Pco₂ in the large intestine of mice, rats, guinea pigs, and dogs and effects of the dietary substrate

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Rasmussen, Henrik, Peyman Mirtaheri, Hubert Dirven, Helge Johnsen, Gunnvald Kvarstein, Tor Inge Tønnessen, and Tore Midtvedt. Pco₂ in the large intestine of mice, rats, guinea pigs, and dogs and effects of the dietary substrate. J Appl Physiol 92: 219–224, 2002; 10.1152/japplphysiol.00190.2001.—Pco₂ in the lumen and serosa of cecum and colon was measured in rats, guinea pigs, and dogs to examine the relationship between serosal Pco₂ and the incidence of intestinal necrotic lesions after administration of gas-carrier contrast agents in rodents. The effects of the dietary substrate were tested in a group of mice maintained on a diet based on glucose as the only carbohydrate source. The anesthetic used was a fentanyl-fluanison-midazolam mixture (rodents) and pentobarbital (dogs). Pco₂ was measured in vivo and postmortem, and the kinetics of the postmortem serosal Pco₂ [transmural CO₂ flux (JCO₂)] was calculated. Pco₂ in the cecal serosa and lumen, respectively, was 64 ± 4 and 392 ± 18 Torr in rats, 67 ± 3 and 276 ± 17 Torr in guinea pigs, and 73 ± 6 and 137 ± 7 Torr in mice on glucose-based diets. In the colon serosa and lumen of dogs, Pco₂ was 30 ± 6 and 523 ± 67 Torr, respectively. Serosal Pco₂ increased rapidly after death in rats and slowly in guinea pigs and mice, and the slowest change was observed in dogs. Compared with dogs, serosal Pco₂ and JCO₂ of rats and guinea pigs were significantly higher. Serosal Pco₂ of guinea pigs was similar to that of rats, whereas the JCO₂ of guinea pigs was significantly lower. These data suggest a causal relationship between the ability of the cecal and colonic wall to act as a barrier to CO₂ diffusion and the presence of characteristic gas-carrier contrast agent-induced intestinal lesions in mice and rats and their absence in guinea pigs, dogs, and other species.

gas-carrier contrast agents; gas supersaturation; serosa; carbon dioxide

MEASUREMENT OF INTESTINAL tissue Pco₂ (PticO₂) by gastrointestinal tonometry is a well-known diagnostic and prognostic tool in critical care medicine (4–8). With the introduction of miniature Pco₂ sensors, Pco₂ measurements can be carried out in the intestinal lumen, as well as on mesenteric surfaces, in experimental animals (1, 15, 16, 18). It is generally assumed that the Pco₂ measured by intestinal tonometry is produced by, and reflects the status of, the intestinal tissues, increasing during states of ischemia when protons are produced by anaerobic metabolism (17). However, Pco₂ measurements in the cecum of mice indicated that CO₂ produced during bacterial fermentation of the dietary substrate, and not the intestinal wall metabolism, was the main source of CO₂ in intestinal segments with a high bacterial activity. The resulting serosal Pco₂, which may exceed 100 Torr, could represent a condition of gas supersaturation in mice (13). The results in mice indicated that the in vivo serosal Pco₂ is determined by a combination of luminal Pco₂, vascular washout of CO₂, and transmural permeability to CO₂. However, another reason for our measurement of cecal Pco₂ in mice was the observation of characteristic necrotic lesions of cecum, colon, and liver in mice and rats (14) after a single administration of gas-carrier contrast agents (GCAs). GCAs consist of gas microbubbles that are small enough to pass the pulmonary circulation and sufficiently pressure stable to allow passage through the left ventricle after intravenous administration. GCAs provide ventricular and myocardial contrast during ultrasound examination of the heart, as well as other parts of the vasculature.

