\cdot$ $ml^{-1}$, respectively, demonstrating the capability of anti-C5a antibody of binding the ligand ($P$ = 0.018). The corresponding values for the control group were $86.0 \pm 8.8$ ng $\cdot$ $ml^{-1}$ and 94.9 ● 11.3 ng $\cdot$ $ml^{-1}$. Mean C5a values *postdive 2h* were $18.7 \pm 2.9$ ng $\cdot$ $ml^{-1}$ in the anti-C5a group ($n$ = 3) and 104.8 ng $\cdot$ $ml^{-1}$ $\pm$ 9.9 ng $\cdot$ $ml^{-1}$ in the control group ($n$ = 6).

Histologic examination results(left and right lung) from the anti-C5a group and the control group are given in Fig. 1. No significant difference was found between left and right lung, not for the anti-C5a group ($P$ = 0.499) or the control group ($P$ = 0.237). Further, there was no significant difference in PMN in the lung between the two experimental groups ($P$ = 0.277). No significant correlation was found between percent PMN difference in blood count and accumulation of PMN in the lungs (R = 0.3, $P$ = 0.277).

A significant difference was observed in PMN accumulation in the lung between the surviving rabbits ($n$ = 9) and the ones that died early ($n$ = 5), seen in Fig. 2 ($P$ = 0.009). The PMN accumulation was larger in the surviving animals compared to the ones that died early. A significant positive correlation was observed between PMN accumulation and survival time *postdive* (R = 0.7, $P$ = 0.006, $n$ = 14). The results from the pilot study showed that the accumulation of PMN in both lungs for group B was 100% larger than for group A.

Percent lung water content was used to estimate the severity of pulmonary edema. The pulmonary water content was $79.6 \pm 1.4\%$ in the anti-C5a group and 78.5 $\pm$ 1.8% in the control group ($P$ = 0.201). If both groups were tested together, a significant negative correlation (R = 0.7, $P$ = 0.003) was found between PMN accumulation and water content in the lung. The lung water content in the surviving rabbits ($78.1 \pm 1.1\%$, $n$ = 9) was significantly lower ($P$ = 0.003) than in the ones that died early ($80.8 \pm 0.6\%$, $n$ = 5).

Figure 3 shows the mean specific gravity of the brain specimens. Data from two rabbits (one in each experimental group) are missing due to technical problems with the brombenzene/kerosene column. The specific gravity for both gray ($P$ = 0.037) and white matter ($P$ = 0.124) was lower for the group treated with anti-C5a. The specific gravity for gray ($P$ = 0.306) and white matter ($P$ = 0.020) was lower for those rabbits dying early as compared to the surviving rabbits (Fig.4).

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**Table 1: Mean Values ± SD of Leukocytes (WBC), Neutrophil Granulocytes (PMN), Lymphocytes (LY), and Trombocytes (PLT) *Predive, Postdive 1h,* and *Postdive 2h* for the Anti-C5a Group and the Control Group ($n$ = number of rabbits included in each period)**

| | Anti-C5a Group | | Control Group | |
|---|---|---|---|---|
| Hematologic Parameters | *$10^9$/liter | ±SD ($n$) | *$10^9$/liter | ±SD ($n$) |
| WBC *pre* | 5.44 | 1.02 (7) | 5.78 | 1.01 (7) |
| *post 1h* | 3.32 | 1.19 (7) | 3.56 | 0.92 (7) |
| *post 2h* | 4.80 | 0.60 (3) | 5.01 | 1.26 (6) |
| PMN *pre* | 1.59 | 0.41 (7) | 2.05 | 0.58 (7) |
| *post 1h* | 1.75 | 0.67 (7) | 1.85 | 0.72 (7) |
| *post 2h* | 3.07 | 0.65 (3) | 3.14 | 1.20 (6) |
| LY *pre* | 3.35 | 053 (70 | 3.03 | 0.58 (7) |
| *post 1h* | 1.21 | 0.51 (7) | 1.20 | 0.28 (7) |
| *post 2h* | 1.34 | 0.26 (3) | 1.28 | 0.38 (6) |
| PLT *pre* | 374 | 57 (7) | 457 | 64 (7) |
| *post 1h* | 262 | 90 (7) | 431 | 86 (7) |
| *post 2h* | 348 | 75 (3) | 396 | 91 (7) |

