THE UNITED STATES ARMY MEDICAL UNIT

United States Army Medical Research and Development Command

Walter Reed Army Medical Center

Fort Detrick, Maryland

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Section II

STUDIES ON PASTEURELLA TULARENSIS

STUDIES ON PASTEURELLA TULARENSIS

FOREWORD

This constitutes a section of the 1958 Annual Report of the U. S. Army Medical Unit, Fort Detrick. Most of the studies on Pasteurella tularensis were accomplished as preparatory steps to the conduct of studies in man, or as a result of the direct care of accidentally infected laboratory workers.

Practically all animal studies on tularemia during 1957 and 1958 were conducted jointly with Dr. Henry T. Eigelsbach, MB Division, Fort Detrick. Unless otherwise specified, he prepared the challenge strains and the vaccine strains of P. tularensis employed. He has also participated in the experimental design and has been instrumental in providing a wealth of published and unpublished information to us.

The general background on the "viable" P. tularensis vaccine has been outlined in an earlier document. The accumulation of additional information has required a revision of this literature summary. The conclusions reached therein remain essentially unchanged but can now be considered firm. Without question the "viable" vaccine described in the accompanying reports is a substandard product. However, the results obtained with this product are such as to make further development promising.

The 1957 animal exposures were accomplished by Aerobiology Branch personnel and utilized the conventional techniques of that group. This permitted initiation of familiarization studies with this infection prior to completion of our own facilities.

STUDIES ON PASTEURELLA TULARENSIS

CONTENTS

STUDIES ON PASTEURELLA TULARENSIS

CHALLENGE OF MACACA MULATTA WITH 100,000 PASTEURELLA TULARENSIS ORGANISMS BY THE RESPIRATORY ROUTE

(Overholt, Hughes, Hornick)

I. PURPOSE

Part I of this initial pilot study was for the purpose of determining the effect of streptomycin used prophylactically and therapeutically in Macaca mulatta monkeys following respiratory exposure to 100,000 Pasteurella tularensis cells (streptomycin-sensitive strain).

Part II was for the purpose of determining the protection afforded by a living attenuated P. tularensis vaccine in monkeys challenged by the respiratory as in Part I.

II. METHOD

A. EXPOSURE

Each animal was given sodium pentothal intravenously in an amount sufficient to render it unconscious and limp. The animal was then placed in a Plummer exposure chamber and exposed for one minute to an aerosol of P. tularensis with a particle size of 0.7 micron or less.

Exposure methods on October 1, 1957, were identical for the 20 monkeys (11 in Part I and 9 in Part II). The nominal dose desired was 100,000 cells per animal; calculated dosages based on weights of the animals ranged from 65,500 to 143,000. The average number of organisms per liter of aerosol was 83,500.

Control animals were common to both Parts I and II.

B. STUDIES AND EXAMINATIONS POST-EXPOSURE

Studies on all animals included temperatures every 6 hours; physical examinations every 6 hours throughout the first week, then once daily; weights weekly; and chest roentgenograms daily throughout the first two weeks, and thereafter, twice daily for the next two weeks, and once weekly for the surviving animals. The severity of lung disease was classified as negative or ± to 5+:

±: questionable infiltrate.
1+: minimal infiltrate, diffuse or localized.
2+: definite infiltrate, diffuse or localized. If diffuse - miliary or 1-cm bronchopneumonic patches; if localized - an ill-defined 1 to 2 cm bronchopneumonic patch.
3+: diffuse 0.5-to 1-cm or well-defined 2-to 3-cm bronchopneumonic patches.
4+: multiple 1-cm infiltrates or early lobar consolidation.

5+: coalescence of the above multiple infiltrate with or without extensive lobar consolidation, obscuring both mediastinum and heart shadow.

Blood cell count and differential, hemoglobin, hematocrit, and C-reactive protein (CRP) determinations were performed daily throughout the first week, thereafter at least once every two weeks. Sedimentation rates were similarly performed but the micro-sedimentation rate determination used the first 3 to 4 days was found to be inaccurate; thereafter, the Wintrobe method was used. Correction factors used for man were applied. Blood cultures were performed daily throughout the first week. Agglutinin titers for F. tularensis were done weekly. Detailed autopsies were performed on all dead animals.

III. PART I - EFFECT OF STREPTOMYCIN AS PROPHYLAXIS AND THERAPY

A. MATERIAL

The 11 animals were randomly distributed by sex and weight (range 2.25 to 3.95 kg). They were divided into three groups: Group 1 - Controls (3), Group 2 - Prophylaxis (4), and Group 3 - Therapy (4). In the latter two groups dihydrostreptomycin (50 mg intramuscularly every 6 hours) was given for 10 days. In the Prophylaxis group, the drug was started 10 hours after exposure, in the Therapy group, after two consecutive rectal temperatures of 104.5°F or greater.

B. RESULTS

1. Incubation Period (the time between exposure and onset of fever) The incubation periods are shown in Table I.

TABLE I. INCUBATION PERIODS

GROUP	HOURS POST-EXPOSURE
1. Controls	40,40[a]
2. Prophylaxis	No illness
3. Therapy	48,50,58,64

a. 1 apparently uninfected.

One control animal did not become infected. The monkeys in the prophylaxis group did not become ill. Temperatures are shown in Figures 1 through 3.

2. Physical examination

a. Control Group: With the onset of fever there was anorexia. By the second or third day of fever, lethargy, mild dehydration and rapid grunting respirations were apparent which progressed to death. Two or three days prior

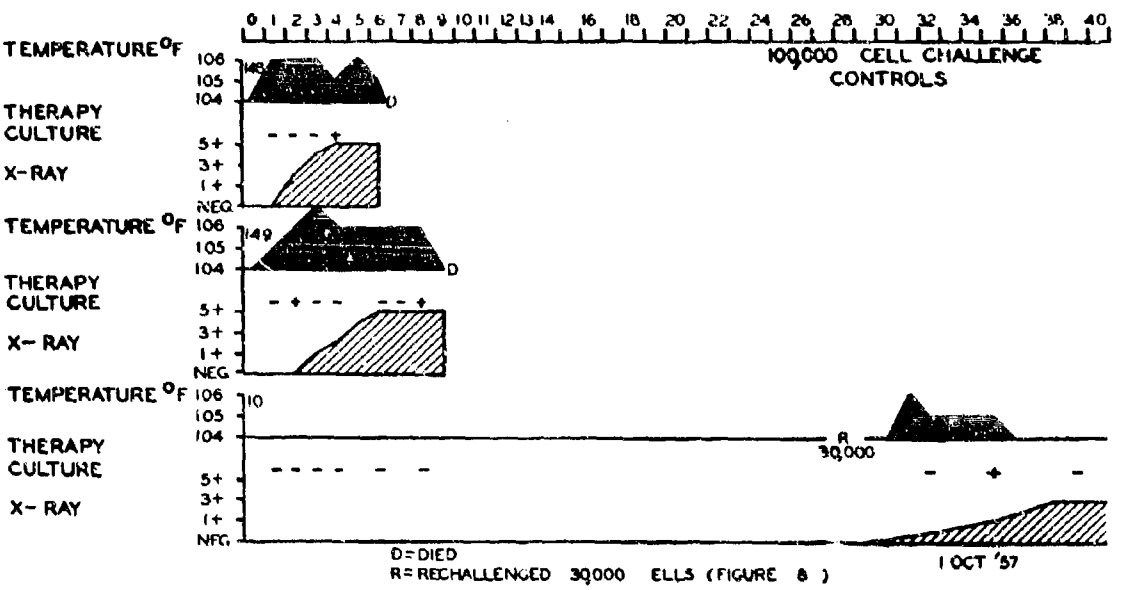


FIGURE 1. TEMPERATURE, BLOOD CULTURES, AND X-RAY STATUS IN CONTROLS.

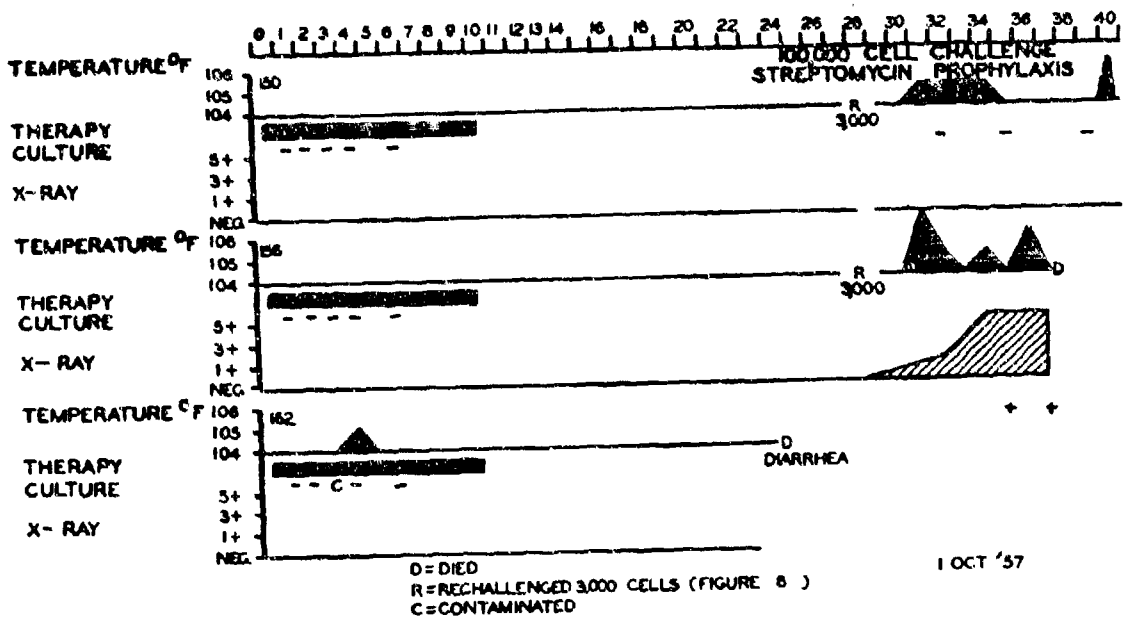


FIGURE 2. TEMPERATURE, BLOOD CULTURES, AND X-RAY STATUS IN STREPTOMYCIN PROPHYLAXIS GROUP.

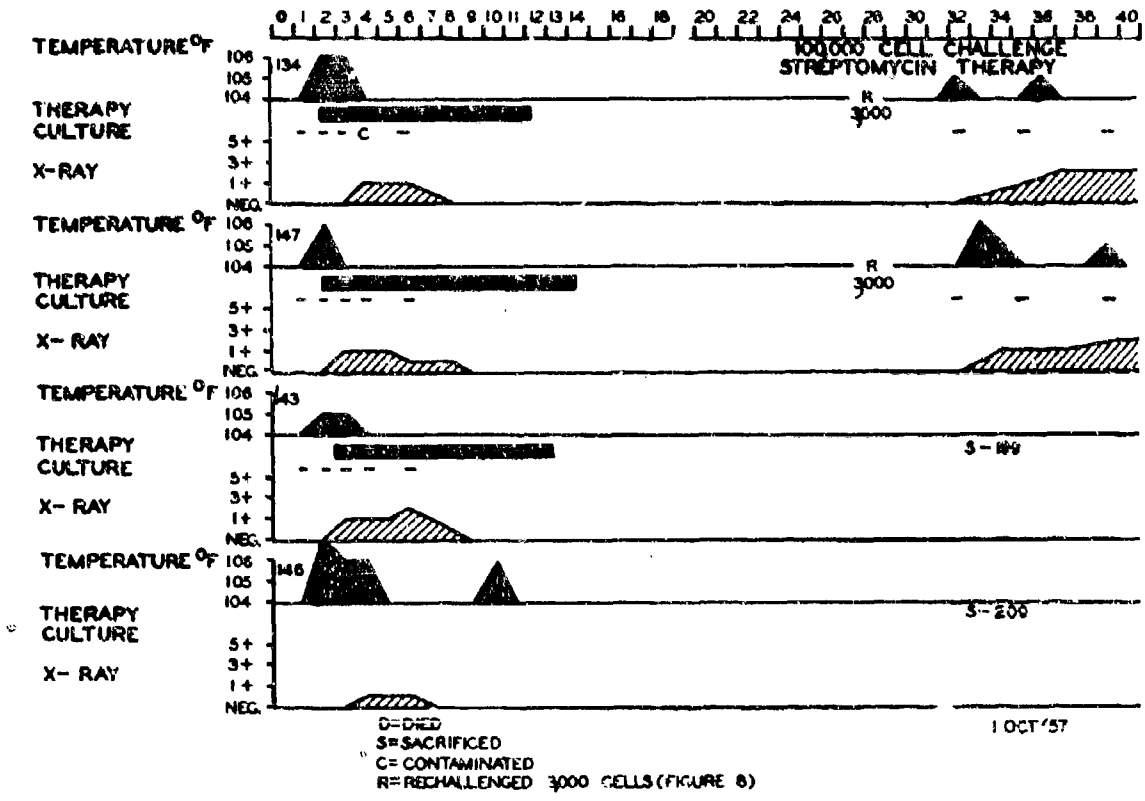


FIGURE 3. TEMPERATURE, BLOOD CULTURES, AND X-RAY STATUS IN THERAPY GROUP.

to death coarse rales could be heard throughout the lungs but these were strikingly disproportionate to the extensive radiographic abnormalities and dyspnea. There was no significant peripheral adenopathy or hepatosplenomegaly. Terminally the monkeys were lethargic, dyspneic, moderately cyanotic, pale, and lay on the bottom of the cage. One control animal was not infected and showed no evidence of disease.

b. Prophylaxis Group: The animals remained asymptomatic and physical examinations were normal.

c. Therapy Group: These animals had only mild anorexia on the first day of illness. No physical abnormalities were found. All became afebrile within 12 to 30 hours after onset of therapy. One animal had a brief fever on day 18 due to a secondary skin infection which responded to penicillin.

3. Laboratory Studies

a. Chest roentgenographic examination showed (Figures 1 - 3):

(1) Controls: Within 24 hours of onset of fever, a reticulated infiltrate was seen throughout all lobes. This rapidly progressed through a miliary phase, thence to multiple bronchopneumonic patches to early consolidation which obscured the heart and mediastinal shadows.

(2) Prophylaxis: No abnormalities seen.

(3) Therapy: Radiographic abnormalities appeared but did not increase beyond a reticulated to miliary phase. They were normal by the completion of therapy.

b. Clinical and Bacteriological Studies

In Control and Therapy groups the total leukocyte count (Figure 4) usually exceeded 20,000/cu mm within 24 hours of fever onset. No leukocytosis was noted in the Prophylaxis group. There was terminal leukopenia in the Controls, but in the Therapy group there was prompt return to normal levels. Within 24 hours of exposure and prior to fever, CRP (Figure 5) became mildly elevated in all but one of the challenged animals. This probably was the result of non-specific trauma as a result of exposure, i.e., anesthesia or handling. In the infected Controls the CRP remained elevated to death. In the Prophylaxis group it rapidly fell after the post-exposure rise. In the Therapy group there was a reversion to near normal at the completion of streptomycin. The sedimentation rate (Figure 6) became abnormal within 24 hours of fever onset and followed the acute phase of the disease. There was no elevation in the Prophylaxis group. Blood cultures were positive in only the two infected controls (Figure 1 - 3). Agglutinin titers (Figure 7) were not detected prior to death in infected controls. In the Prophylaxis group two animals developed low grade titer rises (1:10 and 1:20). In contrast, animals in the Therapy group developed diagnostic titers in the second week.

4. Deaths and Autopsy findings

A summary of autopsy findings is shown in Table II. Two control animals died on days 5 and 9 post-exposure. Autopsy revealed extensive

FIGURE 4. WHITE BLOOD CELL COUNTS (100,000 CELL CHALLENGE, STREPTOMYCIN STUDY)

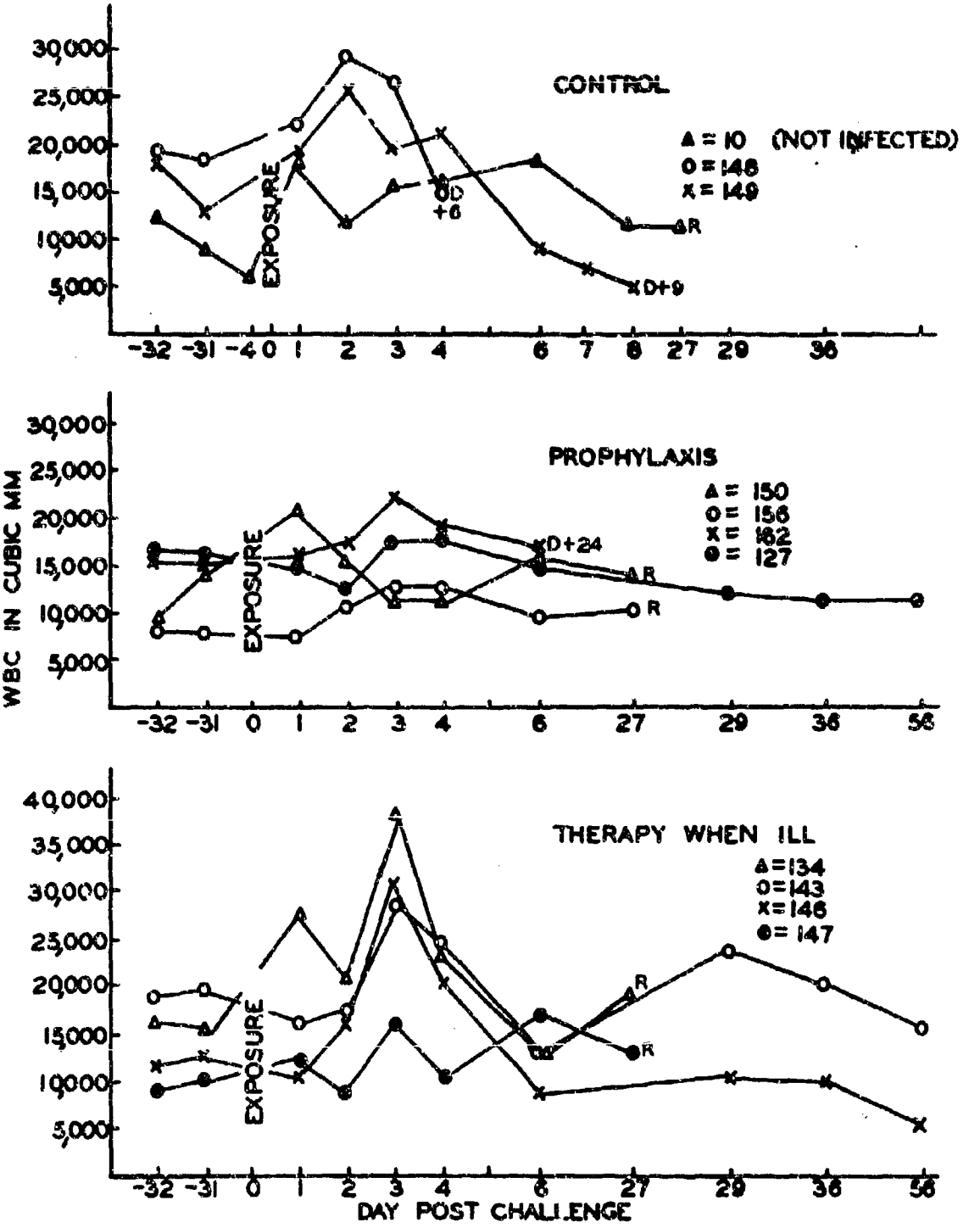


FIGURE 5. C-REACTIVE PROTEINS (100,000 CELL CHALLENGE, STREPTOMYCIN STUDY)

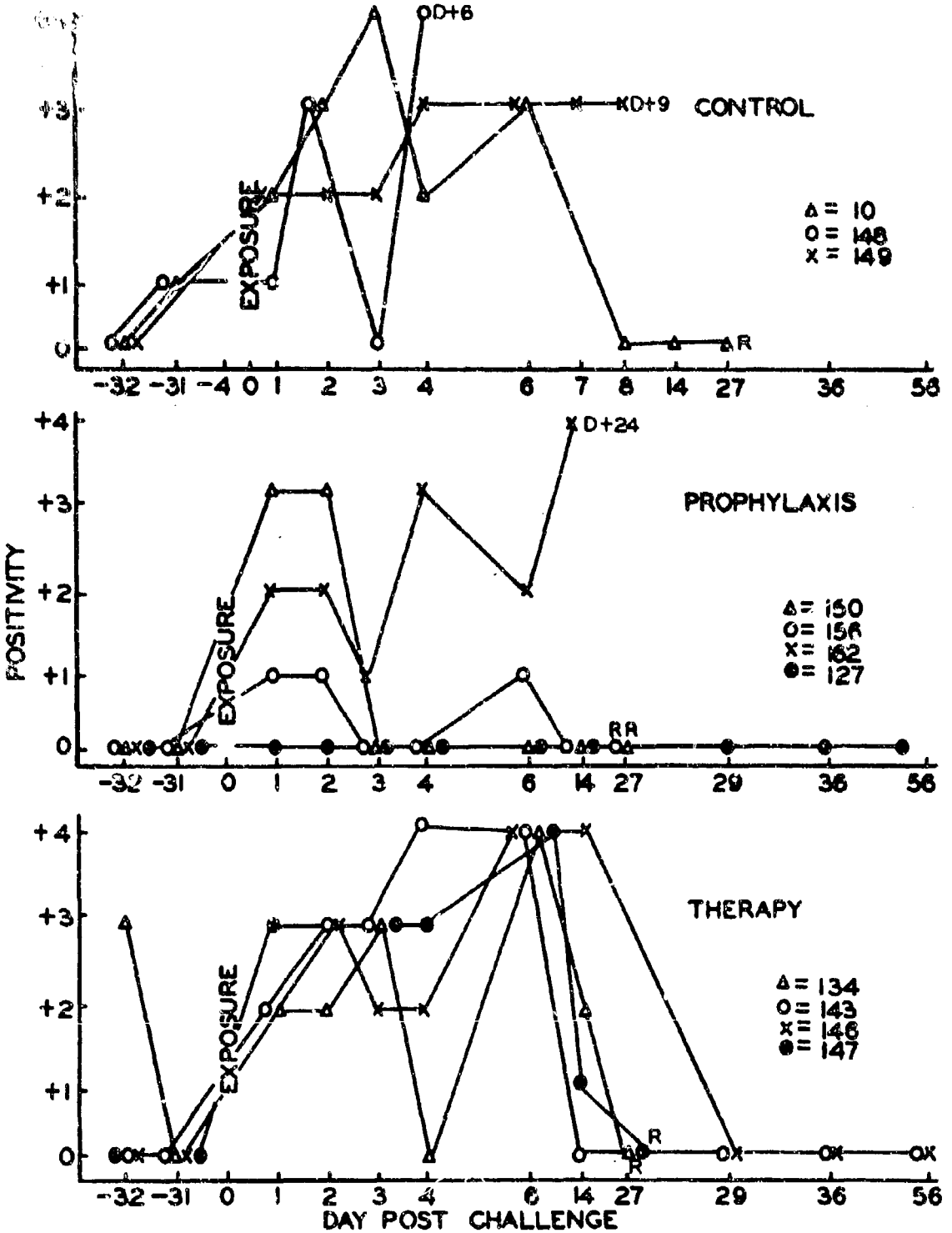


FIGURE 6. SEDIMENTATION RATES (100,000 CELL CHALLENGE, STREPTOMYCIN STUDY)

