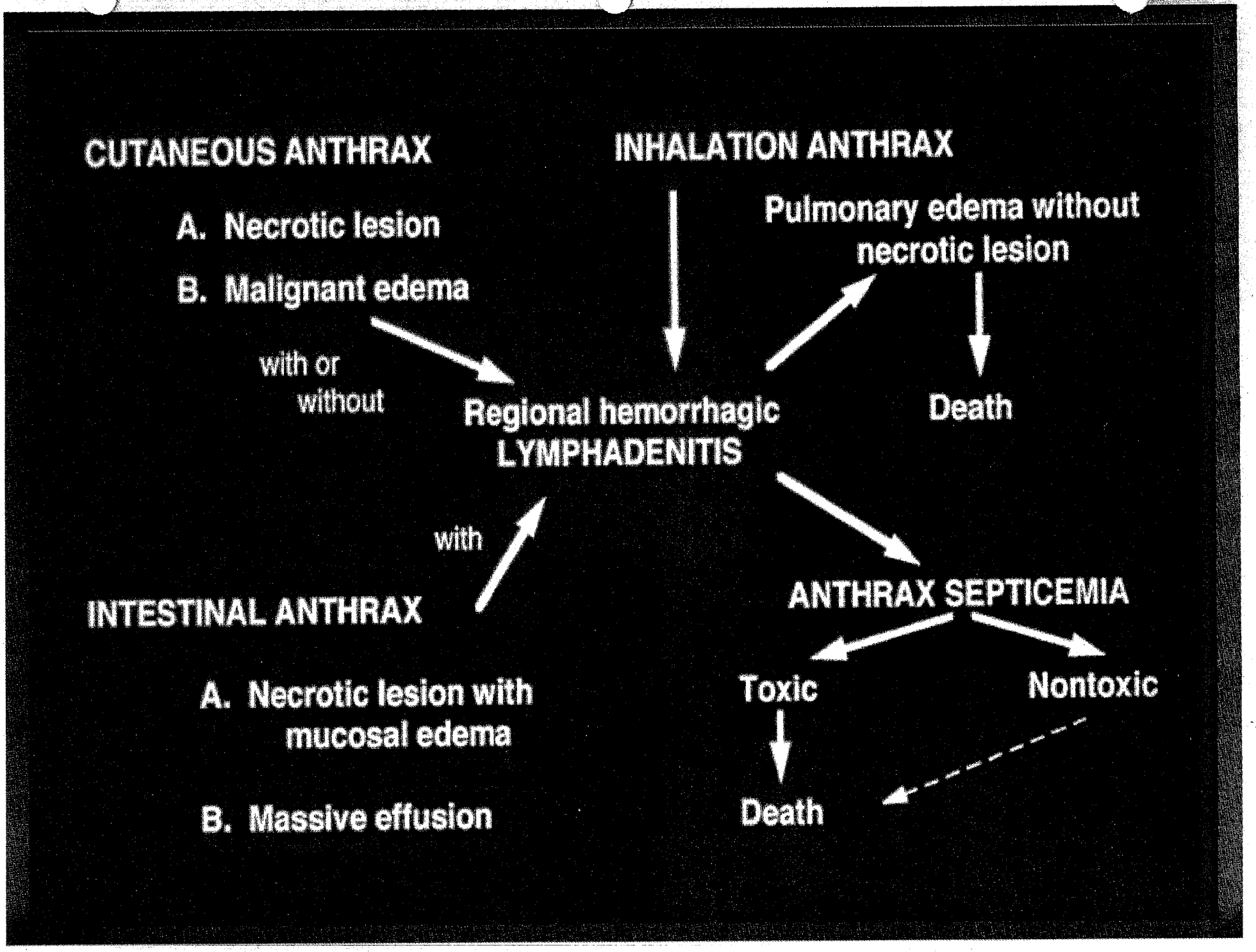
Non-Human Primate Model of Inhalational Anthrax

Arthur Friedlander, M.D.

Pathogenesis

- Spore enters skin, GI tract, or lung
- Germinates in macrophage locally or is transported to regional lymph nodes
- Local production of toxins leads to edema and necrosis
- Spread from node with bacteremia and toxemia


CUTANEOUS ANTHRAX
A. Necrotic lesion
B. Malignant edema
with or without
INHALATION ANTHRAX
Pulmonary edema without necrotic lesion
Death
Regional hemorrhagic LYMPHADENITIS
with
INTESTINAL ANTHRAX
A. Necrotic lesion with mucosal edema
B. Massive effusion
ANTHRAX SEPTICEMIA
Toxic
Nontoxic
Death

