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Acute Hemorrhagic Hypotension and its Effect on the Pulmonary Clearance of Helium Instilled into the Rabbit Colon[1]

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ABSTRACT. The study investigates the effect of acute and incremental posthemorrhagic hypotension on pulmonary clearance of helium (CHe) introduced into the colon. Eighteen New Zealand White rabbits were cannulated and connected to a respirator at constant minute ventilation. A helium mass spectrometer was used to monitor airway gas. After 30 min stabilization, 10 ml/kg of helium were injected rectally while CHe and mean aortic blood pressure (BPm) were continuously monitored. Control animals (group 1, $n = 5$) achieved constant CHe (0.8–3.0 μl/kg/min) by 20 min, with CHe and BPm continuing unchanged over a 90-min period. Group 2 animals ($n = 5$) underwent acute blood loss of 12 ml/kg with reinfusion after 30 min. Group 3 animals ($n = 8$) underwent incremental blood loss of 4 ml/kg up to a maximum of 28 ml/kg without reinfusion. Two animals in group 3 had electromagnetic flow probes placed around their distal abdominal aortae. At 12 ml/kg blood loss, group 2 and 3 animals experienced falls in BPm of 46 and 58% along with simultaneous falls in CHe of 33 and 53%, respectively. These changes were statistically significant ($p < 0.05$). Reinfusion (group 2) caused initial parallel increases in CHe and BPm. However, CHe remained elevated as BPm returned to baseline, a finding consistent with colonic reperfusion hyperemia. At blood loss of more then 12 ml/kg (group 3), BPm and electromagnetic flow stabilized while CHe continued to decrease. Under these conditions CHe appeared to reflect shunting of intestinal blood flow away from the mesenteric bed. These data indicate that CHe responds predictably to alterations in BPm, and may thus provide a sensitive yet noninvasive measure of disturbances in intestinal blood flow. (*Pediatr Res* 22:595–598, 1987)

Abbreviations

IBF, intestinal blood flow
NEC, necrotizing enterocolitis
CHe, pulmonary clearance of helium
BPm, mean aortic blood pressure
EMF, electromagnetic flow
ANOVA-RM, analysis of variance for repeated measures

Regional IBF is subject to considerable physiologic and potentially pathologic change during the perinatal period. Being able to detect alterations in IBF is of considerable importance, in particular because bowel ischemia may play a prominent role in the etiology of NEC (1). Clinical assessment of IBF with currently available laboratory techniques (2, 3) is not feasible as such tests are not adaptable for use as bedside tools.

We have recently described a noninvasive technique to measure relative changes in colonic IBF using the CHe as an index (4). During hypoxic hypoxia we noted a close temporal relationship between the reduction of arterial oxygen tension and the pulmonary excretion of colonic helium measured by gas chromatography.

The present set of experiments were undertaken to further study CHe as an index of IBF under conditions of reduced colonic blood flow caused by controlled hemorrhage. Additionally, in order to monitor CHe in "real-time," we explored the utility of mass spectrometry as an alternative to gas chromatography for the quantitation of pulmonary helium excretion.

METHODS

Eighteen adult New Zealand White rabbits ranging in weight from 3.3 to 5.0 kg were anesthetized and surgically prepared in the following manner. Anesthesia was induced by the intramuscular injection of diazepam (5 mg/kg) and ketamine (30 mg/kg). Under local xylocaine anesthesia a tracheotomy was performed and a 3.5-mm endotracheal tube secured in place using two circumferential tracheal ties. An ear vein was cannulated for the administration of intravenous fluids (4–5 ml/kg/h) and to maintain anesthesia using ketamine (15 mg/kg) and xylazine (2.5 mg/kg) at 30-min intervals.

After tracheotomy the animal was mechanically ventilated using a time-cycled, pressure-limited neonatal ventilator (Healthdyne Infant Ventilator, Marietta, GA), adjusted to yield normal blood gas and pH values. Because the level of anesthesia was chosen to produce apnea, and because there was no airleak around the endotracheal tube, minute ventilation was determined from tidal volume given by the ventilator's tidal volume calculator, and ventilator rate.

A soft red rubber catheter was introduced rectally 12–15 cm into the colon and verified on postmortem examination to be located in the sigmoid or descending colon. A stopcock was attached to the proximal end of the catheter to allow for injection of helium. A helium mass spectrometer (Porta-Test, model 938-41, Varian/Vacuum Products Division, Lexington, ME) was used to monitor exhaled helium by continuous aspiration. The spectrometer was attached to the side port of the endotracheal tube connector (RSP Products, Newport Beach, CA). Mass spectrometer readings were scaled to room air helium concentration (5 ppm) at an aspiration rate of approximately 100 μl/min. CHe was then calculated from minute ventilation and helium concentration as detailed previously (4).