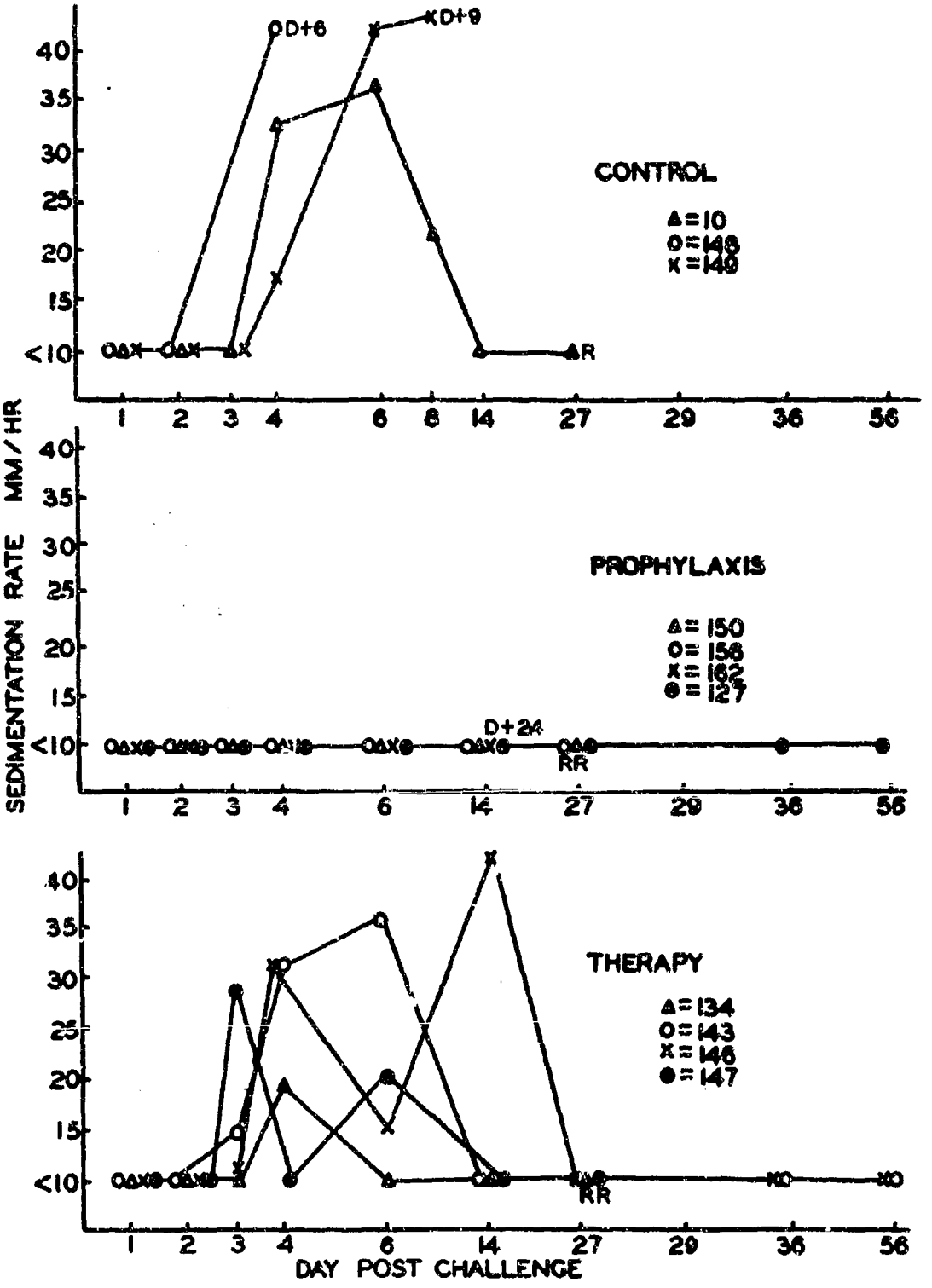
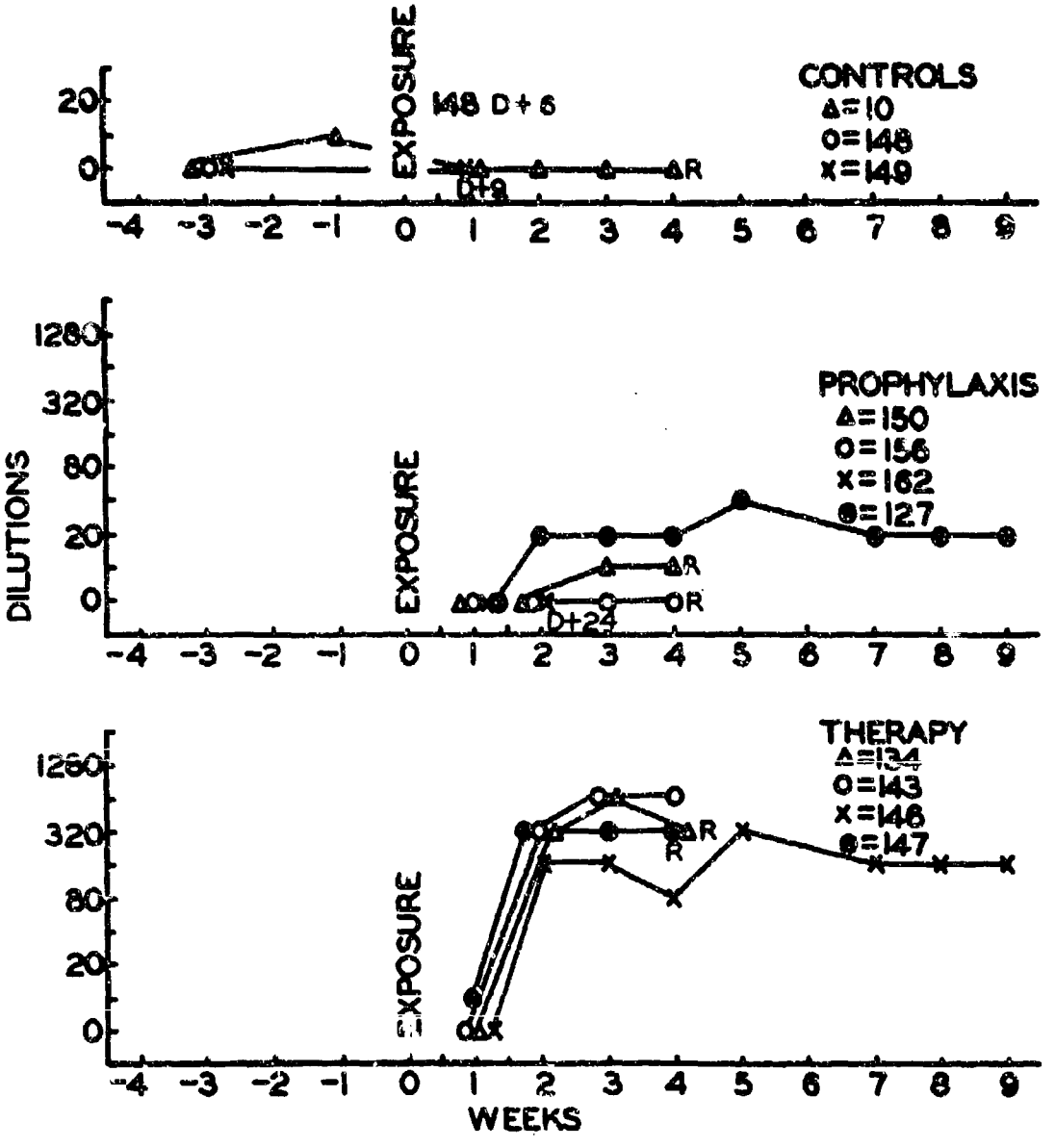


FIGURE 7 TULAREMIA AGGLUTININ TITERS
(100,000 CELL CHALLENGE, STREPTOMYCIN STUDY)



grey-yellow focal lesions throughout the lung parenchyma. These nodules were 1 to 2 mm in size, quite firm and contained thick caseous material but had not yet developed into abscesses. Fibrinous pleurisy was also present. Hilar lymph nodes were enlarged but contained no gross lesions. Remaining viscerae were unaffected grossly. One Prophylaxis group monkey died on day 24 post-challenge of a non-specific diarrhea; no evidence of tularemia was found. Three other animals were sacrificed after 6 to 7 months follow-up and showed no evidence of tularemia.

TABLE II. SUMMARY OF AUTOPSY FINDINGS IN MONKEYS CHALLENGED WITH 100,000 P. TULARENSIS CELLS

GROUP	MONKEY		PATHOLOGY			BACT'Y	COMMENT
	No. (Day a/)	Wt. kg	Thoracic Cavity	Lungs	Other	Culture	
Control	148 (6)	3.4	Adhesions, dense, fibrinous	Bronchopneumonia, miliary w/congestion, atelectasis, emphysema	NVL b/	Blood: +	Tularemia, acute
	149 (13)	2.9	Same as No. 148	Same as No. 148	NVL	Blood: + Eye: -	Tularemia, acute
Prophylaxis	162 (23)	2.45	Fluid & adhesions, bilateral	Bronchopneumonia w/congestion & atelectasis	GI: Trichomonas	Blood, lung, & eye: -	Diarrhea
	156 (10 c/)	3.15	Adhesions, fibrinous	Bronchopneumonia, miliary, lobar, w/congestion & atelectasis	NVL	Blood, lung, & spleen: +	Tularemia, acute
	127 (178)	4.0	Adhesion, L. small	Atelectasis & emphysema	NVL	ND d/	Sacrificed day 178
Therapy	143 (199)	ND	Adhesions, few, bilateral	NVL	NVL	ND	Sacrificed day 199
	146 (209)	2.6	Adhesions, left	Mites	Mesentery: parasitic tumors	ND	Sacrificed day 209

a. Day post-challenge of autopsy.
b. NVL indicates no visible lesions.
c. Day post-rechallenge.
d. ND indicates no data.

C. SUMMARY (Table III)

Monkeys exposed to approximately 100,000 P. tularensis cells (SCHU S4) by the respiratory route became ill within 40 to 64 hours. Two infected controls died days 5 and 9. Streptomycin begun 10 hours after exposure prevented clinical and laboratory evidence of illness. Streptomycin given 6 to 12 hours after onset of illness promptly controlled fever, x-ray evidence of disease, and abnormal CRP, sedimentation rate, and leukocytosis. Diagnostic agglutinin

titers were noted only in the Therapy group. There was no clinical or laboratory evidence of relapse in animals given dihydrostreptomycin.

TABLE III. SUMMARY OF RESULTS OF STREPTOMYCIN THERAPY AND PROPHYLAXIS IN TULAREMIA IN MONKEYS (100,000 cell dose)

GROUP a/	MONKEY No.	AGGLUTININ TITER Base/Peak	DAY POST-exposure Onset	DAY POST-exposure Afebrile	MAXIMUM SEVERITY	WBC > 20,000	ADRENAL CRP	ADRENAL ESR > 10	BLOOD CULTURE +or-	DRUG Hours Post-Exposure	DAY OF DEATH	COMMENT
1	10	0/0	-	-	-	-	+	+	-	None	-	Rechallenged
	148	0/0	2	5	5+	+	+	+	+	None	5	Tularemia
	149	0/0	2	9	5+	+	+	+	+	None	9	Tularemia
2	127	0/40	-	-	-	-	-	-	-	10	-	
	150	0/10	-	-	-	-	+	-	-	10	-	Rechallenged
	156	0/0	-	-	-	-	+	-	-	10	-	Rechallenged
	162	0/0	-	-	-	+	+	-	-	10	24	Diarrhea
3	134	0/640	2	4	1+	+	+	-	-	48	-	Rechallenged
	143	0/2560	3	4	2+	+	+	+	-	70	-	
	146	0/320	2	5	±	+	+	+	-	76	-	
	147	0/320	2	3	1	-	+	+	-	48	-	Rechallenged
RECHALLENGED WITH 3,000 cells												
2	150	10/640	3	20	3+	ND b/	+	+	-	None	-	
2	156	0/ND	2	9	5+	ND	+	+	+	None	9	Tularemia
3	134	320/2560	4	8	2+	ND	-	-	-	None	-	
3	147	320/5120	5	22	3+	ND	+	+	-	None	-	
RECHALLENGED WITH 30,000 cells												
1	10	0/5120	4	15	3+	ND	+	+	+	None	-	

a. Groups are (1) Controls; (2) Prophylaxis; (3) Therapy.
b. ND indicates no data.

D. ADDENDUM

Rechallenge

Four monkeys were re-exposed by the respiratory route to a nominal 3,000 organism dose of P. tularensis four weeks after the above challenge. (Figure 8)

Two of these animals (Nos. 150 and 156) were in the Prophylaxis group. Base-line agglutinin titers at rechallenge were 1:10 and 0 respectively (Figure 8). Both became ill on the third day after the second exposure. No. 156 died on the 9th post-challenge day; the other remained febrile for two weeks and recovered.

Two monkeys (Nos. 134 and 147) from the previous Therapy group were rechallenged. One remained well; the other had a low grade fever for two days.

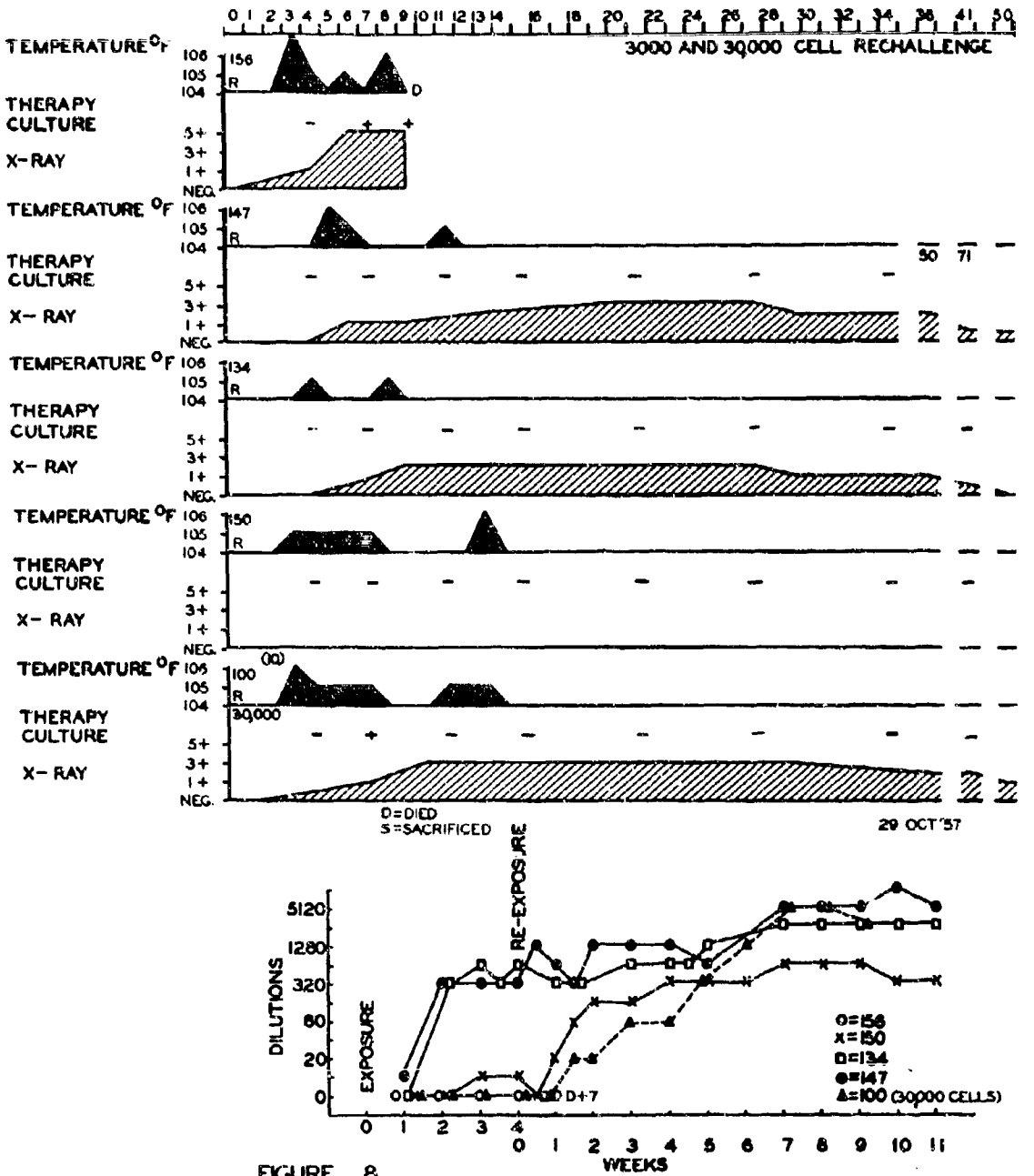
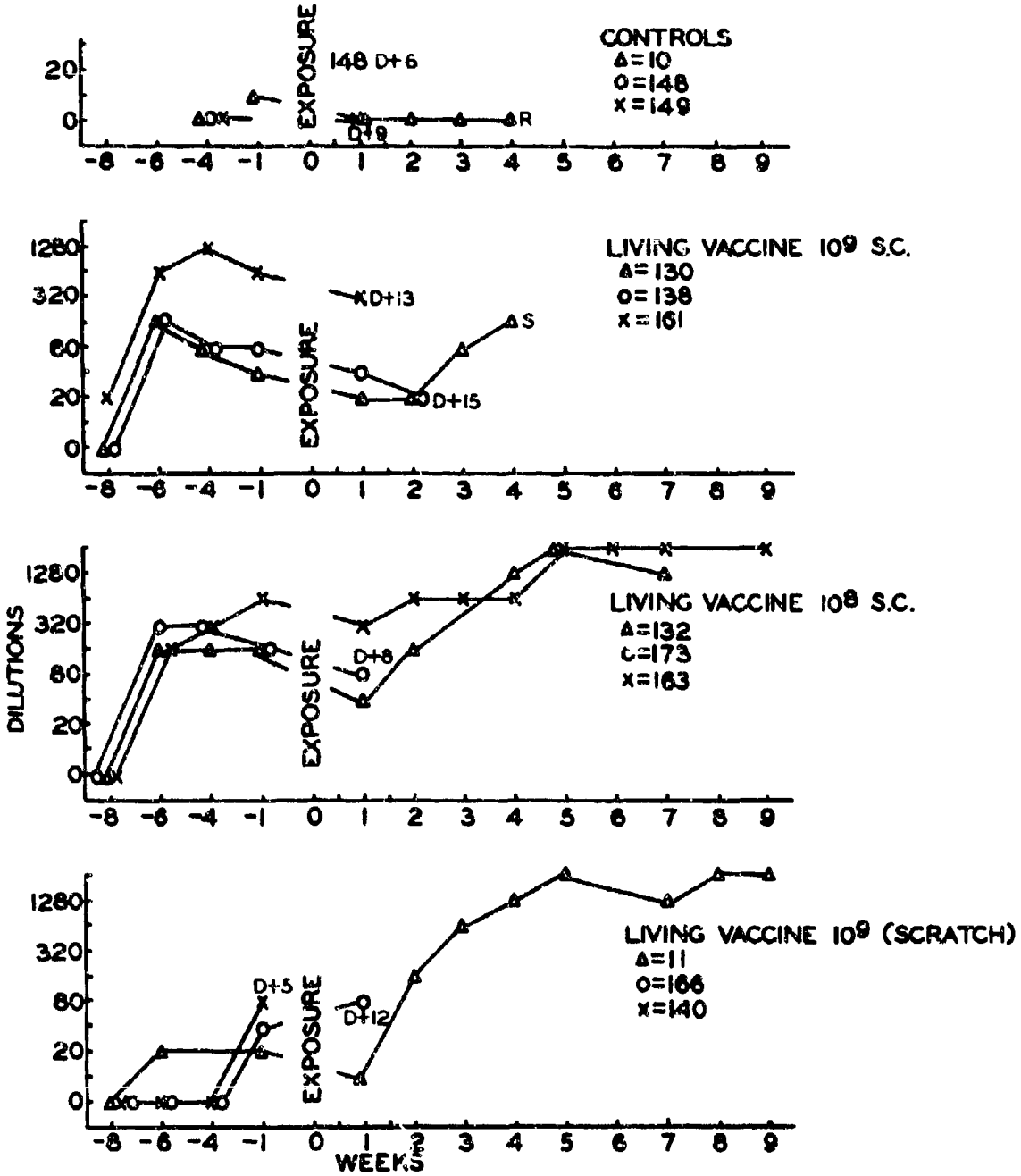


FIGURE 8.
TEMPERATURE, BLOOD CULTURES, X-RAY STATUS AND TULAREMIA AGGLUTININ TITERS IN RECHALLENGED MONKEYS.

FIGURE 9 TULAREMIA AGGLUTININ TITER (100,000 CELL CHALLENGE, VACCINE STUDY)



Monkey No. 10, a control monkey which initially had not become infected, on rechallenge with 30,000 organisms had a severe febrile illness lasting for 10 days (Figure 8).

