TABLE I

Tissue*	Mean glucose uptake (mg/100 mg of tissue) C–B	Control, R–B	P
Diaphragm (14)	.446	.307(±.027)†	P = .01
Adipose (15)	.293	.184(±.025)	P = .01
Kidney (18)	.757	.408(±.075)	P = .01
Brain (11)	.677(±.070)†	.628(±.073)	P > .05

	Mean respiration (μl O_2/100 mg tissue/hr) C–B	Control, R–B	
Brain (11)	207.7(±7.90)	162.2(±7.10)	P < .01

* Figures in parentheses are number of experiments.

† Standard error of the mean.

humoral factor is produced during muscular contraction which enhances the entry of glucose into tissues. We are now investigating the nature of this factor and its possible physiological role.

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Development of Hyaline Membranes and Atelectases in Experimental Chronic Respiratory Acidosis.* (27657)

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Carbon dioxide retention has been considered a primary factor in the development of pulmonary hyaline membranes in the asphyctic newborn(1). Atelectases associated with hyaline membranes is a frequent cause of death in the newborn(2). Exposure of guinea pigs to 14-17% CO_2 in 21% O_2 for 8-22 hours was found to result in hyaline membrane formation while ammonium chloride acidosis (3-24 days) failed to produce such effects(3). On this basis it was concluded that hyaline membrane formation is caused specifically by CO_2 rather than by acidosis(3). However, during our studies of chronic CO_2 toxicity it was observed that incidence of hyaline membrane formation progressively decreased in guinea pigs during prolonged exposure to CO_2 after compensation of the respiratory acidosis. This supports the concept of non-specific acidosis.

Material and methods. Male guinea pigs (400-600 g) of the Hartley strain and male albino rats from the colony of the Harvard Biological Laboratories (age 75-120 days) were exposed to various CO_2 concentrations for different periods. Animals in any particular group were of the same age. They were sacrificed after exposure to CO_2 and after recovery periods of various lengths.

Anaerobic blood samples were taken through heart punctures. pH was measured at 37°C by a Model G, Beckman pH meter. Whole blood CO_2 content was determined with the Van Slyke apparatus and CO_2 tension was calculated using the nomogram of Van Slyke and Sendroy(4).

Exposure times were divided into 2 periods corresponding to the phases of uncompensated and compensated respiratory acidosis. The former was found to last 23-28 days under 1.5% CO_2, 4-5 days under 3% CO_2, and 2 days under 15% CO_2(5). Furthermore, a third period of extended exposure was included. Recovery periods of one day and 2 weeks were selected. The histological data presented show incidence of pulmonary path-

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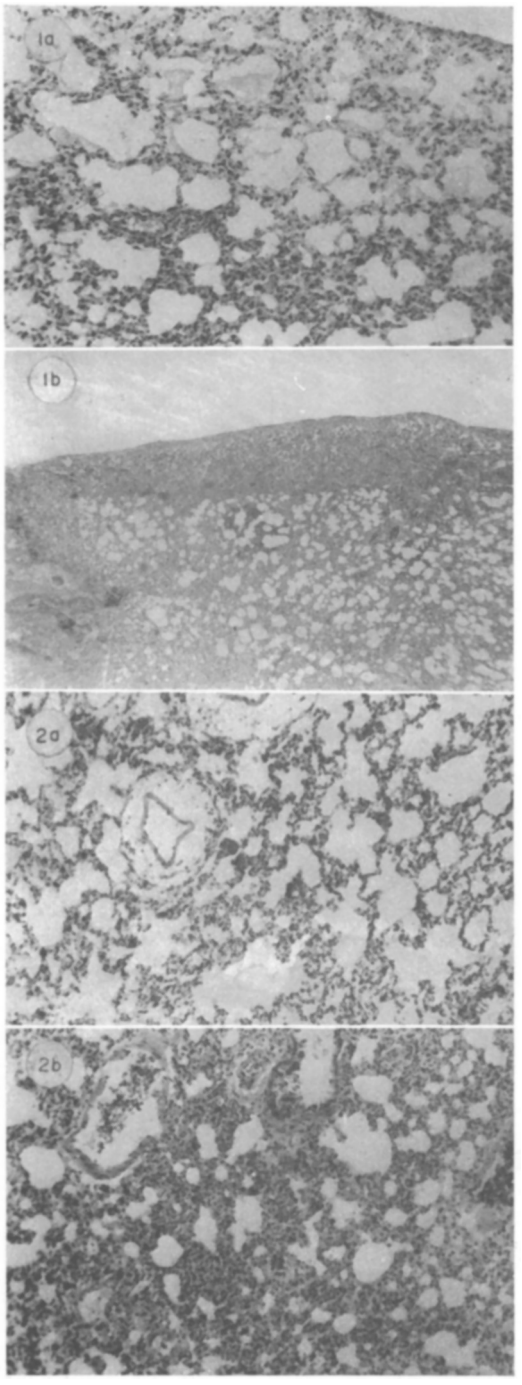