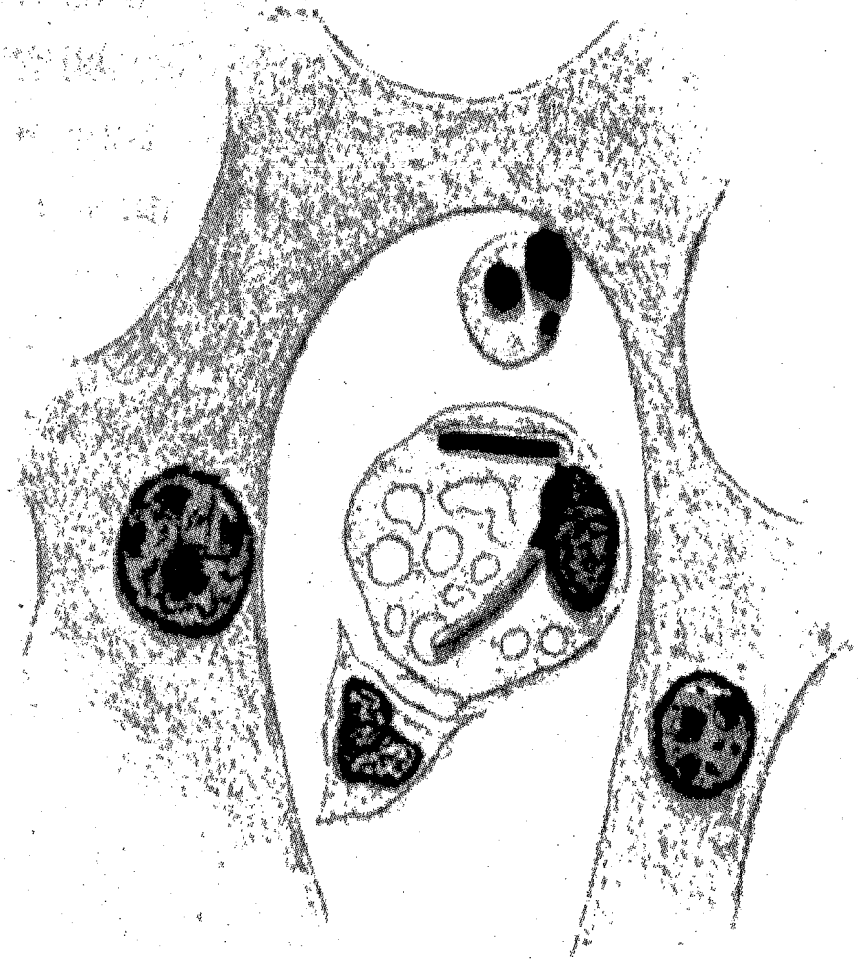


Fig. 22. Macrophage from the liver of a rat affected with anthrax.

Fig. 23. Macrophage containing bacilli, from the liver of a rat affected with anthrax.

"The disease was most marked near the bifurcation of the trachea and in the large bronchi."
"Extending thence, either directly into the mediastinum and causing mediastinal cellulitis, or by the way of the bronchial glands, producing in them intense lymphadenitis and hemmorrhage."

"...great swelling of the bronchial glands, these being sometimes completely broken down by hemorrhage, and transformed into blood clots; extensive cellulitis, together with hemorrhagic effusion, around the bronchial glands and in the mediastinum generally; serous pleural effusion, often in great amount, pretty equally in both pleura, usually unaccompanied by any signs of pleural inflammation."

"In the lungs the changes are but slight."

Supplementary Report on the Woolsorters' Disease
in the Bradford District by W. S. Greenfield, 1882

Table 1. Comparative Summary of the Principal Lesions of Inhalational Anthrax[a]