E. CONCLUSION

This study suggested that prophylactic streptomycin so effectively arrests the tularemia infection and the host's immunity response that on rechallenge these animals respond as controls. In contrast, animals receiving therapeutic streptomycin 6 to 12 hours after onset of illness, acquire significant immunity. This is shown by high convalescent titers after initial illness and mild or no illness on rechallenge one month later (Figure 8).

The previously uninfected control on rechallenge developed typical illness with positive blood culture.

III. PART II - EFFECT OF LIVING VACCINE IN TULAREMIA IN MONKEYS

Details concerning the vaccine employed are given elsewhere in these reports. It was a suspension of viable attenuated P. tularensis.

A. METHOD

The nine monkeys used were randomly distributed by sex and weight (range 2.45 - 4.45 kg). They were divided into three groups and vaccinated two months prior to exposure: Group 1 - Subcutaneous vaccination (1 ml of a 10^9 dilution), (3), Group 2 - Subcutaneous vaccination (1 ml of a 10^8 dilution) (3), and Group 3 - Scratch method (3). In the last group the method used was abrasion of the skin by scalpel and application of two drops of a 10^9 dilution of vaccine; two failed to develop tularemia agglutinin titers and were re-vaccinated using a multiple puncture method two weeks pre-exposure. Groups 1 and 2 had pre-exposure agglutinin titers of 1:160 to 1:640; Group 3, 1:20 to 1:80 (Figure 9).

B. RESULTS

1. Clinical observations

The incubation periods are shown in Table IV.

TABLE IV. INCUBATION PERIODS

GROUP	HOURS POST-EXPOSURE
Controls	40,40
1. Vaccine 10^9 s.c.	52,60,70
2. Vaccine 10^8 s.c.	52,70,94
3. Vaccine 10^9 scratch	40,46,58

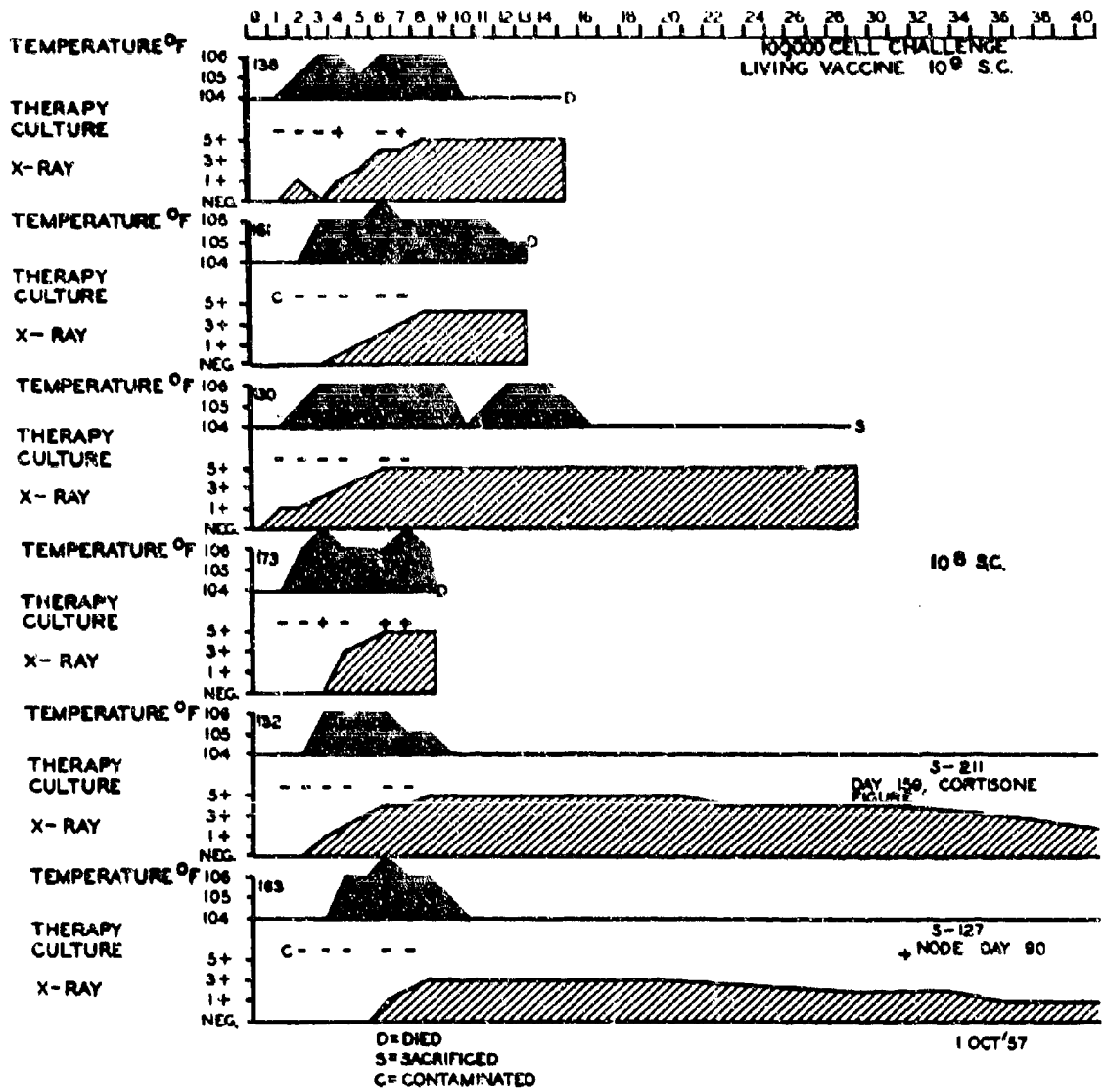


FIGURE 10. TEMPERATURES, BLOOD CULTURES, X-RAY STATUS IN VACCINE GROUPS 10^9 AND 10^8 S.C.

Temperatures for the three vaccine groups are shown in Figures 10 and 11. In the three animals surviving from Groups 1 and 2, fever lysed between the 9th and 15th days. In Group 3 one animal survived, fever lysing by the 11th day.

On physical examination animals dying exhibited similar findings to those found in Controls. Surviving animals in all groups appeared as ill as those dying; however, with the return of the temperature to normal there was prompt clinical improvement. One animal in Group 2 by the 90th day developed a suppurating cervical lymph node from which P. tularensis was isolated.

2. Laboratory Studies:

Chest roentgenographic examination showed similar involvement and comparable progression as the Controls (Figures 10 and 11). Of the four survivors, one developed multiple cavitation throughout all lobes by the 28th day and was sacrificed. The remainder were followed for 90 days; there was gradual clearing to what appeared to be slight fibrosis.

White blood counts (Figure 12) showed leukocytosis comparable to that found in Controls; CRP (Figure 13) and sedimentation rate (Figure 14) showed similar trends. In surviving animals, these abnormal findings had returned to normal by the end of the acute phase of disease or shortly after. The agglutinin titers are shown in Figure 9. Blood cultures were negative in surviving animals and positive in 3 of 5 which died (Figures 10 and 11).

3. Deaths and Autopsy Findings

Deaths occurred between 5 and 15 days post-exposure.

Autopsy findings in these monkeys were similar to those of Controls (Table V). However, there was one vaccinate (Monkey #138, Group 1) that died on 15th day post-challenge and had hepatic, brain and probably splenic involvement plus the usual pulmonary lesions. Although the surviving animals were clinically well at the time of sacrifice, there were residuae of previous tularemia infection. Monkey #130 (Group 1) was sacrificed after one month because of x-ray evidence of extensive cystic pulmonary lesions; these were 5 to 7 mm in diameter with a gray-tan firm tissue surrounding the cavity. The mucoid material in the center of these lesions appeared translucent. Smaller, grey-yellow, nodular lesions were also present. Monkey #163 (Group 2) developed a draining axillary lymph node 3 months post-challenge which contained P. tularensis. At autopsy he had a paravertebral abscess due to tularemia. The remaining two animals sacrificed at 7 months had only pleural adhesions after having had extensive pulmonary involvement.

C. SUMMARY (Table VI)

Monkeys vaccinated with viable P. tularensis and then exposed to approximately 100,000 virulent cells became ill within 40 to 94 hours. Five vaccinated animals died between 5 and 15 days as compared to 5 and 9 days in controls. There was no apparent difference between controls and vaccinates

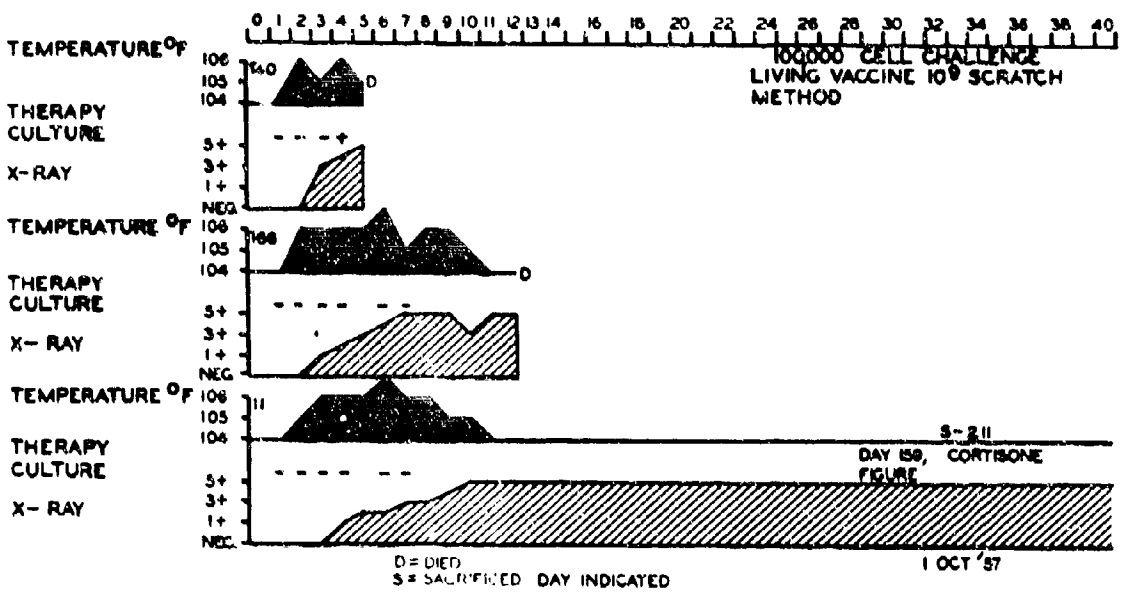


FIGURE 11. TEMPERATURES, BLOOD CULTURES, X-RAY STATUS IN VACCINE GROUPS 10^9 SCRATCH

FIGURE 12. WHITE BLOOD CELL COUNTS (100,000 CELL CHALLENGE, VACCINE STUDY)

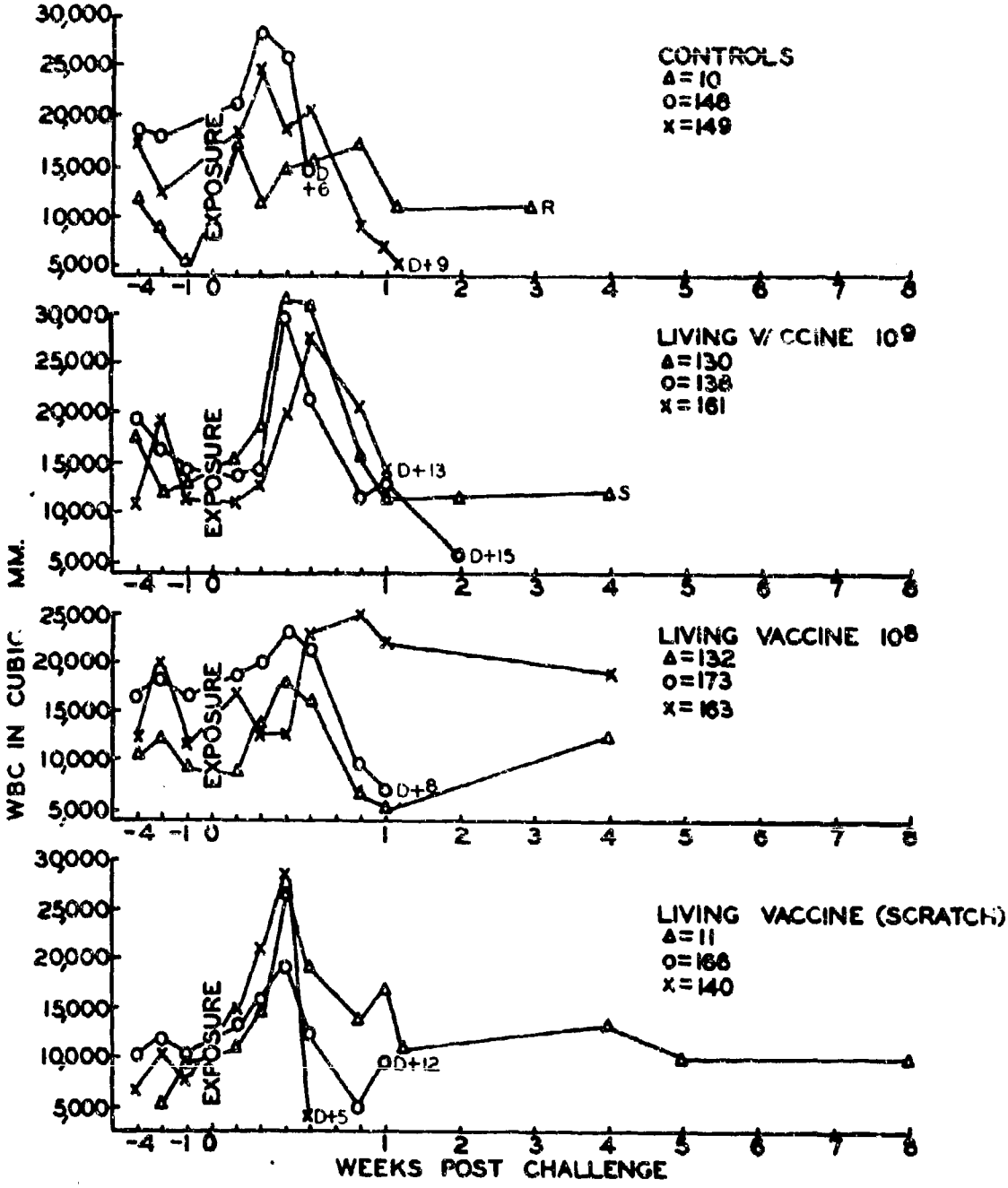


FIGURE 13. C-REACTIVE PROTEINS (100,000 CELL CHALLENGE, VACCINE STUDY)

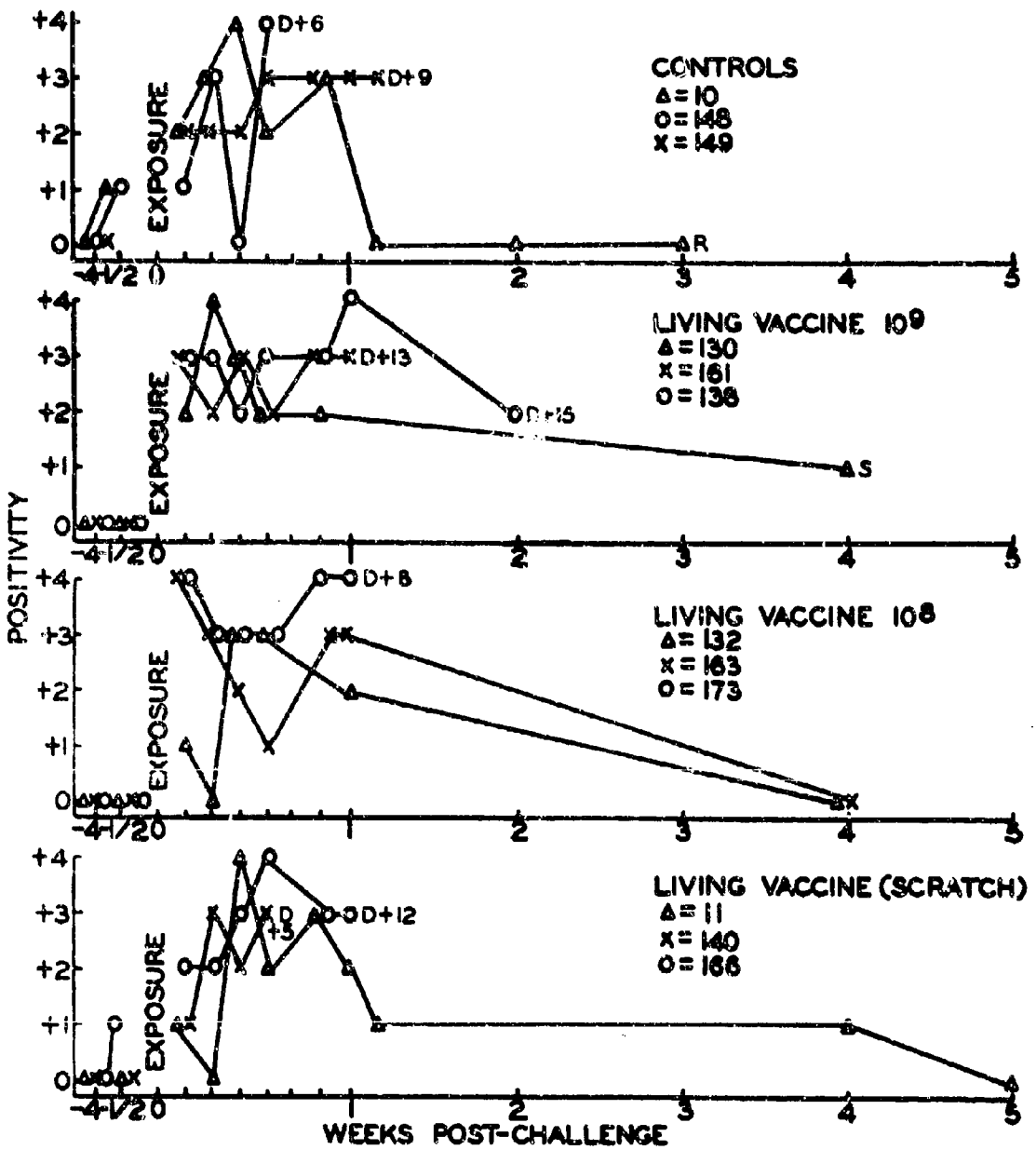


FIGURE 14. SEDIMENTATION RATES (100,000 CELL CHALLENGE, VACCINE STUDY)

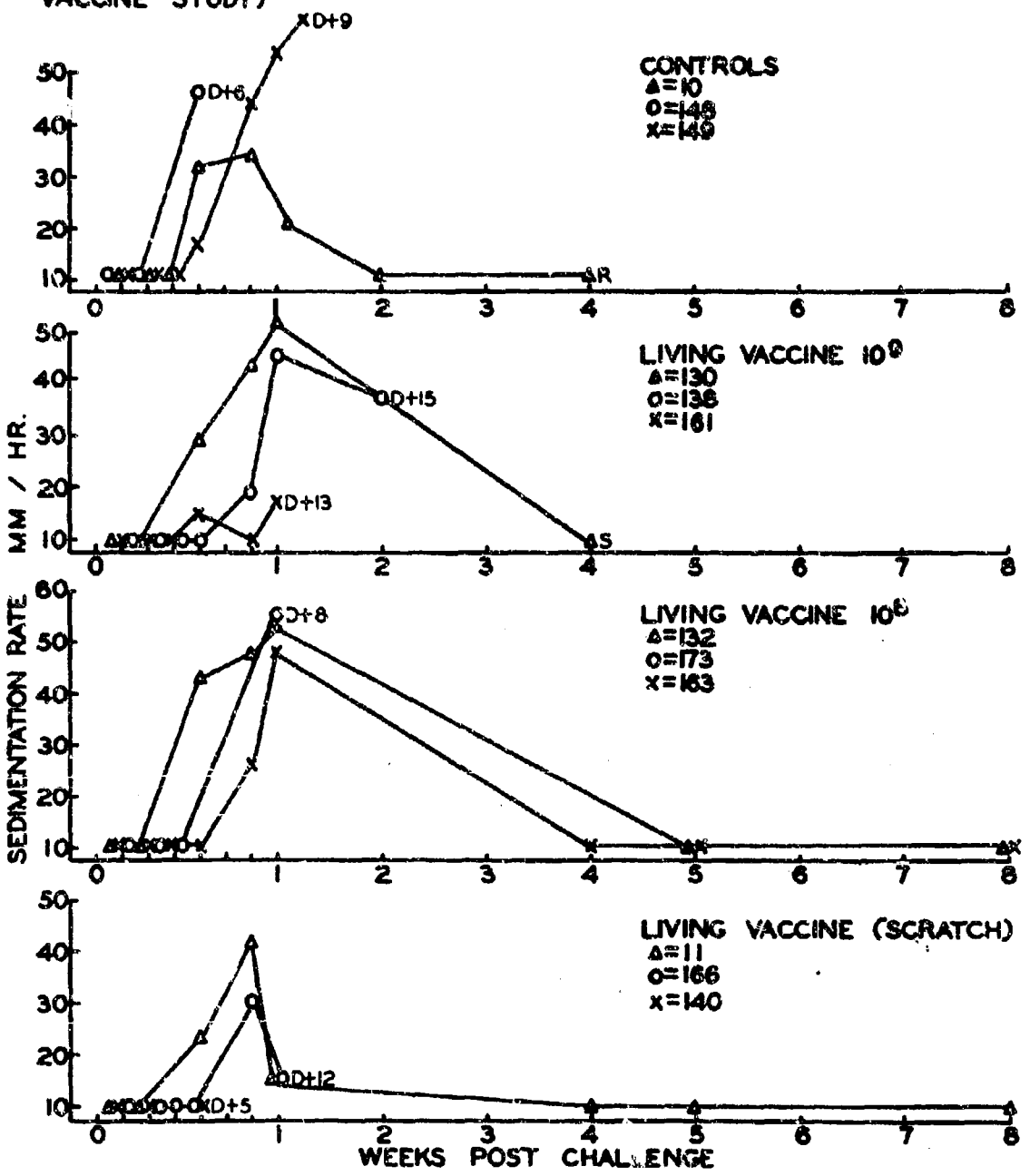


TABLE V. SUMMARY OF AUTOPSY FINDINGS IN MONKEYS CHALLENGED WITH 100,000 F. TULARENSIS CELLS

GROUP	MONKEY No. (Days[a])	Wt. kg	PATHOLOGY Thoracic Cavity	Lungs	Other	BACT'Y Culture	COMMENT
Control	148 (6)	3.4	Adhesions, dense, fibrinous	Bronchopneumonia, miliary w/congestion, atelectasis, emphysema	NVL[b]	Blood: +	Tularemia, acute
	149 (13)	2.9	Same as No. 148	Same as No. 148	NVL	Blood: + Eye: -	Tularemia, acute
Vaccine 10^9 s.c.	161 (13)	3.15	Adhesions, fibrinous	Bronchopneumonia, miliary w/congestion, atelectasis, emphysema	Spleen: congested Liver: abscesses	Blood & eye: -	Cerebral abscess
	138 (15)	3.75	Same as No. 161	Same as No. 161	Liver: congested L. nodes: enlarged	Blood & eye: -	
	130 (29)	2.5	Adhesions, dense fibrinous	Bronchopneumonia, miliary w/cavitation	Media. L nodes: enlarged	Blood, lung, spleen, etc.: -	Sacrificed day 29. Clinically well.
Vaccine 10^8 s.c.	173 (9)	3.75	Adhesions, dense, fibrinous	Bronchopneumonia, miliary w/congestion, atelectasis	Liver: congested, Media. L. nodes: enlarged	Blood: + Eye: -	Tularemia, acute
	165 (127)	2.1	Abscess, subpleural	Bronchopneumonia	Peritonitis unrelated	Blood & Lung: - Nodes & Paravertebral abscess: +	Sacrificed day 127
	132 (211)	5.0	Adhesion	NVL	Intestinal parasites	ND[c]	Tularemia, chronic
Vaccine 10^9 Scratch	140 (6)	2.9	Adhesions, scattered, fibrinous	Bronchopneumonia, miliary w/congestion	Media. L. nodes: enlarged	Blood: + Eye: -	Tularemia, acute
	166 (12)	2.5	NVL	Same as No. 140	NVL	Blood & Eye: - Pericardial fluid: +	Tularemia, acute
	11 (211)	4.0	Adhesions	Mites	NVL	ND	Sacrificed day 211

a. Day post-challenge of autopsy.
b. NVL indicates no visible lesions.
c. ND indicates no data.

with respect to infectivity, incubation period and severity of the acute disease. The mortality rate for vaccinates was only 55 per cent. Survivors showed evidence of a prolonged infection.