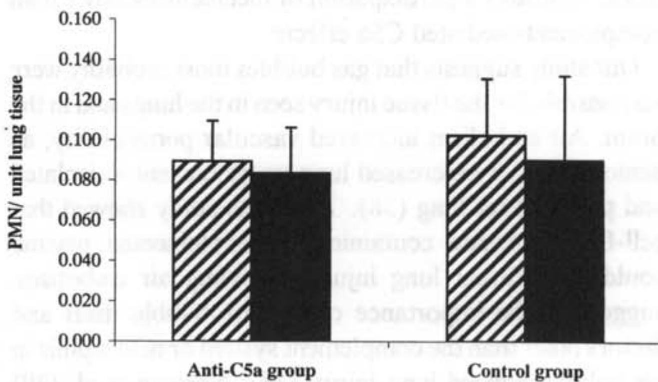


FIG. 1—Number of PMN per unit of lung tissue for left (*hatched bars*) and right (*solid bars*) lungs from the anti-C5a group ($n$ = 7) and the control group ($n$ = 7), presented as mean ± SD.

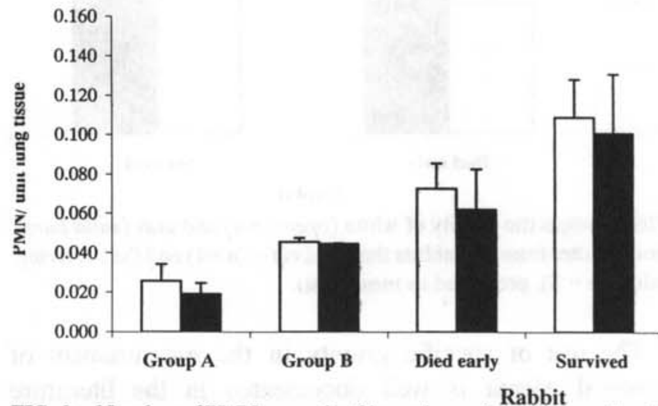


FIG. 2—Number of PMN per unit of lung tissue for left (*open bars*) and right (*solid bars*) lungs from group A ($n$ = 2), group B ($n$ = 2), the rabbits that died early ($n$ = 5), and the surviving rabbits ($n$ = 9), presented as mean ± SD.

## DISCUSSION

Our hypothesis was that anti-C5a antibody would reduce lung PMN accumulation and cerebral edema in decompressed rabbits by eliminating circulating C5a and presumably thereby reducing the effects of C5a on PMN. The effect of anti-C5a antibody would theoretically be associated with diminished vascular permeability changes in the lung and brain.

In our study, pretreatment with anti-C5a did not attenuate neutrophil accumulation or pulmonary edema compared to the rabbits without anti-C5a treatment. Lung injury was measured both as infiltration of neutrophils assessed by histopathology and by edema formation measured as difference in wet- and dry-tissue weight. Histologic examinations revealed extensive inflammation in both groups compared to the anesthetic, nonexposed rabbits in the pilot study. In a previous study we found no significant effect of anti-C5a antibody in preventing endothelial damage in the pulmonary artery (Nossum et al., unpublished data). Supporting our negative finding, Broome et al. (32) demonstrated that pretreatment with sCR1, which blocks C3 and C5 convertase activity, had no effect. They found no significant change in mortality rate or incidence of severe decompression sickness between treated and non-treated rats. Mulligan et al. (33), however, demonstrated reduced lung vascular permeability and neutrophil accumulation in sCR1 pretreated rats after complement activation. Our study clearly demonstrated that C5a was blocked by anti-C5a antibody pretreatment but not with the control antibody.

