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Immune Responsiveness in Chlorine Exposed Rats
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16. Abstract (Limit: 200 words) A mouse model was developed to test the hypothesis that acute exposure to high levels of chlorine (7782505) gas can increase sensitivity to inhaled antigen. This initial survey was designed to assess whether there was an increase in sensitivity to antigen and whether the effect was transient or of extended duration. BALB/c-mice were exposed to 200 or 150 parts per million (ppm) chlorine for 1 hour. The animals were then exposed to 1% aerosolized bovine serum albumin (BSA) immediately or 1, 3, 7, and 30 days after exposure. Exposures were for 30 minutes a day for 5 consecutive days. The findings indicated that in mice, acute exposure to 200ppm followed immediately by exposure to BSA resulted in increased levels of antigen specific immunoglobulin-G when compared to control values. Mice exposed acutely to lower levels of chlorine or to antigen at more distant time points following the irritant exposure did not demonstrate any increased antibody levels.

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LIST OF ABBREVIATIONS

Cl_2	Chlorine
BSA	Bovine serum albumin
ppm	Parts per million
BA	Breathing air
BALF	Bronchoalveolar lavage
ELISA	Enzyme linked immunosorbent assay
Ig	Immunoglobulin
RT	Room temperature
OD	Optical density

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The significant finding of these experiments is that, in BALB/c mice, acute exposure to high concentrations of Cl_2 (200 ppm) followed immediately by aerosolized exposure to bovine serum albumin (BSA) leads to increased levels of antigen-specific IgG, when compared with controls. Mice exposed acutely to lower levels of Cl_2 or to antigen at more distant time points following the irritant exposure did not demonstrate increased antibody levels.

INTRODUCTION

Chlorine gas is frequently involved in industrial and transportation accidents. Inhalation of chlorine can injure airway epithelial cells directly or secondarily following lung inflammation. Either of both of these events can disrupt mucosal exclusion barriers which have evolved to protect the host from inhaled antigen and, thus, increase contact between exogeneous materials and immunocompetent cells. This interaction may lead to altered immune function, particularly increased production of antibodies following exposure to inhaled antigen. To test the hypothesis that acute exposure to high levels of chlorine gas can increase sensitivity to inhaled antigen, a mouse model was developed. This initial study was designed to assess whether there was an increase in sensitivity to antigen and whether the effect was transient or of extended duration.

MATERIALS AND METHODS

Modification of the chlorine exposure chamber: An exposure chamber was modified extensively for chlorine use (Figure 1). Chlorine gas (A), controlled by a rotameter and solenoid valve (B), is metered into the system (C). Chlorine/dilution air pass through triple diffusion screens to mix and achieve uniform flow prior to entering the exposure area (D). Air is removed from the chamber through additional screens. Sample ports (E) can access the environment for measurement of gas concentrations. Air from the chamber is exhausted through a wet gas scrubber (G) (Table 1). The column is filled with a high performance wet scrubber packing material. Scrubbing liquid is sprayed at the top of the column, while contaminated air enters the bottom. Liquid collects at the bottom of the column and recirculates via a high pressure pump (H). Pressure drop across the column is continuously monitored by a manometer (I). A HEPA filter (J) collects aerosol particles generated in the column; pressure drop across this filter is monitored by a manometer (I). Air flow rate through the exposure system, measured using an orifice meter (K), is controlled by a ball valve (L). Flow rate is measured using a third manometer (I). A water trap (M) was installed in-line before the air blower (N) to collect water vapor. The entire system is exhausted by an EG&G Rotron, Spiral Simplex Regenerative Air Blower, Model SL 2P2. Chlorine monitoring probes (Anacon Instruments) were substituted for the MDA Gas Monitor, since they can simultaneously monitor room air (F1), chamber (F2), and exhaust (F3).

Sixty four mice can be exposed individually using compartmentalized stainless steel mesh cages.

Chlorine exposures: To determine the highest possible chlorine concentration which would induce sub-lethal injury, a dose response curve was run. Groups of mice (n=10) were exposed for 1 hour to Cl_2 to 50-250 ppm. Sub-lethal concentrations of Cl_2 for exposure were selected following preliminary lethality testing. The final concentrations were 150 ppm and 200 ppm; control mice were exposed, in the chamber itself, to breathing air. All test exposures were 1 hour.