Preformed gas nuclei grow rapidly under conditions of gas supersaturation (2, 3, 9, 10, 19). As GCAs are ideal examples of gas nuclei, we stipulated that gas supersaturation in the cecal wall of mice and rats could explain the GCA-induced lesions. Similarly, lower serosal gas tensions in other species could explain why GCA-induced lesions do not occur in guinea pigs, rabbits, and dogs after a single administration of GCAs (14). In this study, we have measured the serosal Pco₂ levels in the large intestine and the jejunum of rats, guinea pigs, and dogs. To study what factors influenced the serosal Pco₂, we measured the luminal Pco₂ and the postmortem transmural CO₂ flux (JCO₂). In addition, as GCA-induced lesions in mice were absent in...
mice maintained on a diet without dietary fibers and glucose as the only carbohydrate source (14), we measured PCO2 in the cecum of mice maintained on this diet. These data were compared with our previously published data of mice maintained on a standard rodent diet (13) to determine whether the serosal PCO2 in mice was due to unique bacterial activity, physiology, or anatomy. Rats, guinea pigs, and mice were included, as these rodent species are often used in research, whereas the dog represented a monogastric carnivore with an intestinal tract comparable to that of humans. Based on our previous experience in mice and on knowledge that the cecum is a major site of diet fermentation in rodents, high luminal PCO2 levels were expected in both rats and guinea pigs. In the dog, we had no indications from either the literature or prior experiments as to what levels of PCO2 to expect. Based on the bacterial counts in the intestinal tract, we expected the jejunum and large intestine (cecum and/or colon) to be the most relevant segments of low and high bacterial activity, respectively, to examine.

MATERIALS AND METHODS

Animal model. Male rats, guinea pigs, mice, and dogs of both sexes were included in the experiments approved by the Amersham Health Institutional Animal Care and Use Committee. Mol:SD rats were supplied by Mollegaard AS (L1. Skensved, Denmark), HsdHan:NMRI mice and HsdPoc:DH guinea pigs were supplied by Harlan (Bicester, UK), and mongrel dogs were supplied by a local breeder. The rats were 7–8 wk old and 266 ± 4 (SE) g at study start, the guinea pigs were 5–6 wk old and 294 ± 4 g at study start, and the mice were 4–6 wk old and 32 ± 1 g at study start. The rats, guinea pigs, and mice were housed two to five animals per cage (polycarbonate size III, B&K International, Sollentuna, Sweden) with autoclaved aspen tree bedding (B&K International) in a controlled environment (21 ± 2°C, 55 ± 10% relative humidity, 12:12-h light/dark cycle in phase with natural daylight) for an acclimatization period of at least 4 days after delivery. The rats received SDS RM1 (E) SQC diet (Special Diets Services, Essex, UK), the guinea pigs received Rabbit and Guinea Pig Maintenance diet (B&K International), and the mice received a custom-made diet with glucose as the only carbohydrate source (diet 4012.01, Hope Farms, Woerden, The Netherlands) ad libitum. Details about the composition of these diets have been described previously (14). The dogs were 10–30 mo old and weighed 20–24 kg at study start. The dogs received Purina Fit and Trim diet (Purina) until the night before the experiment and were included in the study on the day of delivery. The rats, guinea pigs, and mice were anesthetized with intraperitoneal injections of a 1:1:2 mixture of Hypnorm (fentanyl citrate, 0.315 mg/ml and fluanisone, 10 mg/ml; Janssen-Cilag), Dormicum (midazolam, 5 mg/ml; Roche), and sterile water (0.15–0.20 ml/100 g in rats, 0.50–0.60 ml/100 g in guinea pigs, and 0.1 ml/10 g in mice). Pentobarbital (25 mg/kg induction, 17.5 mg/kg−1·h−1·iv) was used to anesthetize the dogs. The animals were maintained on a deep surgical plane anesthesia and placed on a 39°C heating pad to maintain normal body temperature. Miniature PCO2 sensors (type MI 720, Microelectrodes, Bedford, NH) were introduced into the jejunal and cecal lumen, as well as on the cecal serosa of rats, guinea pigs, and mice, as previously described (13). The PCO2 sensors used in the various segments of the gut were consistently used for the same segment and not interchanged during the day of measurement. A midline abdominal incision exposed the jejunum, cecum, and cranial colon in dogs. Intraluminal PCO2 sensors were introduced into these intestinal segments through antimesenteric incisions, whereas the serosal PCO2 sensor was placed on the colonic serosa. Unless otherwise noted, the tip of the intraluminal PCO2 sensor was placed in the center of the intestinal contents (at the cecocolonic junction of the colon), avoiding contact with the mucosa. In two dogs, in which the PCO2 sensor was positioned perpendicular against the mucosa, mucosal PCO2 was also recorded. The tip of the serosal PCO2 sensor was placed perpendicular on the serosal surface, avoiding pressure on the intestinal wall and preventing atmospheric air from entering the serosa-gas membrane interface. All saline used for keeping the intestines moist during measurements was 39°C.

