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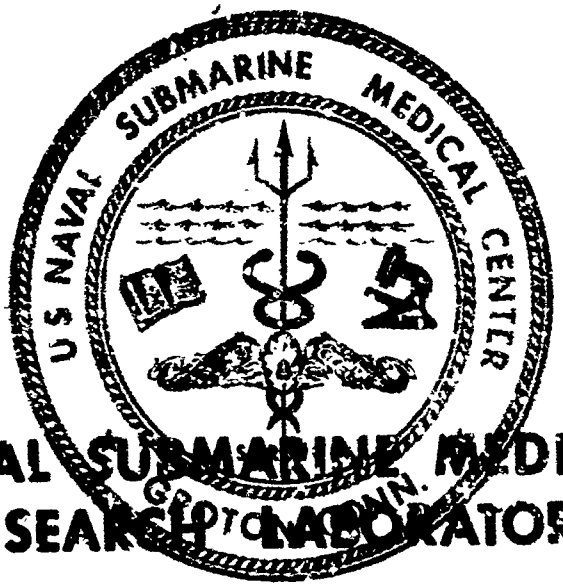
K. E. Schaefer, et al

Naval Submarine Medical Research Laboratory
Groton, Connecticut

4 March 1971



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CHRONIC CO_2 TOXICITY: SPECIES DIFFERENCE IN PHYSIOLOGICAL AND HISTOPATHOLOGICAL EFFECTS

by

K. E. Schaefer, H. Niemoeller, A. Messler, E. Heyder and J. Spencer

Bureau of Medicine and Surgery, Navy Department
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J. E. Stark, CAPT MC USN
COMMANDING OFFICER
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13. ABSTRACT

Guinea pigs were found to have a much higher susceptibility to carbon dioxide than rats. During exposure to CO_2 concentrations ranging from 1-50% CO_2, marked species differences were observed in mortality, growth curves, organ/body weight ratios and serum enzyme responses.

The difference in tolerance to CO_2 between guinea pigs and rats has been related to their differing buffer capacity. Guinea pigs showed higher levels of hydrogen ion concentrations for every exposure.

No evidence of tissue necrosis in heart, liver, and other organs was obtained in guinea pigs or rats exposed for prolonged periods to 15% CO_2. The increased levels of serum enzymes (GPT, GOT, LDH) observed in guinea pigs under these conditions were interpreted as signs of increased permeability caused by hypercapnia.

An organ specific pattern of fat accumulation was observed in chronic hypercapnia.

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Transmitted by:

Karl E. Schaefer

K. E. Schaefer, M.D.
Chief, Biomedical Sciences Division

Reviewed and Approved:

Charles F. Gell

Charles F. Gell, M.D., D.Sc.(Med)
Scientific Director
NAVSUBMEDRSCHLAB

Reviewed and Approved:

Joseph D Bloom.

J. D. Bloom, CDR MC USN
Officer-in-Charge
NAVSUBMEDRSCHLAB

Approved and Released by:

J. E. Stark

J. E. Stark, CAPT MC USN
COMMANDING OFFICER
Naval Submarine Medical Center

I-C

SUMMARY PAGE

THE PROBLEM

To determine physiological and correlated histopathological effects of chronic exposure to carbon dioxide with particular emphasis on low levels of CO_2 as may be potentially found in Navy submarine and diving facilities. To clarify the causes of species difference in tolerance to CO_2.

FINDINGS

Guinea pigs were found to have a much higher susceptibility to carbon dioxide than rats. During exposure to CO_2 concentrations ranging from 1-50% CO_2 marked species differences were observed in mortality, growth curves, organ/body weight ratios and serum enzyme responses.

The difference in tolerance to CO_2 between guinea pigs and rats has been related to their differing buffer capacity. Guinea pigs showed higher levels of hydrogen ion concentrations for every exposure.

No evidence of tissue necrosis in heart, liver, and other organs was obtained in guinea pigs or rats exposed for prolonged periods to 15% CO_2. The increased levels of serum enzymes (GPT, GOT, LDH) observed in guinea pigs under these conditions were interpreted as signs of increased permeability caused by hypercapnia.

An organ specific pattern of fat accumulation was observed in chronic hypercapnia.

APPLICATIONS

These findings are of interest to cognizant personnel concerned with medical aspects of exposure to CO_2 at levels which may be encountered in submarine, diving, space and other closed environments.

ADMINISTRATIVE INFORMATION

This investigation was conducted as part of a Bureau of Medicine and Surgery Research Work Unit MF12.524.006-9028BA9K-Time Concentration Exposure Limits of CO_2 in Navy Submarine and Diving Facilities. The present report is No. 8 on this Work Unit. The manuscript was approved for publication on 4 March 1971 and designated as Naval Submarine Medical Research Laboratory Report Number 656.

Dr. Niemoeller carried out these histopathological studies during his work at the Department of Pathology, Yale University School of Medicine under a contract of the Office of Naval Research.

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ABSTRACT

Guinea pigs and rats were exposed for prolonged periods of time to CO_2 concentrations ranging from 1.0-1.5% CO_2 to 20% CO_2. Rats tolerated these exposures well, in contrast to guinea pigs, 30-50% of which died during exposure to 15% CO_2. Growth curves and organ/body weight ratios obtained in guinea pigs showed marked effects during prolonged exposure to CO_2 concentration as low as 3% CO_2. According to data in the literature, organ weight/body ratios of rats are not affected by prolonged exposures to 11% CO_2 and normal growth is only slightly reduced at these CO_2 levels. Further species differences in the response to CO_2 were observed in serum enzyme responses. GOT and GPT concentrations increased greatly in guinea pigs during exposure to 3% and 15% CO_2 while the enzyme levels in rats were not affected even at 20% CO_2.

Since there was no incidence of tissue necroses, the increased serum enzyme levels in guinea pigs have been interpreted as signs of increased permeability caused by hypercapnia. Studies of acid-base balance showed that guinea pigs had higher levels of hydrogen ion concentration than rats for every arterial CO_2 level investigated. The time course of adaptation to CO_2 was, however, similar. The differences in tolerance to CO_2 between guinea pigs and rats has been related to their differing buffer capacity.

No species differences were observed with regard to histopathological effects. There was a lack of severe tissue damage during exposure to 15% CO_2. No necroses in liver, heart or muscles were found in guinea pigs or rats under these conditions, although lungs, spleen, intestine and kidneys exhibited hemorrhages during the first 24 hours. Exposure to 15% CO_2 caused an inhibition of mitosis in liver cells of rats and an arrest in spermatogenesis in both guinea pigs and rats.

An organ specific pattern of fat accumulation was observed in chronic hypercapnia.

CHRONIC CO_2 TOXICITY: SPECIES DIFFERENCE IN PHYSIOLOGICAL AND HISTOPATHOLOGICAL EFFECTS

INTRODUCTION

Tolerance to CO_2 has been studied by a number of investigators under conditions of sudden and gradual exposure.[3,6,19,21,25-27,30,31-41,43,44,48] Some general effects related to certain ranges of CO_2 are clearly established. In addition, species differences have been found to be quite large. Below 10% CO_2 no narcotic effect can be detected and animals survive for prolonged periods. In most cases, concentrations above 30% CO_2 induce narcosis and the animals do not survive exposure to these levels of CO_2. Subnarcotic concentrations of CO_2 produce a biphasic response during prolonged exposure. Barbour and Seevers[3] found an initial decrease in weight and oxygen consumption which returned to normal values as soon as an acclimatization was reached in rats exposed to 11% CO_2, 15% CO_2, and 20% CO_2. During prolonged exposure of guinea pigs, mice, and rats to CO_2 concentrations ranging from 3% to 24%, biphasic reactions were reported by Schaefer et al[31-33] for body weight, respiration, oxygen consumption and CO_2 excretion, blood sugar, alkali reserve and motor activity. These biphasic responses appear to be associated with the uncompensated and compensated phases of respiratory acidosis. Supporting evidence for this notion was reported with respect to changes in brain excitability found during prolonged exposure of rats to 10% CO_2.[36]

To interpret chronic CO_2 effects it is obviously necessary to know the time course of acid-base regulation that leads to a compensation of the respiratory acidosis. The absence of systematic investigations of pH changes during prolonged exposure to various CO_2 concentrations has also hampered meaningful correlations among the few and incomplete studies of the histopathological effects of CO_2.[19,44,49]

This study was undertaken to establish time-concentration relationships in chronic CO_2 toxicity and to provide a frame of reference for the incorporation of histopathological responses to CO_2. In the course of these studies it was observed that the kidney is a target organ in chronic hypercapnia showing distinct histopathological changes even during exposure to concentrations as low as 1.5% CO_2. The kidney histopathology was found to be related to the involvement of calcium-phosphate metabolism in the regulation of acid-base balance in chronic hypercapnia. A separate report on this subject has been prepared in which details of the time-concentration relationships of pH, bicarbonate and phosphate buffer are included.[41]

The mortality of guinea pigs varied between 25% - 60% during chronic exposure to 15% CO_2. The mortality averaged about 50% during one hour exposure to 30% CO_2 and 100% for exposure to 30% CO_2 for two hours. In contrast, there was generally no mortality in rats during the time-concentration range of CO_2 listed above. Therefore, additional investigations

were undertaken to determine whether these species differences in tolerance to CO_2 are reflected in serum enzyme changes produced by prolonged exposure to various CO_2 concentrations.