TABLE VI. SUMMARY OF RESULTS OF VACCINATION OF MONKEYS WITH LIVING ATTENTUATED VACCINE CHALLENGED WITH 100,000 CELLS
Illness not treated.

GROUP a/	MONKEY No.	AGGLUTININ TITER Base/Peak	DAY POST-EXPOSURE Onset	Afebrile	MAXIMUM SEVERITY	WBC > 20,000	ABNORMAL CRP	ABNORMAL ESR > 10	BLOOD CULTURE +or-	DAY OF DEATH	COMMENT
Control	10	0/0	-	-	-	-	+	+	-	-	Rechallenged
	148	0/0	2	5	5+	+	+	+	+	5	Tularemia
	149	0/0	2	9	5+	+	+	+	+	9	Tularemia
1	130	40/160	2	14	5+	+	+	+	-	-	Sacrificed 28
	161	640/320	3	13	4+	+	+	+	-	13	Traumatic
	138	80/20	2	10	5+	+	+	+	+	15	Tularemia
2	132	640/1280	3	9	5+	-	+	+	-	-	
	163	160/2560	4	9	3+	+	+	+	-	-	L. node + 90 b/
	173	160/80	2	8	5+	+	+	+	+	8	Tularemia
3	11	20/2560	2	10	5+	+	+	+	-	-	
	166	40/80	2	12	5+	-	+	-	-	12	Tularemia
	140	80/ND c/	2	5	5+	+	+	+	+	5	Tularemia

a. Groups are (1) Living vaccine (s.c.) 10^9 dilution July 31, 1957;
(2) Living vaccine (s.c.) 10^8 dilution July 31, 1957;
(3) Living vaccine (scratch method) 10^9 dilution July 31, 1957; and for Monkeys 166 and 140, same material (multiple puncture method) September 19, 1957.

b. P. tularensis isolated from lymph node on 90th day post-exposure.

c. ND indicates no data.

IV. CONCLUSIONS Parts I and II

(1) Dihydrostreptomycin was highly effective prophylactically and therapeutically in preventing and controlling infection in M. mulatta monkeys exposed to a high dosage of P. tularensis, and (2) Living P. tularensis vaccine was effective in reducing mortality in disease resulting from a high aerosol challenge dosage of virulent organisms.

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STUDIES ON PASTEURELLA TULARENSIS

LIVING VACCINE AND TETRACYCLINE THERAPY EVALUATION IN MACACA MULATTA AEROSOL CHALLENGED WITH 60,300, 3,000, AND 30,000 CELLS

(Overholt, Eigelsbach, Hornick, Hughes)

I. INTRODUCTION

The purpose of this study was to evaluate a living-attenuated vaccine for tularemia in Macaca mulatta monkeys exposed to varying doses of Pasteurella tularensis, SCHU 4 strain (particle size 0.7 μ or less) by the respiratory route and to examine therapeutic effect of tetracycline.

This was a cooperative study with Bacteriology II Branch, MB Division. The nominal dose range was 60, 300, 3,000, and 30,000 cells per animal. The untreated animal groups were observed by MB Division personnel, treated animal groups were observed by Medical Unit personnel. All animals were anesthetized and exposed by Aerobiology Division as in the previous study.

II. MATERIALS AND METHODS

The ninety-two monkeys used in this study were distributed in groups as shown in Table I.

TABLE I. DOSAGE AND NUMBER OF MONKEYS IN EACH GROUP

DOSE CELLS	CONTROL		LIVING VACCINE		PHENOLIZED VACCINE		TOTALS
	No Rx	Therapy	No Rx	Therapy	No Rx	Therapy	
60	8	4	8	0	6	0	26
300	8	4	8	0	7	0	27
3,000	1	8	0	7	0	4	19
30,000	1	8	0	7	0	4	20
TOTALS	17	24	16	14	13	8	92

Vaccinations (described elsewhere) were performed approximately one month prior to challenge. Living-vaccine (LV) was given by application of two drops of a suspension containing 10^9 organisms/ml on the shaved skin of the intrascapular area utilizing multiple puncture technique. Phenolized-vaccine (PV) was given subcutaneously, 0.5 ml on each of three successive days.

Therapy consisted of 125 mg of tetracycline hydrochloride, pediatric syrup, every six hours for 7 days, then 125 mg every eight hours for an additional week for a total of 14 days. The drug was administered through a polyethylene

infant feeding tube which had been inserted into the stomach. Therapy was started after two successive temperature readings of 104.5°F (rectally) or a single elevation greater than 106.0°F.

Animals were exposed on October 29, 1957. Therapy monkeys were observed for 163 days. Thereafter they were used for other studies. At autopsy, they were checked for tularemic lesions.

STUDIES AND EXAMINATIONS

Treated animals were evaluated as follows: Rectal temperatures were taken every 6 hours during the first week, every 8 hours during the second week, every 12 hours for the third and fourth week and once per day for the following two months. Physical examinations were performed daily. Chest x-rays were obtained every other day during the first week, twice during the second week and weekly for the following six weeks, and thereafter at 2 to 3 week intervals. X-rays were graded as in the previous study. White blood cell counts, differential, hemoglobin, hematocrit, sedimentation rate and C-reactive protein (CRP) were determined twice weekly during the first week and weekly thereafter. Blood was drawn for culture on days 1 and 4 post-exposure. Agglutinin titers (as described elsewhere) were obtained weekly for the first six weeks, thereafter at least monthly. All animals that died were autopsied.

Animals evaluated by MB Division were followed by chest x-ray and agglutinin titers as above. Temperatures were obtained twice daily. Blood cultures were obtained weekly for 6 weeks.

III. RESULTS

A. 60 ORGANISM GROUP (12 control, 6 FV, and 8 LV monkeys)

Weights ranged from 1.4 to 4.3 kg. Morbidity and mortality rates are shown in Table II.

TABLE II. MORBIDITY AND MORTALITY 60-CELL GROUP

	MORBIDITY		TULAREMIA[a/] MORTALITY	
	No.	%	No.	%
Controls	4/12	33	2/3	66
Phenolized-Vaccine	5/6	83	2/5	40
Living-Vaccine	4/8	50	0/4	0

a. Untreated.

The incubation period ranged from 4 to 9 days, with two exceptions, two FV monkeys developed fever at 27 and 31 days. Fever was of shorter duration in the LV group than the FV group and controls (Figures 1-3). CRP and sedimentation rate were likewise abnormal for a shorter period of time in this group (Figures 4 and 5).

Of three infected control monkeys, two had positive blood cultures and died. Of the 5 FV monkeys that became ill only one had a positive culture and died. Of the 4 LV monkeys infected, one had a positive culture but did not die. All animals with short incubation periods developed roentgenographic changes at about the same time.

Prechallenge tularemia agglutinin titers for all vaccinated animals ranged from 1:40 to 1:160 (Figure 6). Titers on non-vaccinated infected animals showed a rise by the second week of illness and reached a peak level in two survivors at 4 and 9 weeks, ranging from 1:320 to 1:2560. The response in both vaccinated groups was delayed in comparison to the control. Two control monkeys were thought to have subclinical experience with tularemia on the basis of agglutinin titers of 1:10 and 1:20 which appeared during the third week. They failed to develop any febrile illness.

One control animal was treated with tetracycline, becoming afebrile within 12 hours; there was no evidence of pulmonary involvement. Abnormal CRP and sedimentation rate were promptly controlled. An 8-fold titer rise occurred by day 20; there was no relapse.

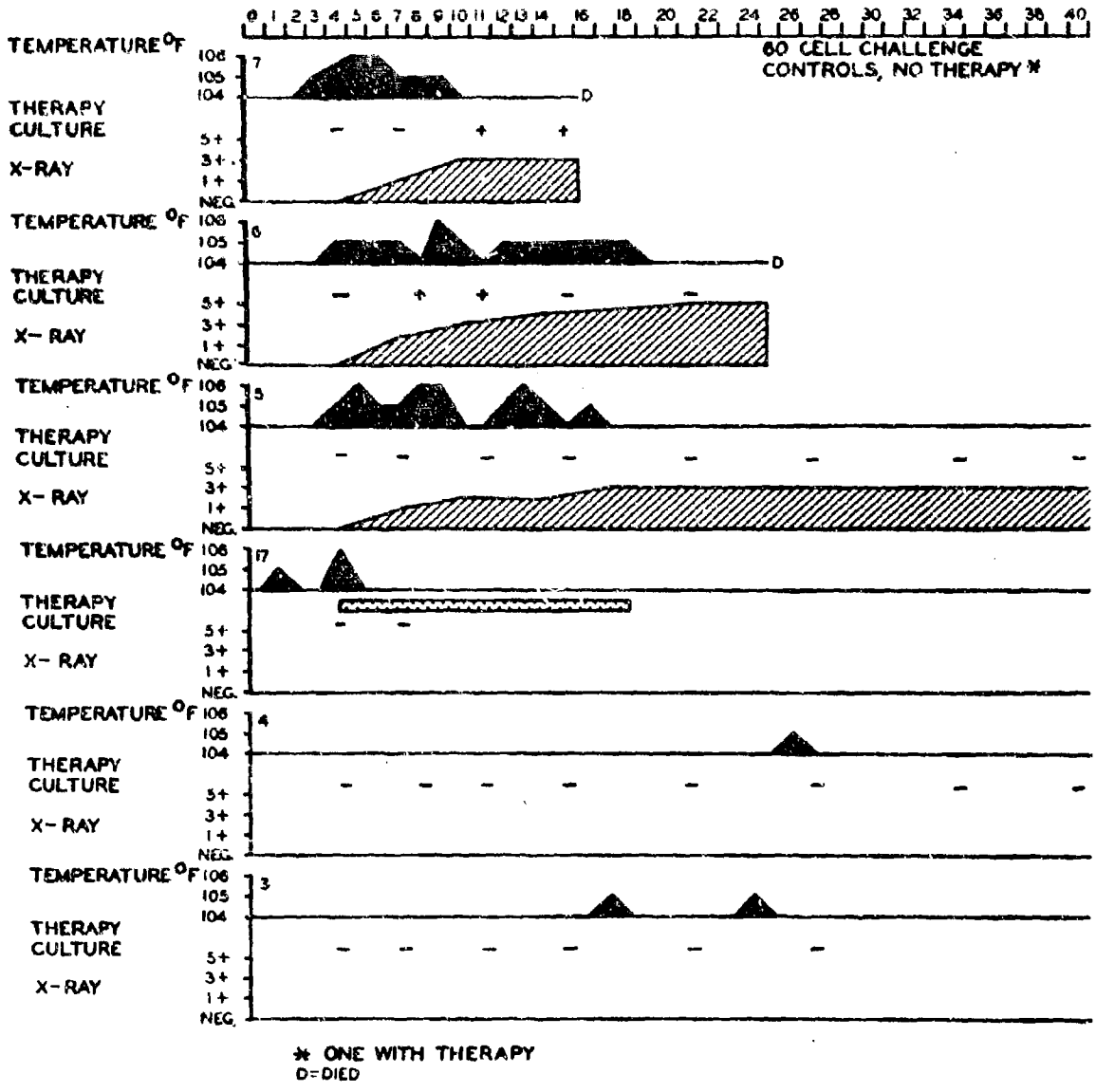


FIGURE 1. TEMPERATURES, BLOOD CULTURES, X-RAY STATUS IN CONTROLS (60 CELL CHALLENGE).

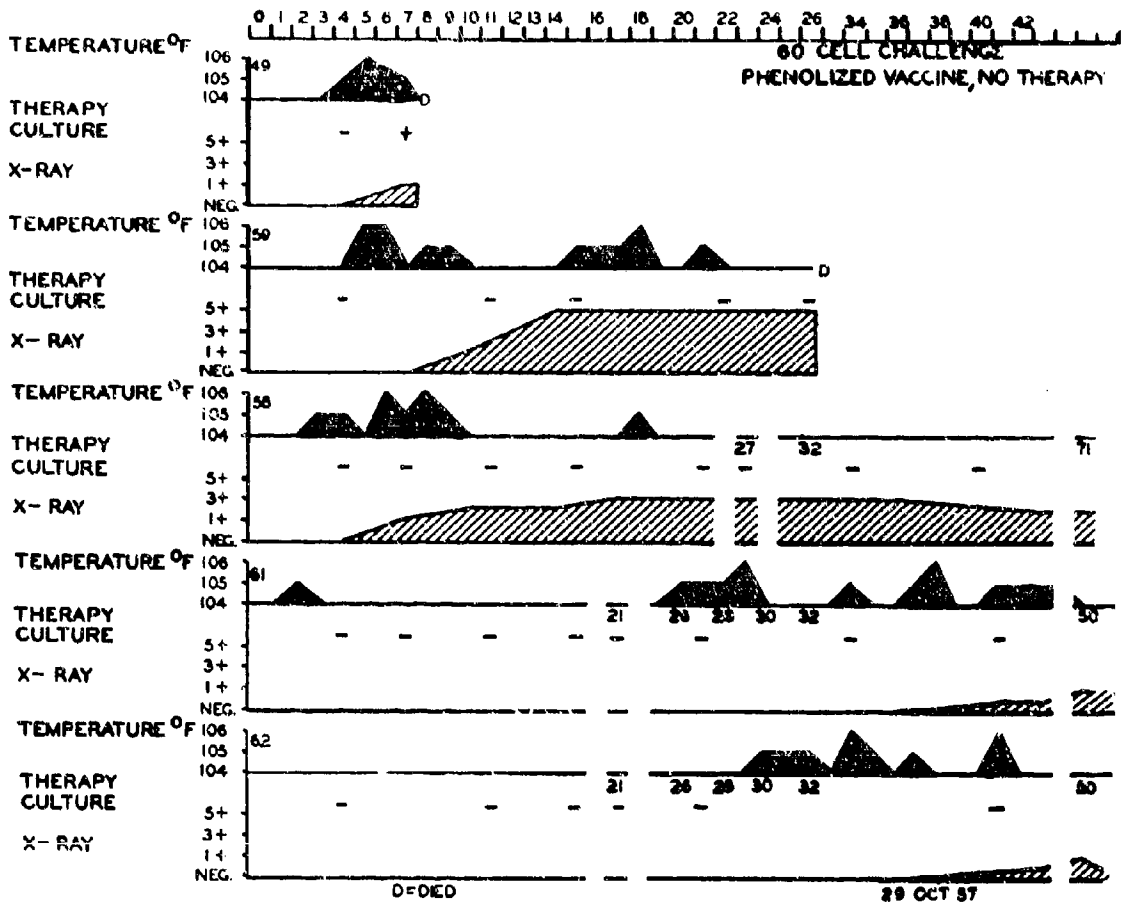


FIGURE 2. TEMPERATURES, BLOOD CULTURES, X-RAY STATUS IN PV GROUP (60 CELL CHALLENGE).

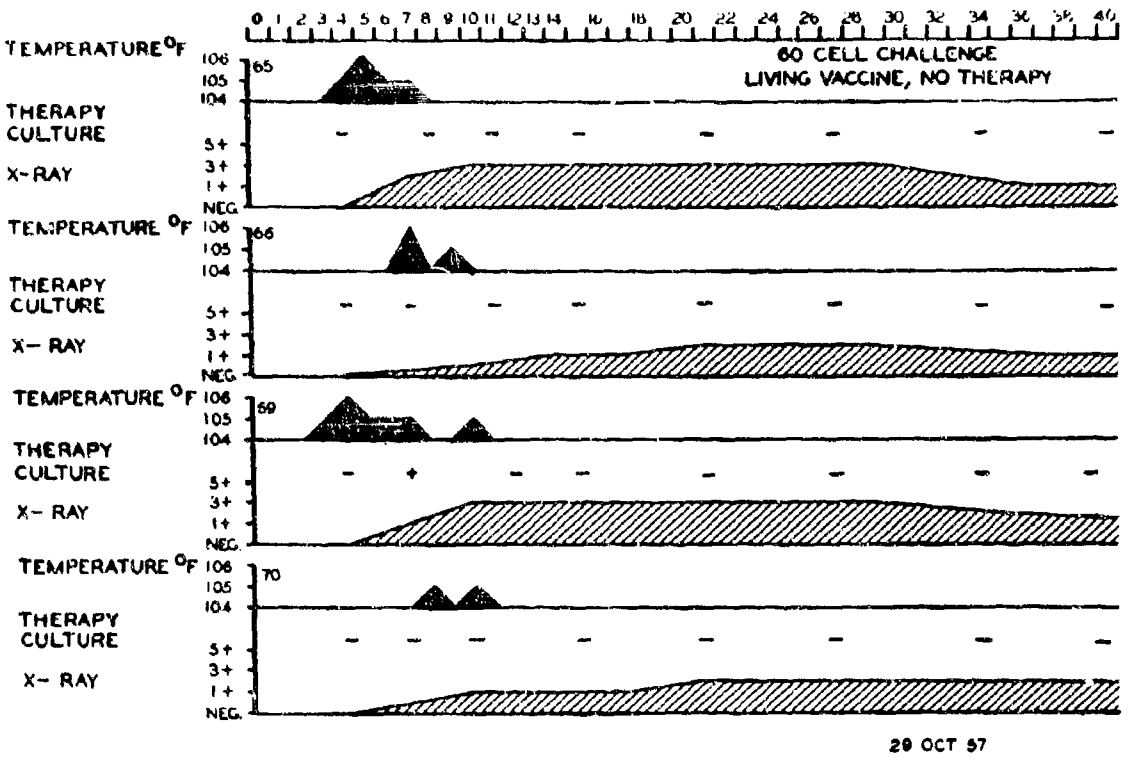
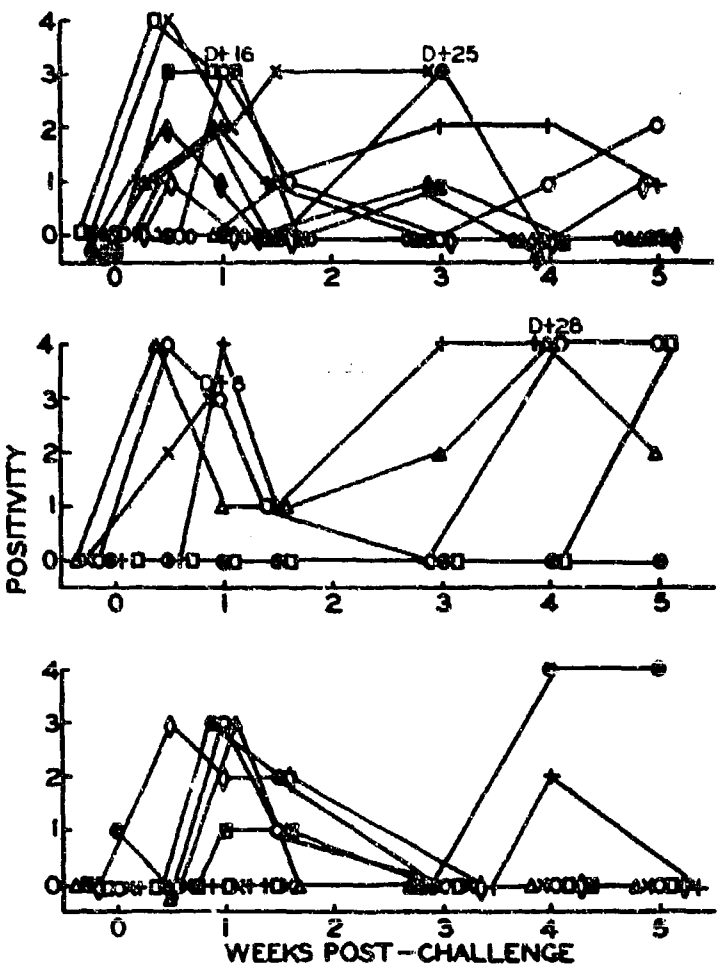


FIGURE 3. TEMPERATURES, BLOOD CULTURES, X-RAY STATUS IN LV GROUP (60 CELL CHALLENGE).