Antigen exposure chamber: A simple system to expose mice to soluble antigen was designed, built, and calibrated. This chamber (Figure 2) is constructed of polystyrene with teflon tubing connections. For nose-only exposures, mice are placed into conical tubes (A) with ends removed; these are inserted into the exposure chamber (B). Antigen is placed in a DV 646 nebulizer (C) which is driven by compressed air (D). The system is closed and a continuous flow of aerosolized antigen is achieved by pumping air out of the system using a vacuum pump (F): antigen flows into a mixing chamber (E) and then into the exposure chamber. Flow rates for the air cylinder and vacuum pump are assessed using a rotameter (I). A filter paper disc (G) (Whatman #1), positioned at the outlet (H), collects antigen. The antigen used was 1% bovine serum albumin (BSA).

Chlorine/antigen exposures: Animals were exposed to 200 or 150 ppm Cl_2 or breathing air (BA) for 1 hour. Immediately; 1, 3, 7, and 30 days post exposure, animals were exposed, by nose only, to 1% aerosolozed BSA. Exposures were for 30 minutes a day for 5 consecutive days.

Collection of fluid samples: Test sera and bronchoalveolar lavage (BALF) are obtained as follows. Mice are anesthetized with ether; using sterile technique, the thoracic cavity is exposed carefully without damage

to the lung tissue; and the animal is exsanguinated by severing the abdominal aorta. Following clotting, sera are separated and frozen until use. Because of the small volume, sera from the same time points and exposure groups were pooled.

Lungs are perfused through the right ventricle with with RPMI 1640 (GIBCO; Grand Island, NY) containing 12mM lidocaine HCl. The trachea is cannulated with PE-260 polyethylene tubing approximately 6.5 cm long attached to a 5 cc syringe; the tubing is tied into place with suture thread. The lung is washed 4 times with 1.0-1.5 cc aliquots of physiologic saline. Retrieved aliquots from each animal are pooled, centrifuged, and the supernatant is concentrated by Amicon ulltrafiltration (B 15). Concentrated BALF is frozen until quantitation of antibodies.

Quantitation of BSA-specific antibodies by the enzyme-linked immunosorbent assay (ELISA): Specific antibodies were quantitated using a commercially available kit (Kirkegaard and Perry, Gaithersburg, MD). Briefly, Immunon 1 (Dynatech) plates are coated with 0.5 mg/ml BSA in phosphate buffered saline (PBS) (pH 8.5) and incubated overnight at 4° C. Coating solution is removed and unreacted sites are blocked with PBS with 1% BSA for one hour. One hundred ul undiluted BALF or sera (1:5 for IgG and neat for IgA an IgM) are added and incubated for one hour at room temperature (RT). Following washing, plates are incubated with peroxidase-labelled goat anti-mouse anti-immunoglobulin (IgG 1:1000, lgA 1:1000, IgM 1:1000) for 1 hour at RT. Following washing, 100 ul ABTS-H_2O_2 ae added to wells and color is developed in the dark at RT fo IO min. The reaction is stopped by the addition of 100 ul 5% sodium dodecly sulfate. The OD of each well is read on a Dynatech ELISA reader at 410nm.

RESULTS

Evaluation of the Cl2 exposure system: The chlorine exposure chamber can achieve and maintain target concentrations, within 5% (Figure 3). Uniformity of gas concentration within the chamber (Table 2) was evaluated using 16 chlorine passive dosimeters constructed from 37 mm polystyrene aerosol monitor cassettes with teflon membrane filters as diffusion screens. Dosimeters were placed in cage compartments and exposed to 100 ppm Cl_2 for one hour. Cl_2 was quantitated using the sulfamic acid-iodine specific ion electrode technique (OSHA Method ID-101). We had problems preparing standards for quantitation and, therefore, modified the technique. In our modification, iodate standards (chlorine surrogate) must react at least 2 minutes prior to dilution. This appears to eliminate the problem of overestimating Cl_2 in samples. A representative standard curve for the sulfamic acid method is presented in Figure 4. Side by side sampling using the methyl orange method for quantitation of airborne chlorine was used to validate the sulfamic acid-specific ion electrode technique. Concentrations were generally within 7% of each other (Figure 5).