MATERIAL AND METHODS

Experiments were carried out with mature male guinea pigs of the Hartley strain weighing between 400 and 600 grams (gs). Mature male rats between 75 and 120 days of age from the Harvard Biological Laboratory and later the Charles River Laboratory were also used. The animals were exposed to gas mixtures containing 21% O_2 and 1.0% CO_2, 1.5% CO_2, 3% CO_2, 15% CO_2, 30% CO_2 balance N_2; also to mixtures of 30% CO_2 in 70% O_2, and 50% CO_2 in 50% O_2, for various periods of time. The gas mixtures were obtained by mixing 100% CO_2, supplied from a bank of cylinders charged to 1800 psi, with air delivered by pump. Oxygen was delivered to the gas inlet from high pressure oxygen cylinders. The rather short experiments with 30% and 50% CO_2 were carried out in small plastic chambers and pre-mixed gas concentrations in high pressure cylinders were used. Because of the larger number of experiments, three exposure chambers with different CO_2 levels were utilized simultaneously in some of the experimental series.

The exposure chambers were constructed of wood with lucite side walls. The chambers were fitted with a cooling system which consisted of copper pipes through which tap water circulated continuously. The chambers also contained a closed circuit system which continuously circulated chamber air through silicagel containers. Under these conditions, the temperature in the exposure chambers was kept at 78° F and the humidity between 65 and 75%. Ammonia vapor was absorbed by boric acid spread at the floor of the cages. The carbon dioxide level in the chambers was continuously monitored with a Beckman infrared CO_2 meter and the oxygen content with a Beckman - E 2 oxygen analyzer. An automatic gas sampling device switched every 20 minutes from one exposure chamber to another and recordings of gas concentrations were made.

The bottom of the chambers was made of wire mesh through which urine and faeces of the animal drained into a tray containing kitty litter. The tray could be removed through a slit in the side of the chamber without changing the CO_2 concentration within the chamber. Food could be put in the chamber through an opening in the side wall. For the refilling of the large water bottles, the top of the chamber had to be opened every morning for a period of about 20 minutes. By flushing the chambers with high CO_2, the concentrations used during the chronic exposure could be reestablished within 30 minutes. In later experiments commercially built environmental control chambers with automatic temperature and humidity controls were used.

At the time of sampling, the animals were anesthetized with pentobarbital and blood was withdrawn from the abdominal aorta while the animals breathed, through a mask, the same CO_2 gas mixture to which they had been exposed.

Serum values of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), malic dehydrogenase (MDH), isocitric dehydrogenase (ICDH), and cholinesterase were determined using Boehringer enzymatic test kits.[2] GOT and GPT were also measured in heart and liver of guinea pigs exposed to 15% CO_2. Blood pH was measured with an Instrumentation Laboratory ultramicro pH and blood gas analyzer (Model 113). The pCO_2 was measured with a Severinghaus pCO_2 electrode and hematocrit was determined by the microcapillary method.

Tissue specimens from each group of experimental animals were fixed in buffered formalin, embedded in paraffin, sectioned and routinely stained with hematoxylin and eosin. In certain cases Masson stain was also used. A card was prepared for each animal on which the results of the histological examination of the various organs were entered. Following this, another evaluation was made comparing the findings of individual organs in a whole experimental series. The principal histological changes were graded from 1 to 3 and charted. Tissues from 279 animals (guinea pigs and rats) were examined.

A special additional study of 32 guinea pigs was undertaken to clarify the histological effects of 15% CO_2 on the myocardium. Groups of eight guinea pigs were exposed to an atmosphere containing 15% CO_2 and 21% O_2, for periods of one hour, one day and seven days prior to sacrifice. Adjacent transverse sections of both ventricles were taken from each of these animals and from eight controls. One section was immediately frozen in dry ice and the other section was fixed in 10% buffered formalin. The formalin fixed specimens were imbedded in paraffin and 5 micron sections were cut. The prepared slides were stained with hematoxylin and eosin, PAS, diastase digestion followed by PAS and by acid fuchsin. Six micron cryostat-sections of the frozen material were stained with Oil Red 0.

RESULTS

Bodyweights

Figure 1 shows effect on body weights of exposure to 1.5%, 3% and 15% CO_2. It can be seen that the normal growth curve of guinea pigs is depressed for about 25 days during exposure to 1.5% CO_2; after this time, they show a weight increase but at a lower rate than that of the controls.

Since the animals exposed to 1.5% CO_2 start at a higher weight than the control animals comparable weight ranges (700-814 gm) of the two groups of animals have been marked by dotted lines. The control animals gained 114 gm of weight within 24 days or 4.75 gm per day. Following the initial period of weight loss and weight stabilization, guinea pigs exposed to 1.5% CO_2 reached 700 gm of weight at the 38th day of exposure and required 52 days until they attained 814 gm of weight at a rate of 2.20 gm. per day.

During 3% CO_2 exposure, various cycles in weight changes can be observed. Approximately 35 days are required for the weight of the animals to increase above the initial level.

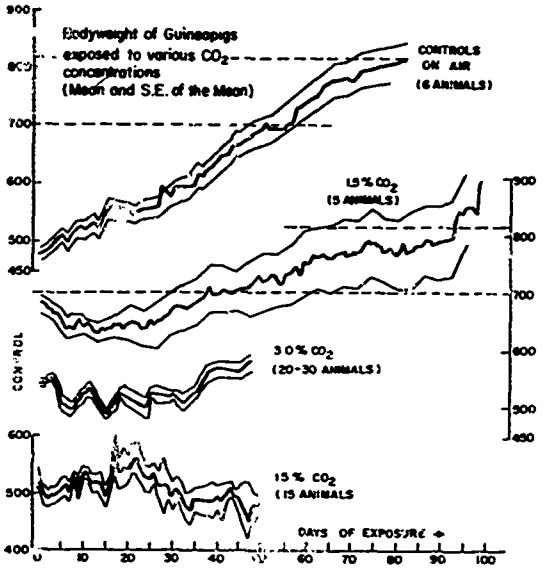

Fig. 1. Growth curves of guinea pigs exposed to various CO_2 concentrations for prolonged periods. Mean and S.E. of the mean of body weights. Number of animals listed in the graph.

During exposure to 15% CO_2, a loss of weight occurs the first day. This loss is followed by a gain in weight for about 20 days and then by a low decrease from 20 to about 50 days. During a longer exposure period to 15% CO_2, the animals continue to lose weight.

Tables I and II show the results of exposure of guinea pigs to 3% CO_2 and 15% CO_2 on organ weight as expressed in percent body weight. The effects on adrenals, thymus, spleen and peri-arterial nodes were reported elsewhere.[39]

Prolonged exposure to 3% CO_2 does not produce any significant changes in organ weights other than an increase in the weight of testes. Testicular weight returns to initial levels after 35 days of recovery.

However, chronic hypercapnia induced by inhalation of 15% CO_2 causes an elevation in kidney weight which is maintained throughout the CO_2 period while an increase in lung weight is limited to the first days of exposure. The liver exhibits a transitory increase after seven days of exposure and on the first day of recovery in air following the seven days' exposure. This increase is followed by a decrease in weight during the subsequent two days. After 11 days of recovery in air another increase in weight is noted.

Detailed studies of the effects of prolonged exposure to 1.0-1.5% CO_2, 2% CO_2 and 15% CO_2 on blood pH of guinea pigs and rats are reported in an accompanying paper.[41] In all subsequent rise of the pH, which returns to control levels during exposure to the two lower CO_2 concentrations. However, during exposure to 15% CO_2 for periods up to 14 days, the pH remains considerably below the control levels although an apparent steady state developed within three days after which the pH rose very slowly. Since the stress response subsides by three days[39], we have considered the acidosis to be "physiologically compensated" at this point. Surprisingly, however, it took a much longer time to reach compensation with lower CO_2 concentrations, specifically 5 days during 3% CO_2 and approximately 25 days during 1.0-1.5% CO_2 exposure.