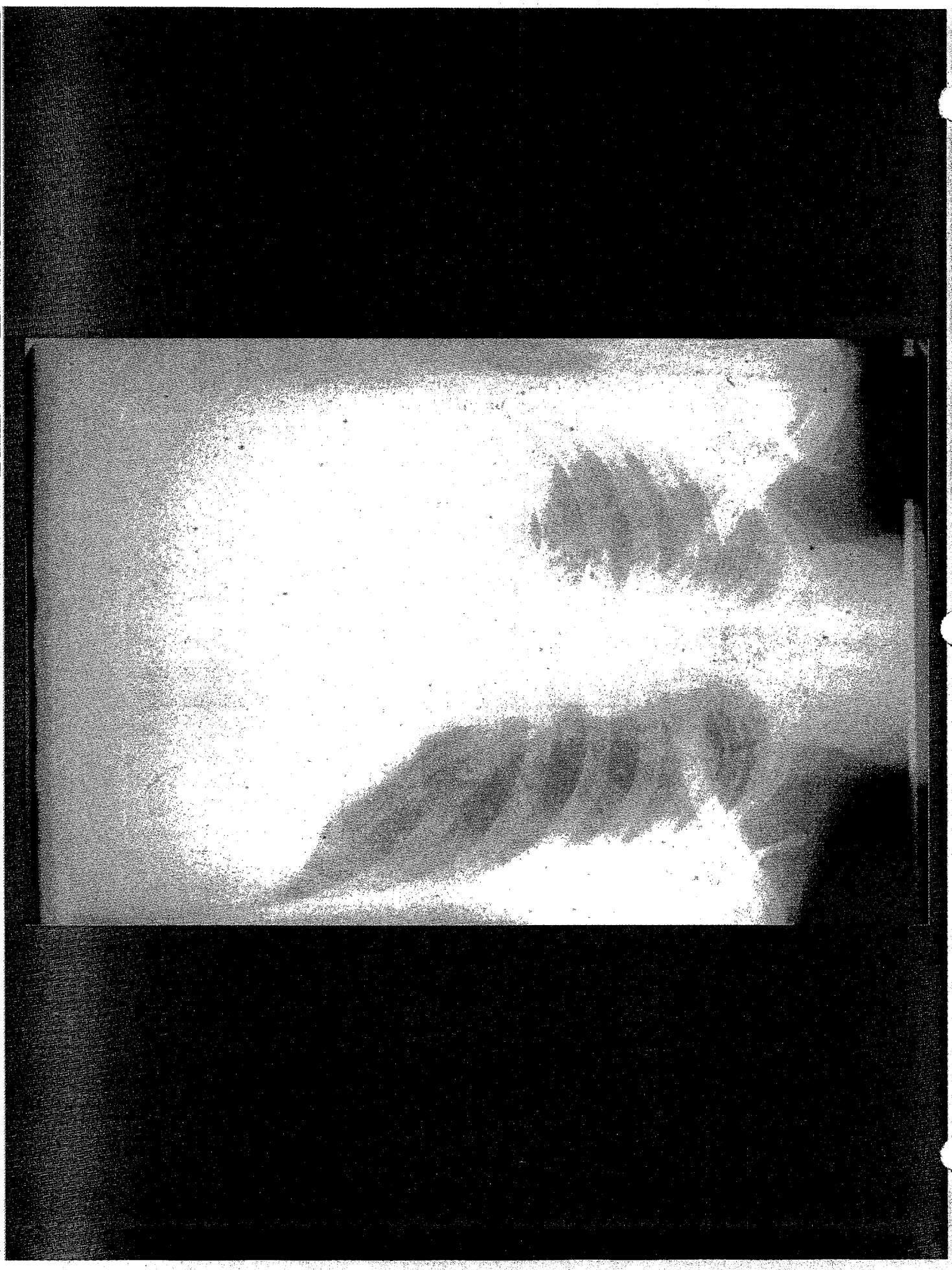
Organ / Findings	Species		
	Human	Rabbit	Rhesus Monkey
	Percent Affected (n)	Percent Affected (n)	Percent Affected (n)
Lung			
edema	67 (30[b])	95 (22)	60 (25)
hemorrhage	50 (30[b])	9 (22)	56 (25)
pneumonitis/pneumonia[c]	30 (72)	9 (22)	16 (25)
Mediastinum			
inflammation, hemorrhage, edema, enlarged[d]	78 (72)	36 (22)	40 (25)
Intrathoracic Lymph Nodes			
inflammation, necrosis, hemorrhage, enlarged, edema, bacilli	89 (72)	100 (22)	80 (25)
Brain/Meninges			
hemorrhage, edema, noninflammatory	14 (64[e])	18 (22)	21 (24[f])
hemorrhage, inflammation	38 (64[e])	0 (22)	33 (24[f])
total CNS involvement	52 (64[e])	18 (22)	54 (24[f])
Gastrointestinal Tract			
edema, erosion, ulceration, hemorrhage, inflammation	71 (72)	54 (22)	52 (25)
Mesenteric Lymph Nodes			
inflammation, necrosis, hemorrhage, enlarged, bacilli	15 (72)	59 (22)	72 (25)
Spleen			
enlarged, hemorrhage, inflammation, necrosis, congestion, bacilli	85 (72)	100 (22)	100 (25)
Liver			
inflammation, hemorrhage, necrosis	17 (72)	0 (22)	36 (25)
Adrenal			
hemorrhage		73 (22)	26 (19[g])
Bone Marrow			
inflammation, depletion		41 (22)	22 (18[g])
Thymus			
necrosis		14 (22)	0 (8[g])
Mean survival (days)	4.74 POST-ONSET	2.36 post-exposure	4.76 post-exposure