Chlorine exposure trials: Results from the concentration trials are in Table 3. It is important to note that for the 200 ppm trial, no deaths occurred in the chamber; however, one mouse died 4 days post-exposure and one died 5 days post-exposure. 200 ppm was selected as the initital exposure dose.

Evaluation of antigen exposure chamber: This chamber is calibrates using changes in filter weight over time (Table 4). As can be seen, a clear dose response for BSA can be generated using this system.

Antibody studies: Exposure to 200 ppm chlorine, followed by immediate exposure to antigen, resulted in an elevated serum specific IgG response (Table 5). Results from other time points were less clear cut. However, the general observed pattern suggests that the further away antigen exposure is from irritant exposure, the less likely a specific antibody response will be mounted. Exposure to 150 ppm chlorine followed by antigen exposure did not result in elevated specific antibody levels.

CONCLUSIONS

During this study, accurate exposure systems were developed whereby BALB/c mice could be exposed to high levels of chlorine and subsequently to antigen. Our data suggested that only exposure to the highest possible sub-lethal concentration of chlorine followed immediately by antigen exposure could induce a modest increase in circulating IgG antibodies. This information and the experience obtained during these experiments, can however, establish a basis for future studies. In a grant application currently in the preparation stage, we will modify our exposure schedule and choice of antigen to favor synthesis of IgE antibody production and expand our schedule to look at three strains of mice (high, medium, and low IgE responders). Moreover, we will examine the secondary antibody response. This should favor production of higher levels of antibodies which should be easier to quantitate and more subtle differences should be detectable. Lastly, to facilitate statistical analysis, no samples will be pooled. Together, these modifications should allow us to explore further the relationship between chlorine and increased antigen sensitivity.

TABLE 1 CHLORINE SCRUBBER COLUMN CHARACTERISTICS

Dimensions	6 ft. (length) x 8 inches (diameter)
Construction Material	Schedule 40 PVC
Bed Material	Jaeger Nor-Pak, 5/8" polypropylene
Scrubber Solution	0.1-0.15 M Sodium Thiosulfate (4 gal)
Recirculation Rate	0.16 gpm
Airflow Rate	28 cfm
Pressure Drop (@ 28 cfm)	2.2 inches of water
Input Cl_2 concentration	40 to 700 ppm
Output Cl_2 concentration	< 1 ppm
Efficiency	> 99%

TABLE 2 HORIZONTAL DISTRIBUTION OF CHLORINE GAS CONCENTRATION. Values are ug chlorine collected by dosimeter.

Pos. No.	Shelf			
1	613	582	753	613
2	590	618	674	738
3	608	600	689	695
4	661	692	501	800
5	N.D.	556	669	690
6	586	505	720	705
7	666	676	704	679
8	622	686	900	721
9	581	618	684	695
10	N.D.	627	699	585
11	608	637	715	659
12	697	697	659	617
13	604	613	715	603
14	618	661	829	655
15	627	656	699	664
16	671	681	726	572
Mean	625	632	709	670
S.D.	35	53	83	59
C.V.	5.6%	8.45%	11.7%	8.8%

N.D.= not determined

Table 3. Dose response for determining experimental exposure concentrations.

Cl_2 expected conc.	Cl_2 actual conc.	Survival (%)
50 ppm hrs	47.6 ppm hrs	100
100 ppm hrs	94.6 ppm hrs	100
150 ppm hrs	153.3 ppm hrs	100*
200 ppm hrs	184 ppm hrs	80
250 ppm hrs	249 ppm hrs	20