The initial fall in pH during exposure to both 15% CO_2 and 3% CO_2 is greater in

Table I. Effect Of Exposure To 15% CO_2 On Organ Weights

		Control	Exposure to 15% CO_2				Exposure to 15% CO_2 for 7 Days followed by Recovery on Air	
			24 Hours	2-3 Days	4-7 Days	20-73 Days	1 Day	5-11 Days
Thyroid	Mean	.0142	.0120	.0133	.0151	.0151	.0183	.0142
	S.D.	.0091	.0025	.0021	.0036	.0061	.0038	.003
	N	31	28	16	22	10	10	17
	P		.1	.1	.1	>.1	.1	.1
Testes	Mean	.439	.479*	.456*	.447	.452	.431	.522*
	S.D.	.091	.017	.121	.124	.148	.102	.009
	N	30	29	16	22	8	10	17
	P		.05	.02	.1	>.1	.1	.001
Pituitary	Mean	.0030	.0026	.0030	.0030	.0056	.0032	.0027
	S.D.	.0006	.0006	.0007	.0011	.0061	.0010	.0007
	N	31	29	16	22	8	10	17
	P		.05	.1	.1	>.1	.1	.1
Liver Percent of total body weight	Mean	3.558	3.611	3.670	4.188	3.580	4.625*	4.092
	S.D.	.591	.417	.571	.872	.031	.571	.871
	N	11	16	25	13	6	10	12
	P		.1	>.1	.1	>.1	.001	.1
Kidney Percent of total body weight	Mean	.718	.785*	0.745	1.008*	1.049*	.973*	.744
	S.D.	.062	.084	0.371	.154	.121	.247	.093
	N	11	17	20	13	10	10	11
	P		.05	>.1	.001	<.001	.01	.1
Heart Percent of total body weight	Mean	0.35	0.33	0.37	0.35	0.38	0.41*	0.36
	S.D.	.09	.05	.09	.07	.07	.01	0.04
	N	8	13	12	8	11	8	5
	P		>.1	>.1	.1	>.1	<.001	>.1
Body weight in grams	Mean	513.0	466.8	464.0	473.3	455.8	475.1	566.0*
	S.D.	65.0	114.4	68.9	72.5	68.8	75.7	75.7
	N	42	29	16	22	11	17	17

Table II. Effect Of Exposure To 3% CO_2 In 21% O_2 On Organ Weights

			Exposure to 3% CO_2 in 21% O_2				Recovery on Air Following exposure to 3% CO_2 for 21 Days	
		Control	1 Day	3 Days	7 Days	21 Days	14 Days	35 Days
Adrenals	Mean	.030	.038	.037		.038*	.032	.031
	S.D.	.012	.013	.014		.010	.010	.012
	N	58	17	19		28	10	6
	P					(.05)		
Thymus	Mean	.027	.019	.021*		.026	.034*	.024
	S.D.	.006	.003	.008		.009	.007	.011
	N	22	9	12		24	8	8
	P		(.01)	(.05)			(.05)	
P.A.N.	Mean	.0049	.0043	.0048		.0065	.0036	.0052
	S.D.	.0031	.0021	.0020		.0040	.0007	.0010
	N	15	11	12		20	4	3
	P							
Spleen	Mean	.149	.148	.144		.119*	.141	.118*
	S.D.	.031	.020	.061		.040	.023	.011
	N	15	15	11		14	4	7
	P					(.05)		(.05)
Thyroid	Mean	.015	.015	.013		.015	.012	.012
	S.D.	.008	.001	.002		.005	.001	.002
	N	36	15	12		22	4	8
	P							
Testes	Mean	.440	.588*	.588*		.612	.480	.463
	S.D.	.091	.100	.042		.101	.111	.081
	N	30	13	5		19	6	9
	P		(.01)	(.01)		(.01)		
Liver	Mean	4.02	3.80	3.88		3.87	4.15	3.05
	S.D.	.71	.44	.52		.63	.22	.40
	N	16	16	12		24	4	9
	P							
Lungs	Mean	.684	.690	.730*		.664	.757*	.670
	S.D.	.021	.101	.120		.211	.100	.171
	N	16	16	12		24	4	8
	P			(.05)			(.05)	
Kidney	Mean	.768	.820*	.741		.776	.782	.703
	S.D.	.08	.140	.121		.040	.042	.101
	N	15	16	12		24	4	9
	P		(.05)					

guinea pigs than in rats. This is particularly pronounced during 15% CO_2 exposure, and suggests a marked difference in buffer capacity between guinea pigs and rats. This difference is demonstrated in Figure 2, in which the time course of the standard bicarbonate is plotted.

During exposure to 15% CO_2 and 3% CO_2, standard bicarbonate shows a significant initial fall in guinea pigs and an immediate rise in rats. During the subsequent period, the standard bicarbonate remains significantly higher in rats during exposure to 3% CO_2. However, after 7 days of exposure to 15% CO_2, standard bicarbonate values are approximately the same in both species.

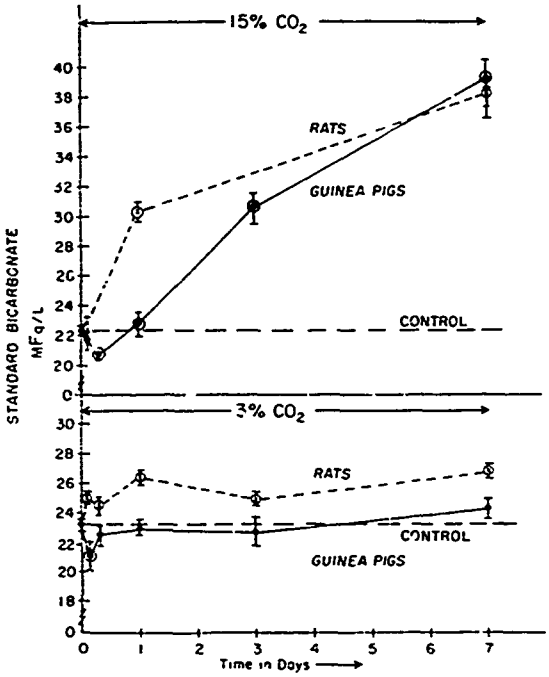

Fig. 2. Standard bicarbonate (mEq/L) of guinea pigs and rats during prolonged exposure to 3% CO_2 and 15% CO_2. Means and S.E. of the means. Number of animals. Guinea pigs: 3% CO_2 - control group 27, exposure group 6-17. 15% CO_2 - control group 11, exposure group 10-20. Rats: 3% CO_2 control group 12, exposure group 9-14. 15% CO_2 - control group 8, exposure group 11-15. Statistically significantly different from controls. Under 3% CO_2, the standard bicarbonate values of rats are statistically significantly higher than those of guinea pigs.

Serum Enzyme Changes

Exposure of guinea pigs to 15% CO_2, 21% O_2 balance N_2 for seven days results in a striking increase in (GOT) and (GPT) during the first three days of exposure. After seven days both GOT and GPT return to initial values (Figure 3). These changes in serum enzyme activities follow the time course of the pH changes which is shown in Figure 3. The observed increase in serum enzyme activities is limited to the uncompensated phase of respiratory acidosis, and indicates a pH dependence. This conclusion is supported by the results obtained from a group of animals which were unable to compensate the respiratory acidosis after 3 days of exposure. The animals exhibit very low pH values, corresponding to those found after 6 hours exposure, and have greatly elevated GOT and GPT values. These values are significantly higher than those obtained from animals able to compensate the respiratory acidosis after 3 days of exposure (Figure 3).

Heart and liver values of GOT and GPT of guinea pigs measured during prolonged exposure to 15% CO_2 are presented in Table III together with the corresponding blood data. It can be seen that both GPT and GPT activities increase in heart and in liver, particularly after 7 days of exposure.

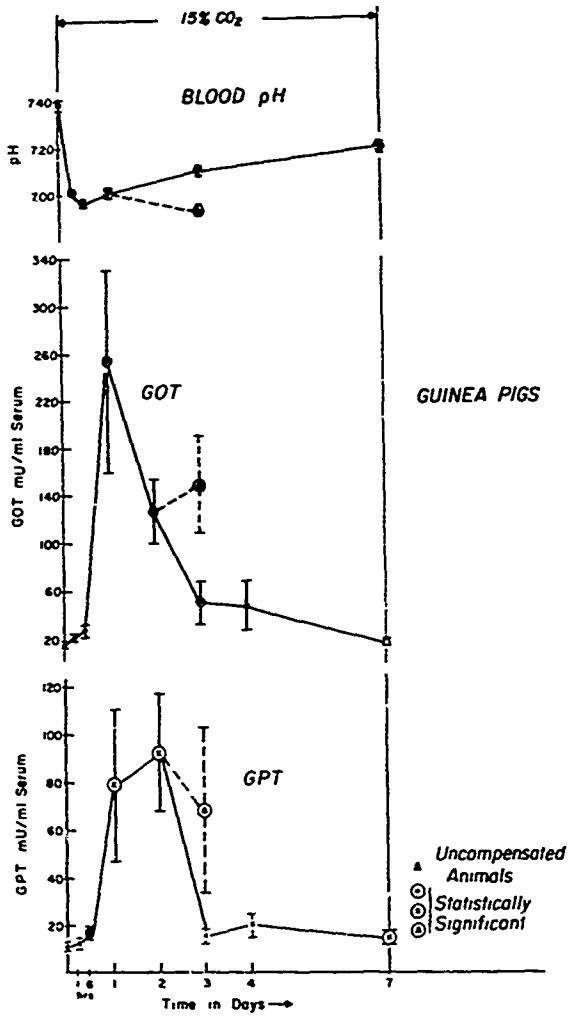

Fig. 3. Effect of prolonged exposure to 15% CO_2, on blood pH and serum levels of GOT and GPT in guinea pigs. Points connected by dotted line signify guinea pigs which remained uncompensated after three days of exposure. Circled symbols are data points significantly different from controls at the 5% level and better.