PCO2 measurement and calibration of PCO2 sensors. When stable PCO2 values of all sensors had been recorded in vivo [time (t) = 0], the animals were killed, and the PCO2 values during the resulting no-flow ischemia were recorded. The rats, guinea pigs, and mice were killed with an intracardiac overdose of pentobarbital, and the dogs were killed with intravenous KCl. PCO2 was recorded 30, 60, 90, and 120 s, and 3, 4, and 5 min postmortem in the rats, guinea pigs, and mice; and 60 and 120 s and 3, 4, and 5 min postmortem in dogs. Stability of the PCO2 values before killing was indicated as a standard feature of the PCO2 sensors when the dPCO2/dt decreased below a fixed value. Stable PCO2 values were usually obtained from −5–10 min after introduction, depending on the positioning of the sensors. Before the experiments, the PCO2 sensors were calibrated with a three-point calibration in saline solutions of known PCO2, with a measuring span from 47.3 to 168.8 Torr at 39°C. The saline solutions were made by bubbling with 100% CO2 gas. The PCO2 tensions were then measured by sampling some of the solution and analyzing it in a blood-gas machine (AVL 995, Biomedical Instruments; and 278 Blood Gas System, CibaCorning Diagnostics). At the end of the experiments, another three-point PCO2 check, equivalent to the one performed before the experiment, was carried out to determine any detectable drifting. According to the previously published specifications, the PCO2 sensors are linear and valid for PCO2 measurements up to 630 Torr at 37°C, corresponding to 659 Torr at 39°C ([solubility CO2, solubility CO2 in water]−630 Torr) (11).1 Data from the sensors were corrected for drift, assuming that it was linear according to previous experience (18). The sensors drifted <29% during a total of 5 h of measurements in rats, guinea pigs, and mice and <10% during the 8- to 30-min measurements in dogs.

Statistics. All data values in the text, Fig. 1, and Tables 1–3 are means ± SE. Unless otherwise stated, all reference to results in the text are in vivo PCO2 levels at t = 0. Parametric ANOVA and nonparametric Kruskal-Wallis ANOVA followed by t-test or pairwise Mann-Whitney rank sum test were applied on the serosal and luminal PCO2 at t = 0 and jCO2 of rats, guinea pigs, mice, and dogs (Mann-Whitney rank sum test when normality or equal variance test of the data failed). As the intention of using the special diet in the mice of the present study was to compare the effect of the diet on PCO2, these results were only compared with previously published PCO2 levels in mice on a standard rodent diet (13). Jandel Sigmapost version 2.0 was used for the statistical analyses. The chosen levels of significance (P ≤ 0.05 and P ≤ 0.01) were corrected (Bonferroni) for multiple

1With PCO2 microsensor linearity up to 659 Torr at 39°C, values above this level were included in the mean as 659 Torr (one dog).
comparisons \((n = 7)\) to \(P \leq 0.007\) and \(P \leq 0.001\). The postmortem \(J_{CO_2}(\text{Torr/min}) = \frac{dP_{CO_2}_{\text{serosa}}}{dt}\) was calculated as \(P_{CO_2}\) serosa, \(t = 5\) min \(- P_{CO_2}\) serosa, \(t = 0\)/5 min for 5 min postmortem.