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In all animals an internal carotid artery was cannulated for continuous blood pressure measurement, intermittent blood gas sampling, and for phlebotomy and blood reinfusion. Two animals in group 3 (see below) underwent laparotomy with isolation of the abdominal aorta just distal to the inferior mesenteric artery. An appropriate sized (2–4 mm) EMF probe cuff was calibrated and then applied to the distal aorta. Retracted bowel was gently reapproximated and the EMF signal allowed to stabilize.

Heart rate, arterial blood pressure, rectal temperature, arterial blood gases, helium concentration by mass spectrometer, and EMF were monitored and recorded at preset intervals. Baseline values were obtained immediately following instrumentation and stabilization. Helium was then slowly injected in a bolus of 10 ml/kg through the rectal tube.

Animals were randomly assigned to one of three experimental groups. In control animals (group 1, $n = 5$), CHe was monitored for a period of 90 min with no manipulation of the blood volume. In group 2 (acute blood loss with reinfusion, $n = 5$) 30 min following helium injection a total of 12 ml/kg of blood representing approximately 25% of total blood volume (5) was drawn into a heparinized syringe over a 1-min period. The animals were monitored for 30 min, following which the withdrawn blood was reinfused over a 1-min period. Monitoring was continued for an additional 30 min and the animal was then euthanized. In group 3 (incremental blood loss without reinfusion, $n = 8$) 4 ml/kg aliquots of blood were withdrawn from the arterial catheter every 3–5 min starting 30 min after the helium was injected. Aliquots were not drawn until the arterial blood pressure had begun to stabilize. This process was continued until a total of 24 ml/kg of blood had been withdrawn, at which time the animal was euthanized.

Percent change from baseline values were calculated beginning 30 min following helium instillation. Changes in CHe and BPm over time were compared using ANOVA-RM. Comparisons at specific time points were made using paired t test analysis.

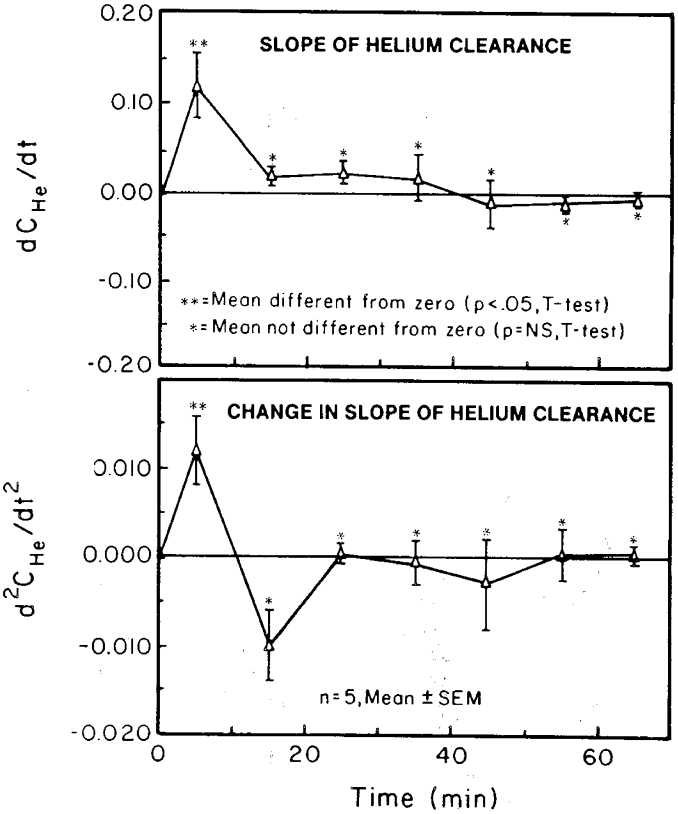

Fig. 1. Slope analysis of baseline CHe. *Top*, plot of first derivative of CHe (dCHe/dt). Note that in control animals (group 1) slope is not different from zero by 20 min following colonic helium instillation. *Bottom*, plot of second derivative of CHe (d^2CHe/dt^2). There is no statistical change in the slope following 20 min, indicating a constant baseline clearance rate.

RESULTS

Heart rate, rectal temperature, and arterial blood gases did not vary significantly between groups during the experimental period.