Data obtained from additional studies of other enzyme activities in guinea pigs during exposure to 15% CO_2 (LDH*, MDH, ICDH, and cholinesterase) are shown in Figure 4. Blood pH values are also included in this figure. All enzyme activities increase significantly during the first three days of exposure which corresponds to the uncompensated phase of respiratory acidosis. The activities return to control level after 7 days (compensated phase of respiratory acidosis).

In Figure 5, GOT and GPT activities found in rats exposed to 15% CO_2 for 7 days are shown. In contrast to guinea pigs, no changes were observed in GOT and GPT values, and the pH fell less during exposure to 15% CO_2.

To determine the threshold concentrations of CO_2 required to produce an increase in serum enzyme activities, guinea pigs were exposed for periods up to 7 days to 3% CO_2 in 21% O_2, balance N_2, for periods up to three days. The results are presented in Table IV. Although the decrease in pH caused by exposure of guinea pigs to 3% CO_2 is less than half of that measured during exposure to 15% CO_2, the serum GOT activity increases significantly after a one-hour exposure and remains at the elevated level for three days. The value returns to near control levels after 7 days. GPT levels in guinea pigs did not change throughout the 7-day exposure to 3% CO_2.

Exposure of rats to 20% CO_2 for three days did not produce an increase in GOT or GPT.

Histopathology

Liver

In three series of experiments prolonged exposure of guinea pigs and rats

**LDH data were previously reported[8] and are here included for comparison.*

Table III. Effect of Prolonged Exposure of Guinea Pigs to 15% CO_2 on GOT and GPT Levels in Blood, Heart and Liver

Conditions		GOT			GPT		
		Blood mU/ml	Heart mU/g	Liver mU/g	Blood mU/ml	Heart mU/g	Liver mU/g
Control	Mean	16.5	58.9	22.0	11.8	6.5	2.7
	S.E.	.9	3.3	1.9	.7	.7	.4
	N	13	8	8	15	8	8
15% CO_2							
1 Hour	Mean	22.4	74.9	28.1	13.1	5.8	3.1
	S.E.	2.1	11.3	2.7	1.0	.6	.7
	N	5	6	6	6	6	6
6 Hours	Mean	27.3	75.9	26.6	17.6*	6.1	2.2
	S.E.	5.7	10.2	3.3	1.1	1.0	.4
	N	6	6	6	6	6	6
24 Hours	Mean	246.1*	48.8	38.23*	78.9*	4.8	3.4
	S.E.	103.3	4.3	1.83	3.7	.4	.4
	N	6	6	6	6	6	6
3 Days	Mean	50.0*	71.7	35.5	16.1	5.9	4.0
	S.E.	14.0	11.0	4.1	3.1	.8	.5
	N	5	7	7	10	7	7
7 Days	Mean	16.4	81.2*	27.3	15.7*	59.3*	11.7*
	S.E.	.7	8.9	1.7	1.0	4.1	.7
	N	10	6	6	12	6	6

* Differences from controls statistically significant at the 5% level and better

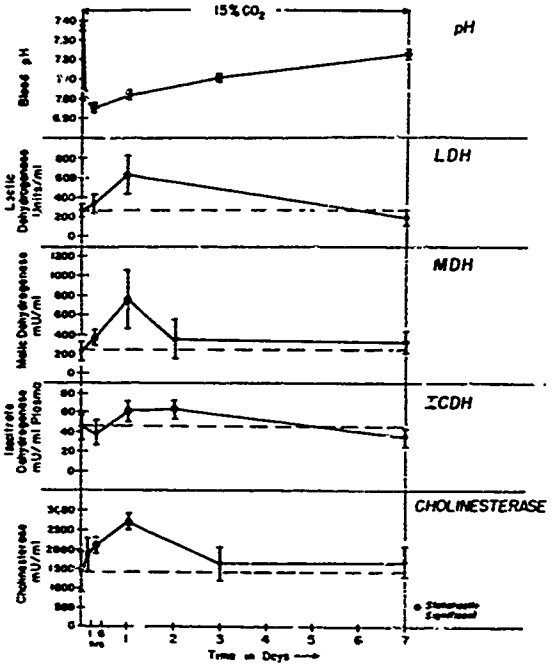

Fig. 4. Effect of prolonged exposure to 15% CO_2 on blood pH, lactic dehydrogenase (LDH), malic dehydrogenase (MDH), isocitrate dehydrogenase (ICDH), and cholinesterase activities in guinea pigs. Circled symbols are data points significantly different from controls at the 5% level and better.

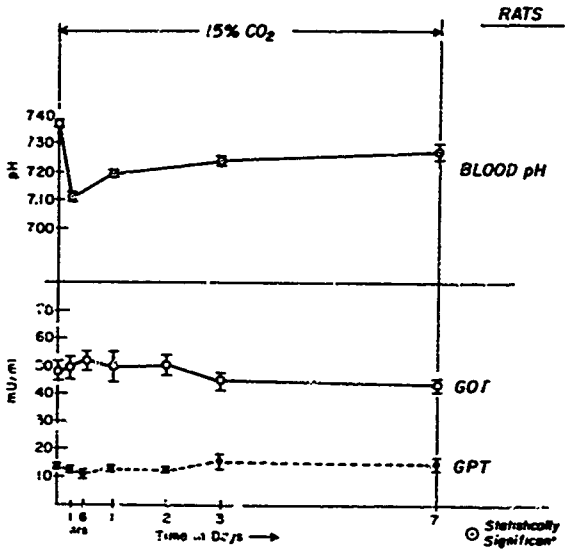

Fig. 5. Effect of prolonged exposure to 15% CO_2 on blood pH and serum levels of GOT and GPT in rats. Filled symbols significantly different from controls at the 5% level and better.

to 1.5%, 3% and 15% CO_2 produced no significant pathological findings in the liver. Rats exposed to 30% CO_2 for 5 hours and 50% CO_2 for 1 hour did not exhibit any histologically demonstrable liver damage. However, certain changes reflecting functional alterations in the liver metabolism under these experimental conditions were noted. The glycogen and fat containing liver cells can be differentiated on the basis of their morphological appearance. According to Aterman[1] the clear spaces, of usually polygonal outlines seen in normal liver cells are filled with glycogen and classified in our study as glycogen type vacuolization. Fat vacuoles, on the other hand, are distinguished by their round outline and the peripheral localization of the cell nucleus. Incidence of basophilic granules in the cytoplasm, considered to represent particulate RNA[1] are also noted.

Figure 6 demonstrates the depletion of glycogen vacuoles and increase in fat vacuoles in liver sections of guinea pigs exposed to 3% CO_2 for 7 days. After 3 weeks of exposure to 3% CO_2 and subsequent recovery for 1 day on air, reaccumulation of glycogen vacuoles is noted.