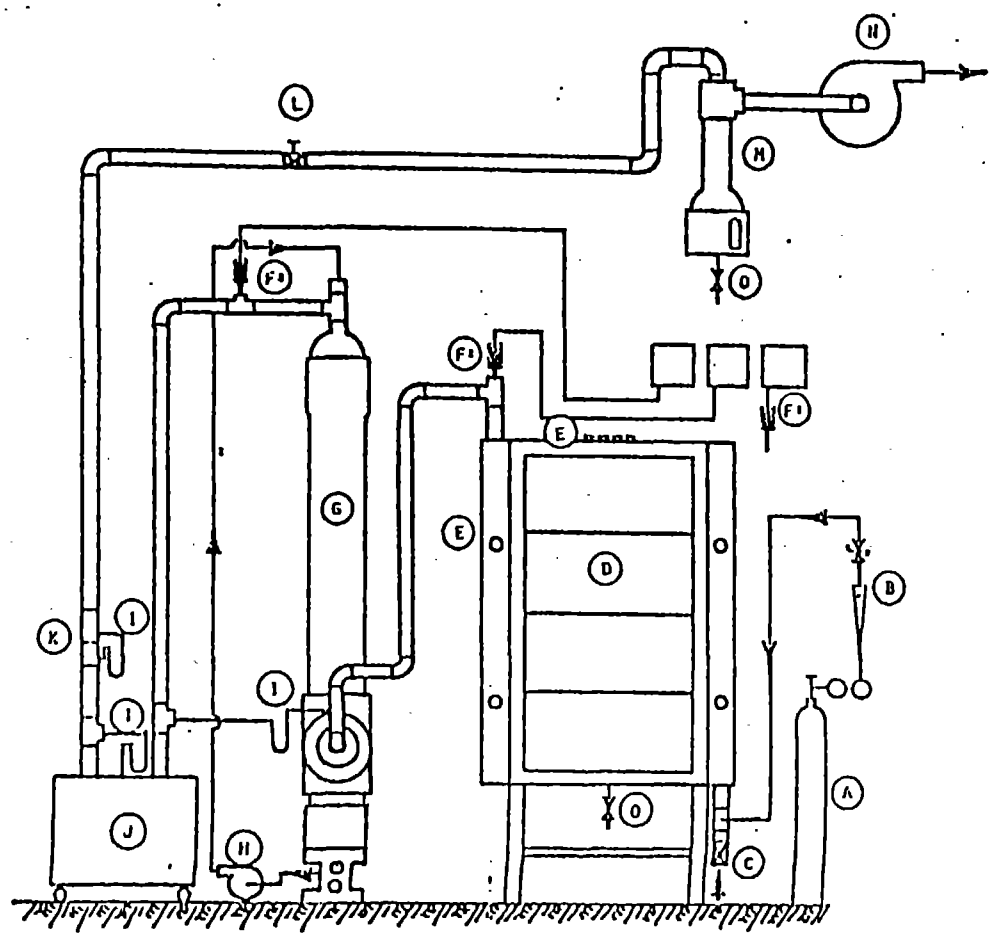
* No deaths were observed in the chamber; one mouse died 4 days post-exposure and the other died 5 days post-exposure. The two highest concentrations were run on the same day; if we had realized that 200 ppm was the LC_{20}, the next highest dose would not have been attempted.

TABLE 4. CALIBRATION OF ANTIGEN EXPOSURE CHAMBER

BSA (mg/ml)	Trial	Filter Wt. (mg)	Time (min)	Actual Conc. (mg/m^3)	Ave. Conc. (mg/m^3)
30	1	23.80	13.00	122.05	120.96
	2	19.50	13.17	98.71	
	3	15.90	10.07	105.26	
	4	21.80	9.21	157.80	
20	1	23.00	14.33	107.00	108.57
	2	24.40	14.39	113.04	
	3	17.30	11.60	99.43	
	4	20.80	12.08	114.79	
10	1	16.20	19.25	56.10	49.82
	2	14.80	19.11	51.63	
	3	10.80	17.50	41.14	
	4	11.30	14.94	50.42	
5	1	10.10	23.21	29.01	27.78
	2	8.70	24.02	24.15	
	3	9.10	20.56	29.51	
	4	9.40	22.04	28.43	

Table 5. Class BSA-specific antibody levels (optical density) of chlorine (Cl2) or breathing air (BA) exposed mice.

Immunization	Sample	Cl2 IgG	BA IgG	Cl2 IgA	BA IgA	Cl2 IgM	BA IgM
Immediate	SERA	0.11	0.02	0.03	0.06	0.04	0.03
Immediate	BALF	0.03	0.03	0.06	0.08	0.01	0.02
1 day	SERA	0.07	0.02	0.05	0.05	0.04	0.03
1 day	BALF	0.05	0.04	0.11	0.05	0.02	0.01
3 days	SERA	0.02	0.03	0.06	0.05	0.02	0.06
3 days	BALF	0.04	0.04	0.08	0.08	0.02	0.01
7 days	SERA	0.03	0.04	0.08	0.05	0.03	0.04
7 days	BALF	0.06	0.05	0.05	0.09	0.02	0.01
30 days	SERA	0.07	0.87	0.04	0.03	0.16	0.09
30 days	BALF	0.01	0.05	0.00	0.04	0.01	0.00