FIGURE 4. C-REACTIVE PROTEINS (60 CELL CHALLENGE).



CONTROLS

●=2	x=6
Δ=3	□=7
○=4	◊=8
+=5	■=17 Rx
	Δ=19
	◊=21
	○=23

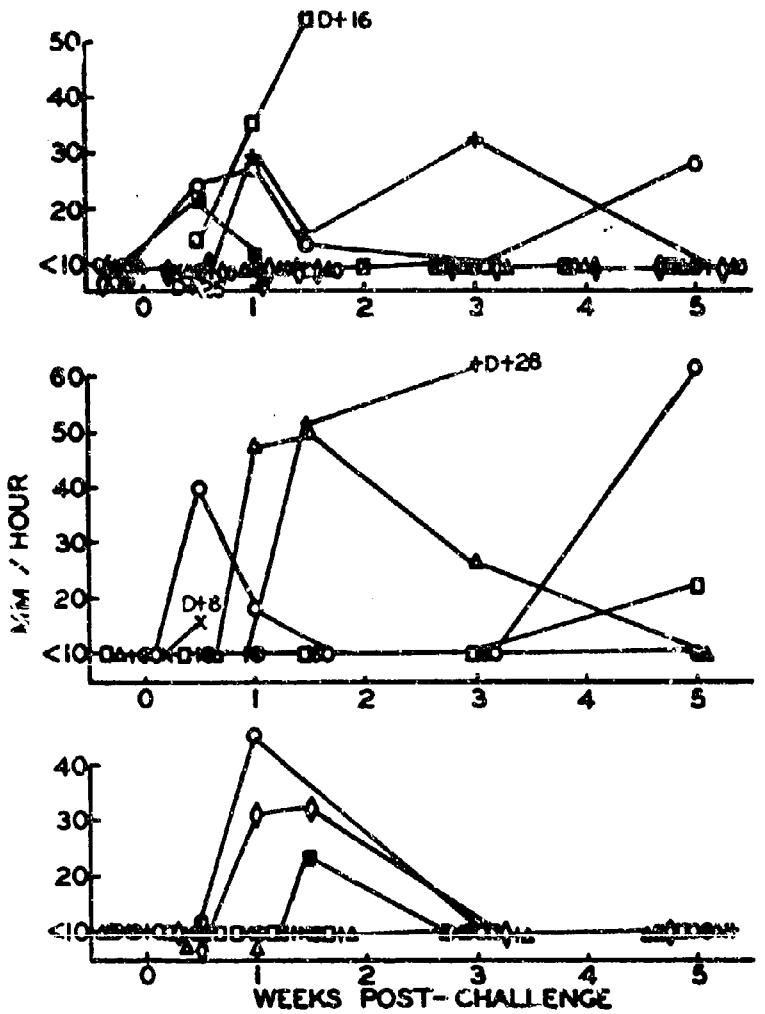
PHENOLIZED VACCINE

●=46	○=61
x=49	□=62
Δ=58	
+=59	

LIVING VACCINE

○=65	◊=69
●=66	■=70
□=67	+=71
x=68	Δ=72

FIGURE 5. SEDIMENTATION RATES (60 CELL CHALLENGE).

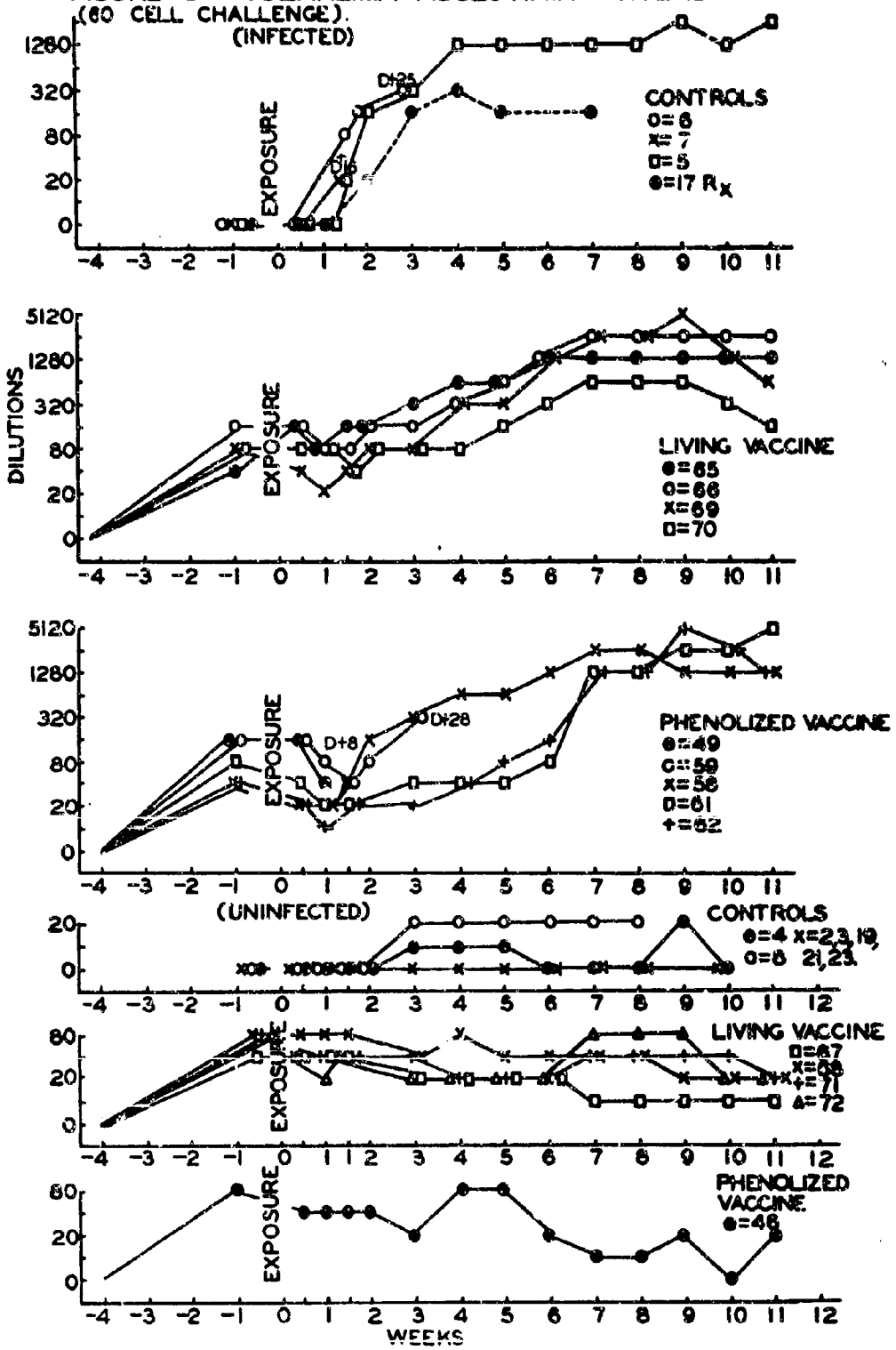


CONTROLS
●=2 ◊=8
▲=3 ⊞=17 Rx
○=4 ▲=19
+=5 ◆=21
x=6 ⊕=23
□=7

PHENOLIZED VACCINE
⊕=48 ○=61
x=49 □=62
▲=58
+=59

LIVING VACCINE
○=65 ◊=69
●=66 ⊞=70
□=67 +=71
x=68 ▲=72

FIGURE 6. TULAREMIA AGGLUTININ TITERS



B. 300 ORGANISM GROUP (12 control, 7 PV, and 8 LV monkeys)

Weights ranged from 1.4 to 4.0 kg. Morbidity and mortality rates are shown in Table III.

TABLE III. MORBIDITY AND MORTALITY 300-CELL GROUP

	MORBIDITY		TULAREMIA[a] MORTALITY	
	No.	%	No.	%
Controls	9/12	75	5/6	87
Phenolized-Vaccine	7/7	100	5/7	77
Living-Vaccine	4/8	50	1/4	25

a. Untreated.

Incubation periods were 3 to 5 days with 3 exceptions, two controls at 15 and 18 days, and one PV monkey at 23 days.

Again fever in the LV monkeys was of short duration, except for the one fatal case, when compared with untreated controls and PV monkeys (Figures 7-9). CRP and sedimentation rate reverted to normal at about the same time as controls (Figures 10 and 11).

Blood cultures were positive in 5 of 6 control monkeys, 2 of 4 LV monkeys, and 5 of 7 PV monkeys. Chest roentgenograms showed similar changes in all three groups as to types and severity of lesions.

Pre-challenge agglutinin titers in LV animals (Figure 12) ranged from 1:40 to 1:640 and in PV animals, from 1:10 to 1:80. Titers on control monkeys began to rise by the first or second week reaching a peak in the surviving two animals of 1:320 to 1:1280 (> 8-fold). LV monkeys showed less agglutinin response, 1:640 to 1:2560 (2- to 6- fold rises), whereas the titers of the surviving PV monkeys reached peak titers of 1:5120 (> 8-fold).

Three additional unvaccinated monkeys were treated with tetracycline (Figure 13). The monkeys were afebrile within 18 to 30 hours and remained so without evidence of relapse. Chest films remained negative and abnormal white blood cell counts (Figure 14), CRP, and sedimentation rate were promptly controlled (Figures 10 and 11).

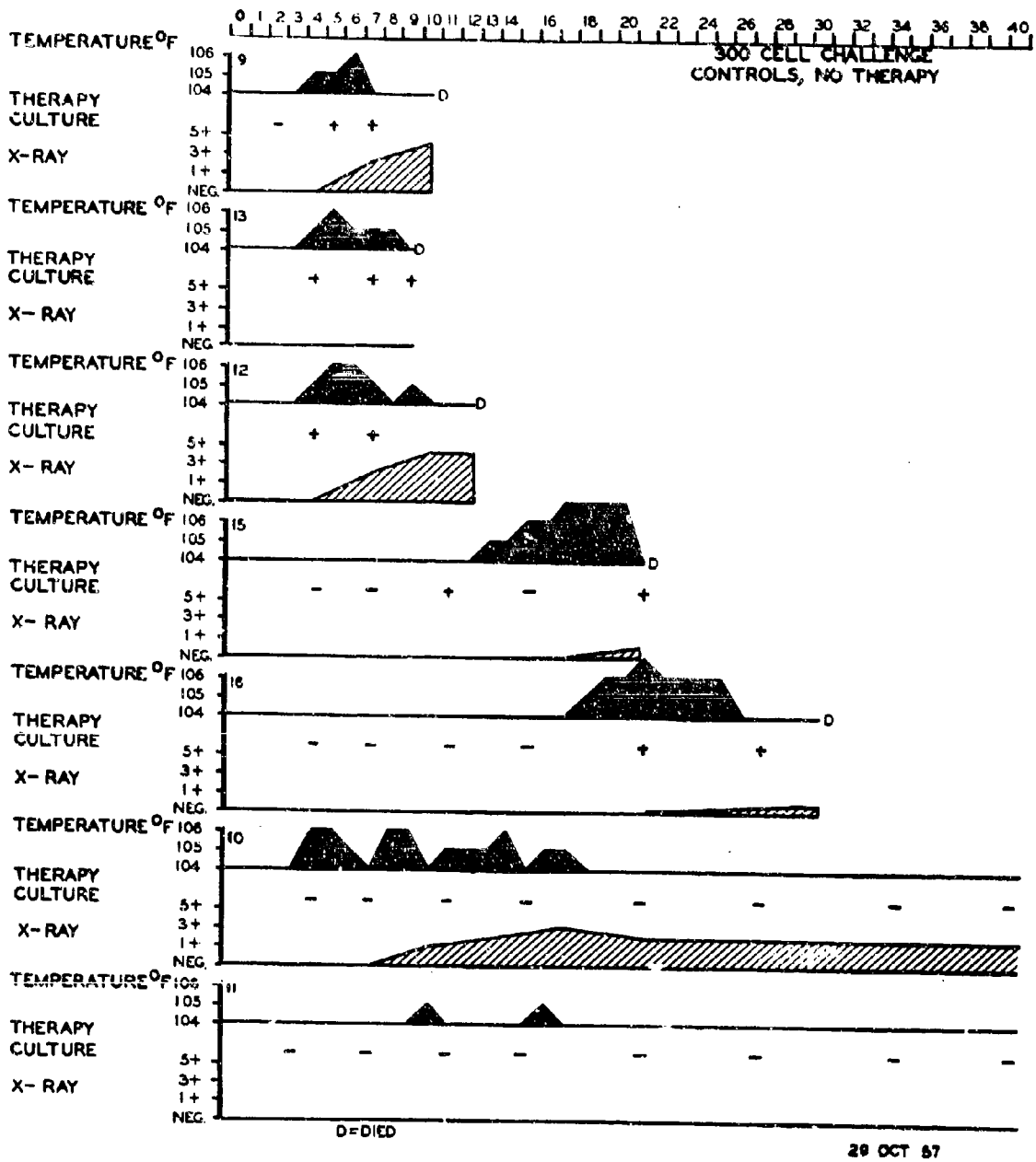


FIGURE 7. TEMPERATURES, BLOOD CULTURES, X-RAY STATUS IN CONTROLS, NO THERAPY (300 CELL CHALLENGE).

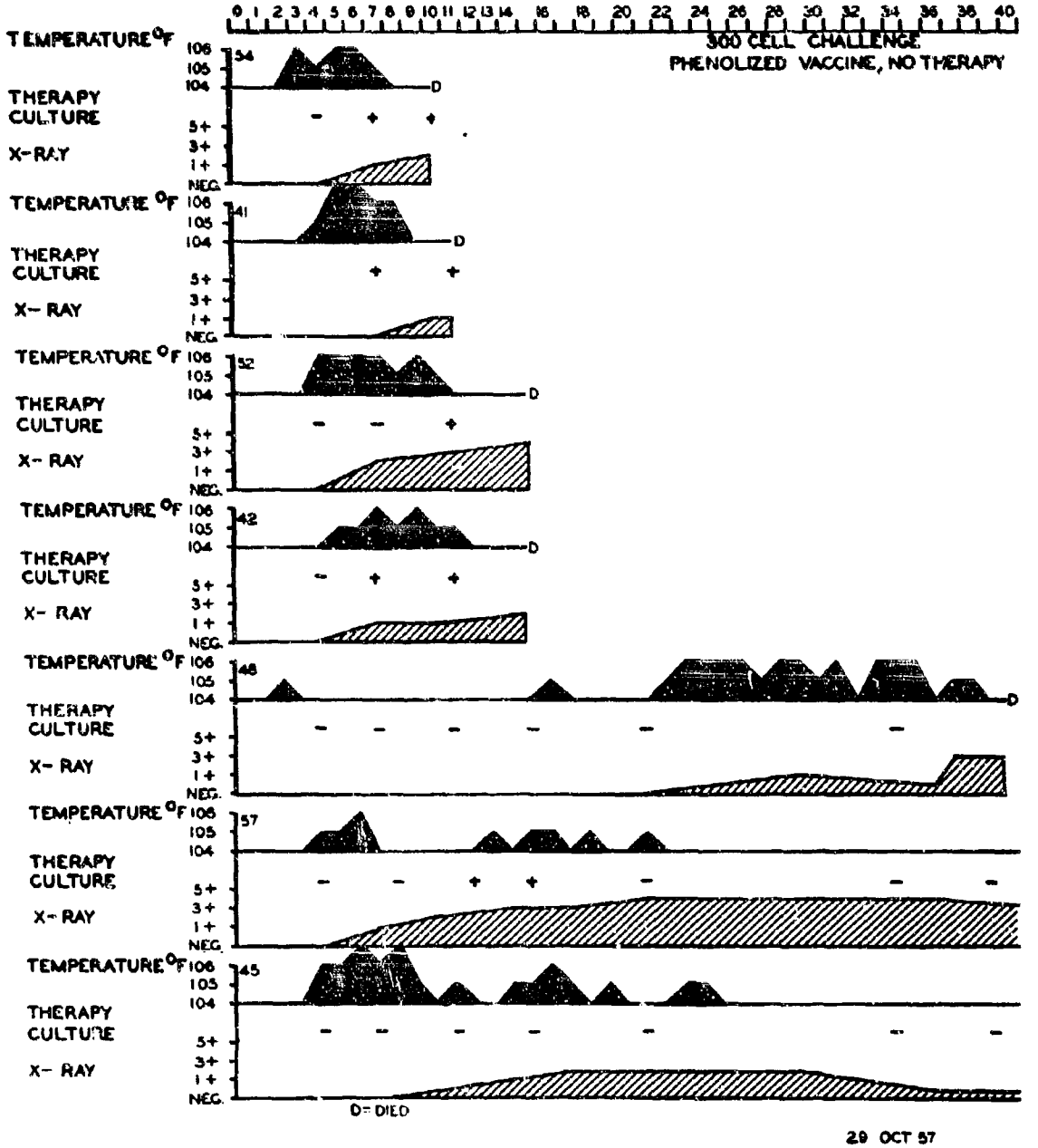


FIGURE 8. TEMPERATURES, BLOOD CULTURES, X-RAY STATUS IN PV GROUP, NO THERAPY (300 CELL CHALLENGE).

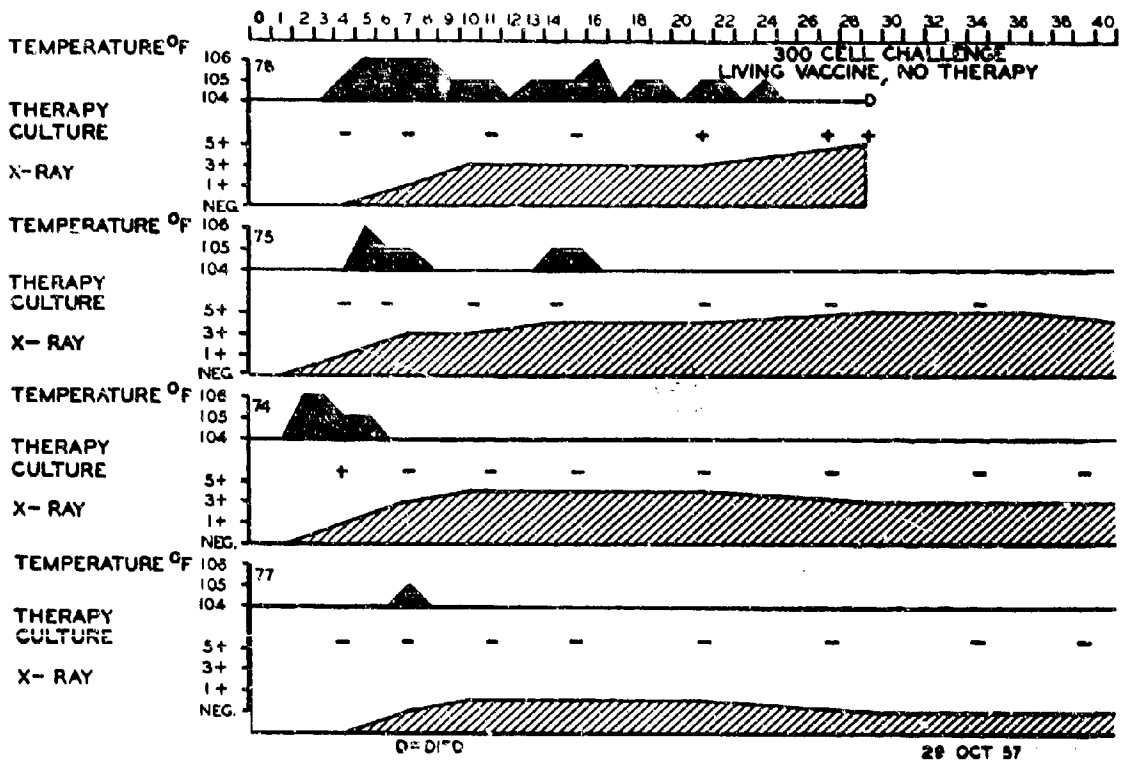
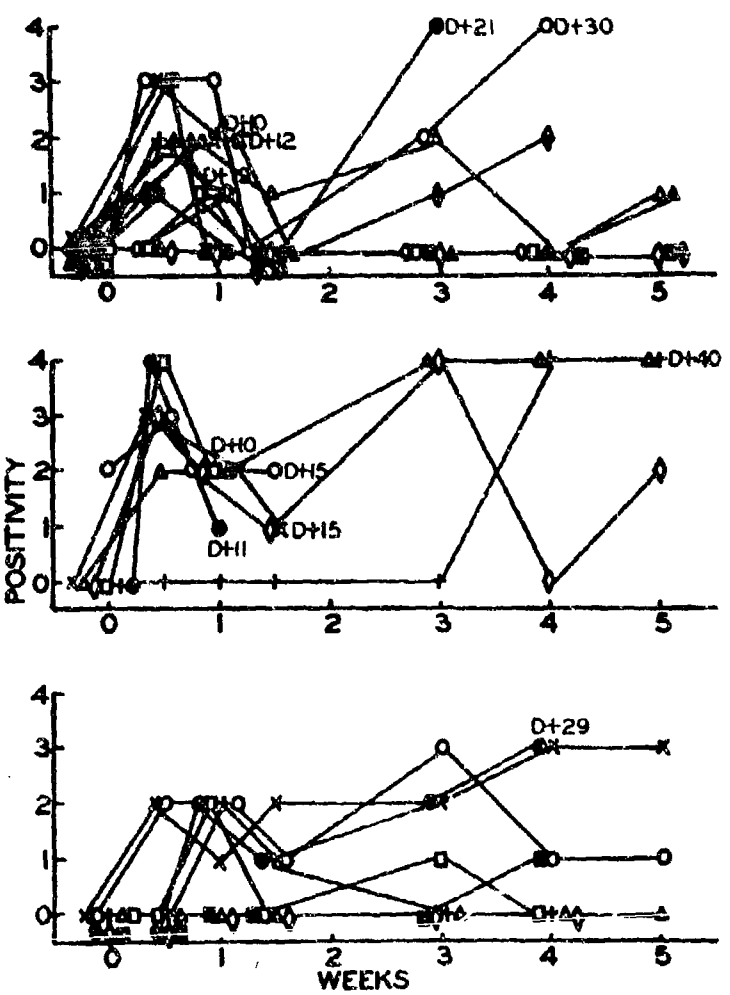


FIGURE 9. TEMPERATURES, BLOOD CULTURES, X-RAY STATUS IN LV GROUP, NO THERAPY (300 CELL CHALLENGE).