RESULTS

Intestinal content was present, and \(P_{CO_2}\) was measured in the cecum of all rats, guinea pigs, and mice, whereas no or very little intestinal content was observed in the cecum of the dogs. Large intestinal \(P_{CO_2}\) was, therefore, measured in the cranial colon of the dogs and compared with cecal \(P_{CO_2}\) of the other species. As luminal \(P_{CO_2}\) varied markedly according to the diet \((13)\) was significantly higher than that in mice on standard rodent diet \((13)\) was the lowest of all the species examined and significantly lower than that in rats \((64 \pm 4\) Torr\) and guinea pigs \((67 \pm 3\) Torr\) (Table 1, Fig. 1). The serosal \(P_{CO_2}\) of 30 \pm 6 Torr in dogs was the lowest of all the species examined and significantly lower than that in rats, guinea pigs, dogs, and mice on \(diet 4012.01\) \((73 \pm 6\) Torr\). No significant difference in serosal \(P_{CO_2}\) between rats and guinea pigs was observed. With a high incidence of GCA-induced lesions in HsdHan:NMRI mice on standard rodent diet \((2)\) and no lesions in HsdHan:NMRI mice on \(diet 4012.01\) \((14)\), the marked and significant difference in serosal \(P_{CO_2}\) in these mice supports the hypothesis that gas supersaturation is a cause for GCA-induced lesions in mice. However, as a low incidence of GCA-induced lesions, despite low serosal \(P_{CO_2}\) levels, is observed in rats, in vivo serosal \(P_{CO_2}\) does not by itself explain the GCA-induced lesions in this species.

To study what factors influenced the different serosal \(P_{CO_2}\) levels, we measured the luminal \(P_{CO_2}\) and the postmortem \(J_{CO_2}\). The postmortem increase in the cecal luminal \(P_{CO_2}\) during the 5-min observation period was insignificant in all species \((data not shown)\). The luminal \(P_{CO_2}\) of 409 \pm 32 Torr in mice on standard rodent diet \((13)\) was significantly higher than that in mice on \(diet 4012.01\) \((137 \pm 7\) Torr\) and guinea pigs \((276 \pm 17\) Torr\) but not significantly different from that in rats \((392 \pm 18\) Torr\) and dogs \((523 \pm 67\) Torr\) (Table 2). The luminal \(P_{CO_2}\) of guinea pigs was significantly lower than that in rats and dogs. The luminal \(P_{CO_2}\) in dogs was the highest of all species and not significantly different from that in rats and mice on standard rodent diet. The lower serosal \(P_{CO_2}\) may be associated with the lower luminal \(P_{CO_2}\) in guinea pigs and mice on \(diet 4012.01\). However, the differences in serosal \(P_{CO_2}\) among rats, dogs, and mice on standard rodent diet, despite equally high luminal \(P_{CO_2}\) levels, are likely to be due to inherent differences in the intestinal wall. These results indicate that the colon wall of dogs and the cecal wall of rats are significantly more impermeable to in vivo \(CO_2\) diffusion than is the case in mice on standard rodent diet.

Vascular removal of \(CO_2\) and the physical barrier to \(CO_2\) diffusion of the intestinal wall are believed to determine the transmural \(CO_2\) diffusion in vivo, whereas only the physical barrier is effective postmor-

![Fig. 1. Serosal \(P_{CO_2}\) and transmural \(CO_2\) flux in Mol:SPRD rats \((n = 8)\), Hsd:DH guinea pigs \((n = 7)\), mongrel dogs \((n = 4)\), and HsdHan:NMRI mice on \(diet 4012.01\) \((n = 8)\) and on SDS diet \((n = 10)\). Rat cecum; guinea pig cecum; mongrel dog colon; HsdHan:NMRI mice on \(diet 4012.01\) cecum; HsdHan:NMRI mice on SDS diet cecum. Values are means \(\pm SE\). Values at time 0 are stable values before killing. Values from 0 to 5 min are postmortem values. When error bars are not shown, they are smaller than the symbols of the graph. The inclination of the serosal \(P_{CO_2}\) line from time 0–5 indicates the postmortem transmural \(CO_2\) flux for each group.

