Infusion of 400 μl air into the left internal carotid artery of five anesthetized rabbits caused transient pial arteriole air embolism, an immediate 41.9±0.8% dilatation of the embolized vessels, suppression of the cortical somatosensory evoked response to 29.4±2.7% of baseline, and a progressive decline in ipsilateral cerebral blood flow (measured by hydrogen clearance) to 46±4.1% of baseline after 2 hours. These values were significantly different from those at baseline and from the responses of 10 control rabbits given equivalent intracarotid saline infusions. Twelve other rabbits were made leukopenic by treatment with 1.5 mg/kg i.v. mechlorethamine 72 hours prior to study. Mean±SEM leukocyte count decreased from 6,320±73/mm³ to 1,890±66/mm³ without any change in the leukocyte differential or erythrocyte and platelet counts. Intracarotid infusion of saline into seven of the leukopenic rabbits caused no changes. In the other five leukopenic rabbits, infusion of 400 μl air caused air embolism but did not produce the anticipated declines in cerebral blood flow or the cortical somatosensory evoked response, both of which remained indistinguishable from baseline values and responses in the seven saline-treated leukopenic controls. Similarly, air-embolized arterioles showed nonsignificant dilatation in leukopenic rabbits. Our data suggest that the decreases in both cerebral blood flow and brain function seen after air embolism require the presence of leukocytes. (Stroke 1991;22:351-354)

Injection of air into the internal carotid artery of rabbits causes significant decrements in function and blood flow on the brain surface.1,2 These changes occur both when bubble transit is rapid1 and when bubbles are temporarily trapped in pial arterioles.2 The effects persist for the duration of the experiment (3 hours after bubbles have cleared from the observed field).

It has been shown in both this rabbit preparation1,2 and in other species3 that such bubbles can pass through the brain capillaries, suggesting that the decrements in brain function and blood flow described above may be due not to vessel occlusion by bubbles but rather to bubble/blood/blood vessel interactions.1,4 However, because all measurements of cerebral blood flow (CBF) have limited resolution, it cannot be established that these functional and flow decrements are not due to small bubbles’ being trapped in intraparenchymal vessels.5

To test further the hypothesis that brain dysfunction and reduced CBF after air embolism is due to mobile bubbles damaging endothelial cells and activating leukocytes,1,4 we studied the effect of mechlorethamine treatment and the resulting leukocyte depletion in embolized rabbits.

Materials and Methods

This study was performed in accordance with the guidelines of and with the approval of the Animal Ethics Committee of the Institute of Medical and Veterinary Science (Adelaide). New Zealand White rabbits weighing 2.1-2.4 kg were used because the behavior of pial vessels in this species parallels that of intraparenchymal brain vessels of similar size.6 Seventy-two hours prior to the experiment, 12 rabbits were treated with 1.5 mg/kg i.v. mechlorethamine (Boots Pharmaceutical, London, England). None of these rabbits showed a subsequent decline in condition or body weight. A further 15 untreated rabbits acted as controls. Anesthesia was induced with a 2-g/kg i.v. bolus of urethane.7 All 27 animals were prepared in an identical manner. Rectal temperature was maintained at 38°C with a heating pad. A tracheotomy was performed. Femoral arterial and venous catheters (Silicone 602-175, Dow-Corning, Corning, N.Y.) were introduced for blood sampling, blood pressure recording, and fluid infusion (3 ml/
kg/hr of lactated Ringer’s solution). The left external and internal carotid arteries were isolated. A silicone cannula was introduced retrogradely into the external carotid artery so that its tip was adjacent to the internal carotid artery, which remained patent. The rabbit was then placed in the sphinx position in a stereotactic frame (David Kopf Instruments, Tujunga, Calif.), connected to a ventilator (Harvard Apparatus, South Natick, Mass.), and paralyzed with 10 mg/hr gallamine triethiodide. The physiologic ranges of PaCO2 and PaO2 were maintained by varying the ventilator stroke volume and rate and the inspired concentration of oxygen.