FIGURE 10. C-REACTIVE PROTEINS (300 CELL CHALLENGE)



CONTROLS

x=9	●=15
Δ=10	o=16
◊=11	▲=18 R_x
+=12	◆=20 R_x
⊕=13	o=22
■=14	□=24 R_x

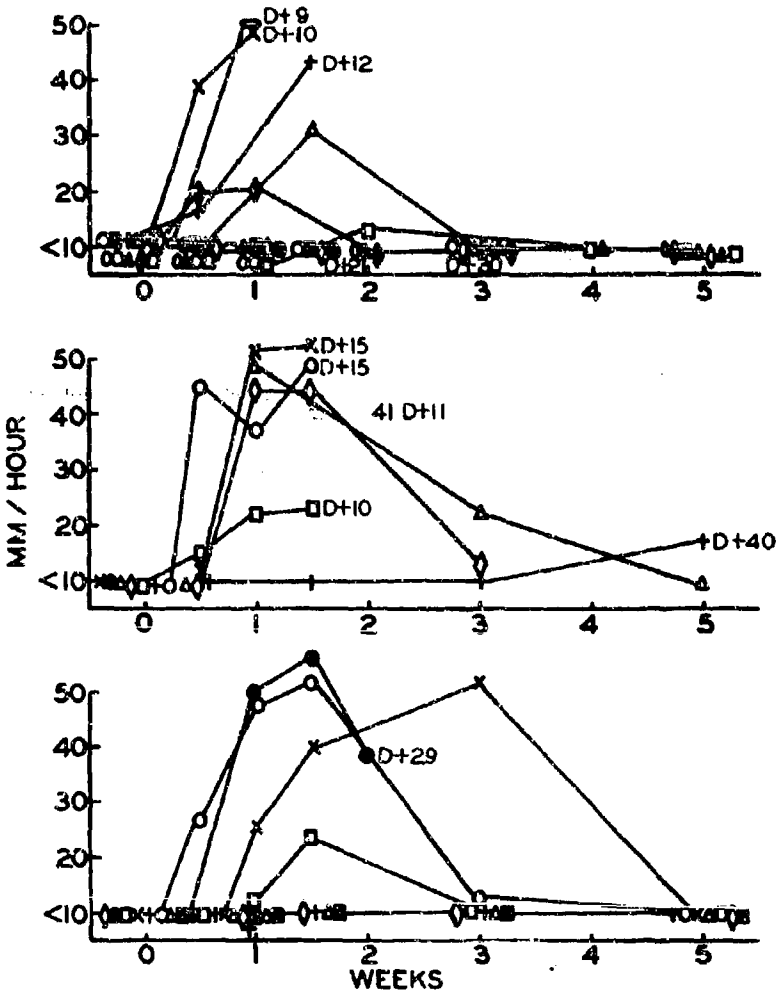
PHENOLIZED VACCINE

●=41	x=52
o=42	□=54
◊=45	Δ=57
+=48	

LIVING VACCINE

+=73	□=77
o=74	●=78
x=75	◊=79
Δ=76	■=80

FIGURE 11. SEDIMENTATION RATES (300 CELL CHALLENGE)

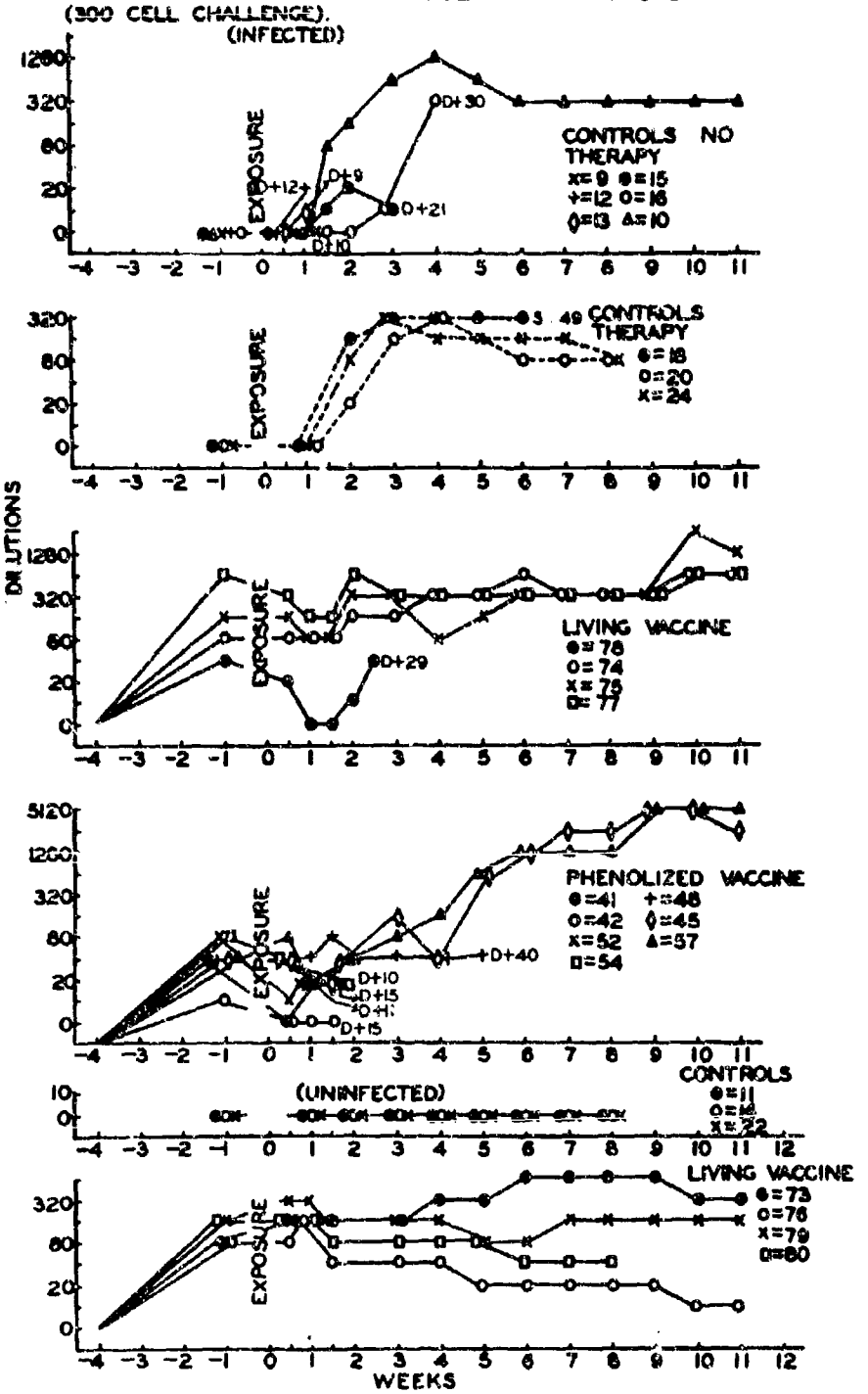


CONTROLS
x=9 ●=15
▲=10 ○=16
◊=11 ▲=16 Rx
+=12 ◆=20 Rx
⊠=13 ⊙=22
■=14 □=24 Rx

PHENOLIZED VACCINE
●=41 x=52
○=42 □=54
◊=45 ▲=57
+=48

LIVING VACCINE
+=73 □=77
○=74 ●=78
x=15 ◊=79
▲=16 ■=80

FIGURE 12. TULAREMIA AGGLUTININ TITERS (300 CELL CHALLENGE).



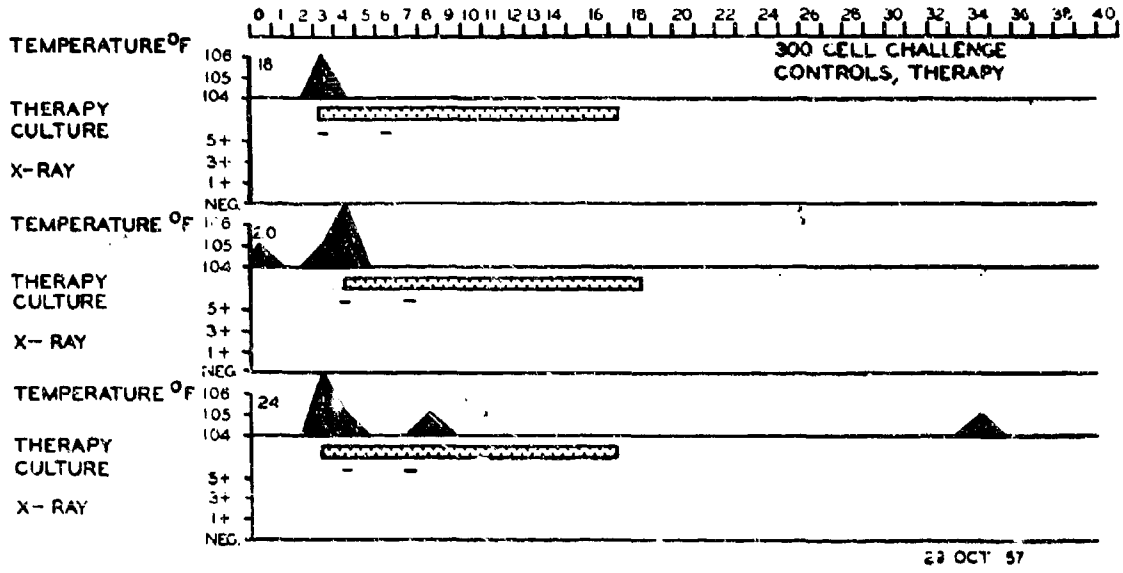
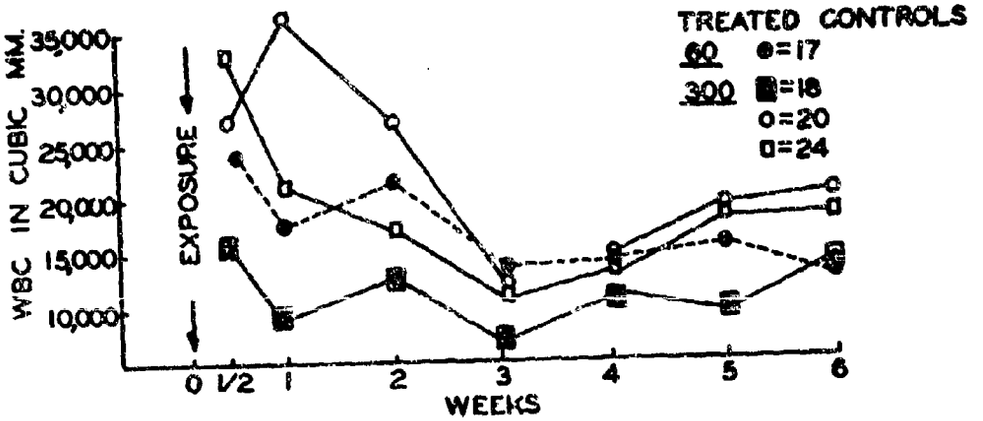


FIGURE 13. TEMPERATURES, BLOOD CULTURES, X-RAY STATUS IN CONTROLS, THERAPY (300 CELL CHALLENGE).

FIGURE 14. WHITE BLOOD CELL COUNTS IN CONTROLS, THERAPY (60 & 300 CELL CHALLENGE).



C. 3,000 ORGANISM GROUP TREATED (8 controls, 4 PV, and 7 LV monkeys)

Weights ranged from 1.4 to 2.6 kg. Morbidity and mortality rates are given in Table IV. All animals were treated with tetracycline.

TABLE IV. MORBIDITY AND MORTALITY 3000-CELL GROUP (Treated)

	MORBIDITY		TULAREMIA MORTALITY	
	No.	%	No.	%
Controls	6/8	75	0/6	0
Phenolized-Vaccine	4/4	100	0/4	0
Living-Vaccine	7/7	100	0/7	0

Incubation periods ranged between 2 and 6 days. All animals were afebrile within 24 hours of onset of therapy (Figures 15-17). With the exception of anorexia during the brief febrile period, physical findings were minimal.

Five animals showed x-ray abnormalities no greater than 3+ in severity which regressed to 1+ or less or cleared by the completion of therapy. It should be noted that 3 of these 5 were monkeys in the LV group. Abnormal white blood cell count (Figure 18), C-reactive protein (Figure 19) and sedimentation rate (Figure 20) were promptly controlled by therapy.

Pre-challenge agglutinin titers in LV monkeys were 1:20 and 1:160 and in PV animals, 1:40 to 1:80. Titers on all infected monkeys began to rise by the second week (Figure 21). Among Controls, only four of the six febrile animals developed significant titer rises to 1:160 or greater. Among LV monkeys, there were small titer rises (2- to 4- fold). In PV monkeys, peak titers were 1:160 to 1:640 (4- to 8- fold). Two control animals were thought to have subclinical experience with tularemia on the basis of agglutinin titers of 1:20 to 1:40, which appeared by the second week (Figure 21).

Relapse and/or complications were noted only in the control group. One animal (No. 28) developed an enlarged cervical lymph node which suppurated and drained on day 98; F. tularensis was cultured from the exudate. Roentgenograms and temperature remained normal. Another animal (No. 38) had a febrile relapse on day 27 with 3+ chest x-ray findings, abnormal CRP and sedimentation rate. On day 77 an enlarged node, on the right foot suppurated and drained. The animal was sacrificed on day 84 and was the only animal in this group to have a positive culture at autopsy (Table VI).

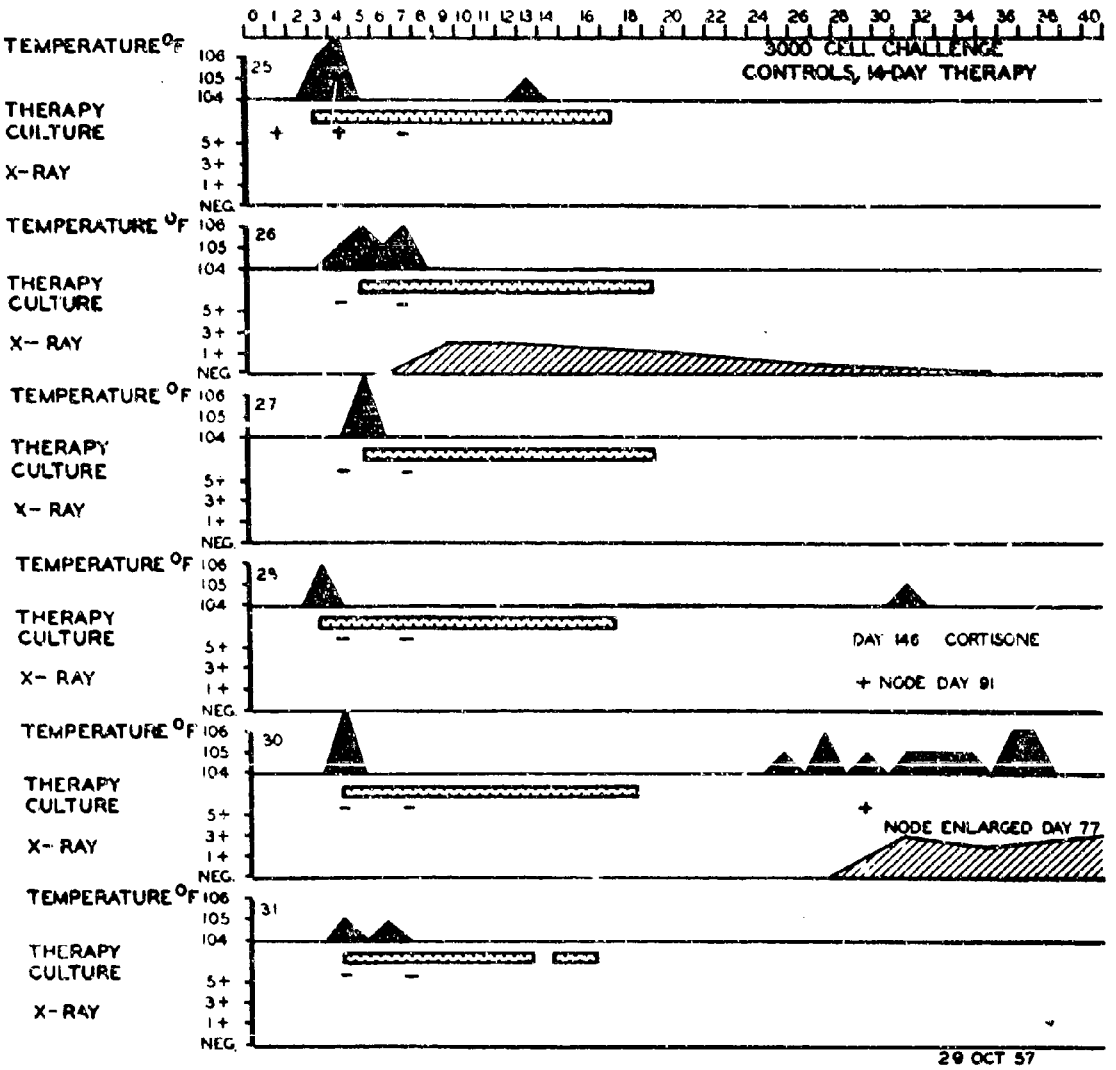


FIGURE 15. TEMPERATURES, BLOOD CULTURES, X-RAY STATUS IN CONTROLS (3000 CELL CHALLENGE).

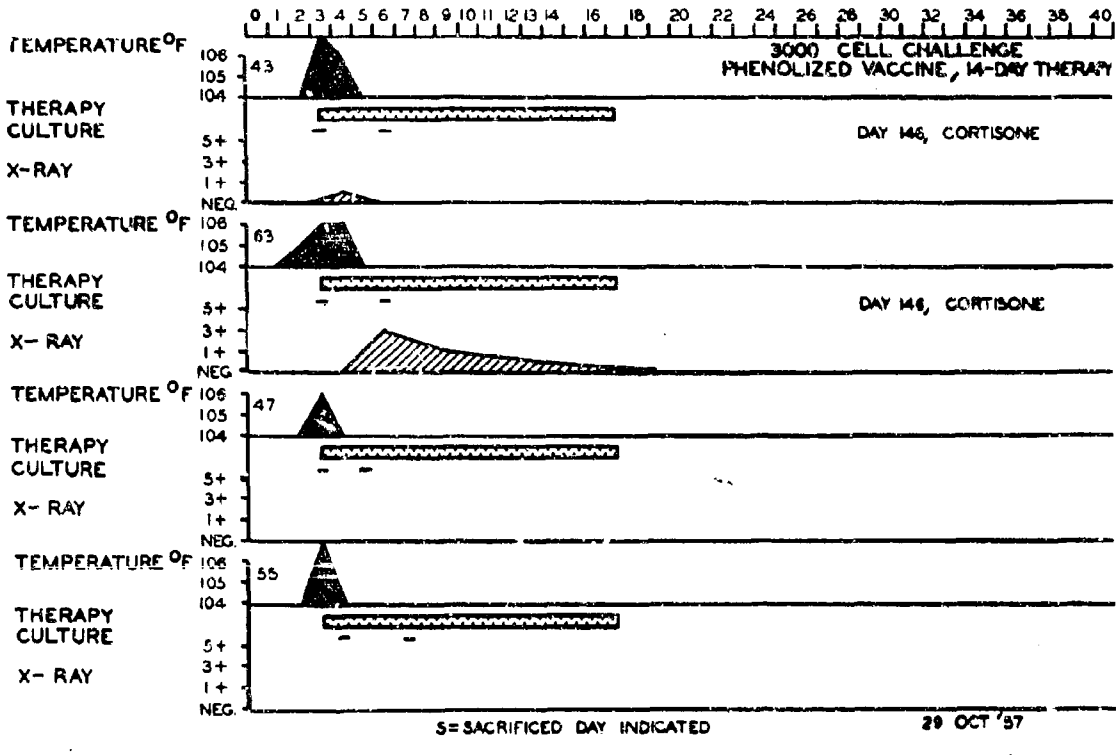


FIGURE 16. TEMPERATURES, BLOOD CULTURES, X-RAY STATUS IN PV GROUP (3000 CELL CHALLENGE).