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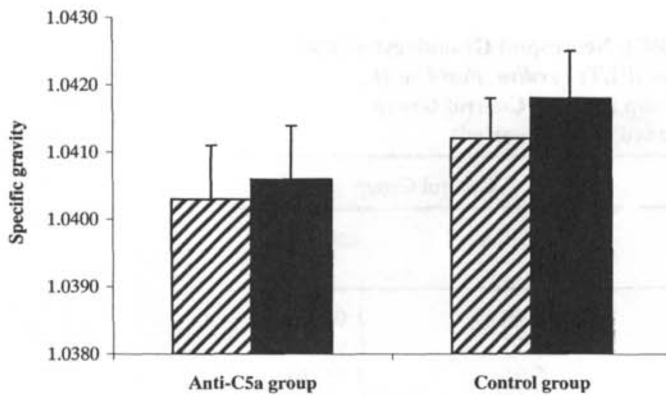


FIG. 3—Specific gravity of white (*hatched bars*) and gray (*solid bars*) brain matter from the anti-C5a group ($n$ = 6) and the control group ($n$ = 6), presented as mean ± SD.

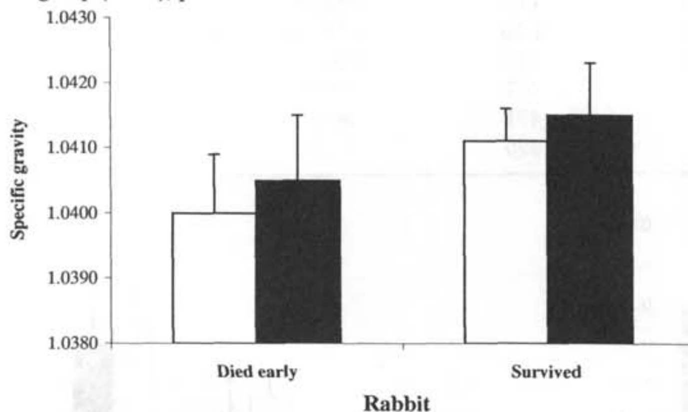


FIG. 4—Specific gravity of white (*open bars*) and gray (*solid bars*) brain matter from the rabbits that died early ($n$ =4) and the surviving rabits ($n$ = 8), presented as mean ± SD.

The use of specific gravity in the measurement of cerebral edema is well documented in the literature (29,30), demonstrating a significant decrease in specific gravity in response to increased water content of the brain tissue. Obviously, a gradual uptake of water leads to a decreased density. In spite of blocking of C5a, a significant decrease in specific gravity in the cortical gray matter of the brain was seen after decompression. There was also a reduction in white matter specific gravity, but this difference was not significant. Thus, anti-C5a antibody did not prevent fluid leakage across the blood–brain barrier. On the contrary, this antibody apparently increased the leakage. In studies on brain edema with microgravimetric techniques it is of utmost importance to minimize shifts of water between the sample and the medium. By using a brombenzene-kerosene density gradient as we did, tissue water is not lost into the hydrophobic medium. The individual density values obtained in this study were well reproducible and resulted in very small standard deviations. On the other hand we cannot exclude the possibiliy that the reduction of specific gravity, in addition to water uptake, is due to some retained gas in the brain tissue, because we have no control of the amount of gas accumulated in this area.

