Effect of blood pH on anionic ferritin transport through rat aortic endothelium[1]

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Summary. Acute lowering of blood pH between 7.4 and 6.9 in rats by ventilation with 10 or 20% CO_2 does not increase the passage of ferritin molecules across the aortic endothelium. These results do not rule out alteration of endothelial permeability to anionic macromolecules in local circulatory disturbances when blood pH drops to levels much lower than 6.9.

The luminal front of blood vessel endothelial cells is negatively charged[4]. This anionic cell coat, rich in sialic acid residues[5], may represent the primary limiting barrier to the transport of anionic macromolecules across the endothelial cell layer[6,7]. It is not unreasonable to suggest that this electrostatic barrier may be affected by alterations in blood pH. Changes in pH as a result of local microcirculatory disturbances have indeed been implicated in producing endothelial cell injury[8]. The present study was designed to examine whether the passage of native anionic ferritin across the aortic endothelium could be influenced by pH changes in the systemic circulation.

Materials and methods. The experimental design is shown in figure 1. Male Wistar rats (250–300 g) were used throughout these studies. All rats were anaesthetized with 50 mg/kg b.wt of nembutol injected i.p., which provided adequate anaesthesia for up to 2 h. The iliac and carotid arteries and jugular vein were cannulated. The blood pressure was measured via the carotid cannula through a pressure transducer on a Narco physiograph. A baseline blood pressure was established. All rats with diastolic pressures greater than 120 mm Hg were discarded from the study. After insertion of a tracheostomy Y-tube, 4 mg/kg b.wt of flaxedil (gallamine), a neuromuscular blocker, was injected as a bolus via the jugular cannula. The rat was ventilated by a Harvard rodent ventilator at a rate of 80 strokes per min with a tidal volume of 2 ml. Muscle paralysis was maintained for 40 min by the constant infusion of flaxedil (4 mg/ml) by a Harvard syringe pump. After 20 min of ventilation time, 50 mg/100 g b.wt of dialyzed native ferritin (horse spleen, cadmium free, Nutritional Biochemicals Corporation, Cleveland, Ohio, USA) was injected via the jugular vein over a 5-min period. The arterial system was perfused with fixative after 20 min ferritin circulation time via the left ventricle of the heart and segments of the abdominal aorta were processed for electron microscopy as described previously[9]. Arterial blood samples were drawn from the iliac cannula in heparinized capillary tubes at 5 min prior to and at 5, 15 and 40 min after initiation of ventilation. These samples were refrigerated until determination of pCO_2, pO_2 and pH by microtechnique using a Model 212 IL blood gas analyzer.

The control group (5 rats) was ventilated with room air. The 2 experimental groups were ventilated with premixed gases composed of 10.18% CO_2, 20.79% O_2 and 68.03% N_2 (3 rats); and 20.00% CO_2, 20.73% O_2 and 59.27% N_2 (10 rats) respectively.

Results. The table illustrates the averaged values of the indices measured for each of the 3 groups during the 40 min of ventilation time. The arterial blood gases and pH-values of each individual rat was determined by averaging the results obtained from 2 readings of 3 separate blood samples (drawn at the times indicated above) – that is a total of 6 readings of 3 blood samples.

There was no significant change in either the diastolic pressure or the pO_2 between the experimental groups and the control group. However, as the percentage of inspired CO_2 was increased from room air concentration in the control group to 10 or 20 in the experimental groups, there was a significant ($p < 0.001$) and progressive increase in pCO_2 and decrease in pH.

Electron microscopy of aortic segments revealed continuous endothelial cell layers with well preserved fine structure in both control and experimental groups. There was no difference found in the distribution of ferritin between groups. Ferritin molecules were identified within some plasmalemmal vesicles and multivesicular bodies of endothelial cells. The subendothelial space was devoid of ferritin in multiple sections; it contained only occasional tracer molecules (figure 2, a–c).

Discussion. The model utilized in this experiment produced consistent alterations of the arterial pCO_2 and pH while maintaining normal pO_2 and diastolic blood pressures.

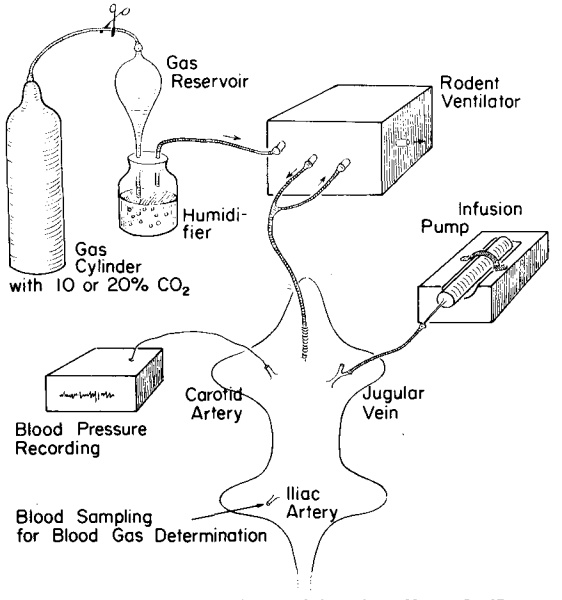

Fig. 1. Experimental design for studying the effect of pH on tracer transport through rat aortic endothelium (see explanation in text).