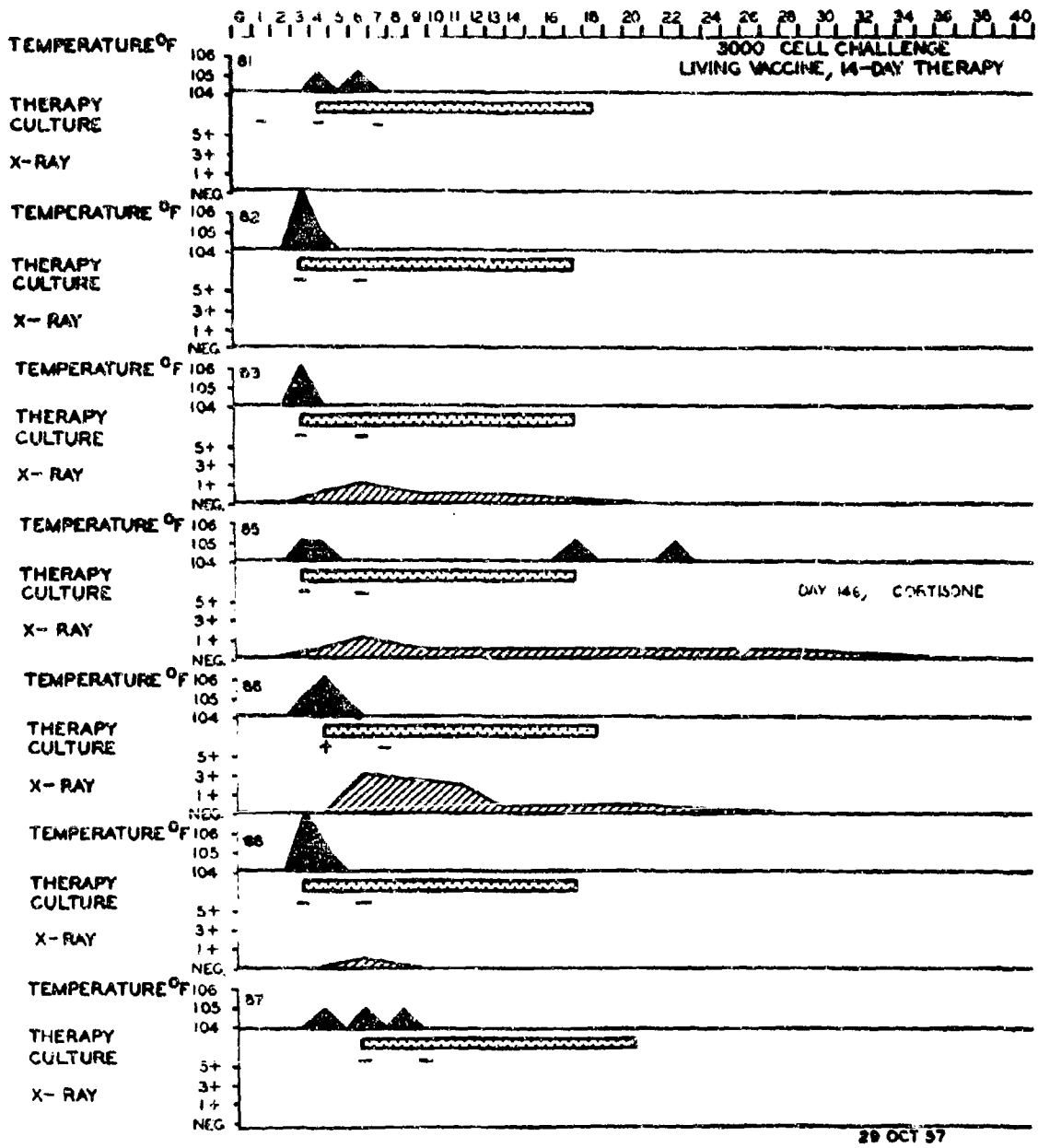


FIGURE 17. TEMPERATURES, BLOOD CULTURES, X-RAY STATUS IN LV GROUP (3000 CELL CHALLENGE).

FIGURE 18. WHITE BLOOD CELL COUNTS (3000 CELL CHALLENGE).

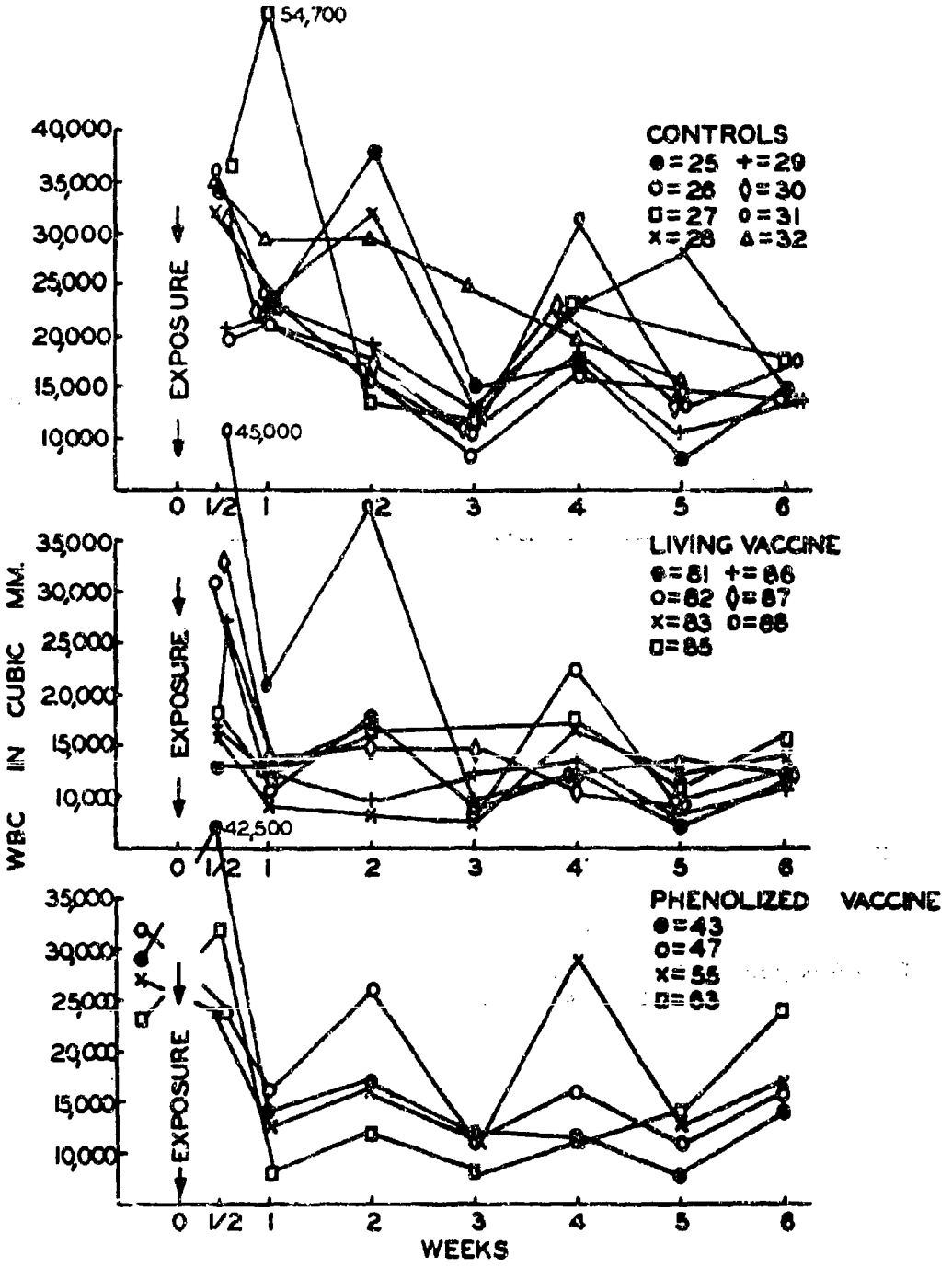


FIGURE 19. C-REACTIVE PROTEINS (3000 CELL CHALLENGE).

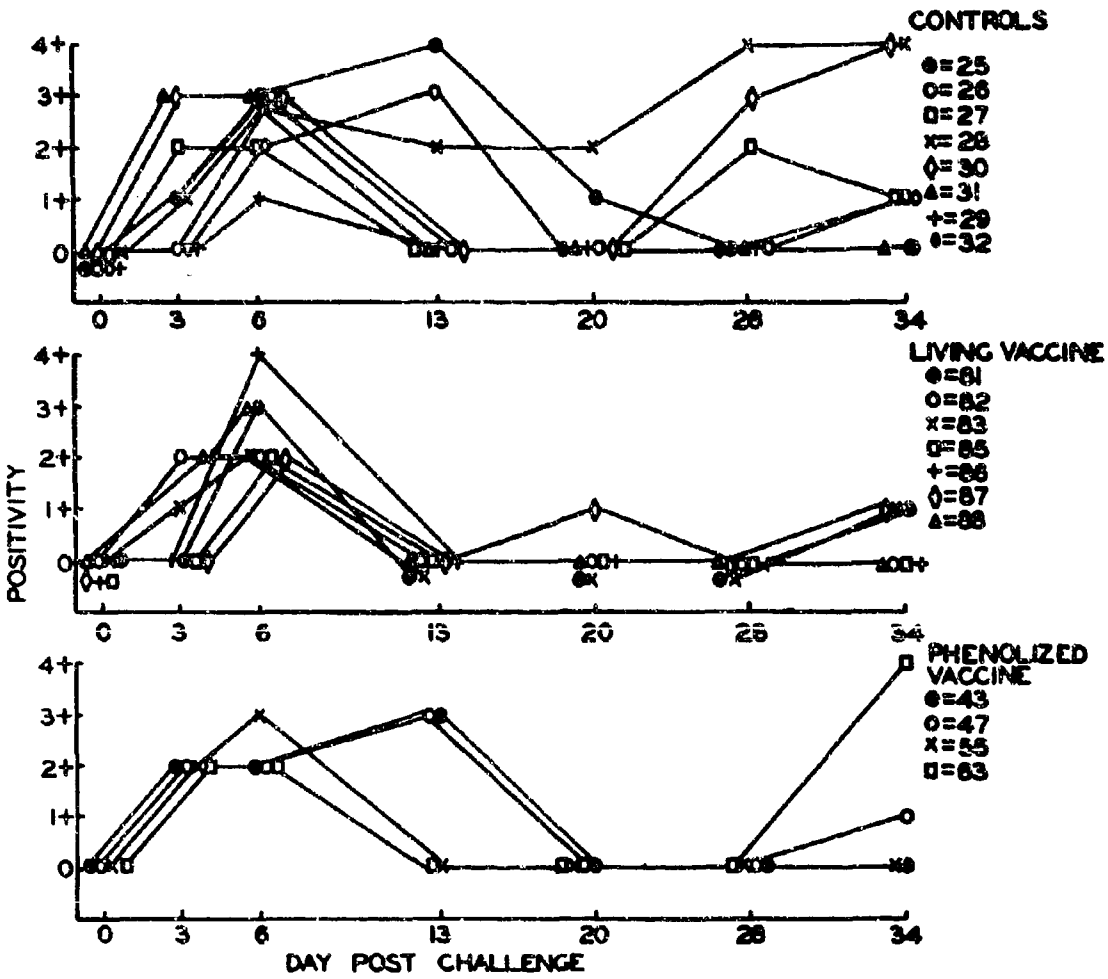


FIGURE 20. SEDIMENTATION RATES (3000 CELL CHALLENGE).

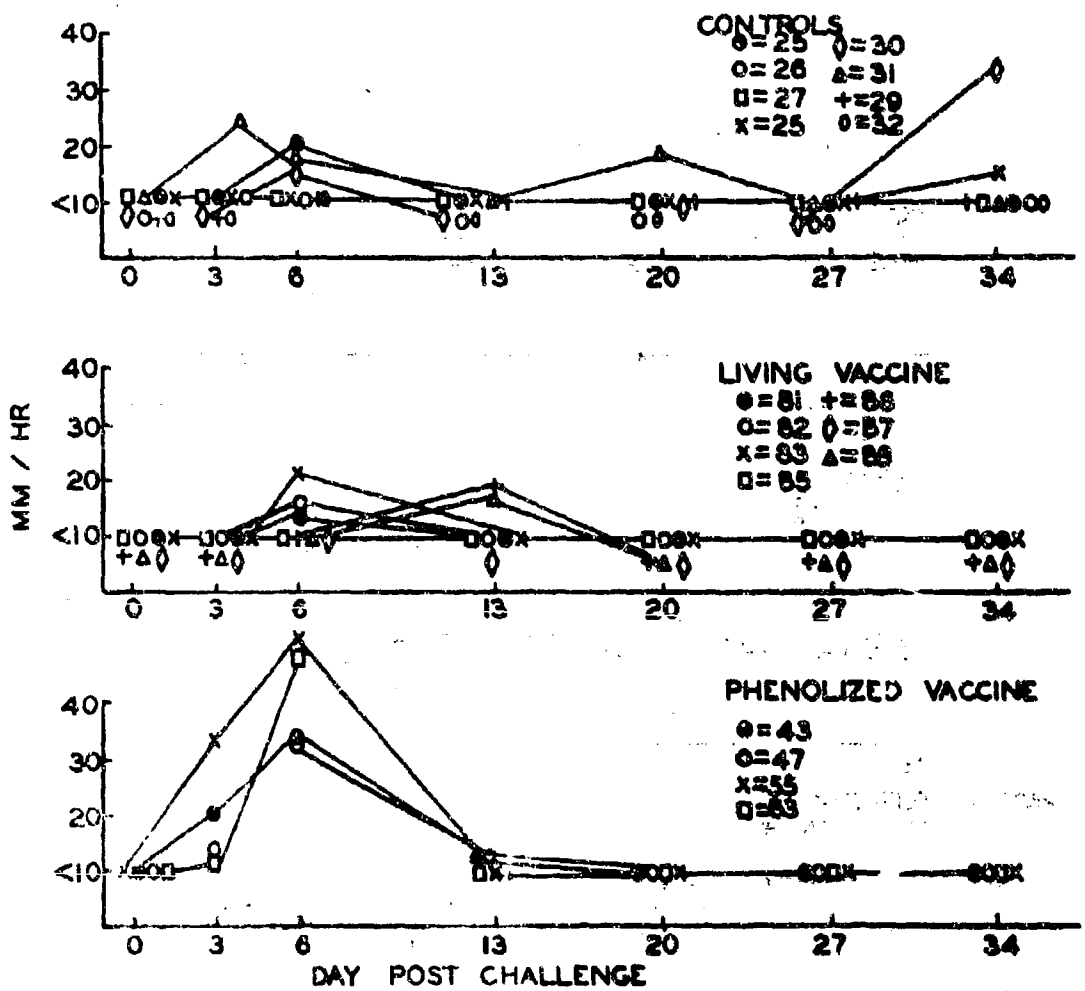
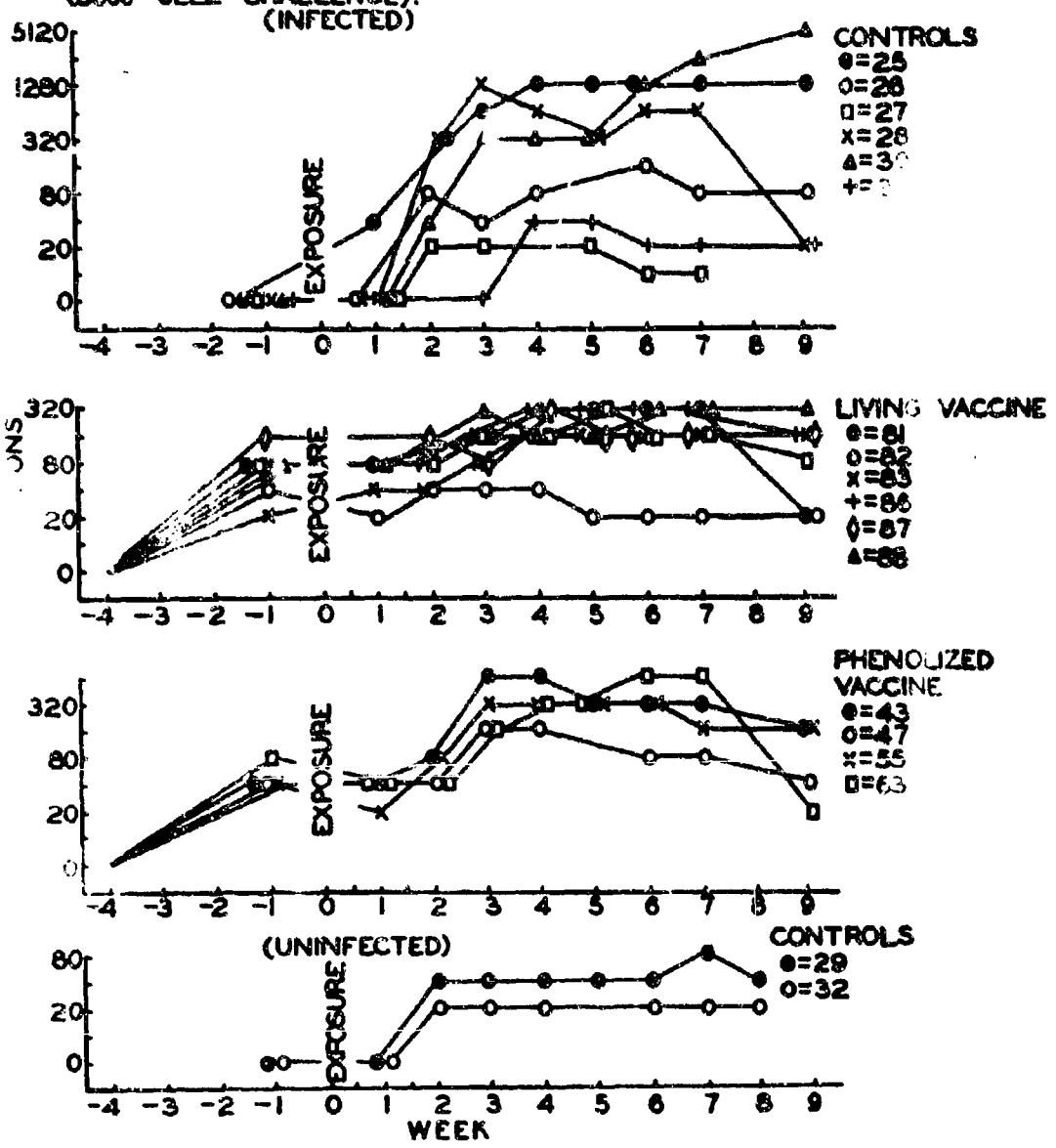


FIGURE 21. TULAREMIA AGGLUTININ TITERS
(3000 CELL CHALLENGE).



D. 30,000 GROUP THERAPY (8 controls, 4 FV, and 7 LV monkeys)

Weights ranged from 1.6 to 2.3 kg. Morbidity and mortality rates are given in Table V.

TABLE V. MORBIDITY AND MORTALITY 30,000-CELL GROUP (Treated)

	MORBIDITY		TULAREMIA MORTALITY	
	No.	%	No.	%
Controls	6/8	75	0/6	0
Phenolized-Vaccine	3/4	75	1/3	33
Living-Vaccine	7/7	100	0/7	0

The incubation period was 2 to 4 days. All were afebrile within 48 hours after onset of treatment (Figures 22-24). Chest rales were noted in only a few monkeys, clearing promptly after start of therapy.

Eight of the 19 animals had abnormal roentgenograms; 6 of these 8 were from the LV group. All x-rays became negative with therapy. Abnormal white blood cell count (Figure 25), CRP (Figure 26), and sedimentation rate (Figure 27) returned to normal by the completion of therapy. LV animals had base-line titers of 1:80 to 1:160, FV monkeys, 1:40 to 1:160 (Figure 28). Among controls there were significant titer rises (8-fold or more) by the third week. LV monkeys demonstrated titer rises (0- to 4-fold). The surviving FV animal had a 10-fold rise in titer. There were two control animals thought to have sub-clinical experience with tularemia because of 1:40 and 1:640 titers (Figure 28).

During the early phases of the study four animals died from non-specific causes. Two were from the control group and one each from the vaccinated groups. Of these, only a control animal dying on day 8 showed lesions of tularemia, limited to the lungs and hilar nodes.

One monkey (No. 56) from the FV group showed an initial favorable response to 14 days of therapy. However, the illness relapsed 4 days after cessation of treatment, with fever, 3+ roentgenographic abnormalities, and elevated sedimentation rate and CRP. The animal died day 39 of extensive tularemia.

Autopsies were performed on all 3,000 and 30,000 cell group animals that died. The numbers with residual tularemia, as compared to totals are summarized in Table VI.

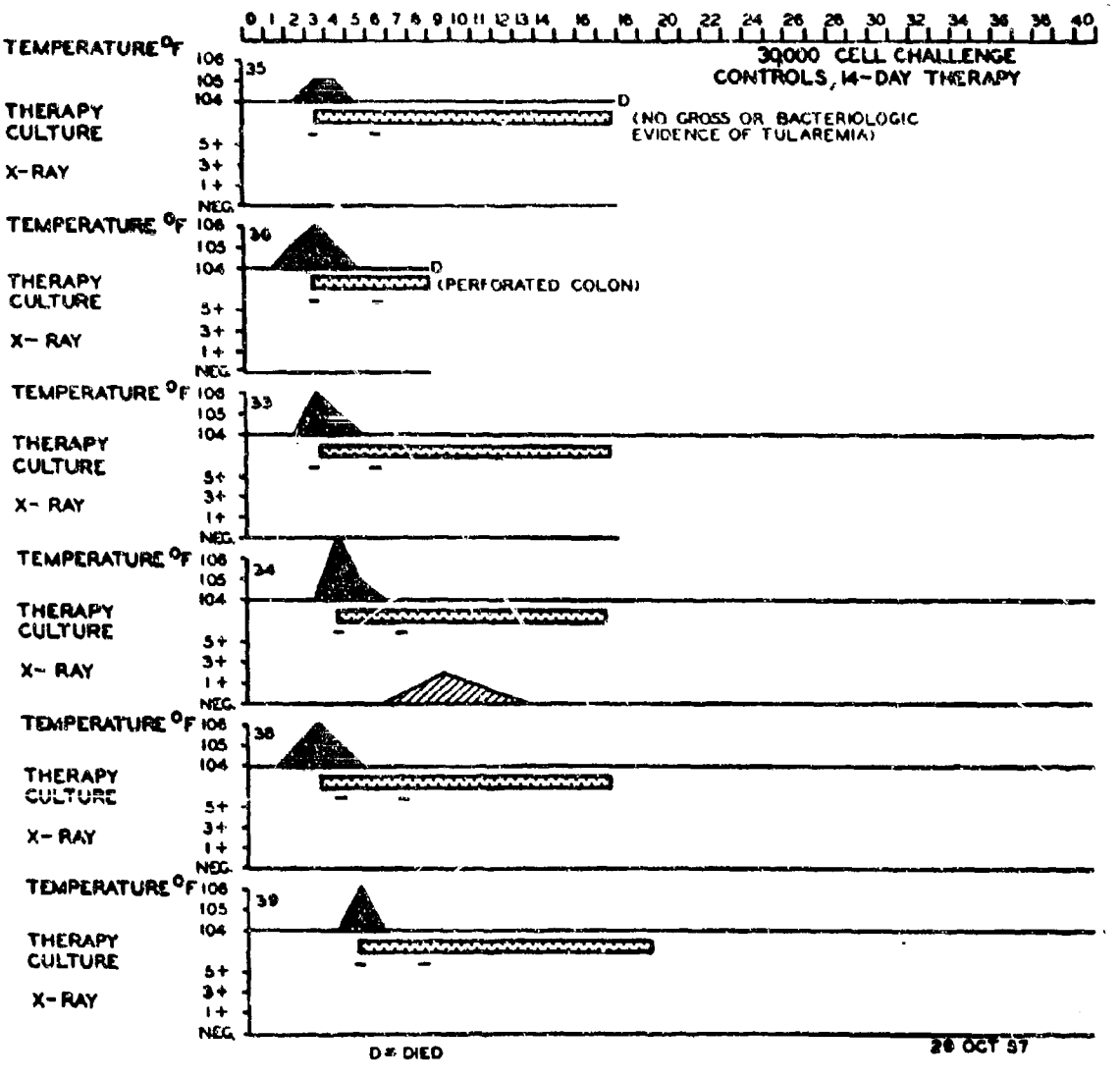


FIGURE 22. TEMPERATURES, BLOOD CULTURES, X-RAY STATUS IN CONTROLS (30,000 CELL CHALLENGE).