The scalp was reflected, and a craniotomy was performed over the left sensorimotor cortex. The dura was removed, and a 1-cm-diameter polypropylene cylinder was cemented to the skull. This cylinder was filled with paraffin oil to a depth of 1 cm to maintain the pH of the pial surface. A 1-mm burr hole was made over the right sensorimotor cortex.

To measure CBF, electrodes were prepared from sharpened 0.2 mm Teflon-coated platinum wire. The bare platinum tips were placed in the right and left cerebral cortices to a depth of 1 mm using an operating microscope (OPMI, Carl Zeiss Pty. Ltd., Adelaide, South Australia) and microelectrode carriers. An indifferent Ag-AgCl electrode was placed subcutaneously in the animal’s back. A two-channel polarographic amplifier system was used to measure hydrogen concentration, and hydrogen gas (approximately 10% by volume) was added to the ventilator inlet for 5 minutes. The first 30 seconds of the subsequent hydrogen clearance was ignored, and CBF as milliliters per minute per 100 g was calculated from the next 90 seconds using the initial slope index method. This method was chosen because it is regional, highly reproducible, and repeatable and does not affect CBF itself. The initial slope index gives an estimate of the fastest flow within the tissue being sampled such that blood flow decrements after air embolism will be underestimated and not overestimated.

Two minutes after the beginning of each hydrogen clearance, 50 μl blood was taken for PaO2 and PaCO2 analysis. Simultaneously, the temperature and mean arterial blood pressure were recorded, and the brain surface was photographed (Ilford FP4 film, Melbourne, Australia). An arteriole with an external diameter of 40–100 μm was selected, and the external diameter of a chosen fixed point of the vessel was measured from the projected film. Arteriole diameter was calibrated against a 35-μm suture thread. These vessels were chosen because if bubbles do become trapped, it is in arterioles of this size.

A silver ball electrode 0.75 mm in diameter was placed on the exposed brain surface to measure the cortical somatosensory evoked response. The animal’s right forepaw was stimulated via needle electrodes for 0.5 msec at a frequency of 1 Hz and a voltage three times that producing a detectable response. The ball electrode was positioned to record the maximum signal from the left somatosensory area I,12 with a frequency response of 5 to 2,000 Hz. A continuous record of the cortical signal was displayed on a chart recorder (Neotrace NEO400ZEF, Neomedix Systems, Sydney, Australia). In addition, 2 minutes after the beginning of each hydrogen clearance, 80 discrete evoked responses were recorded and averaged. In earlier studies,1,2 the second positive wave (P2)12 of the cortical response was found to be the most sensitive to air embolism.

The mechlorethamine-treated rabbits were assigned to either the mechlorethamine control group (n=7) or the mechlorethamine embolism group (n=5). The untreated rabbits were similarly assigned to either the untreated control group (n=10) or the untreated embolism group (n=5). All animals were monitored until at least 60 minutes of stable recordings were established (preinfusion baseline) and then either 400 μl air plus 600 μl saline (embolism groups) or 1,000 μl saline (control groups) were infused into the carotid artery cannula. In a previous study2 we showed that 400 μl air causes sustained deficits in CBF and brain function.

All groups were monitored for 3 hours following the air/saline or saline infusion, and then the rabbits were killed by a barbiturate overdose. All parameters were recorded 2 and 15 minutes after the infusion and then every 15 minutes. For each parameter, the mean of the preinfusion data was assigned a value of 100%. All subsequent data were recorded as a percentage of the preinfusion mean. Data are expressed as mean±SEM and were tested by analyses of variance and t tests. A significance of p<0.05 was chosen, and when simultaneous multiple comparisons were performed, the Bonferroni method was used.

**Results**

Prior to mechlorethamine treatment, hematologic values were consistent with published data (erythrocyte count, 5.7±0.17×1012 cells/μl; leukocyte count, 6.32±0.73×109 cells/μl; 31.5±1.32% neutrophils and 67.6±2.8% lymphocytes; platelet count, 378±60×103 cells/μl).4 Seventy-two hours following mechlorethamine administration, there were no significant changes in the erythrocyte or platelet counts but a significant decrease in the leukocyte count (1.89±0.66×109 cells/μl, p=0.003). The differential of the leukocyte count was unchanged.

