The Role of Acidosis in the Etiology of Pulmonary Emphysema* (34034)

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Experimental pulmonary emphysema has been produced in newborn piglets both acutely and chronically, by a combined technique of hypercapnia and hypoxia (1, 2). During the experimental period in both studies the piglets underwent severe hyperventilation as well as a marked elevation of the partial pressure of carbon dioxide in the blood, marked depression of the partial pressure of oxygen in the blood and a markedly decreased pH of the blood.

Thus, these animals simultaneously experienced hyperventilation, metabolic acidosis, and respiratory acidosis. It is, therefore, important to segregate each of these factors to see if only one is necessary to produce the pulmonary emphysema. The object of this experiment was to produce artifically a metabolic acidosis and respiratory acidosis with no severe concomittant hyperventilation and to then observe whether these changes would induce pulmonary emphysema.

Materials and Methods. Newborn piglet littermates were allowed to suckle for the first 24 hr of life in order to obtain antibodies from the colostrum that they received from nursing on the sow, after which they were removed from the sow and transported to the laboratory in a heated car. Using the piglet technique previously described (1), the piglets were paired by weight into control and test groups and placed into metabolic cages. They all were maintained on water *ad libitum* and fed liquid Similac with iron. An ambient temperature at 92° and humidity control was maintained throughout the entire experiment. The control piglets were maintained on the Similac diet for the entire experimental period. By the tenth day of life, the test piglets were also given NH_4Cl (1 g/liter) and Acetazolamide (Diamox) (0.75 g/liter) b.i.d., dissolved in the standard Similac diet, using a technique previously reported (3). This regimen was maintained for a 6-week period. After this dietary regimen was stopped, the animals were allowed to live for a few days before being sacrificed. Immediately before being sacrificed, the piglets were given light anesthesia, then cardiac punctures were done to determine arterial pH, pCO_2, pO_2, hematocrit, total CO_2, and bicarbonate. Blood analyses were done as previously described (1, 2). The piglets were then weighed and sacrificed by a method previously reported (1, 2). The trachea was tied and the lungs were removed and weighed, then preserved for gross and histological study. Urine for calcium determinations were collected from the metabolic cages and terminally by aspiration with a syringe from the bladder. Bone for calcium determinations was obtained by removal of the fourth right rib from each piglet. This bone was then dried in an oven at 100° for 24 hr to dry weight and subsequently ashed in a furnace at 500° for 48 hr (4). Calcium determinations on urine, blood, and fourth right-rib bone ash were done using atomic absorption spectrophotometric techniques (5).

Results. Three sets of piglet littermates were paired by weight and divided into control and test groups. Mean birth weights are shown in Table I to differ by 60 g. The same quantity of liquid Similac with iron and water was offered to each group during the entire experimental period. On Day 10 after birth, the test group received ammonium chloride and acetazolamide dissolved in their routine diet. This acidogenic diet was fed to the test piglets until Week 7 after birth at which time the experiment was terminated. At death the mean body weight of the control group was 117 g greater than that of the test group. The growth curve pattern (Fig. 1) of the test animals was similar, but at a lower level than that of the control piglets. These

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TABLE I. Effect of Chronic Acidosis on Birth Weight, Death Weight, and Terminal Lung Weight.

Piglet litter	Birth wt (g)	Death wt (g)	Lung wt (g)
Control			
1	1.225	2.500	28.31
2	1.320	1.800	22.05
3	1.296	2.300	26.49
$\bar{X}$	1.280	2.200	25.62
± SD	0.049	0.361	3.22
Test			
1	1.347	2.400	30.92
2	1.287	1.550	20.75
3	1.022	2.300	26.92
$\bar{X}$	1.220	2.083	26.19
± SD	0.184	0.465	5.123

weights did not differ significantly by statistical analysis.