In Table V glycogen vacuolizations, fat vacuolization and cytoplasmic granules are listed for the various time

Table IV. Effect of Prolonged Exposure of Guinea Pigs to 3% CO_2 on Blood pH and Plasma Levels of GOT and GPT

Conditions		Blood pH corrected to 37°C	GOT mU/ml	GPT mU/ml
Control	Mean	7.394	18.4	11.1
	S.E.	.012	1.89	.92
	N	6	6	6
3% CO_2				
1 Hour	Mean	7.281*	40.3*	10.5
	S.E.	.021	8.21	.49
	N	9	9	9
6 Hours	Mean	7.260*	27.5*	12.7
	S.E.	.020	3.74	1.19
	N	8	8	8
1 Day	Mean	7.278*	17.8	13.5
	S.E.	.023	2.95	.76
	N	8	8	8
3 Days	Mean	7.316*	29.1*	12.7
	S.E.	.014	3.77	1.19
	N	8	8	8
7 Days	Mean	7.350	22.3	13.9
	S.E.	.010	2.53	.82
	N	8	10	10

* Differences from controls statistically significant at the 5% level and better

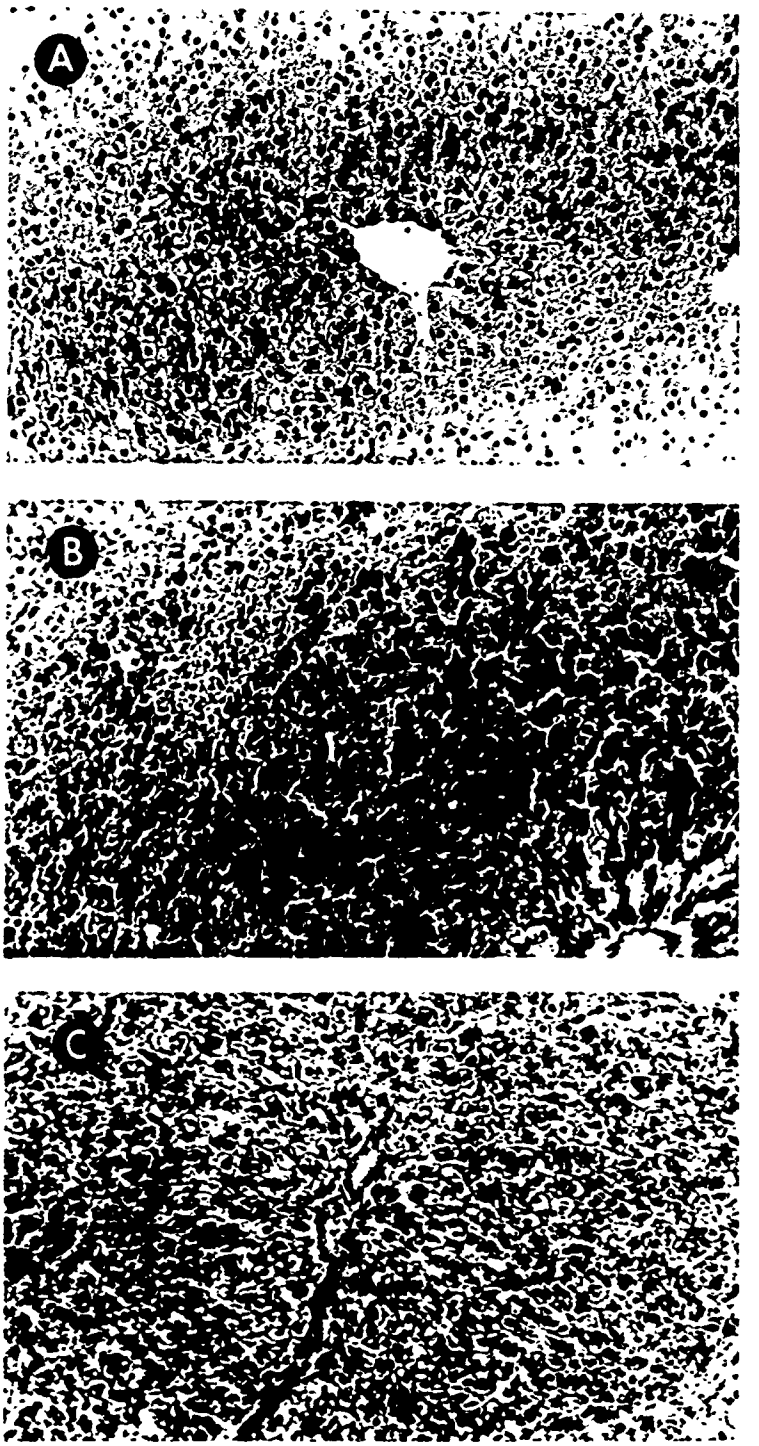

Fig. 6. *A. Liver: Control Guinea pig. H.E. x 100 Normal glycogen vacuolization*
B. Liver Guinea pig exposed for 7 days to 3% CO_2. Moderate depletion of glycogen vacuoles and occurrence of dark cells.
C. Liver, Guinea pig exposed for 3 weeks to 3% CO_2 followed by 1 day recovery on air. Masson x 100. Reaccumulation of glycogen vacuoles.

Table V. Effects of Prolonged Exposure to Different CO_2 Concentrations on Liver Morphology of Guinea Pigs

	No. of Animals	Glycogen Incidence	Vacuolization Grading	Fat Vacuolization Incidence	Fat Vacuolization Grading	Cytoplasmic Incidence	Granules Grading
CONTROLS							
1.5% CO_2 in 21% O_2	(5)	100%	3.0	0	0	100%	0.5
1-23 Days	(2)	100%	1-2+	0	0	100%	0.5
24-42 Days	(6)	84%	1-2	50%	1+	100%	0.5-1.0
Recovery 1	-	-	-	-	-	-	-
Recovery 2	(3)	100%	2-3+	33%	1+	100%	0.5-1.0
II							
CONTROLS							
3% CO_2 in 21% O_2	(5)	100%	3+	0	0	100%	0.5-1
1-5 Days	(2)	70%	0.5-1	0	0	100%	1+
6-42 Days	(12)	75%	0.5-2	25%	0.5-2.0	75%	1-2+
Recovery 1 day	(4)	50%	3+	25%	1+	25%	0.5
Recovery 2 weeks	(9)	100%	.5-2.0	11%	0.5	55%	0.5-2.0
III							
CONTROLS							
15% CO_2 in 21% O_2	(18)	94%	0.5-3+	16%	0.5-2+	94%	0.5-1.0
1-2 Days	(6)	66%	0.5-2+	66%	0.5-2+	83%	1.0
3-7 Days	(8)	74%	0.5-2	75%	1-3	75%	.5-2.0
7- Days	(3)	33%	2+	66%	.5-1.0	66%	1+
Recovery 1 (1 Day)	(8)	87%	1-3+	50%	1.0-2+	63%	.5-1.0
Recovery 2 (11 Days)	(4)	100%	1-2+	25%	1+	100%	.5-1.0

periods of the 15% CO_2 series. Glycogen vacuolization decreases during the exposure to CO_2 and increases again during the recovery period. The degree of glycogen vacuolization agrees well with the chemical glycogen determinations for the various experimental periods (Figure 7). Incidence of cytoplasmic granules follows the pattern of glycogen vacuolization. Fat vacuolization shows the opposite trend and corresponds with chemical fat determination (Figure 7). The data obtained on liver glycogen, fat content, and total nitrogen during and after exposure of guinea pigs to 15% CO_2 are shown in Figure 7. Changes in