Alteration of blood gases associated with CO_2 ventilation*

	Diastolic pressure	pCO_2	pO_2	pH
Control (5)	103±5	33.1±1.9	77.1±2.8	7.40±0.02
10% CO_2 (3)	113±4	69.0±1.3**	72.8±1.8	7.14±0.01**
20% CO_2 (10)	99±5	95.3±1.5**	78.3±1.2	6.96±0.01**

* The values are expressed as the mean ± SEM. Number of observations is indicated in parenthesis. ** $p < 0.001$.

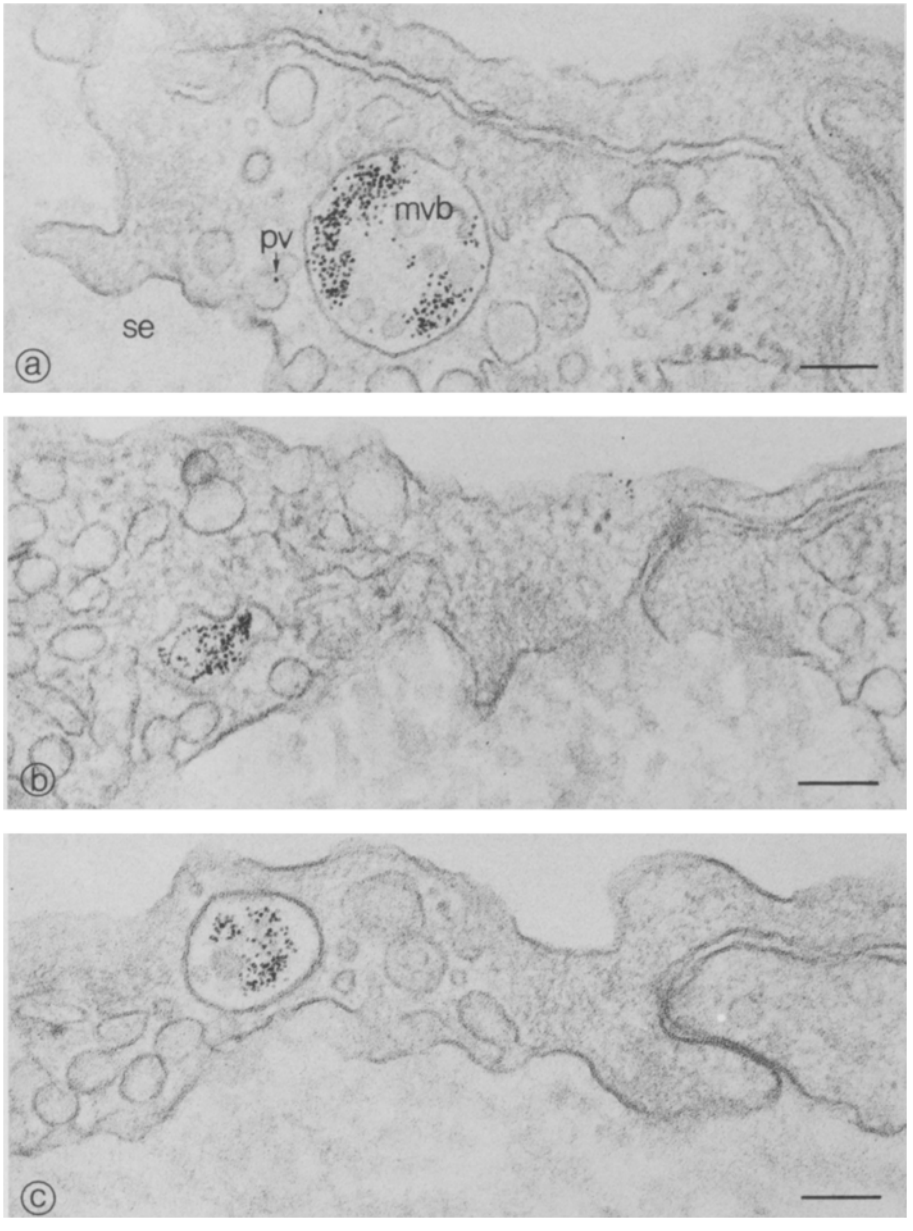

Fig. 2. Comparative electron micrographs of abdominal aortic endothelium, 20 min after ferritin injection, from control rat (a), from rat ventilated with 10% CO_2 (b) and from rat ventilated with 20% CO_2 (c). Ferritin molecules are localized in some plasmalemmal vesicles (pv) and multivesicular bodies (mvb). The subendothelial space (se) is devoid of tracer in both control and treated animals. Uranyl acetate en bloc staining. The scales in a, b and c represent 0.1 μm.

Changes of pH to levels as low as 6.95 however, had no effect on the transport of anionic ferritin across rat aortic endothelium. This negative result has clinical implication as pH range of 7.40 to 6.95 studied in the present model is that usually found in the systemic circulation in various disease states associated with metabolic and respiratory acidosis[10,11].

The pH-alterations produced in the rats were certainly not sufficient to significantly affect the net charge of anionic ferritin molecules (pI 4.1–4.6)[12]. On the other hand, the anionic elements on the endothelial surface coat are probably not affected by small change in blood pH. The pKa of sialic acid is around 2.6, which means that a large percentage of these anionic groups are ionized and probably charged even at very low blood pH. In any case, our results indicate that pH changes in the systemic circulation compatible with life probably do not affect charge-related endothelial barrier function. This function may be altered however in local circulatory disturbances, when blood pH drops to levels much lower than 6.9.

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