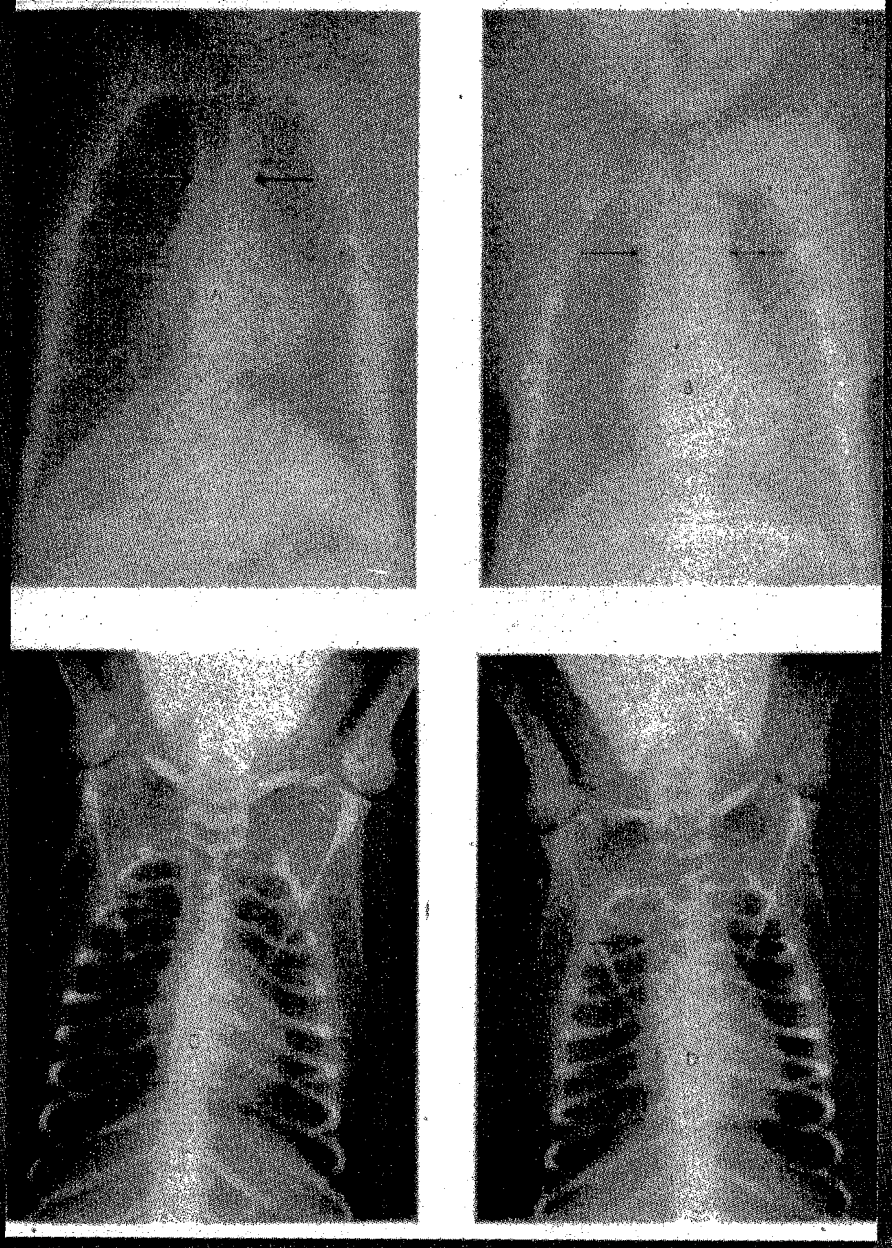
[a]Data represent a compilation of principal gross and histopathologic findings. [b]Does not include Sverdlovsk cases. Lesion was noted as present but specific incidence was unavailable. [c]Histologic confirmation required, only cases which were examined microscopically are included in the value for n. [d]Where findings for an organ are grouped horizontally, a minimum of one of those findings need be present for that organ to be counted as having a lesion. [e]The brain was not examined for eight cases. [f]The brain was not examined for one case.