### Table 1. In vivo serosal \(P_{CO_2}\) levels at time 0 and statistical differences in rats, guinea pigs, dogs, and mice

<table>
<thead>
<tr>
<th>Strain/Species</th>
<th>n</th>
<th>Serosal (P_{CO_2}), Torr</th>
<th>(P) Values of Interspecies Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mol:SPRD rats</td>
<td>8</td>
<td>63.7 (\pm) 3.7</td>
<td>HsdHan:NMRI mice(^a) Mongrel dogs(^a) Hsd:DH guinea pigs</td>
</tr>
<tr>
<td>Hsd:DH guinea pigs</td>
<td>7</td>
<td>66.5 (\pm) 2.9</td>
<td>(&lt;0.001) (&lt;0.001) 0.57</td>
</tr>
<tr>
<td>Mongrel dogs(^a)</td>
<td>4</td>
<td>29.6 (\pm) 6.4</td>
<td>(&lt;0.001) (&lt;0.001) 0.006(^a)</td>
</tr>
<tr>
<td>HsdHan:NMRI mice(^b)</td>
<td>8</td>
<td>73.0 (\pm) 6.1</td>
<td>(&lt;0.001) (&lt;0.001) 0.006(^b)</td>
</tr>
<tr>
<td>HsdHan:NMRI mice(^c)</td>
<td>10</td>
<td>198.9 (\pm) 21.9</td>
<td>(&lt;0.001) (&lt;0.001) 0.006(^c)</td>
</tr>
<tr>
<td>Mongrel dogs(^d)</td>
<td>4</td>
<td>47.6 (\pm) 6.1</td>
<td>(&lt;0.001) (&lt;0.001) 0.006(^d)</td>
</tr>
</tbody>
</table>

Values are means \(\pm SE\); \(n = \) no. of animals. \(^a\)Dogs with intestinal content in colon; \(^b\)mice maintained on \(diet 4012.01\); \(^c\)mice maintained on SDS RM1 (E) SQC diet, data from Ref. 13; \(^d\)dogs without intestinal content in colon. Values were tested by \(t\)-test or Mann-Whitney rank sum test with corrected levels of significance: \(\*P \leq 0.007, \#P \leq 0.001.\)
tem. During a 5-min observation period, the postmortem increase in serosal PCO2 due to inherent proton production during anaerobic metabolism in the cecal wall is negligible in mice (13). Proton production during anaerobic metabolism is likewise considered to contribute only little to the \( J_{CO2} \) in the other species. Differences in \( J_{CO2} \) during the 5-min observation period are, therefore, expected to reflect primarily differences in the physical barrier to CO2 diffusion as the vascular removal of CO2 fails. The \( J_{CO2} \) of 33 ± 4 Torr/min in mice on standard rodent diet (13) was significantly higher than that in the mice on diet 4012.01 (13 ± 2 Torr/min) but not significantly different from that in the jejunum of rats (23 ± 2 Torr/min) and rats (40 ± 5 Torr/min) (Table 3, Fig. 1). The \( J_{CO2} \) of 1 ± 1 Torr/min in dogs was the lowest of all species and was significantly lower than that in mice on standard rodent diet, rats, and guinea pigs. The highest \( J_{CO2} \) was observed in rats, which was significantly higher than that in guinea pigs. However, the serosal PCO2 kinetics appear to be biphasic in rats, with a higher \( J_{CO2} \) of 62 ± 6 Torr/min for the first 3 min postmortem (Fig. 1).