Mean arterial blood pressure, PaCO2, PaO2, and heart rate did not change significantly at any time in these rabbits. Similarly, there were no significant preinfusion changes in CBF, the cortical somatosensory evoked response, or arteriole diameter.

Following the infusion of 400 μl (150–200 μl/kg) air into the left internal carotid artery, bubbles appeared in the pial arteries of all rabbits within 5 seconds. These bubbles were observed in pial arterioles 50–200 μm in diameter and were displaced by blood 40–365 seconds after embolism.

Right CBF did not change significantly at any time in any group (data not shown). In the untreated
Air Embolism After Mechlorethamine

In untreated rabbits, air embolism caused significant arteriolar dilatation ($F=5.8$, $df=93$, $p=0.005$) (Figure 1, top) that persisted for 30 minutes. In mechlorethamine-treated rabbits, a similar vasodilatation was seen (Figure 1, bottom), but arteriole diameter did not differ significantly from the preinfusion mean or from that in the mechlorethamine controls at any time after embolism.

Air embolism also caused significant deterioration in both left CBF ($F=2.85$, $df=69$, $p=0.01$) and AP$_2$ ($F=2.85$, $df=68$, $p=0.01$) in untreated rabbits (Figures 2, top, and 3, top, respectively). These values became significantly different from corresponding values in the untreated controls at 60 and 5 minutes after embolism, respectively. In contrast, no significant changes were seen in either left CBF or AP$_2$ after air embolism in the mechlorethamine-treated animals, and at no time after embolism did either left CBF or AP$_2$ in this group differ from that in the mechlorethamine controls (Figures 2, bottom, and 3, bottom).

Discussion

We have previously reported pial arteriolar dilatation and significant deteriorations in both blood flow and function on the brain surface following infusion of 25–400 $\mu$l air into the internal carotid artery of rabbits.$^{1,2}$ These findings can be explained by the effect bubbles have been shown to have on endothelial cells and leukocytes.$^{15-18}$ Nevertheless, we have not been able to establish that bubbles that clear from the exposed pial arteries do not become trapped in intraparenchymal vessels.$^5$

In a canine model, labeled granulocytes have been shown to accumulate in the brain after air embolism,$^{15}$ and in a subsequent study the recovery of brain function was accelerated in those dogs made agranulocytopenic by pretreatment with mechlorethamine.$^{16}$ Leukocytes affect the microcirculation of capillary beds and are important mediators of local inflammatory responses.$^{16,19}$ Bubbles could either stimulate endothelial cells and/or leukocytes to in-
increase leukocyte binding to vessel walls or could activate leukocytes so that they plug small vascular channels. The hypoperfusion seen after reversible ischemia may be due to leukocytes obstructing capillaries, but this may not require leukocytes to adhere to vascular elements.

To test if leukopenia was protective in our model, we conducted the study reported here using pretreatment with mechlorethamine. This was tolerated very well by the rabbits, and leukopenia was established prior to air embolism. Untreated control rabbits demonstrated the stability of the preparation. Rabbits pretreated with mechlorethamine but not air-embolized did not differ from these untreated controls. Although arterioles in both the mechlorethamine-treated and untreated rabbits were dilated in response to air embolism, the increase in diameter seen in the mechlorethamine-treated group was not significant. Also, the significant deterioration in CBF and brain function seen after air embolism in the untreated rabbits was abolished by pretreatment with mechlorethamine.

This protective effect may be due to the leukopenia and is clearly worthy of further study because it could underlie the development of useful therapies or prophylaxis.

Acknowledgments
We thank Ms. Charlene Carr for technical assistance, Mario Kyriacou for blood cell analysis, and the Pharmacy Department at the Royal Adelaide Hospital for supplies of mechlorethamine.

References

KEY WORDS • embolism, air • mechlorethamine • rabbits
Air embolism of the brain in rabbits pretreated with mechlorethamine.
S C Helps and D F Gorman

doi: 10.1161/01.STR.22.3.351

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/22/3/351