Table 2. Comparative Summary of Inhalational Anthrax in Rabbits and Rhesus Monkeys: Influence of Survival Time on Lesion Incidence[a]

Organ / Findings	Species / Exposure Strain (n)							
	Rabbit[b] Ames (22)	Rabbit[c] Ames (2)	Rhesus Ames (5) Vollum (1)	Rhesus Ames (5) Vollum (1)	Rhesus Ames (3) Vollum (3)	Rhesus Ames (1) Vollum (2)	Rhesus Ames (0) Vollum (3)	Rhesus Ames (0) Vollum (1)
Lung								
edema	95	100	100	33	67	33	33	0
hemorrhage	9	100	50	50	83	33	33	0
pneumonia	9	100	0	0	50	33	0	0
Mediastinum								
inflammation, hemorrhage, edema, enlarged	36	100	17	0	67	100	67	0
Intrathoracic Lymph Nodes								
inflammation, necrosis, hemorrhage, enlarged, edema bacilli	100	NE[d]	67	67	100	67	100	100
Brain/Meninges						n=2		
hemorrhage, edema, noninflammation	18	0	17	17	33	0	33	0
hemorrhage, inflammatory	0	100	0	33	33	50	67	100
total CNS involvement	18	100	17	50	67	50	100	100
Liver								
inflammation, hemorrhage, necrosis	0	0	0	17	67	67	33	100
Mean survival (days)	2.4	7	3	4	5	6	7	8

[a]Data represent a compilation of principal gross and histopathologic findings and are reported as percent affected. [b]Nonimmunized. [c]Partially immunized, correlate of immunity study. [d]Not examined.





One of the main factors in the therapy of inhalational anthrax is the

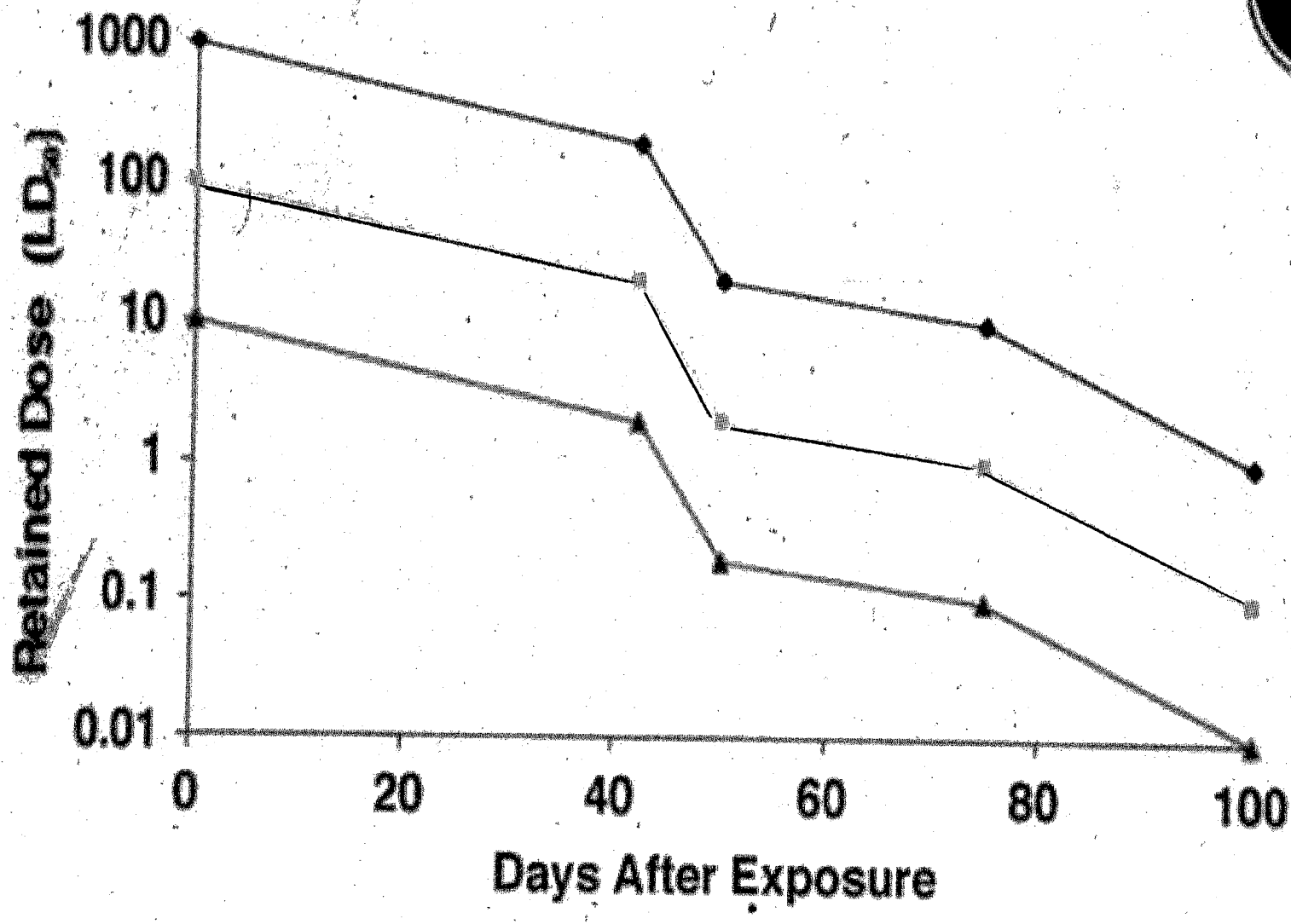
"...persistence of spores in the tissues and their germination after the blood-penicillin level has fallen..."