Group 1. After helium was injected into the colon of the control animals there was an initial rapid rise in CHe followed by a plateau in the rate of helium excretion. By 20 min postinjection each animal established its own steady state excretion level, ranging from 0.6 to 3.3 μl/min/kg. To determine the point at which plateau values were achieved, the first and second derivatives of CHe were plotted (Fig. 1). Both derivatives approached zero by 20 min and were not statistically different from zero by t test analysis for any later time point. Experimental interventions for group 2 and 3 animals were therefore begun 30 min after helium injection, when it could be assured that CHe had reached a constant level. A combined plot of initial CHe (0–20 min) for all animals is shown in Figure 2. For the remainder of the 90-min control period, CHe, BPm, heart rate, temperature, and blood gases remained stable.

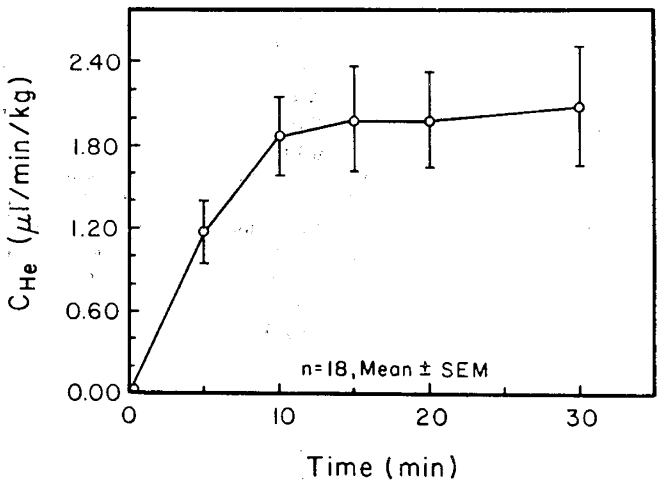

Fig. 2. CHe for all animals during initial 30-min stabilization period following colonic instillation of 10 ml/kg helium. Blood volume manipulations in group 2 and three animals were begun after plateau values had been achieved.

Group 2. In these animals, withdrawal of 12 ml/kg of blood was immediately followed by an acute drop in BPm and CHe (Fig. 3). Values decreased 46 and 33% from baseline for BPm and CHe, respectively. With reinfusion, BPm and CHe showed parallel increases, returning to baseline levels. BPm continued to increase, but then returned again to baseline. CHe also increased above baseline, but remained elevated. Although ANOVA-RM indicated no significant difference between the changes in BPm and CHe, *ad hoc* t test showed significant ($p < 0.05$) initial decreases from baseline in both BPm and CHe at 40 min, and a significant increase ($p < 0.05$) above baseline for CHe at 90 min.

Group 3. Animals undergoing incremental hemorrhage also showed parallel decreases in BPm and CHe, with these values falling 58 and 53% from baseline, respectively, when the incremental loss was 12 ml/kg (Fig. 4). Changes from baseline were statistically significant for both BPm and CHe (t test, $p < 0.05$) at 12 ml/kg volume depletion. However, as blood loss increased above 12 ml/kg, BPm began to stabilize at a new low level,

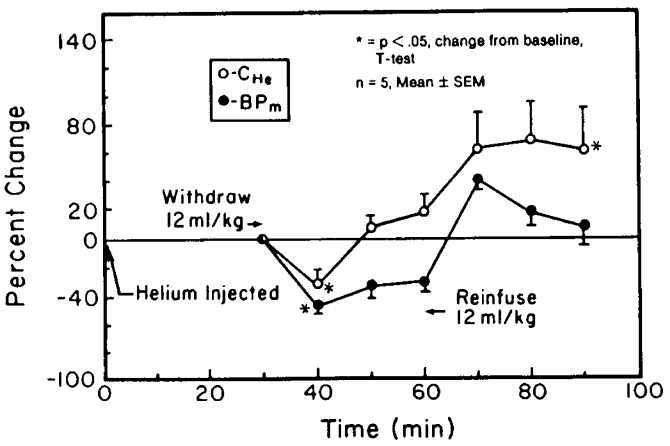

Fig. 3. Changes in CHe and BPm with blood loss of 12 ml/kg. Reinfusion occurred 30 min later. There is no statistical difference between CHe and BPm over this interval (p = NS, ANOVA-RM). *Ad hoc t* test indicates that changes at 40 min are significantly different from baseline for both CHe and BPm, and that at 90 min CHe remains elevated above baseline.