FIG. 1a. Hyaline membranes in lungs of a guinea pig exposed for 3 wk to 3% CO_2 in 21% O_2, H. and E. 50×. 1b. Subpleural atelectasis in a guinea pig exposed for 48 hr to 15% CO_2 in 21% O_2, H. and E. 13×.

FIG. 2a. Perivascular edema in a rat exposed to 50% CO_2 for 1 hr, Masson, 50×. 2b. Massive interstitial infiltration in a rat exposed to 50% CO_2 for 1 hr with subsequent recovery on air for 7 days, H. and E. 50×.

ology and grades of development.

Interstitial infiltration of the lungs was found in a high percentage of control animals and no significance was attached to such findings. However, hyaline membrane formation was never observed under normal conditions in a larger population of guinea pigs and rats, which is in agreement with other investigations(3).

Results. Histopathological data obtained in guinea pigs exposed to 1.5%, 3% and 15% CO_2 are presented in Tables I and II with blood pH and pCO_2 values. Hyaline membrane formation was not seen during exposure to 1.5% but was found during exposure to 3% (Fig. 1a) and 15% CO_2. Incidence was highest during the uncompensated phase of respiratory acidosis in both series of experiments and decreased with further exposure.

Atelectases and hyaline membranes were usually located in the subpleural region (Fig. 1b). Atelectasis followed in general the development of hyaline membranes except for the series of animals exposed to 1.5% CO_2, where it developed during the early as well as the late phase of exposure. Perivascular edema was found only in the 3% and 15% series and showed a tendency to decrease on continued exposure similar to the incidence of hyaline membranes and atelectases. Alveolar edema was noted to a minor degree during exposure to 3% CO_2 and was more pronounced during the uncompensated phase of respiratory acidosis in animals exposed to 15% CO_2. Data on the stress effect of exposure to 15% CO_2 as measured in adrenal cholesterol content were obtained in another series of experiments (Table III).

Adrenal cholesterol content is significantly lower than normal only during one day exposure to 15% CO_2 indicating an increased adrenal activity during this period of uncompensated respiratory acidosis. After compensation of the acidosis, adrenal cortical activity returned to control levels or below.

Fifty-six rats were exposed in several experiments to 1.5% CO_2 and 3% CO_2 corresponding to the exposure times of guinea

TABLE I. Effect of Prolonged Exposure to 1.5% and 3% CO_2 on Formation of Hyaline Membranes and Atelectasis in Guinea Pigs.