J. M. Barnes
J. Path. Bact. 59:113(1947)

"The conditions which govern the germination of anthrax spores *in vivo* remain completely obscure."

J. M. Barnes
J. Path. Bact. 59:113(1947)

USAMRIID


1000
100
10
1
0.1
0.01
Retained Dose (LD_{50})
0
20
40
60
80
100
Days After Exposure

Critical points to consider in prophylaxis or treatment of inhalational anthrax:

- Spore may persist, in a viable but ungerminated state, for extended periods of time.
- Antibiotics, most likely, have no effect on the spore, but rather act on the bacillus.

CHRONOLOGY OF POSTEXPOSURE ANTIBIOTIC PROPHYLAXIS EXPERIMENTS

- Iraq invaded Kuwait on 2 August 1990
- The first challenge in 2 Rhesus monkeys took place on 29 August 1990
- The postexposure prophylaxis experiment began on 13 September 1990

LOGISTICS OF POSTEXPOSURE ANTIBIOTIC PROPHYLAXIS EXPERIMENTS

- More than 60 individuals were involved in the design and implementation of the experiments
- 68 monkeys used: 8 in preliminary experiments and 60 in the post-exposure prophylaxis experiment
- Courses of anesthesia: 3780
- Quantitative blood cultures: 1550
- Parenteral medications given: 720
- Orogastric medications given: 1920
- One animal died from an aspiration pneumonia and one animal died from unknown causes

EXPERIMENTAL DESIGN

Day 0	Challenge with 8 LD_{50}s by aerosol
Day 1	Begin treatment with antibiotic alone, vaccination alone, or antibiotic + vaccination
Day 30	Discontinue antibiotics
Day 131-142	Re-challenge survivors with 50 LD_{50}s by aerosol

EXPERIMENTAL GROUPS

1. CONTROLS: 10 animals given saline as a control solution intramuscularly every 12 hours, beginning 1 day after exposure.
2. PENICILLIN: 10 animals treated with penicillin G intramuscularly at a dose of 180,000 units every 12 hours, beginning 1 day after exposure to anthrax and continuing for 30 days.
3. CIPROFLOXACIN: 10 animals treated with ciprofloxacin at a dose of 125 mg by orogastric tube every 12 hours, beginning 1 day after exposure to anthrax and continuing for 30 days.
4. DOXYCYCLINE: 10 animals treated with doxycycline at a dose of 30 mg by orogastric tube every 12 hours, beginning 1 day after exposure to anthrax and continuing for 30 days.
5. DOXYCYCLINE + HUMAN VACCINE: 10 animals treated with doxycycline beginning 1 day after exposure and with 0.5 ml of the human anthrax vaccine given subcutaneously on days 1 and 15 following the aerosol exposure. The doxycycline treatment was 30 mg by orogastric tube every 12 hours for 30 days.
6. HUMAN VACCINE: 10 animals given water by orogastric tube every 12 hours beginning 1 day after exposure and 0.5 ml of the human anthrax vaccine subcutaneously on days 1 and 15 following the aerosol exposure.

Clinical, microbiological, and pathological studies

Daily blood cultures were obtained from the untreated controls and vaccination groups until death or for 14 days. In the antibiotic-treated groups, blood was cultured every other day until 80% of the controls died, then twice weekly until day 30 when antibiotics were discontinued, then every other day until approximately day 60, and then once a week until re-challenge. The blood from untreated animals was collected in a 1.5 ml Isolator tube (Du Pont Co., Wilmington, DE) and 10-fold dilutions cultured on trypticase soy agar. Blood from antibiotic-treated animals collected in an Isolator 1.5 was cultured undiluted and at a 1:100 dilution on trypticase soy agar. In addition, 1 ml was cultured in a Bactec Peds Plus bottle (Becton Dickinson, Towson, MD). Blood obtained before and at various times after challenge was analyzed for antibodies to the anthrax protective antigen by an ELISA assay.