A. COMPRESSED GAS CYLINDER
B. ROTAMETER AND SOLENOID VALVES
C. INJECTION PORT AND CHECK VALVE
D. EXPOSURE CHAMBER
E. SAMPLING PORTS
F. DIRECT READING SAMPLING PROBES
G. WET GAS SCRUBBER
H. SCRUBBING SOLUTION PUMP
I. MANOMETERS
J. HEPA FILTER
K. ORIFICE METER
L. BALL VALVE
M. WATER TRAP
N. AIR BLOWER
O. DRAIN

FIGURE 1· CHLORINE EXPOSURE SYSTEM

Figure 2. ANtigen exposure chamber.

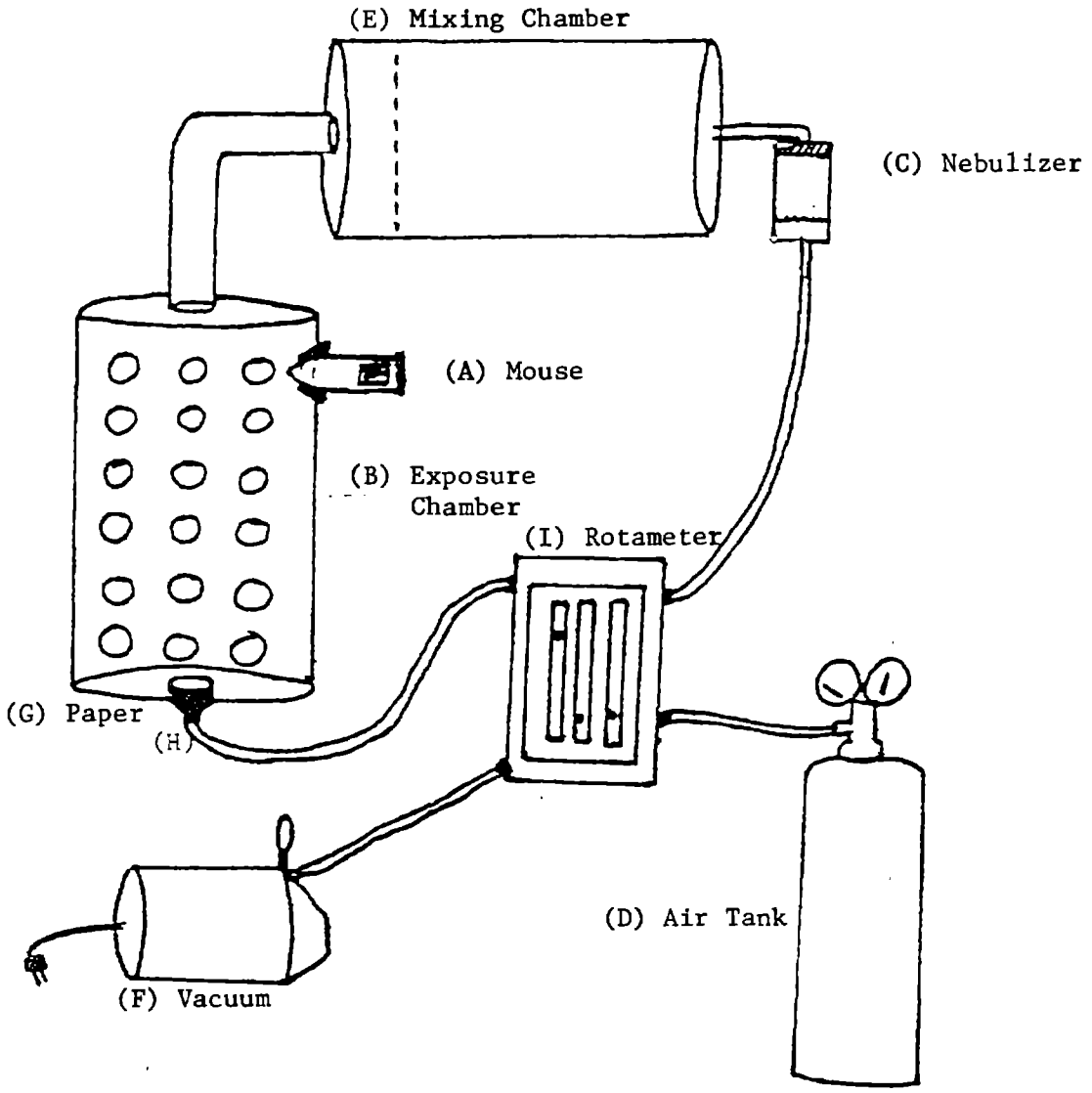


Figure 3.