In addition to the high luminal-to-serosal PCO2 ratio, the effective washout of CO2 in the colon of dogs was also demonstrated by simultaneous measurement of mucosal and luminal PCO2 in two dogs with intestinal content in the colon. Luminal, mucosal, and serosal PCO2 values were 436, 48, and 25 Torr in one dog and 383, 50, and 32 Torr in the other dog, respectively, which demonstrates the efficacy of the mucosal circulation in CO2 removal. In another dog, in which intestinal content was present in the entire colon except for a short segment ~20 cm distal to the cecalocolic junction, luminal PCO2 of the colon was measured at the cecalocolic junction and ~15, 20, and 23 cm distal to this point. PCO2 levels of 383, 190, 47, and 141 Torr, respectively, were recorded in these locations. These data demonstrate that the high luminal PCO2 levels of the colon were associated with local intestinal content and that the luminal PCO2 of the colon appeared to decrease with increasing distance from the small intestine.

The PCO2 levels of the jejunal lumen were low in all species (83 ± 5 Torr in rats, 59 ± 4 Torr in guinea pigs, and 80 ± 3 Torr in dogs) and thus were comparable to the previously observed levels in the jejunum of mice (13) and expected normal Ptico2 of 50–90 Torr (10, 18).

**DISCUSSION**

External influx of CO2 is likely when Ptico2 levels of 150–185 Torr are recorded under conditions of normal tissue metabolism, perfusion, and systemic arterial PO2 and PCO2, as was previously observed in mice (13). Under such conditions, the total pressure of all gases dissolved in the tissues may exceed the hydrostatic pressure, and tissue gas supersaturation can, therefore, occur in areas with a low blood pressure. Mean serosal PCO2 levels as high as previously measured in mice were not measured in any of the species tested in this study, despite the luminal PCO2 levels in rats and dogs (with colon content) being as high as in mice. In the HsdHan:NMRI mice on standard rodent diet (13), the high luminal and serosal PCO2 and \( J_{CO2} \) indicate

### Table 2. In vivo luminal PCO2 levels at time 0 and statistical differences in rats, guinea pigs, dogs, and mice

<table>
<thead>
<tr>
<th>Strain/Species</th>
<th>n</th>
<th>Luminal PCO2, Torr</th>
<th>P Values of Interspecies Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mol:SPRD rats</td>
<td>8</td>
<td>391.6 ± 18.4</td>
<td>0.67</td>
</tr>
<tr>
<td>Hsd:DH guinea pigs</td>
<td>7</td>
<td>275.6 ± 17.3</td>
<td>0.005*</td>
</tr>
<tr>
<td>Mongrel dogs*</td>
<td>4</td>
<td>523.3 ± 67.1</td>
<td>0.006*</td>
</tr>
<tr>
<td>HsdHan:NMRI mice*</td>
<td>10</td>
<td>480.7 ± 31.6</td>
<td>&lt;0.001f</td>
</tr>
<tr>
<td>Mongrel dogs*</td>
<td>4</td>
<td>105.5 ± 20.8</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE, \( n = \) no. of animals. \( J_{CO2} \), transmural CO2 flux; PCO2serosal, serosal PCO2; \( t \), time. \*Dogs with intestinal content in colon; \*mice maintained on diet 4012.01; \*mice maintained on SDS RM1 (E) SQC diet, data from Ref. 13; \*dogs without intestinal content in colon. Values were tested by \( t \)-test or Mann-Whitney rank sum test with corrected levels of significance: \* \( P \leq 0.007 \), \( P \leq 0.001 \).

### Table 3. \( J_{CO2} = dPCO2_{serosal}/dt \) from 0 to 5 min postmortem and statistical differences in rats, guinea pigs, dogs, and mice