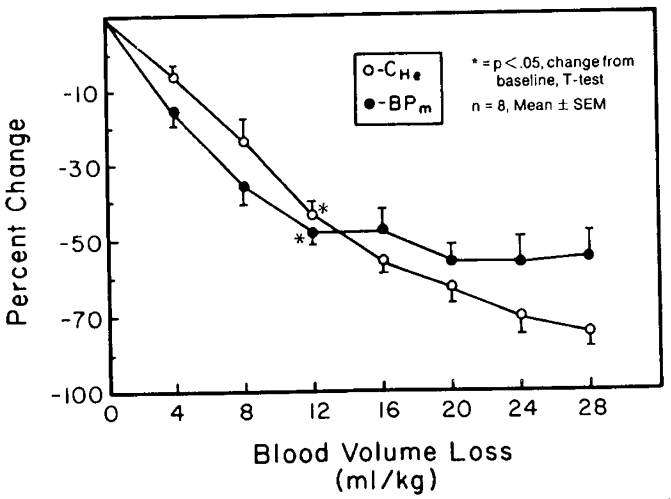

Fig. 4. Changes in CHe and BPm with blood loss increasing by 4 ml/kg aliquots. A statistically significant difference (p = 0.001, ANOVA-RM) is seen between the two parameters for increasing hypovolemia, although no difference exists up to 12 ml/kg volume loss. However, changes at 12 ml/kg are statistically different from baseline ($p < 0.05$, t test). Note that CHe continues to decline for blood loss of more than 12 ml/kg, although BPm stabilizes after a 60% drop.

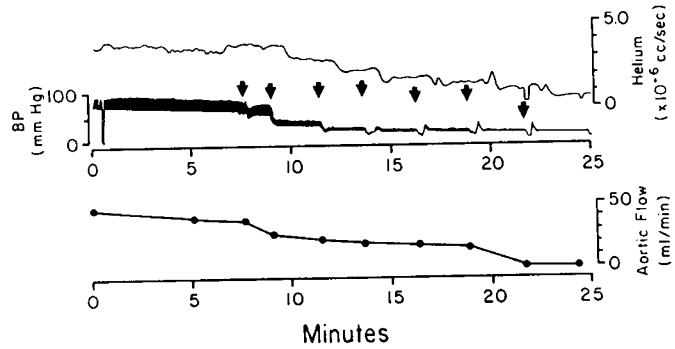

Fig. 5. Typical tracing of mass spectrometer (*top*), blood pressure (*center*), and distal aortic EMF (*bottom*) signals on animal 19 during incremental blood lass. *Arrowheads* indicate each blood withdrawal of 4 ml/kg.

whereas CHe continued to decline. ANOVA-RM indicated a significant difference ($p < 0.001$) over the 90-min time interval between the changes in BPm and CHe for animals in this group. However, similar analysis indicated that this difference was not apparent before a total hemorrhage of 12 ml/kg has been achieved.

EMF values showed changes similar to BPm with average distal aortic flow decreasing 59% (31.0 to 12.8 ml/min) from baseline by 12 ml/kg blood loss. Thereafter, EMF and BPm remained at low but steady levels until 28 ml/kg blood loss when EMF dropped to near zero (Fig. 5).

DISCUSSION

The present observations are an extension of our previous effort to develop a noninvasive method of monitoring relative changes in regional IBF which could be applied in a variety of clinical situations including the monitoring of sick newborn and premature infants. Toward this end the current experiments provide further refinements in the technique and application of CHe monitoring.

Previously we determined CHe using calculations of pulmonary helium excretion based on discrete end-expiratory samples analyzed by gas chromatography (4). Limitations experienced in this previous study using a gas chromatograph and discrete end-expiratory sampling included noncontinuous sampling, inherently large sample volumes, dilutional errors from improper aspiration technique, and difficulties with sample storage. To circumvent these problems we have introduced the use of a mass spectrometer for helium detection. This modification yields several important advantages. Since the spectrometer aspiration tube can be connected directly to the proximal airway, use of a sampling syringe and catheter is unnecessary. The small volume sample size (100 μl/min) is removed from a location set back from the patient airway and thus eliminates any problems with room air contamination and dilutional bias. Results of helium analysis are obtained quickly, with only a 2–4 s response time delay. Accuracy is greatly improved, as the mass spectrometer is capable of detecting helium at a calculated lower limit of 50 parts per trillion. Finally, the device provides "real-time" determinations of pulmonary helium excretion, allowing immediate correlations with physiologic parameters effecting CHe.