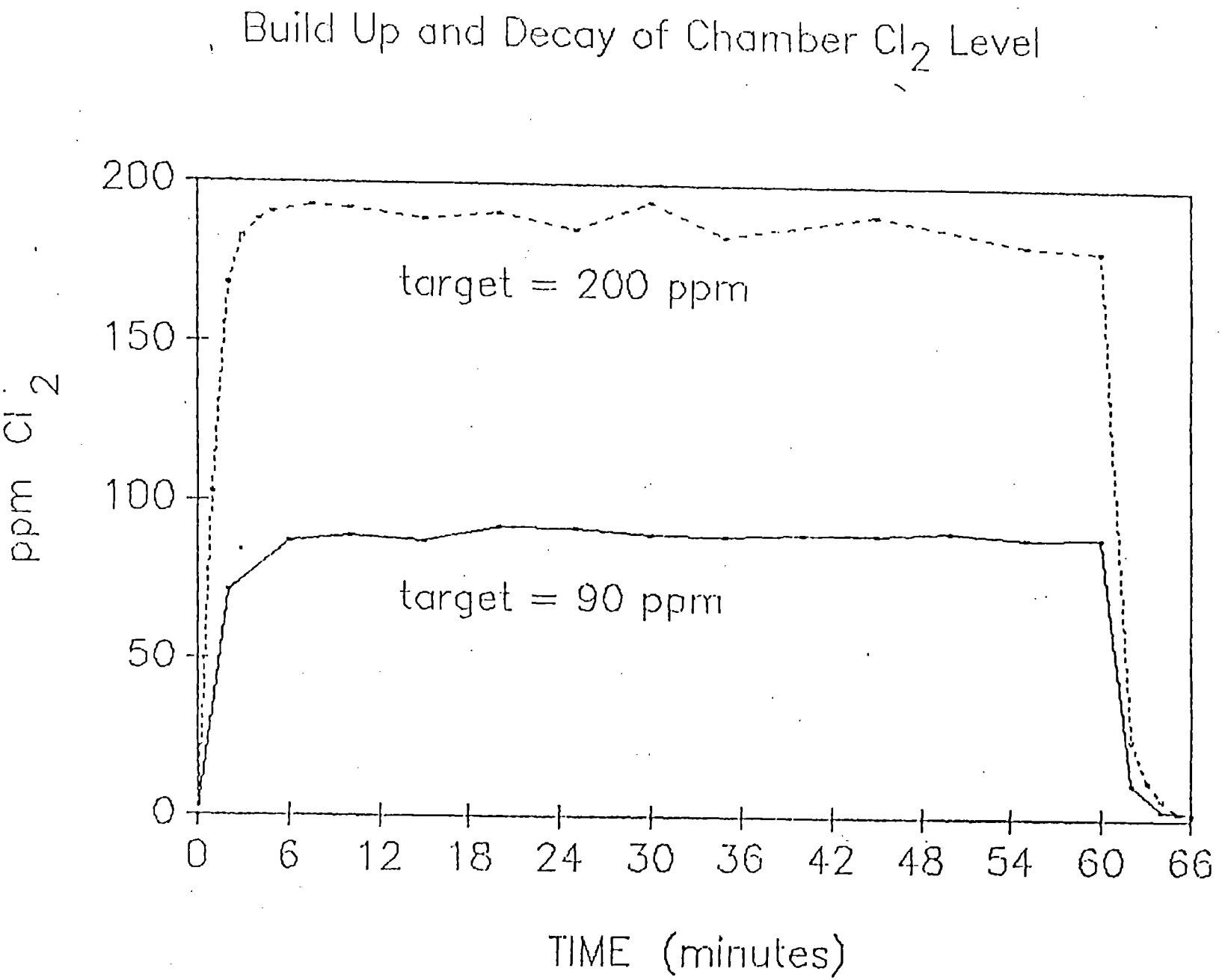

Build Up and Decay of Chamber Cl_2 Level
200
150
100
50
0
ppm Cl_2
target = 200 ppm
target = 90 ppm
0
6
12
18
24
30
36
42
48
54
60
66
TIME (minutes)

Figure 4.