The test piglets showed no gross behavioral differences from the control group. On physical examination the lungs were clear to auscultation. During Week 7 of the piglets' life cardiac puncture was done and blood from the left ventricle was withdrawn anaerobically for analysis. Mean pH value of the control group was 7.355 in contrast to the mean pH value of the test piglets which was 7.195 as shown in Table II. These values are significantly different. The control groups mean pCO_2 (6) was 37.2 mm Hg, whereas the mean of the test group was 34.3 mm Hg; these values were not significantly different. The mean plasma bicarbonate concentration for the control group was 20.3 mEq liter

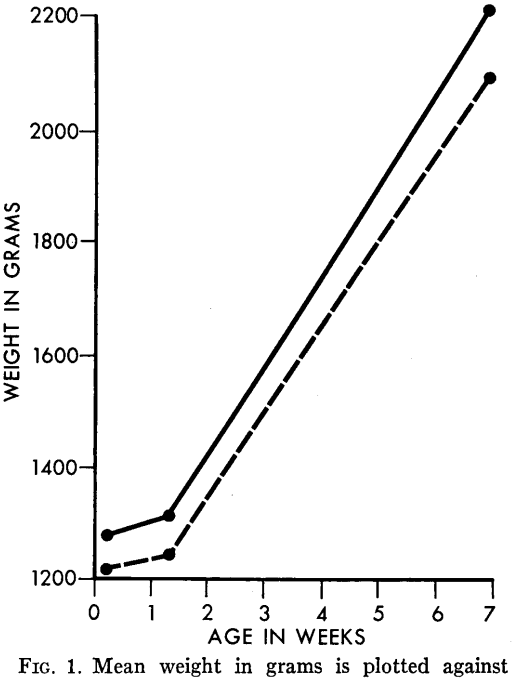

FIG. 1. Mean weight in grams is plotted against age of piglets in weeks. Growth curve for the experimental group is noted by a solid line, that for the control group a broken line.

TABLE II. Effect of Chronic Acidosis on Arterial Blood pH, pCO_2, HCO_3, Total CO_2, Hematocrit, and pO_2.

Piglets	Arterial blood					
	pH	pCO_2	HCO_3	Total CO_2	Hct.	pO_2
Control						
1	7.310	43.0	20.2	21.5	36.0	142.0
2	7.345	38.3	20.2	21.2	34.5	144.0
3	7.410	30.2	20.5	21.4	34.3	147.0
$\bar{X}$	7.355	37.2	20.3	21.4	34.9	144.3
± SD	0.051	6.5	0.17	0.15	0.9	2.5
Test						
1	7.210	35.3	13.8	14.9	28.0	140.0
2	7.290	31.4	14.8	15.7	31.0	139.5
3	7.085	36.3	10.5	11.6	37.2	150.0
$\bar{X}$	7.195	34.3	13.0	14.1	32.1	143.2
± SD	0.103	2.59	2.25	2.173	4.692	5.92
	*		*	*		

* Statistically significant difference between control and test groups.

compared with a mean value of 13.0 mEq/liter for the test group. These values were significantly different. A mean value for plasma CO_2 content was 21.4 mmoles/liter in the control group, whereas the experimental group mean value was 14.1 mmoles/liter; these findings were significantly different. There was no difference between the mean hematocrit value of the control group which was 34.9%, and the test group which was 32.1%. The mean control pO_2 (7) which was 144.3 mm Hg was similar to the mean value of the experimental group which was 143.2 mm Hg.

Each piglet was anesthetized with Nembutal and an incision made in the midline of the neck to enable the trachea to be isolated and tied with a string below the cricoid level (1). The chest was then opened and the lungs of each group observed grossly *in situ*. Control piglet lungs were pink, collapsed, and free of hemorrhage or bullae as were the test piglet lungs. On removal of the lungs from the chest the control lungs (Table I) had a mean weight of 25.62 g while the test lung mean weight was 26.19 g. These values are not significantly different. Cross sections of both groups of piglet lungs were similar. The technique for fixing these small piglet lungs in formalin was described previously (1). Microscopic examination of the control and test lungs showed no significant differences. Normal intact pulmonary structure was evident and the alveolar walls were not markedly dilated nor degenerate in the test piglets.