All animals were observed at least twice daily until death or euthanasia. Moribund animals were euthanized by deep anesthesia (tiletamine/zolazepam, 6 mg/kg) and exsanguination. Autopsies were performed on all animals. A diagnosis of anthrax was confirmed in all animals by isolation of B. anthracis from the blood. In all deaths in which antemortem blood cultures were negative, cultures were obtained at autopsy of the blood, spleen, lung, liver, intrathoracic lymph nodes, and brain.

The experiments were carried out under the guidance of the Veterinary Medicine Division in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to priniciples stated in the Guide for the Care and Use of Laboratory Animals, NIH publication 86-23, 1985 edition. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Antibiotic Sensitivity Testing and Serum Levels

Minimal inhibitory concentrations (MIC) of the *B. anthracis* Vollum 1B strain were determined in Mueller-Hinton broth dilutions using an inoculum of 2.5-3.0 x 10^5/ml in tubes and in a microtiter format. The MIC was 0.08 ug/ml for penicillin, 0.08 ug/ml for ciprofloxacin, and 0.02 ug/ml for doxycycline. The MBC was 0.32 ug/ml for penicillin and 0.08 ug/ml for ciprofloxacin.

Serum antibiotic levels were determined by bioassay. Peak serum levels were determined at 1 h (ciprofloxacin) or 2 h (penicillin and doxycycline) after a dose on days 5, 9, 20, and 30. Trough levels were determined 12 h after a dose on days 3, 5, 9, and 20.

A
Serum Penicillin Concentration (µg/ml)
100
10
1
.1
ND
B
Serum Ciprofloxacin Concentration (µg/ml)
10
1
.1
.01
C
Serum Doxycycline Concentration (µg/ml)
100
10
1
.1
.01
3
5
9
20
5
9
20
30
Trough
Peak
Days After Starting Treatment

Ciprofloxacin Serum Levels

The geometric mean peak levels of ciprofloxacin were between 0.98 to 1.69 ug/ml while the trough levels were between 0.12 to 0.19 ug/ml. The MIC and MBC were 0.08 ug/ml.

CLINICAL AND PATHOLOGICAL FINDINGS IN UNTREATED CONTROL GROUP

1. 9/10 control animals died within 3 to 8 days following exposure (mean ± SE = 5.6 ± 1.1 days).

2. Animals were ill for 1 to 4 days before death, demonstrating decreased spontaneous activity, weakness, and anorexia.

3. Bacteremia at levels of 10^1 to 10^5 colony forming units (CFU)/ml, was present for a mean of 1.8 ± 0.9 days before death.

4. Terminal bacteremias were usually from 10^4 to 10^9 CFU/ml. The one animal with a low terminal bacteremia of 2×10^2 CFU/ml had meningitis with 2×10^7 CFU/gm of brain tissue.

5. 5/9 animals had gross findings of mediastinitis and intrathoracic hemorrhagic lymphadenitis.

6. Meningitis was present in 5/9 animals and was hemorrhagic in 3 of the cases.

7. The one animal which survived had persistently negative blood cultures.

SURVIVAL AFTER POST-EXPOSURE TREATMENT OF INHALATION ANTHRAX

	Anthrax deaths	P value (vs control)
Control untreated	9/10	
Vaccine alone	8/10	>0.1
Penicillin	3/10	<0.02
Ciprofloxacin	1/9*	<0.002
Doxycycline	1/10	<0.002
Doxycycline + vaccine	0/9**	<0.0002

*1 animal died 5 days after exposure from an aspiration pneumonia and had no evidence of anthrax on autopsy. This animal was excluded from analysis. A 2nd animal died 73 days after discontinuance of ciprofloxacin due to urethral obstruction and had no evidence of anthrax at autopsy. This animal is included in the statistical analysis as a survivor.

**1 animal died 6 days after discontinuing doxycycline with no evidence of anthrax on autopsy. The cause of death remains unknown and the animal was excluded from statistical analysis.

CONCLUSIONS

1. Vaccination alone, begun after exposure to anthrax spores, did not protect animals.

2. All the antibiotics provided complete protection when given after aerosol exposure to anthrax spores as long as the animals remained on treatment.