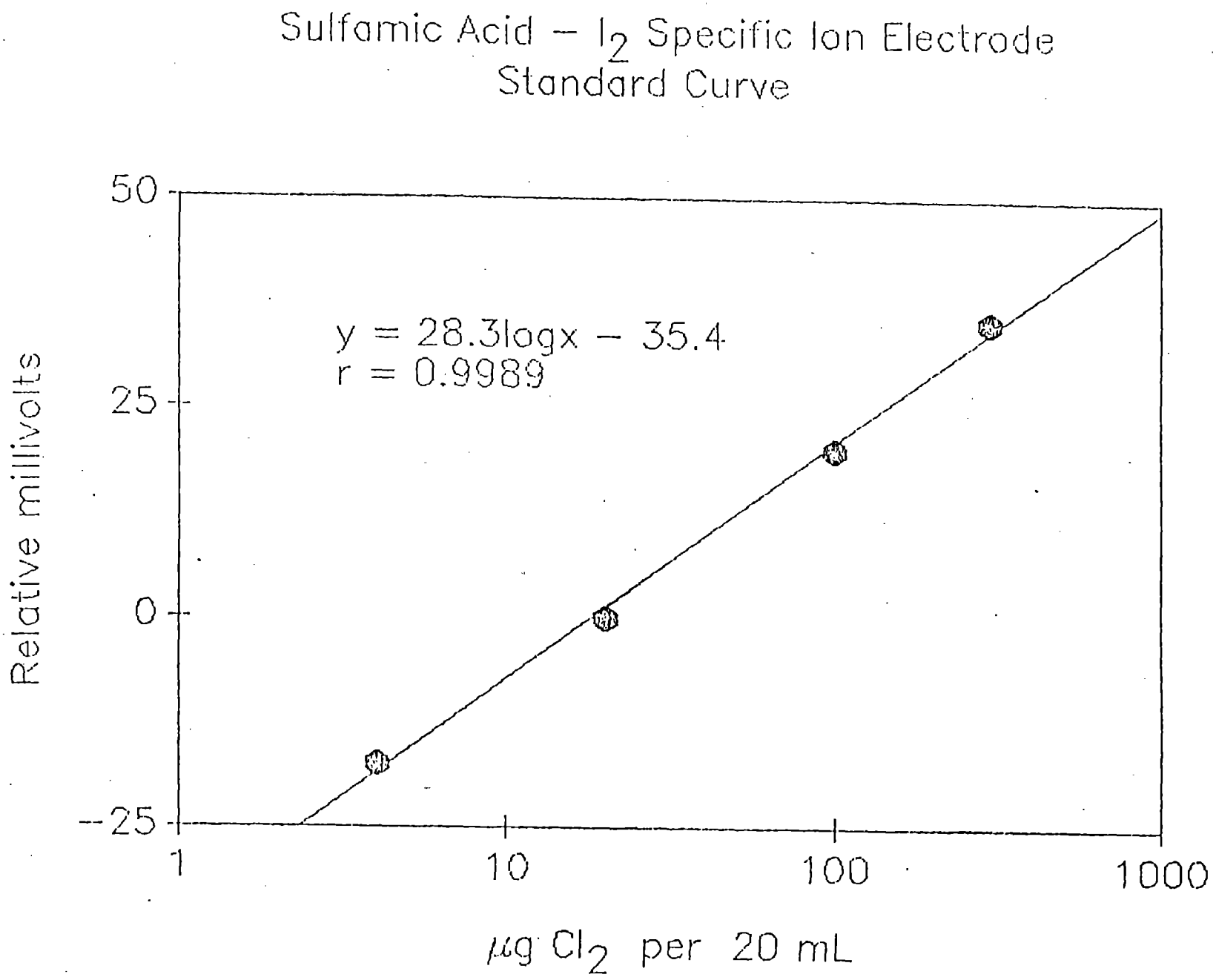
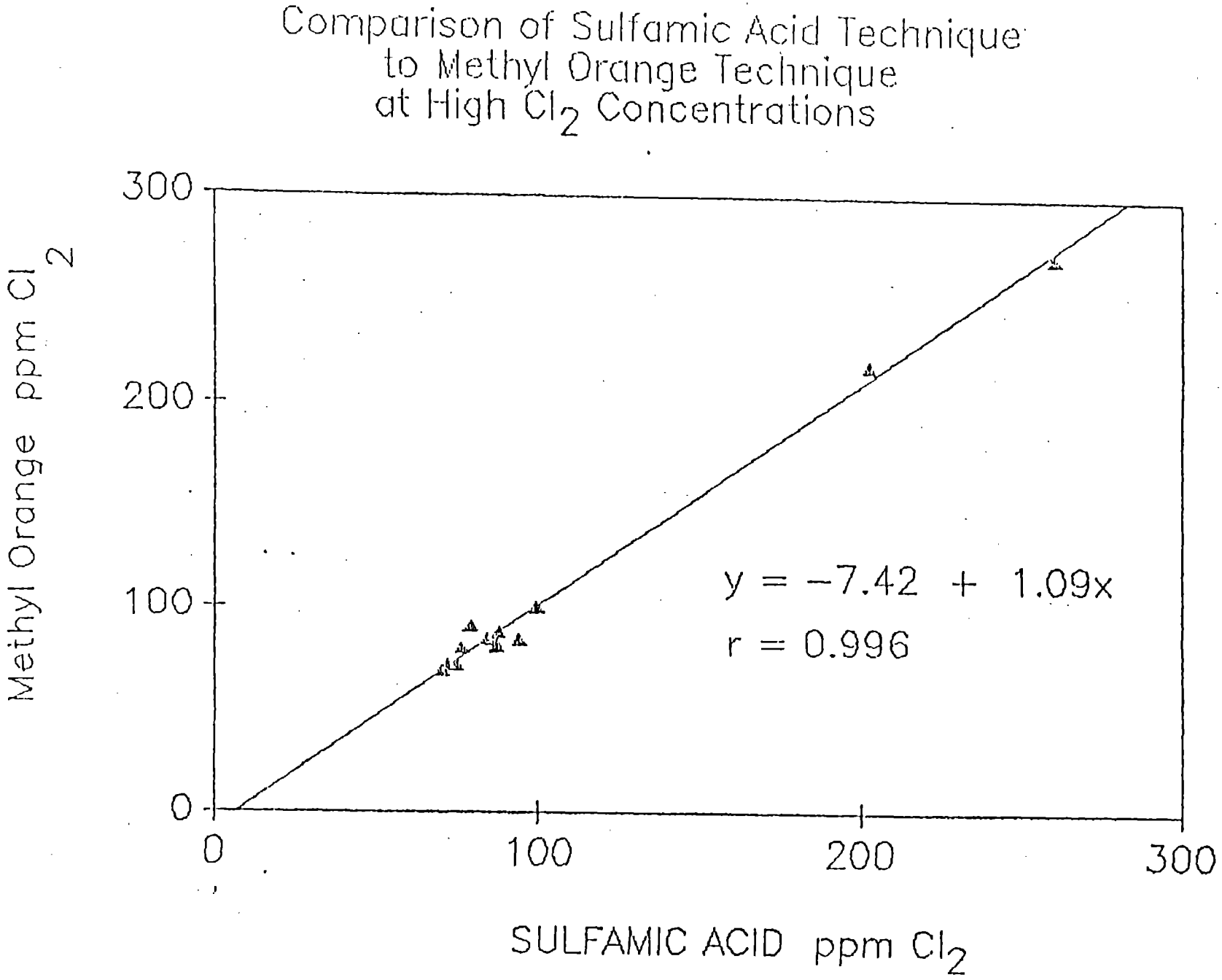

Sulfamic Acid – I_2 Specific Ion Electrode
Standard Curve
y = 28.3logx – 35.4
r = 0.9989
Relative millivolts
50
25
0
–25
1
10
100
1000
μg Cl_2 per 20 mL

Figure 5.


Comparison of Sulfamic Acid Technique
to Methyl Orange Technique
at High Cl_2 Concentrations
Methyl Orange ppm Cl_2
300
200
100
0
0
100
200
300
SULFAMIC ACID ppm Cl_2
y = −7.42 + 1.09x
r = 0.996