Calcium levels given in Table III showed the control mean serum calcium level was 4.46 ppm whereas the test piglets had a serum mean calcium level of 5.20 ppm. No significant difference was found. The mean urine calcium level of the control piglets was 9.2 ppm in contrast to the experimental piglet value of 336 ppm; which is significantly different. The mean percentage of calcium of bone ash for the control piglets was 28.97 whereas the mean value for the test piglets was 26.10. These values are significantly different.

TABLE III. Effect of Chronic Acidosis on the Calcium Levels in Serum, Urine, and Bone.

Piglets	Calcium levels		
	Serum (ppm)	Urine (ppm)	Bone % Ca/Ash
Control			
1	4.80	16.0	28.10
2	4.30	0.5	29.90
3	4.30	11.0	28.90
$\bar{X}$	4.46	9.2	28.97
$\pm$ SD	0.29	7.911	0.902
Test			
1	4.60	296	24.90
2	5.50	320	26.00
3	5.50	394	27.40
$\bar{X}$	5.20	336	26.10
$\pm$ SD	0.52	51.1 *	1.25 *

* Statistically significant difference between control and test groups.

Discussion. At the onset of this experiment, this study was at a bifurcation of a logic tree as to the etiology of experimental pulmonary emphysema (8). The purpose of this experiment was to explore the effects of acidosis on the pulmonary parenchyma and its interrelationship with pulmonary emphysema. The first object was to get a set of experimental animals in a state of chronic acidosis. That this was achieved is seen by looking at the significantly lowered pH, the significantly altered total carbon dioxide, and bicarbonate concentration as direct evidence. As indirect evidence of acidosis the significantly elevated urine calcium and the significantly decreased percentage of calcium of bone ash can be considered. The values that are not expected to change with acidosis, such as blood calcium concentration and hematocrit, have not changed. Having established that this is a truly chronically acidotic animal, now consider the lung of this animal and its function. The piglets had all grown normally, as can be seen from the birth and death weights (Table I) and normal growth pattern (Fig. 1). Looking more specifically at the lung, there was no significant difference in lung weights at the time of death (Table I). The gross examination of the lung showed no bullae, and examination of the lung sections, both grossly and microscopically,

showed no difference in the appearance of the lung parenchyma. In neither the control group nor the test group were there dilated alveoli with thinned and fragmented wall that is characteristically seen in animals with experimental pulmonary emphysema.

A significant physiological measurement of this lung normality was the fact that the pO_2 and pCO_2 were normal in both groups, even though the experimental group was given acetazolamide. This is in marked contrast to the animals in which experimental pulmonary emphysema had been induced by hypercapnia and hypoxia. Test piglets exposed to hypercapnia and hypoxia had blood studies with an increased pCO_2 and decreased pO_2 even when breathing air; histologically their lungs showed dilated alveoli with thinned and fragmented walls.

Therefore, on the basis of both gross and histological studies, as well as physiological measurements of the blood gases, it can be seen that chronic acidosis, both metabolic and respiratory, does not appear to be the necessary and sufficient etiological factor for the production of experimental pulmonary emphysema.

Summary. The object of this experiment was to evaluate the role of acidosis in the etiology of pulmonary emphysema. Piglets were fed a diet of NH_4Cl and acetazolamide for 6 weeks to induce a chronic metabolic and respiratory acidosis.

At autopsy, 3 days after the termination of this acidogenic diet, the animals still had acidosis as determined by arterial blood pH. The animals had maintained a normal growth curve. Their urinary calcium was markedly elevated and their bone calcium depressed, as would be expected in chronic acidosis. The lungs, however, were normal in color and size with no gross bullae. On microscopic examination the alveoli were normal in size and the alveolar walls were also normal. It, therefore, appears that chronic acidosis is not a major etiological factor in pulmonary emphysema.

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