All decompressions using standard decompression tables may lead to intravascular gas bubbles (34). The lung is an efficient filter for gas bubbles (35,36), thus protecting the cerebral and coronary circulations from embolization. Gas bubbles may break through the lung filter if the lung is overloaded (35,36), enter the cerebral circulation, and thus mediate the bubble-induced tissue injury on the BBB. Air bubbles can produce their effects indirectly by activating the complement system or directly by mechanical obstruction. The present investigation failed to confirm earlier observations reported in the literature of the protective effect of different antibodies toward one of the complement proteins in the lung (17,18,33,37) or in the brain (22). However, the protective effect descibed in these studies did not involve dysbaric exposures with gas bubble formation. Thus, the lack of an effect of anti-C5a antibody in our model indicates a participation of mechanisms other than complement-mediated C5a effects.

Our study suggests that gas bubbles most probably were responsible for the tissue injury seen in the lungs and in the brain. Air embolism increased vascular permeability, as demonstrated by increased lung water content in isolated and perfused rat lung (38). The same study showed that cell-free perfusate containing decomplemented plasma could not prevent lung injury following air embolism, suggesting the importance of the air bubble itself and factors other than the complement system or neutrophils in air bubble-induced lung injury (38). Nossum et al. (39) found a positive correlation between endothelial damage in the pulmonary artery in pigs and the number of gas bubbles produced upon decompression. In pig lungs after dysbaric exposure generating a moderate number of gas bubbles, considerable leukocyte invasion was observed (Brubakk, unpublished data). However, the exact mechanism by which bubbles lead to tissue damage is still inadequately understood.

It seems that the number of bubbles developed upon decompression and the duration of the observation time postdive had a crucial impact on the results in our animal model. From the pilot study, the rabbits killed after 2 h (group B) had twice as many PMN counted compared to those killed immediately after preanesthetic treatment (group A). PMN accumulation was significantly higher in the surviving rabbits compared to the rabbits that died early, thus indicating an increasing PMN accumulation by time which was independent of bubble count. Pulmonary and cerebral edema was higher in the rabbits that died

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early compared to the surviving rabbits, suggesting a direct mechanical injury of blood vessels or permeability changes by gas bubbles. These observations indicate that PMN infiltration increased with time and was unaffected by the bubble grade, whereas edema was a direct result of a higher bubble count.

The specific gravity of the white and gray matter in the surviving rabbits was comparable to the values seen in a group of control rabbits in a previous study at our laboratory (40). The rabbits treated with anti-C5a antibody constituted almost 80% of the rabbits that died early and 35% of the surviving rabbits. The rabbits that died early, died due to massive bubbling, and the increase in cerebral and pulmonary edema was probably a result of a direct mechanical damage, unaffected by anti-C5a antibody. A highly unpredictable variability in bubble formation occurs after decompression, both in humans and animals. There is no reason to believe that the anti-C5a antibody caused bubble production. By coincidence, the rabbits that produced most bubbles belonged to the anti-C5a group. Only the surviving rabbits lived long enough to produce a secondary effect resulting in infiltration of PMNs. This biochemical effect was hypothesized to be inhibited by blocking complement activation. In our study however, the majority of the surviving rabbits were those treated with the control antibody, not anti-C5a antibody. Based on these observations, one cannot exclude a protective effect of this antibody if the treated rabbits had not died. A possible protective effect may thus be masked by the mechanical injury caused by the gas bubbles.

The postdive lymphocytopenia and granulocytosis seen in this study are common findings postoperatively following general anesthesia (22,41,42). Such stress associated with anesthesia was also seen in the rabbits in the pilot study, which were not exposed to pressure (data not shown). The reduction in circulating platelets observed is a common hematologic finding both in decompression sickness and in incident-free decompression (43,44). It is suggested that intravascular bubbles act as foreign surfaces to cause platelet adhesion and aggregation (44,45).

To conclude, we were unable to prove a protective effect on the BBB and BLB by injecting anti-C5a antibody, but one cannot rule out such an effect of the antibody. A possible effect of anti-C5a antibody may be masked by the mechanical damage caused by the gas bubbles. No conclusive evidence was found in this study to distinguish between direct mechanical action by the bubbles and indirect biochemical factors via complement activation in changing pulmonary or cerebral vascular permeability.

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