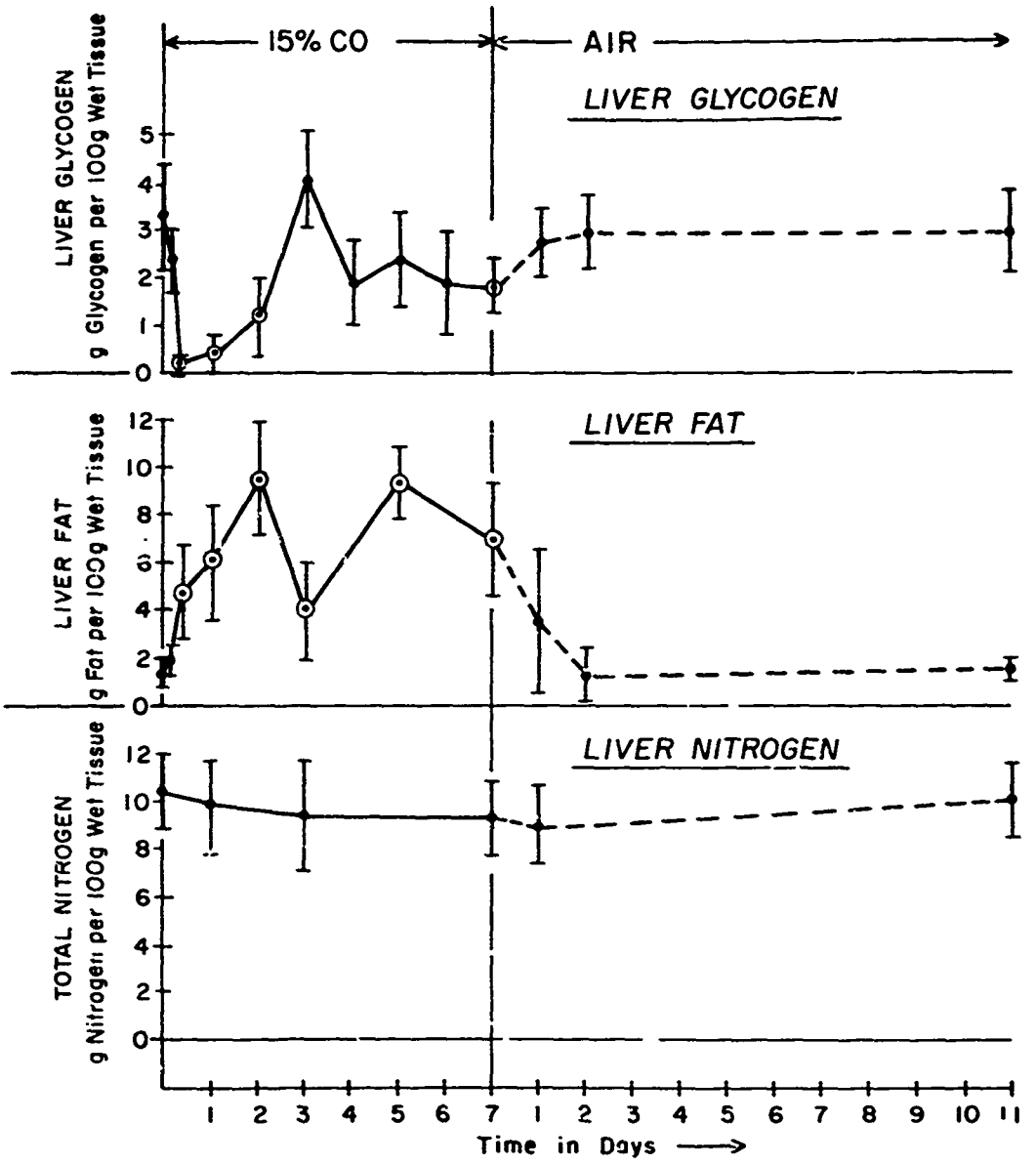

Fig. 7. Effect of prolonged exposure to 15% CO_2 on liver glycogen, fat content and total nitrogen content of guinea pigs. Means and S.E. of the mean. Number of animals. Control Group, 15; exposure groups range from 12-22.

fat content of lungs and muscle and corresponding pH changes are presented in Figure 8. It is apparent that the time course of lipid accumulation in lung and muscle is different from that observed in liver.

In an experiment in which young male rats weighing between 100 and 120 grams were used, a significant depression of liver mitosis was found during the exposure to 15% CO_2 (Figure 9). After a recovery period of 12 hours following exposure to 15% CO_2 for 7 days, a slight increase of mitosis was noted. This effect could not be demonstrated in rats exposed to 3% CO_2 for prolonged periods.

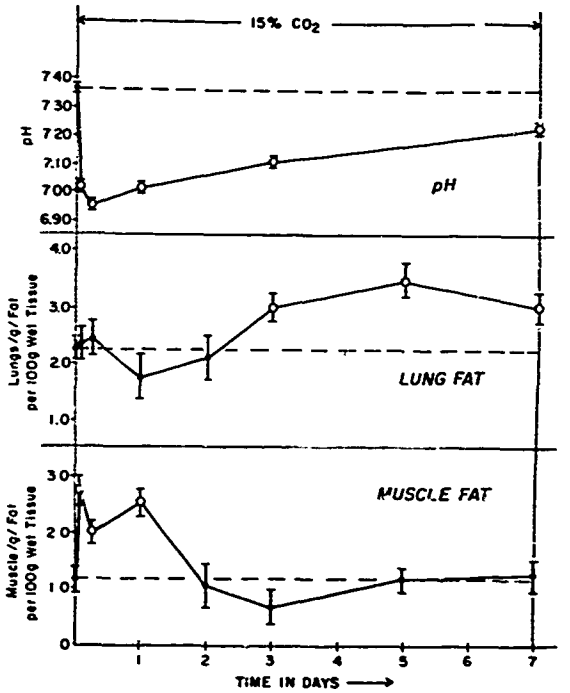

Fig. 8. Time course of pH and of lipid accumulation in lungs and muscle during prolonged exposure to 15% CO_2 in guinea pigs.

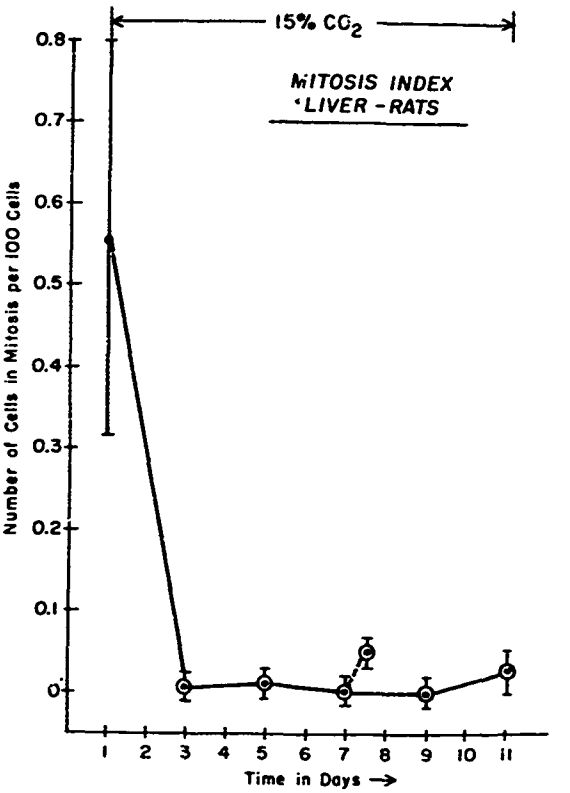

Fig. 9. Mitosis index of the liver in rats exposed to 15% CO_2 for periods up to 11 days. 1 Group Recovery of 1 day following 7 days of exposure to 15% CO_2. Number of animals. Control group 8; exposure groups 7-12.

Testes

A concentration of 15% CO_2 has a marked effect on spermatogenesis. The first changes in spermatogenesis are noted after 48 hours of exposure to 15% CO_2. At this time, a marked reduction in the number of mature spermatocytes becomes apparent and is associated with an increase in their precursors, Figure 10. After 3-7 days' exposure, multinucleated giant cells are seen. These

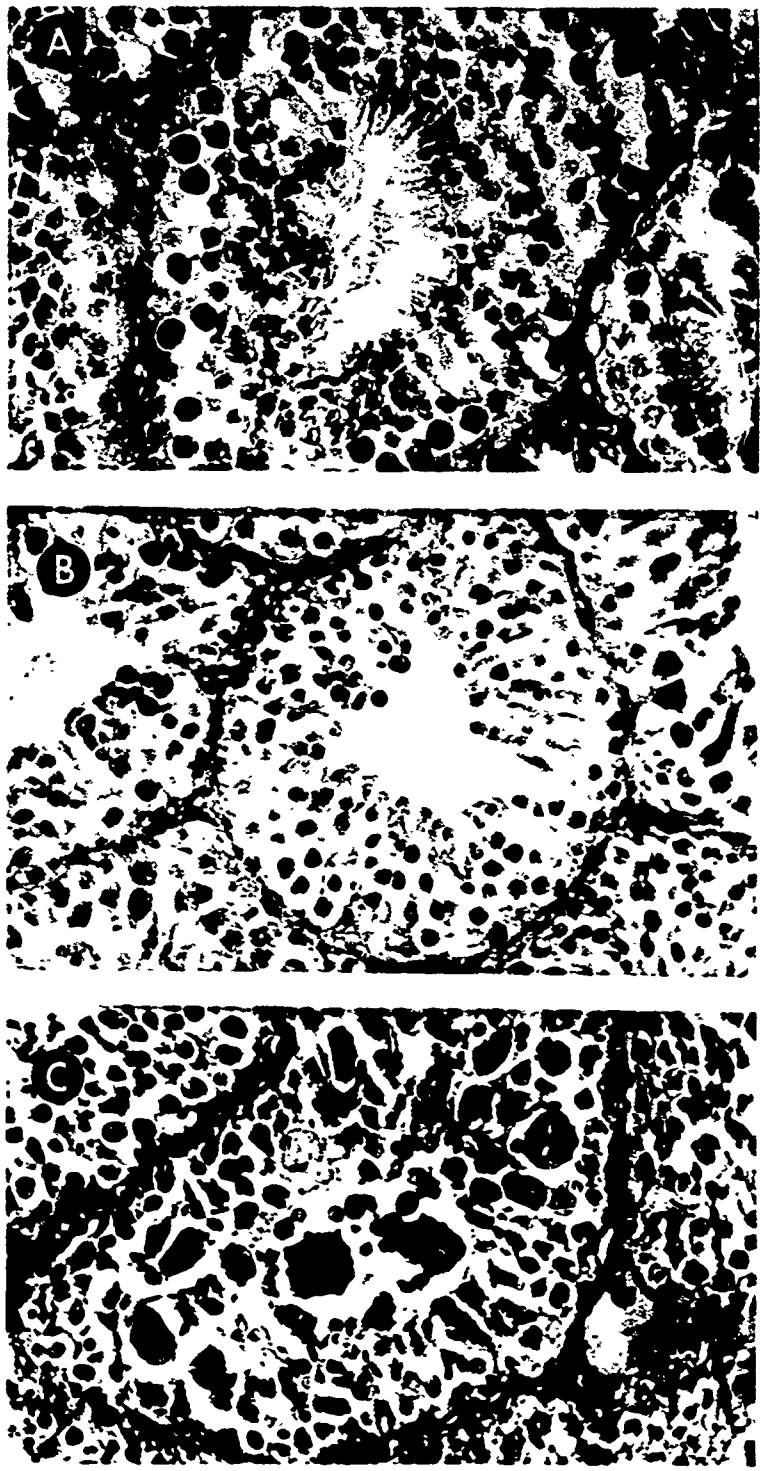

Fig. 10. A. Testes - Guinea pig exposed to 15% CO_2 for 24 hours. H and E x 250. Spermatogenesis still active.
B. Testes - Guinea pigs exposed to 15% CO_2 for 48 hours. Early spermatogenic arrest.
C. Testes - Guinea pigs exposed to 15% CO_2 for 7 days. Spermatogenic arrest with giant form.

were observed in 71% of a group of 7 animals. Following a 7-day exposure to 15% CO_2, the transition to air, which represents an additional stress, leads to a further increase in giant cell formation (9 out of 10 animals). Prolonged exposure to 1.5% CO_2 and 3% CO_2 did not produce any spermatogenic arrest in guinea pigs and rats.