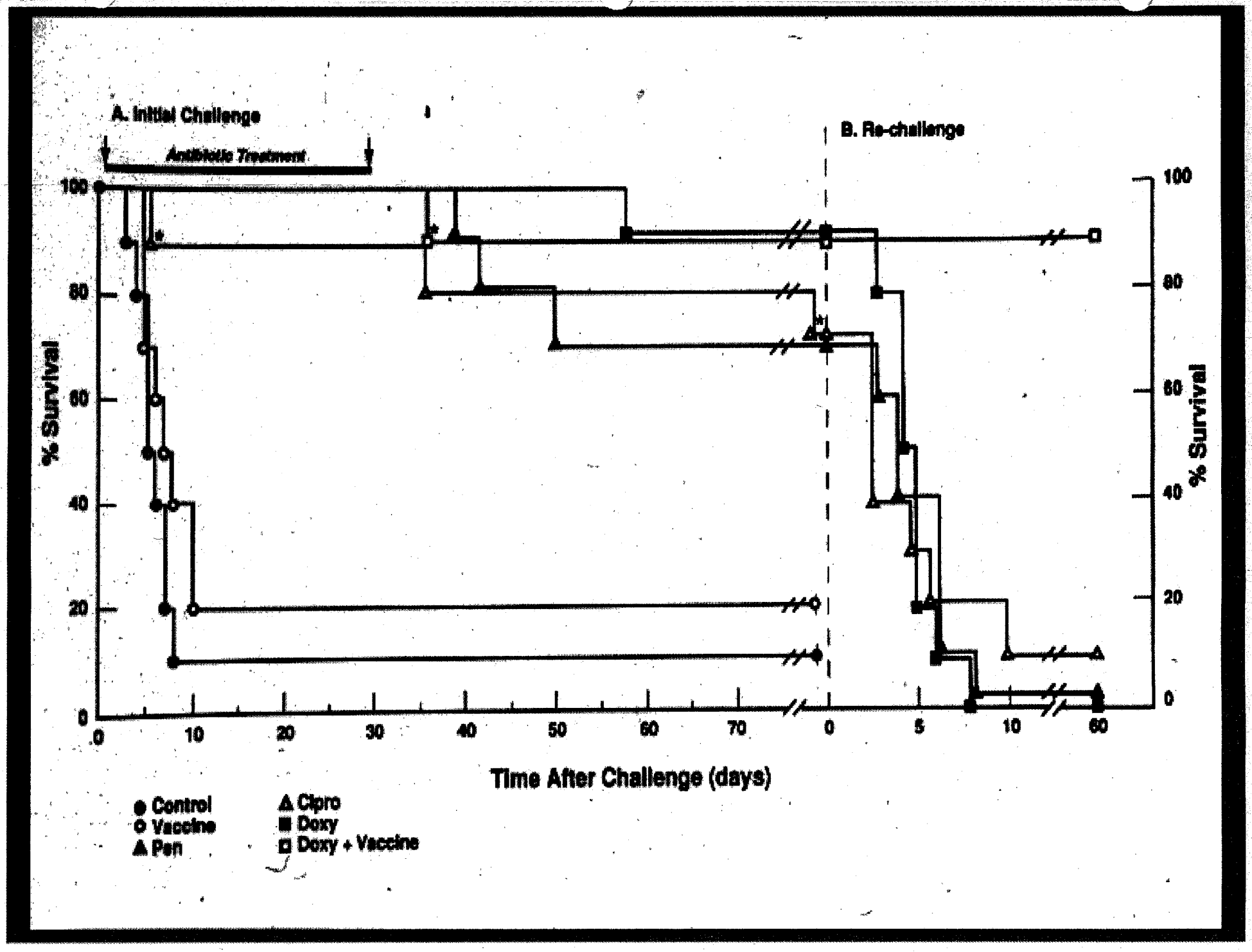
3. Extended treatment for a 30 day period with either penicillin, ciprofloxacin, or doxycycline alone, provided significant long-term protection upon discontinuance of therapy, with from 70-90% of the animals surviving.

4. Post-exposure vaccination combined with doxycycline treatment protected all animals from anthrax upon discontinuance of the antibiotic.

RESISTANCE OF SURVIVORS TO RE-CHALLENGE

	Anthrax deaths	P value (vs control)
Control*	4/5	
Penicillin	7/7	>0.1
Ciprofloxacin	6/7	>0.1
Doxycycline	9/9	>0.1
Doxycycline + vaccine	0/9	0.005

*Controls consisted of 5 additional animals not previously exposed to anthrax.


A. Initial Challenge
Antibiotic Treatment
B. Re-challenge
% Survival
100
80
60
40
20
0
0
10
20
30
40
50
60
70
0
5
10
60
Time After Challenge (days)
Control
Vaccine
Pen
Cipro
Doxy
Doxy + Vaccine

SUMMARY AND CONCLUSIONS

- Post-exposure antibiotics which protect against an aerosol challenge with anthrax spores appear to prevent infection and the development of an effective immune response. Animals treated in this way remain susceptible to re-challenge.
- Post-exposure vaccination when combined with antibiotic therapy protects animals against an aerosol challenge and leads to the development of an effective immune response. These animals are resistant to re-challenge.
- The most effective post-exposure treatment of experimental inhalational anthrax consists of suppressive antibiotic therapy combined with vaccination.