<table>
<thead>
<tr>
<th>Strain/Species</th>
<th>n</th>
<th>( J_{CO2} ), Torr/min</th>
<th>P Values of Interspecies Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mol:SPRD rats</td>
<td>8</td>
<td>39.7 ± 4.7</td>
<td>0.24</td>
</tr>
<tr>
<td>Hsd:DH guinea pigs</td>
<td>7</td>
<td>22.7 ± 1.5</td>
<td>0.033</td>
</tr>
<tr>
<td>Mongrel dogs*</td>
<td>4</td>
<td>1.4 ± 0.9</td>
<td>0.007f</td>
</tr>
<tr>
<td>HsdHan:NMRI mice*</td>
<td>8</td>
<td>12.8 ± 1.5</td>
<td>&lt;0.001f</td>
</tr>
<tr>
<td>HsdHan:NMRI mice*</td>
<td>10</td>
<td>32.6 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>Mongrel dogs*</td>
<td>4</td>
<td>2.5 ± 1.5</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE, \( n = \) no. of animals. \( J_{CO2} \), transmural CO2 flux; PCO2serosal, serosal PCO2; \( t \), time. \*Dogs with intestinal content in colon; \*mice maintained on diet 4012.01; \*mice maintained on SDS RM1 (E) SQC diet, data from Ref. 13; \*dogs without intestinal content in colon. Values were tested by \( t \)-test or Mann-Whitney rank sum test with corrected levels of significance: \* \( P \leq 0.007 \), \( P \leq 0.001 \).
that transmural diffusion is a normal event in vivo due to insufficiency of both the physical and vascular barrier to CO₂ diffusion. This is the reason for the gas supersaturation in the cecal wall of mice. The in vivo luminal and serosal Pco₂ levels in the rat indicate that the cecal wall and/or blood circulation is an effective barrier of transmural CO₂ diffusion. However, the high \( J_{\text{CO₂}} \), not least during the first 3 min after death, indicates the inefficiency of the cecal wall alone to serve as a physical barrier to transmural CO₂ diffusion (as the circulation stops). Indirectly, this implies that the vascular removal of CO₂ in rats is an important and generally efficient part of the barrier of CO₂ diffusion, which enables the mean in vivo serosal Pco₂ to be significantly lower than in mice, despite equally high luminal Pco₂. In guinea pigs, low serosal Pco₂, low luminal Pco₂, and low \( J_{\text{CO₂}} \) are observed. This indicates that the vascular removal of CO₂ in guinea pigs may be equally important and effective as in rats. However, the lower serosal Pco₂ may also be a result of a more effective physical barrier to CO₂ diffusion and the lower luminal Pco₂ in guinea pigs. In the mice on diet 4012.01, the low serosal Pco₂ and \( J_{\text{CO₂}} \) are explained primarily by the low luminal Pco₂, as the vascular and physical barrier to CO₂ diffusion is expected to be identical with that of mice on standard rodent diet. Although lowering the luminal Pco₂ may have effects on serosal Pco₂ in species in which the vascular and physical barriers to CO₂ diffusion are as poor as in mice, this is not a relevant consideration in species such as dogs in which the vascular and/or physical barriers are very efficient. The highest luminal Pco₂, the lowest serosal Pco₂, and the lowest \( J_{\text{CO₂}} \) in any of the species examined were recorded in dogs with intestinal content in the colon. With comparable luminal Pco₂ in dogs, rats, and mice (on standard rodent diet), the significantly lower serosal Pco₂ of dogs indicates how the colonic wall and/or blood circulation is a much more effective barrier to transmural CO₂ diffusion than that in rats and mice. The very low \( J_{\text{CO₂}} \), which is comparable to the low values seen in germ-free mice (13) (with no luminal-serosal CO₂ gradient), illustrates the effectiveness of the colonic wall as a physical barrier to transmural CO₂ diffusion in dogs but also obscures an evaluation of the vascular barrier to transmural CO₂ diffusion. However, as luminal Pco₂ in dogs has been shown to have marked effects on, and is primarily removed by, the intestinal circulation (12), the importance and efficacy of the blood supply may, in this respect, be as important as the physical characteristics of the dog colon. The observation of mucosal Pco₂ levels of 50 Torr (comparable to normal PtiCO₂) in two dogs, despite luminal Pco₂ levels of 380–435 Torr, illustrates the efficacy of the vascular absorption and removal of CO₂ in the mucosa.