	Condition	N	pH Mean	pH S.D.	pCO_2 Mean	pCO_2 S.D.	Hyaline membranes Incid.	Hyaline membranes Grade	Atelectasis Incid.	Atelectasis Grade	Perivascular edema Incid.	Perivascular edema Grade	Alveolar edema Incid.	Alveolar edema Grade
	A						%		%		%		%	
1	Controls	(4)	7.40	.01	40.8	2.0	0	0	0	0	0	0	0	0
	1.5% CO_2 in 21% O_2													
2	1-23 days	(2)	7.37	.02	49.0*	1.5	0	0	50	1.0	0	0	0	0
3	24-42 days	(6)	7.38	.03	50.1*	3.0	0	0	33	1.0	0	0	0	0
4	6 mo	(4)	7.39	.02	51.5*	3.5	0	0	75	1.0	0	0	0	0
5	Recovery 2 wk after 42 days of exposure	(4)	7.38	.02	43.7	2.1	0	0	0	0	0	0	0	0
	B													
1	Controls	(7)	7.38	.02	40.5	3.0	0	0	0	0	0	0	0	0
	3% CO_2 in 21% O_2													
2	2 days	(4)	7.27*	.03	62.0*	6.2	0	0	50	.5–2	25	.5	25	.5
3	4 days	(6)	7.31*	.04	56.0*	5.8	50	.5–1.0	30	1–2	0	0	16	.5
4	6-42 days	(8)	7.36	.03	55.7*	5.3	25	.5–1.0	25	.5–1.0	0	0	50	.5–1.0
5	Recovery 1 day after 42 days of exposure	(7)	7.37	.07	43.4	4.8	29	.5–1.0	29	.5	0	0	57	.5
6	Recovery 2 wk after 42 days of exposure	(10)	7.39	.04	42.0	4.5	0	0	10	.5	0	0	0	0

* Statistically significant from controls at 5% level and greater.

pigs. In addition 2 groups of animals were exposed to 30 and 50% respectively. Interstitial infiltration was present in control as well as experimental animals. In contrast to guinea pigs, hyaline membrane formation was not found in rats under prolonged exposure to 3% CO_2. Perivascular edema and alveolar edema increased during exposure to 3% CO_2 and disappeared after 2 weeks recovery on air following 3 weeks exposure to 3% CO_2. Both forms of edema were particularly striking after exposure to 30% CO_2 for 5 hours, and

TABLE II. Effect of Prolonged Exposure to 15% CO_2 on Development of Hyaline Membranes and Atelectasis in Guinea Pigs.

	Condition	N	pH		pCO_2		Hyaline membranes		Atelectasis		Perivascular edema		Alveolar edema	
			Mean	S.D.	Mean	S.D.	Incid.	Grade	Incid.	Grade	Incid.	Grade	Incid.	Grade
	C						%		%		%		%	
1	Controls	(6)	7.38	.03	42.6	3.4	0	0	16	.5	16	1.0	0	0
	15% CO_2 in 21% O_2													
2	1-2 days	(3)	7.23*	.02	90.6*	4.0	100	.5–2.0	66	1.0	66	2.0	66	1–2+
3	3-7 days	(7)	7.34	.05	88.9*	6.5	71	.5–2.0	86	.5–1	14	1.0	0	0
4	14-93 days	(5)	7.36	.03	85.9*	7.3	0	0	20	.5	0	0	0	0
5	Recovery 1 day on air after 7 days of exposure	(12)	7.35	.04	49.4	3.8	50	.5–1	50	.5–1	25	1+	9	1+
6	Recovery 2 wk on air after 7 days of exposure	(8)	7.37	.04	43.0	3.0	0	0	12	.5	0	0	0	0

* Statistically significant from controls at 5% level and greater.

50% CO_2 for one hour (Fig. 2a). They disappeared, however, after 7 days recovery following one hour exposure to 50% CO_2, at which time they had been replaced by a widespread interstitial and alveolar infiltration (Fig. 2b).

Discussion. The development of hyaline membranes and atelectases during chronic CO_2 exposure was roughly parallel. Both were predominantly located in the subpleural areas and showed a corresponding time sequence. They developed during the uncompensated phase of respiratory acidosis and decreased during the compensated phase. Hyaline membranes disappeared completely during exposure to 15% CO_2 lasting from 14 to 93 days. During the latter period CO_2 tension remained at double the control value while pH was not significantly different from initial levels, which strongly suggests that this type of pulmonary pathology is caused by a non-specific acidosis effect.