Heart

No evidence of a permanent myocardial damage was seen at autopsy in guinea pigs, either in those who expired during the period of acute acidosis or were sacrificed at one day or seven days. Examination of the formalin fixed material revealed no significant variation between the control groups and any of the experimental groups. The Oil Red 0 stain revealed no positive material in the control or one hour groups. However, a small amount of lipid was seen in one animal at one day and by seven days positive material was seen in five of ten animals. Although this stain probably revealed significant fat deposition in the myocardium after chronic exposure, no acute effect was seen. With the methods used, no changes in the myocardium of guinea pigs exposed to 15% CO_2 could be detected except delayed fat deposition.

Pancreas

Changes in the exocrine portion of the pancreas were observed. Significant findings pertain to the relationship between the eosinophilic zymogen granules and the basophilic ergastoplasm of the acinar cells. Generally it can be stated that zymogen granules increase and the ergastoplasm decreases during exposure to 3% and 15% CO_2 in both guinea pigs and rats. The reverse trend occurs during the recovery from CO_2. On short exposure of rats to extreme concentrations of 30% CO_2 and 50% CO_2 there is a relative increase of ergastoplasm at the expense of zymogen granules. In addition, clear vacuoles varying in size from 3 - 10 microns appear in some of the acinar cells. These vacuoles are identical in their morphological appearances to those described by Leblond and Sergeyewa[15] during thyroxin overdosage, and acetylcholine administration.

Spleen

During exposure to 15% CO_2, congestion and hemorrhages were observed in most of the animals, guinea pigs and rats, throughout the 7 days of exposure. Deposition of pigments, (hemosiderin) was increased while the lymphoid elements were reduced. Under 3% CO_2 no hemorrhages were found, and the incidence of pigment deposition was somewhat elevated.

Intestine

During exposure to 15% CO_2, hemorrhages were observed regularly after 24 hours of exposure in guinea pigs and rats. These hemorrhages disappeared after 3 and 4 days of exposure.

DISCUSSION

Species Difference in Tolerance to Chronic Hypercapnia

Body weight changes of guinea pigs in chronic hypercapnia have not been reported in the literature.

The results of our experiments show that guinea pigs are very sensitive to CO_2. The average normal weight gain per day was about 4.0 grams under control conditions. There was no weight gain during the first 25 days of exposure to 1.5% CO_2. This period coincided with the uncompensated phase of respiratory acidosis. Following this period, the weight gain resumed at the lowered rate of 2.5 grams per day.

During exposure to 3% CO_2, there were periods of weight gains osciliating with periods of weight loss which resulted in no net weight gain during a period of 35 days. Guinea pigs exposed to 15% CO_2 showed a continuous decline in weight after 25 days, until the 52nd day at which time the experiment was terminated. These results are in strong contrast to the reported weight changes in rats during chronic hypercapnia. Rats were only slightly affected during 30 days of exposure to 11.6% CO_2 (60 mm Hg) showing a reduction in weight gain of 3.6 grams/day as compared to 4.3 grams/day for control rats.[26] Robinson[30] exposed rats to CO_2 concentrations ranging from 5.7% - 11% and found a retardation of normal growth while organ-weight and body weight ratios were unaffected. No significant histopathological changes were observed.

The adrenal/body weight ratio, and thymus/body weight ratio have been used as indices of stress. These parameters measured under 15% CO_2 have been previously reported.[39]

A typical stress response which later subsided with the increasing compensation of the respiratory acidosis was observed during the first 3 days of exposure to 15% CO_2. During exposure to 3% CO_2, the adrenal/body weight ratio increased while the thymus/body weight ratio declined. Both indices returned to control values during the recovery. In rats exposed to 11.6% CO_2 for 32 days, the adrenal/body weight ratio did not differ from that of controls.

The specific changes observed in the lungs during chronic hypercapnia induced by 15% CO_2 and 3% CO_2 have also been previously reported.[25,43]

Occurrence of pulmonary edema and hyaline membranes after 1 day of exposure to 15% CO_2 was found to be associated with a marked increase in lung/body weight ratio.

During 3% CO_2 exposure there was a small incidence of hyaline membrane formation. The lung/body weight ratio was slightly increased after 3 days of exposure. The heart weight body weight ratio was not affected by 15% CO_2.

The kidney/body weight ratio increased during exposure to 15% CO_2 and 3% CO_2. Moreover, the testes/body weight ratio also increased during hypercapnia induced by 15% CO_2 and 3% CO_2. Specific histopathological changes were also observed in the testes.

The species difference between guinea pigs and rats in tolerance to chronic hypercapnia as observed in the changes of growth curves and stress indicators like the adrenal/body weight ratio are most likely related to differences in

buffer capacity. At every level of exposure to CO_2 the acidosis is greater in guinea pigs than in rats, in other words, more hydrogen ions are formed at a given level of arterial P_{CO_2} in guinea pigs. Rats are able to increase their standard bicarbonate immediately upon exposure to CO_2; in contrast to guinea pigs (Figure 2). This means that rats have a larger quantity of bicarbonate buffer at their immediate disposal for the compensation of the acidosis. Guinea pigs with their lower buffer capacity suffer a greater acidosis during the initial period of exposure to 15% CO_2 until renal regulations of bicarbonate reabsorption come into full operation after 1 day of exposure to 15% CO_2. The standard bicarbonate response of rats to 15% CO_2 (110mm Hg CO_2) is similar to those of dogs to exposure to 60 and 90 mm Hg CO_2.[27]

The species difference in response to CO_2 is further expressed in serum enzyme responses. The CO_2 tolerant rats exhibit no increase in serum GOT and GPT during prolonged exposure to 15% and 20% CO_2 whereas guinea pigs show a marked increase of serum enzymes during the initial phase of exposure to 15% CO_2 and even to 3% CO_2. The fact that the increases in serum enzyme activities in guinea pigs exposed to 15% CO_2 for 7 days was limited to the first 3 days of uncompensated respiratory acidosis indicate the pH dependence of this reaction. If it is established that the rise in serum enzymes is pH dependent, and further, that there is a marked species difference in the acidosis produced, one would expect to find a threshold concentration of CO_2, which would also cause an increase in serum enzymes in rats. The very fact that the acidosis produced in rats by exposure to 20% CO_2, which is similar in magnitude to that caused in guinea pigs by exposure to 15% CO_2, does not result in a rise in serum enzymes suggests that there are other factors involved.

The question arises whether the increased serum enzyme activity observed during prolonged exposure of guinea pigs to 15% CO_2 represents an indication of cell necrosis or is a sign of altered cell permeability without involving any structural changes of the organs.

The increase in the serum specific enzyme, pseudocholinesterase, shows that the synthetic ability of the liver cells has not been depressed. If liver cell damage had occurred a decrease in serum cholinesterase would have resulted.[42] Moreover, the observed increase in liver and heart GOT and GPT does not indicate the existence of a necrosis.

Results of histopathological studies of guinea pigs and rats, under the same experimental conditions did not show any significant morphological changes of the liver and heart during exposure to 15% CO_2.

Increased membrane permeability caused by respiratory acidosis is, therefore, the most likely explanation for the increase in serum enzyme activities. Evidence for increased permeability has been found in the lungs of guinea pigs exposed to the same experimental conditions.[38] These lungs show congestion and pulmonary edema during the uncompensated phase of respiratory acidosis.