When maintained on a standard rodent diet, the incidence of GCA-induced lesions in HsdHan:NMRI mice was high (14). When the same strain was maintained on diet 4012.01, which markedly reduces the supply of dietary substrate to the large intestine, GCA-induced lesions were absent (14). On the basis of Pco₂ levels observed in the present study, these results illustrate the association among bacterial CO₂ production in the cecal lumen, transmural CO₂ diffusion, serosal Pco₂ levels, and GCA-induced lesions in mice, with an ineffective barrier to transmural CO₂ diffusion. Whereas the same dietary control may also be effective in other strains of mice and rats, dietary differences are without relevance in species with thicker intestinal walls and more efficient barriers to CO₂ diffusion, as seen in guinea pigs and dogs. If high serosal Pco₂ levels and gas supersaturation in the cecal wall are considered to be related to the incidence of GCA-induced lesions, the Pco₂ levels in mice on different diets, guinea pigs, and dogs correlate very well with the incidence of GCA-induced lesions. Individual, local, and/or temporal differences in cecal circulation may occur as normal events, in both rats and mice. Considering that the \( J_{\text{CO₂}} \) of rats was as high as that in mice (and higher during the first 3 min post mortem), the same mechanism of intravascular gas bubble growth as that in mice (14) is therefore suggested to account for the GCA-induced pathology observed at low incidence in rats. It should be noted that, once initiated, the proposed mechanism of intravascular gas bubble growth will rapidly promote further bubble growth (14).

Although the very high Pco₂ levels in the colon lumen and the low levels of serosal Pco₂ and \( J_{\text{CO₂}} \) in dogs cannot exclude that the most centripetal layers of the colon wall could be affected by gas supersaturation, mucosal Pco₂ levels comparable to normal PtiCO₂ suggest that gas supersaturation does not occur in the colon wall of normal dogs. These results, in a species with an intestinal tract comparable to that in humans, reflect the efficacious blood supply of the mucosa and the thickness of the intestinal wall, in particular the muscular layer, through which any CO₂ not absorbed by the mucosal circulation must diffuse. The difference in colon luminal Pco₂ correlates with the bacterial fermentation activity of the intestinal content, which both decrease the further one measures distal in the colon. In addition, if the colon is without intestinal content (as seen 20 cm distal to the cecocolonic junction in one dog), the Pco₂ levels decrease markedly. This illustrates that, when present, bacterial fermentation of the intestinal content is the main source of CO₂ produced in the colon of dogs, and the same is expected to be the case in the cecum of rats and guinea pigs. Although no relevant references are available to support this assumption, the wall of the cecum and colon is expected to be thickest in dogs, followed by guinea pigs and/or rats, and thinnest in mice. However, on histomorphological examination, the muscular layer of the intestines is prominent in dogs, whereas it is barely discernible in mice (H. Rasmussen, unpublished observations). This may suggest that possible intramural gas supersaturation due to CO₂ production in the cecum and colon lumen and the incidences of GCA-induced lesions are related to the thickness of the intestinal wall, which again is related to the size of the species.
In conclusion, the very high luminal P\textsubscript{CO2} levels observed in all species, not least in the colon of dogs, suggest a cautious interpretation of tonometry in intestinal segments with a high bacterial activity. These data indicate that the intrinsic ability of the cecal and colonic wall as a barrier to CO\textsubscript{2} diffusion, due to differences in intestinal blood flow and/or intestinal morphology, constitutes the basis for the species differences in transmural CO\textsubscript{2} diffusion and serosal P\textsubscript{CO2}. The transmural CO\textsubscript{2} diffusion in the cecum, which in mice results in P\textsubscript{CO2} levels indicative of gas supersaturation, is related to and most likely is the cause of the intestinal and hepatic lesions observed in mice and rats after a single administration of GCAs. On the basis of these data, it is reasonable to assume that the lower transmural CO\textsubscript{2} diffusion explains the absence of similar lesions or clinical signs thereof in guinea pigs, dogs, and other species.

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