The stress effect of CO_2 as measured in increased adrenal cortical activity was found to be associated with the period of uncompensated respiratory acidosis in guinea pigs exposed to 15% CO_2 for prolonged periods.

Stimulation of adrenal glands is known to play a role in producing pulmonary lesions in oxygen toxicity(6). Protection of animals against oxygen toxicity has been accomplished by hypophysectomy(6) and adrenalectomy (7). Although no studies with adrenalectomized animals were carried out during prolonged exposure to carbon dioxide to determine a possible reduction in pulmonary lesions, it can be assumed that the stress effect of CO_2 contributed to the development of pulmonary pathology during CO_2 exposure. The development of hyaline membranes and atelectases during the uncompensated phase of respiratory acidosis and the subsidence of this pulmonary pathology during the compensated phase of respiratory acidosis appear to have a bearing on physiological findings of alterations in gas exchange during these 2 phases obtained in men during prolonged exposure to 1.5% CO_2. Compared with the data collected during the uncompensated phase of respiratory acidosis, ventilatory efficiency of the CO_2 transport was found improved during the compensatory phase which appeared to be related to a decrease of the physiological dead space following a significant increase of the latter during the uncompensated phase(8).

Findings of hyaline membranes in CO_2 experiments have been reported by Schulz(9)

TABLE III. Effect of Prolonged Exposure to 15% CO_2 on Adrenal Cortical Activity as Measured in Adrenal Cholesterol Content of Guinea Pigs.

			Exposure to 15% CO_2				Recovery on air after 7 days exposure to 15% CO_2	
		Control	1 day	3 days	4-7 days	20-73 days	1 day	5-11 days
Adrenal cholesterol, mg %	Mean	6.1	4.0*	6.2	7.3	5.4	4.7	5.3
	S.D.	.6	1.1	1.4	1.7	1.9	1.2	.8
	N	10	20	13	16	14	17	13

* Statistically significant from controls at the 5% level.

and Kloos and Wulf(1) after exposure to 7% CO_2 in 93% O_2 for 20 hours, after intermittent exposure to various CO_2 concentrations by Meessen (10) and after continuous exposure by Zinck(11). Experimental conditions in Zinck's study were similar to those reported here. However, the correlated physiological measurements published separately (12) did not include pH determinations. Therefore, no relationship of hyaline membrane formation to phases of uncompensated and compensated respiratory acidosis were established. Failure of NH_4Cl acidosis to produce hyaline membranes(3) cannot be considered conclusive evidence for a specific CO_2 effect because of the associated hypocapnia.

The negative findings obtained in rats exposed for prolonged periods to 3% CO_2 indicate a higher resistance of this species to experimental hyaline membrane formation, which is in agreement with other reports(13). This species difference has been explained by the presence of lung plasminogen-activator activity in rats, while the lungs of guinea pigs and rabbits were found totally deficient of this enzyme(14).

Summary. Guinea pigs exposed to 1.5%, 3% and 15% CO_2 for prolonged periods developed, under 3% and 15% CO_2, hyaline membranes associated with atelectases which were predominantly located in subpleural areas. Simultaneous blood pH and pCO_2 measurements indicated that these pulmonary lesions were related to a nonspecific acidosis effect rather than to a specific CO_2 effect. Incidence of hyaline membranes declined from 100% during the uncompensated phase of respiratory acidosis induced by 1-2 days of exposure to 15% CO_2 to 0% during 14-93 days of exposure to 15% CO_2. Under the latter condition blood pH was compensated but blood pCO_2 was elevated to a level twice normal. Rats did not exhibit hyaline membranes during prolonged exposure to 1.5% CO_2 and 3% CO_2.

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