Previously reported densitometric analyses of LDH isozymes released in guinea pigs during exposure to 15% CO_2 showed an increase in the proportion of LDH -5 (M-type) after 24 hours together in addition to an increase in total LDH.[8] After 7 days of exposure, all values returned to normal. The muscle contribution to the total LDH increased 500% while that of the H-type (Heart) rose only 30%. These findings suggest that leakage from the muscles rather than leakage from the heart causes the rise in LDH during exposure to 15% CO_2. No overt damage of skeletal muscle fibers or of heart muscle was observed during exposure to 15% CO_2. Under the same experimental conditions, Tappan[45] observed, in guinea pigs, a large increase in creatine phosphokinase activity in the plasma beginning after 6 hours of exposure with a return to normal by 7 days. Since muscle tissue is the primary source of creatine and phosphocreatine measurements were also made to determine any alterations in the energy stores. Tappan also found no significant changes except a transient increase in phosphocreatine after 3 days.

These findings also speak against necroses in the muscles since one would have expected to see changes in the energy stores, if necroses would have developed.

It is therefore concluded that the high rise of serum creatine phosphokinase observed during 15% CO_2 exposure are not correlated with tissue damage in the sense of necrosis but reflect permeability changes. This view is supported by findings of Kilburn[13] who related increased plasma potassium levels to leakage from the muscles in patients with respiratory acidosis. This loss was found to stop upon return to normal CO_2 tensions.

We have also observed an increase in plasma potassium levels during exposure of guinea pigs to 15% CO_2. Moreover measurements of intracellular pH carried out with the DMO method under the same conditions showed that muscle tissue retains the highest level of hydrogen ion concentration during prolonged exposure to 15% CO_2 or, in other words, has the lowest buffer capacity of all tissues. The species differences in tolerance to CO_2 observed in difference of mortality, retardation of growth ratio, organ body weight ratios and serum enzyme responses of guinea pigs and rats have been related to basic physiological differences in buffer capacity. Another example of marked species differences in response to CO_2 was reported by Stein et al[43] who exposed monkeys (Macaca mulatta) to 3% CO_2 in air for 93 days and did not observe any demonstratable change in body weight and hemotocrit, found in guinea pigs and rats during exposure to 3% CO_2 and 1.5% CO_2 (Schaefer, et al[34]).

Specific Histopathological Effects of CO_2

A systematic differentiation between histopathological effects produced by chronic exposure to increased CO_2 concentration and effects due to anoxia is still missing. Evidence is accumulating which appears to indicate that CO_2 is a specific rather than an anoxic agent. In the older literature, CO_2 is considered to have nonspecific effects. A similar

view is held by Stephens[44] who exposed rats to concentrations of 11% CO_2 and reported nonspecific histopathological changes in the nerve cells resulting from cellular anoxia. This is in contrast to findings of Meesen[19] who shows irreversible damage to the nerve cells in the cerebral cortex after 48 hours of exposure to 16% CO_2 in air. Meesen used rabbits, dogs, rats and guinea pigs in his experiments and various CO_2 concentrations from 4.5% to 16% CO_2. He reports irreversible morphological changes in lungs, liver, kidney and brain due to prolonged CO_2 intoxication. Zinck[48,49] who studied guinea pigs after prolonged exposure to 3% CO_2 and up to 24% also finds characteristic morphological and histological changes in the liver, kidney, heart and striated muscle. He commends on the particular blood distribution. In anoxia, hyperaemia is found in the muscles and splanchnic area. During prolonged exposure to CO_2 these organs and regions are more or less anemic, whereas the respiratory tract and heart have an increased blood content. He states that the observations made under conditions of CO_2 intoxication are "fundamentally different from those produced by anoxia of any origin." Meesen[19] comes to a similar conclusion. Combined physiological and morphological investigations of the adrenals in guinea pigs during prolonged exposure to 3% CO_2 also demonstrated specific effects physiologically as well as morphologically[31-33].

The histopathological studies reported here show the absence of severe histopathological changes which one might have expected, e.g., of necrosis in liver and heart during exposure of guinea pigs to 15% CO_2 for prolonged periods. Increased uptake of acid fuchsin has been reported[28,16] to detect earlier myocardial changes than hematoxylin and eosin, however, in other hands[22,12] this stain has proved to be erratic. Enzyme histochemical techniques produce uncertain results[7,22] and are not readily adaptable to routine histology. Glycogen depletion demonstrated by PAS staining with and without prior diastase digestion has demonstrated infarction or foci of ischaemia in experimental animals[3,5] but in autopsy material glycogen staining in the heart is minimal and its loss is not reliable as a parameter.[22] The four histological stains used in this study (H and E, acid fuchsin, PAS and Oil Red 0) failed to demonstrate any signs of myocardial damage in guinea pigs exposed for periods up to 7 days to 15% CO_2. However, hemorrhages were observed during the first 24 hours in lungs[38] spleen/kidney and intestines. After 2 days of exposure to 15% CO_2 the hemorrhages disappeared, which suggests that an acute acidosis effect caused a transient increase in vascular permeability.

Intestinal hemorrhages were also observed by deBellis et al[6] in dogs exposed for 6 hours to 10% CO_2, 21% O_2, balance nitrogen. Moreover inhalation of 15% CO_2 for 15 minutes was found to produce an increased permeability of the brain capillaries in rabbits as indicated in tryptan blue staining of cerebral tissue following dye injections.[5]

What seems to be more important are the results of histopathological studies showing the adaptive changes in

liver, kidney, and testes in chronic hypercapnia.

The functional changes in liver of glycogen depletion and fat accumulation during chronic exposure to 15% CO_2 and, to a certain extent, during exposure to 3% CO_2 points to important changes in fat metabolism produced by hypercapnia. Nahas and coworkers[24] have demonstrated that acidosis inhibits lipolysis and one could therefore expect an increase in fat since it would not easily be mobilized. Nahas and Poyart[24] observed that adrenalin - induced lipolysis and calorigenesis is inhibited in dogs when breathing a mixture of 10% CO_2 and 25% O_2 in N_2 which results in an average pH of 7.0 and an average $PaCO_2$ of 100 mm Hg.

In subsequent in vitro experiments using rat epididymal adipose tissue, Triner and Nahas[46] demonstrated that acidosis in the medium inhibited the lipolytic activity induced by noradrenalin, glycogen and ACTH - which suggest an inhibiting effect of H+ on cyclic 3'5'=AMP formation. When cyclic 3'5'=AMP dibutyrate (10^{-3} M) is added to the medium it combines with noradrenalin and reverses the inhibitory effect of the acidosis[29]. However, the findings of Longmore et al[18] which showed an increased fat synthesis in the perfused liver, when the level of CO_2 is raised in the medium, suggest that another factor adds to the large increase in fat content found in the liver of guinea pigs exposed to 15% CO_2. Since similar changes in glycogen and fat vacuolization were observed in both guinea pigs and rats even under lower concentrations (3% CO_2), it would seem that changes in fat metabolism are of special significance in hypercapnia and require a more detailed investigation. Lipid accumulation during chronic exposure to 15% CO_2 shows a specific pattern for different organs. Fat content of muscle is increased only during the first 2 days, that of lungs during the period from 3 to 7 days while the lipid content of the liver is greatly elevated throughout the exposure period.

During each of these time periods different factors dominate the acid-base status in the tissue, all of which are known to exert specific effects on metabolic regulations.

During the first 2 days of exposure to 15% CO_2 a maximal extracellular acidosis is observed which is complicated by a superimposed metabolic acidosis between 24 and 48 hours.[40] The fall of 0.4 pH units during this period is sufficiently large to produce inhibition of pH dependent enzymes causing a glycolytic and lipolytic inhibition. The former is expressed in a marked fall of phosphofructokinase and lactate[9,10] and increase in blood glucose resulting in a decrease in 2-3 diphosphoglycerate.[20] Evidence for lipolytic inhibition is seen in the failure of glycerol and triglycerides in the blood to respond to the simultaneously existing stress reaction involving adrenaline release.[38]

Storage of fat in muscle and liver during the first 2 days of exposure is considered to be indicative of pH dependent lipolytic inhibition although CO_2 facilitated lipid synthesis might also play simultaneously a role in the

large fat storage in the liver. During the period of 3-7 days of exposure a partial compensation of the respiratory acidosis has been accomplished and the pH has returned to a level where glycolytic and lipolytic inhibition as well as the stress reaction subside. Then changes should bring the lipid content of the organs back to control levels which is the case in muscle but not in liver and lung. The increased fat content in both liver and lungs during this period suggest a CO_2 dependent stimulation of lipid synthesis in these organs, which is not present in muscle.

This would require the assumption of organ specific CO_2 effect on metabolic pathways in lipid synthesis, for which Longmore[18] has obtained some evidence. He found that [U ^{14}C] glucose incorporation into total phospholipids and into the phosphatidyl choline fraction was increased by CO_2 in the perfused lung but not in the perfused liver. In the latter acetate -1 ^{14}C incorporation into triglycerides and phospholipid fatty acids was increased by CO_2.

Studies reported in the literature have shown that high concentrations of CO_2 inhibit metabolic processes in spermatozoa[17] while low concentrations have a stimulatory effect[23] This is in line with the observation in this study, where inhibition of spermatogenesis is limited to the high concentrations of CO_2.

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