The question of the stability of CHe excretion rates over time was readdressed in control animals (group 1) of this protocol. In our previous experiments (4) we noted that approximately 20 min were required for CHe to increase and stabilize after colonic injection. Using a more detailed method (slope analysis) this observation has been statistically validated in the present study. From 20 min through 90 min the slope of CHe was not different from zero and no statistical change in the slope could be identified, indicating a constant, persisting level of pulmonary helium excretion. Similar observations about the stability of pulmonary gas levels during a 40-min period were noted by Perman *et al.* (6) with hydrogen insufflation of the isolated canine ileum and by Bjorneklett and Jenssen (7) during a 30-min period with hydrogen insufflation of the isolated adult human jejunum and colon. Theoretical work (4, 8) suggests that the rate-limiting step in absorption of gas from the gastrointestinal tract is gastrointestinal blood flow, implying that constant pulmonary gas excretion should occur for conditions of undisturbed IBF. Control data from the present study support this hypothesis.

The physical properties of helium and the small amounts of helium gas absorbed across the colon in the present experiments were such that in the absence of a right to left intracardiac shunt virtually all the helium transported from the colon would be cleared in a single pass through the lungs. Left to right cardiac shunts should not disturb CHe since portal venous return continues to pass to the pulmonary circulation. However, both intracardiac right to left shunting and abnormalities in alveolar

ventilation/perfusion matching have the potential to decrease helium expiratory clearance and prolong total helium excretion time. These fractional shunts could decrease CHe but would have less effect on relative changes. Such abnormalities, if present, are likely to be of fairly constant magnitude during the experimental period and are unlikely to cause the acute changes observed in CHe. The acute fluctuations of CHe which were seen in the present experiments are therefore more likely to be due to variations in the rate of helium being transported from the colon than from changes in pulmonary physiology.

The association between changes in CHe and IBF seen in animals from groups 2 and 3 are in keeping with expected physiologic responses. BPm is a traditional measure of perfusion pressure and was thus used in this study as an indirect estimate of IBF. Other studies quantifying colonic IBF during hypovolemic hypotension (9, 10) have shown a near monotonic relationship between drops in BPm and colonic blood flow. In the rabbit, acute withdrawal of 28 ml/kg blood volume reduced BPm and colonic blood flow by 37 and 50%, respectively (10). Our use of BPm to estimate IBF would at worst underpredict any decreases in colonic flow. Actual decrease in IBF in the face of hypovolemic hypotension is likely to be greater than would be reflected by the change in BPm. Although as yet only inferential, the association between changes in gas excretion to changes in BPm and IBF is a reasonable one and has been previously investigated (6).

In group 2 animals we noted CHe increasing above baseline value following reperfusion, with sustained elevation in excretion levels up to the end of the observation period. This same phenomena has been described following release of mesenteric vascular occlusion in the dog (6). An increase in hydrogen clearance was noted to coincide with direct visual observation of bowel hyperemia. We, therefore, would interpret the sustained elevation in CHe noted in this experiment following blood reinfusion to be due to reperfusion hyperemia associated with the observed transient increase in BPm.

After 12 ml/kg blood loss in group 3 animals, CHe continued to decline despite stabilization of BPm and EMF at a lower level. Similar decreases have been seen in other measures of IBF in dog models during progressive hypovolemic shock using xenon (9) or hydrogen (6) washout or surface oximetry (2). It appeared that during incremental hemorrhage in this rabbit model, CHe also reflected a continuing decline in colonic blood as IBF became maximally diverted to maintain perfusion to more vital organs.

Recent work by Karna *et al.* (11) and Nowicki *et al.* (12) has begun to expand and redefine earlier work by Touloukian *et al.* (13) on the role of gastrointestinal ischemia in the newborn animal and its relationship to the development of lesions similar to those found in the human infant with NEC. Although intestinal ischemia is considered to be an essential component in the development of NEC in the human neonate (1), no direct blood flow data are available to support this hypothesis. Inferential data have also not been helpful in this regard. Epidemiological studies of neonates with NEC indicate that of the factors likely to contribute to the etiology of the disease, *e.g.* hypoxemia, asphyxia or shock, none adequately discriminate the predisposed patient (14, 15). From a clinical point of view, the greatest handicap at present is the inability to measure or quantify the effect of ubiquitous deleterious insults on the immature gastrointestinal tract. A noninvasive means to describe functionally the state of bowel perfusion may aid greatly in sorting out the relationship between insult and disease.

The results of our present and previous observations indicate that alterations in CHe are reflective of changes in IBF. Further investigation is required to corroborate and quantitate this relationship. However, the application of helium mass spectrometry to this technique has added the benefits of increased resolution, ultra small sample volumes, fast response, and real-time data collection, all necessary prerequisites for an effective clinical tool. Measurement of the pulmonary clearance of helium gas instilled into the colon offers promise as a clinically applicable noninvasive measure of regional IBF.

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