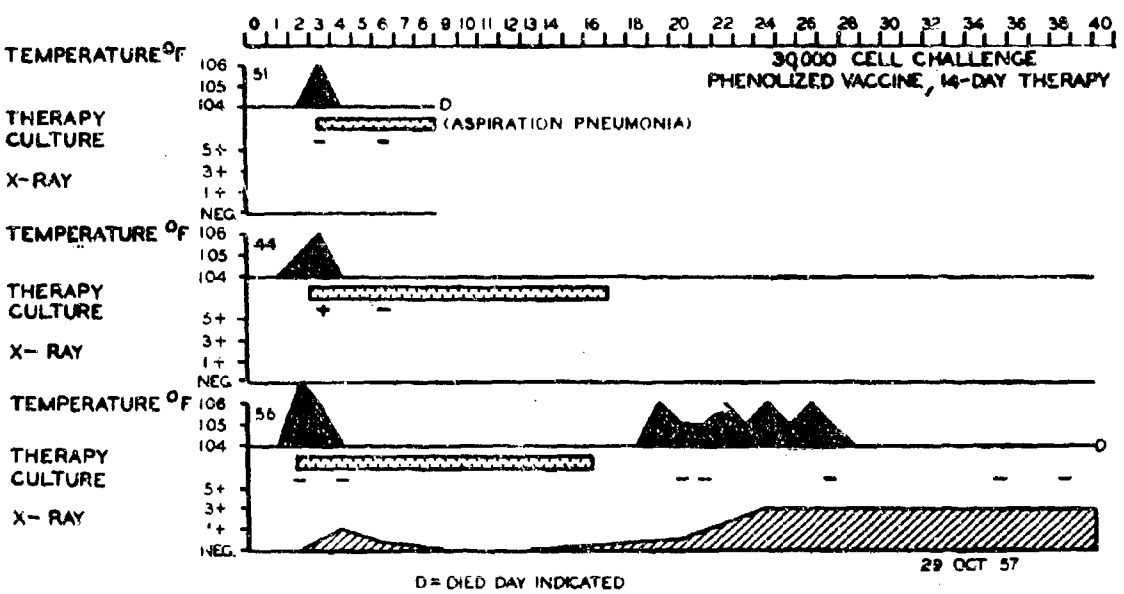


FIGURE 23. TEMPERATURES, BLOOD CULTURES, X-RAY STATUS IN PV GROUP (30,000 CELL CHALLENGE).

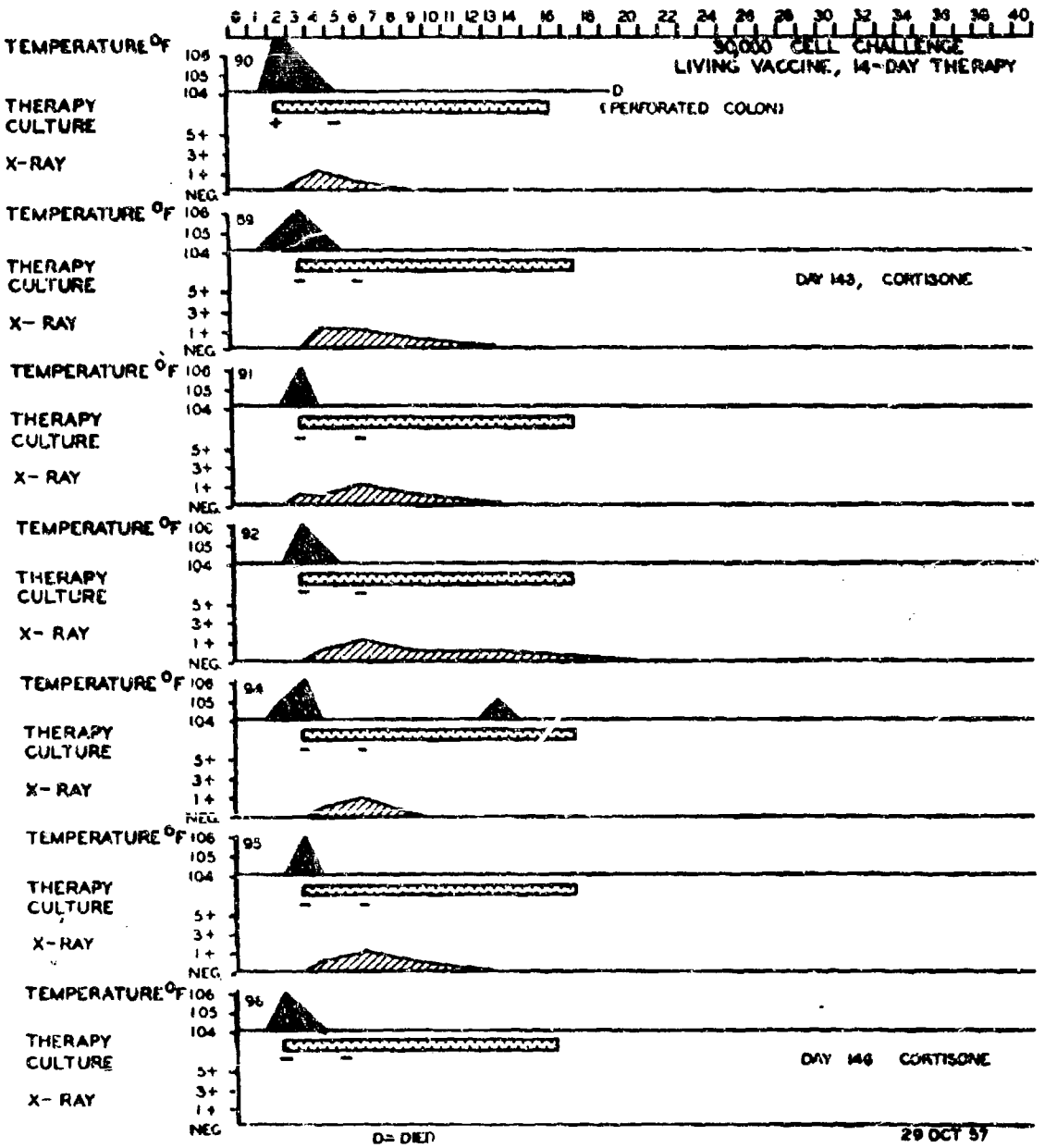


FIGURE 24. TEMPERATURES, BLOOD CULTURES, X-RAY STATUS IN LV GROUP (30,000 CELL CHALLENGE).

FIGURE 25. WHITE BLOOD CELL COUNTS (30,000 CELL CHALLENGE).

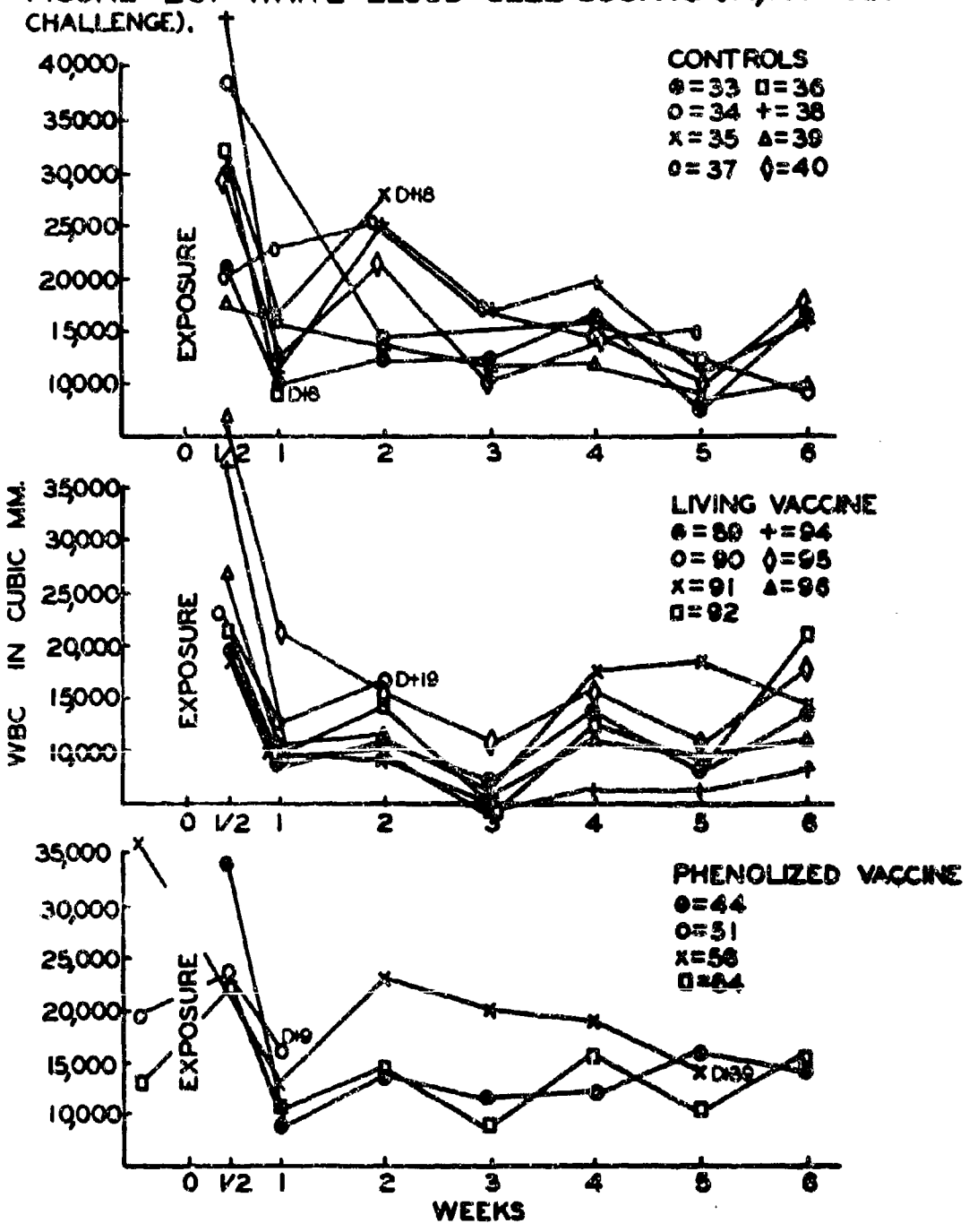


FIGURE 26. C-REACTIVE PROTEINS [illegible] CHALLENGE

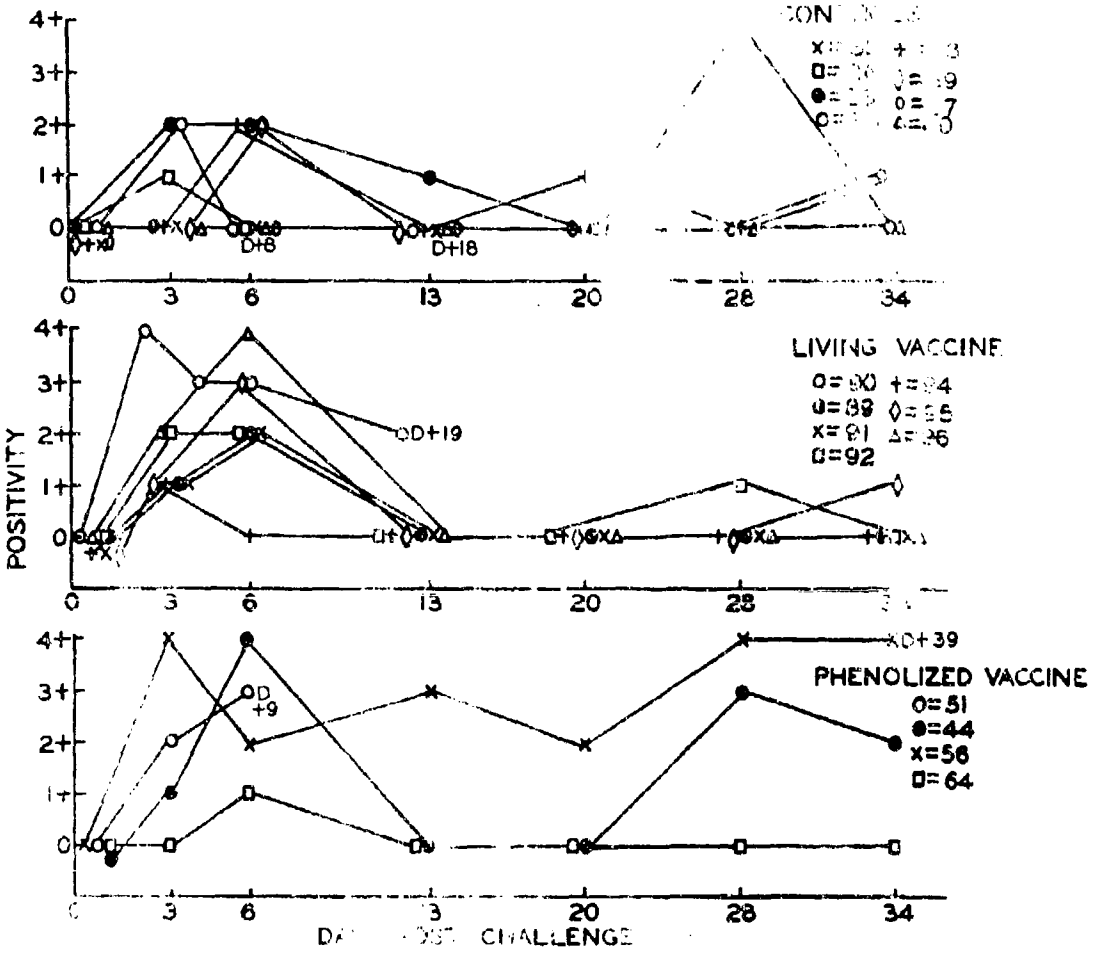


FIGURE 27. SEDIMENTATION RATES (30,000 CELL CHALLENGE).

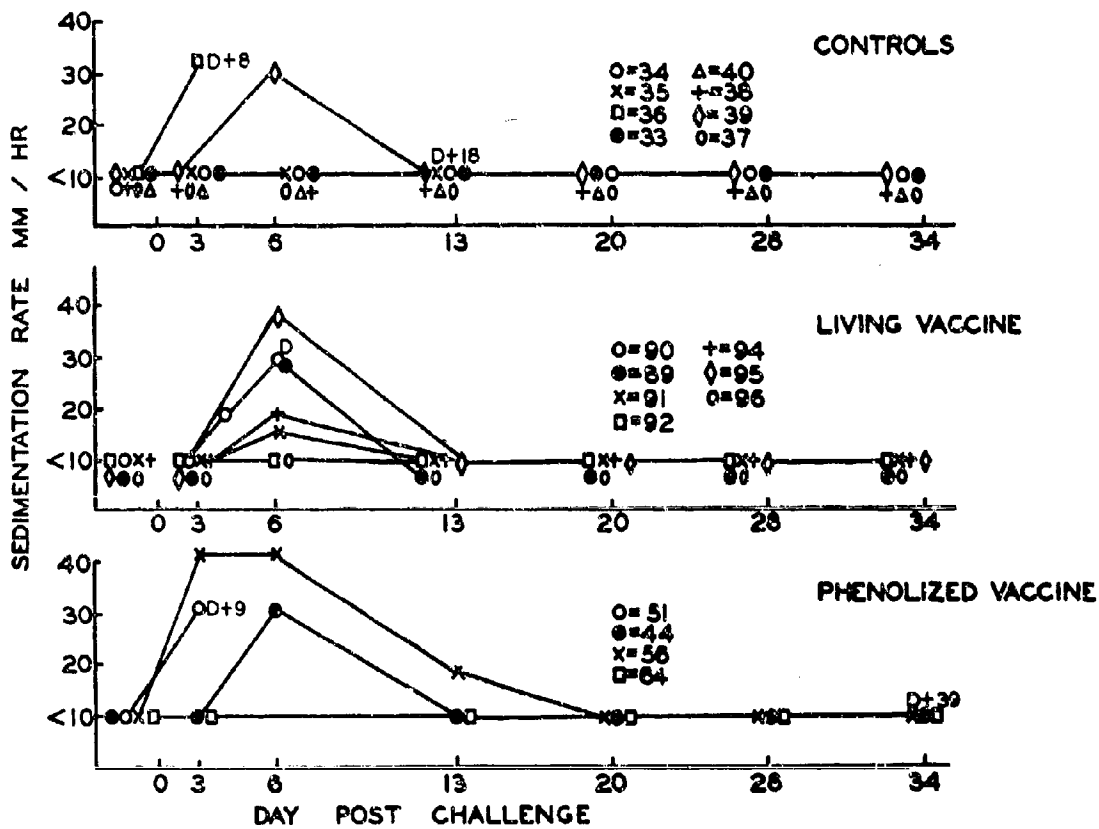


FIGURE 28. TULAREMIA AGGLUTININ TITERS
(30,000 CELL CHALLENGE).
(INFECTED)

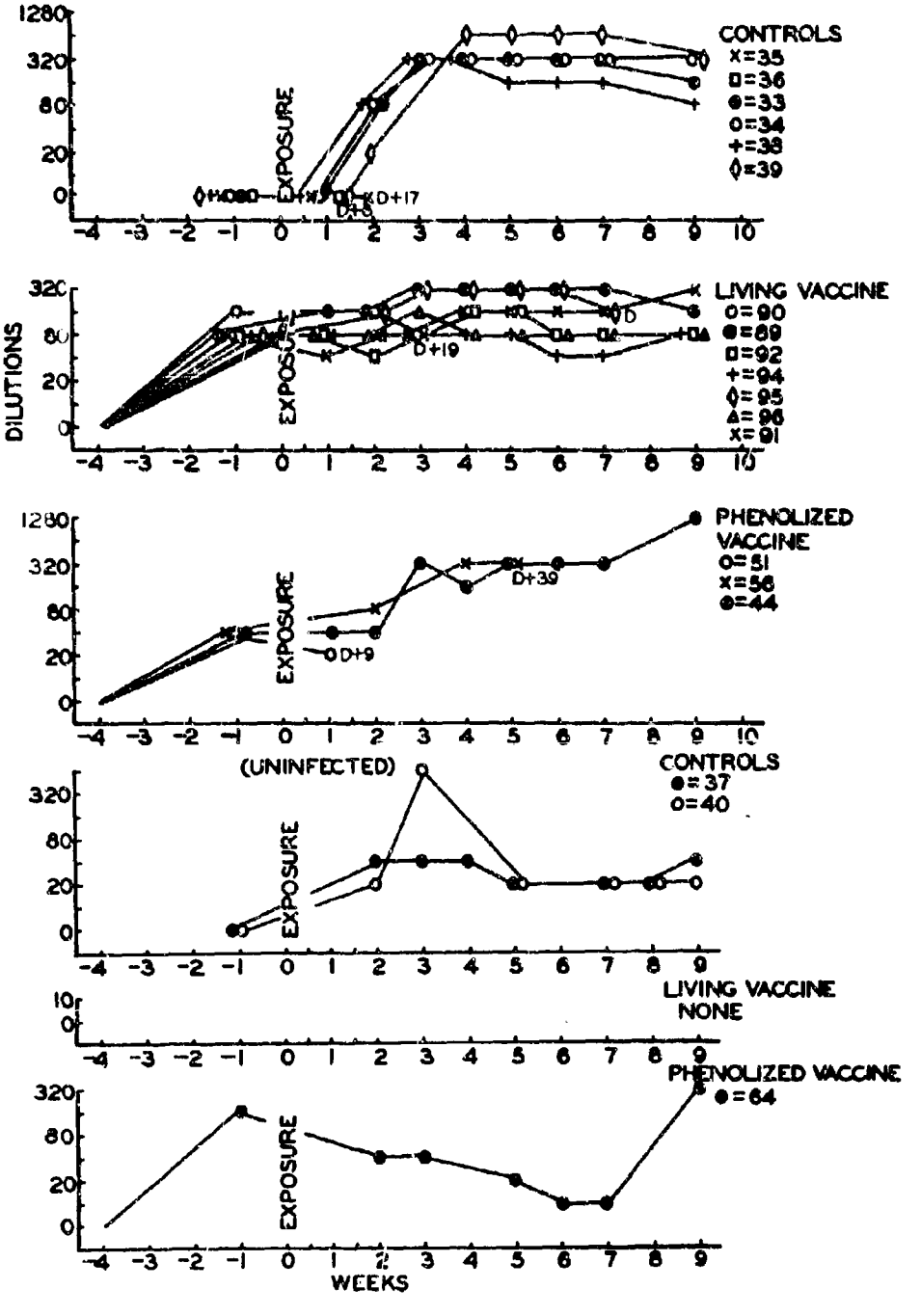


TABLE VI. DAY OF AUTOPSY IN RELATION TO RESIDUAL TULAREMIA (GROSS AUTOPSY)

EXPOSURE DOSE	SUB-GROUP (Treated a/)	TOTAL AUTOPSIES	NO. W/GROSS LESIONS W/OR W/O + CULTURE / NO. AUTOPSIED / Range of days post-exposure					RESIDUAL TULAREMIA
			8-18	38-64	83-121	145-149	175-188	Number
3,000	Control	8			1/2		0/6	1
	PV	4				0/1	0/3	0
	LV	7		0/1	0/2	0/1	0/3	0
30,000	Control	8	1/2			0/4	0/2	1
	PV	4	0/1	1/1	0/1	0/1		1
	LV	7	0/1	0/1	0/1	0/1	0/3	0

a. Therapy: Tetracycline, 125 mg every 6 hours for 7 days, then 125 mg every 8 hours for 7 days.

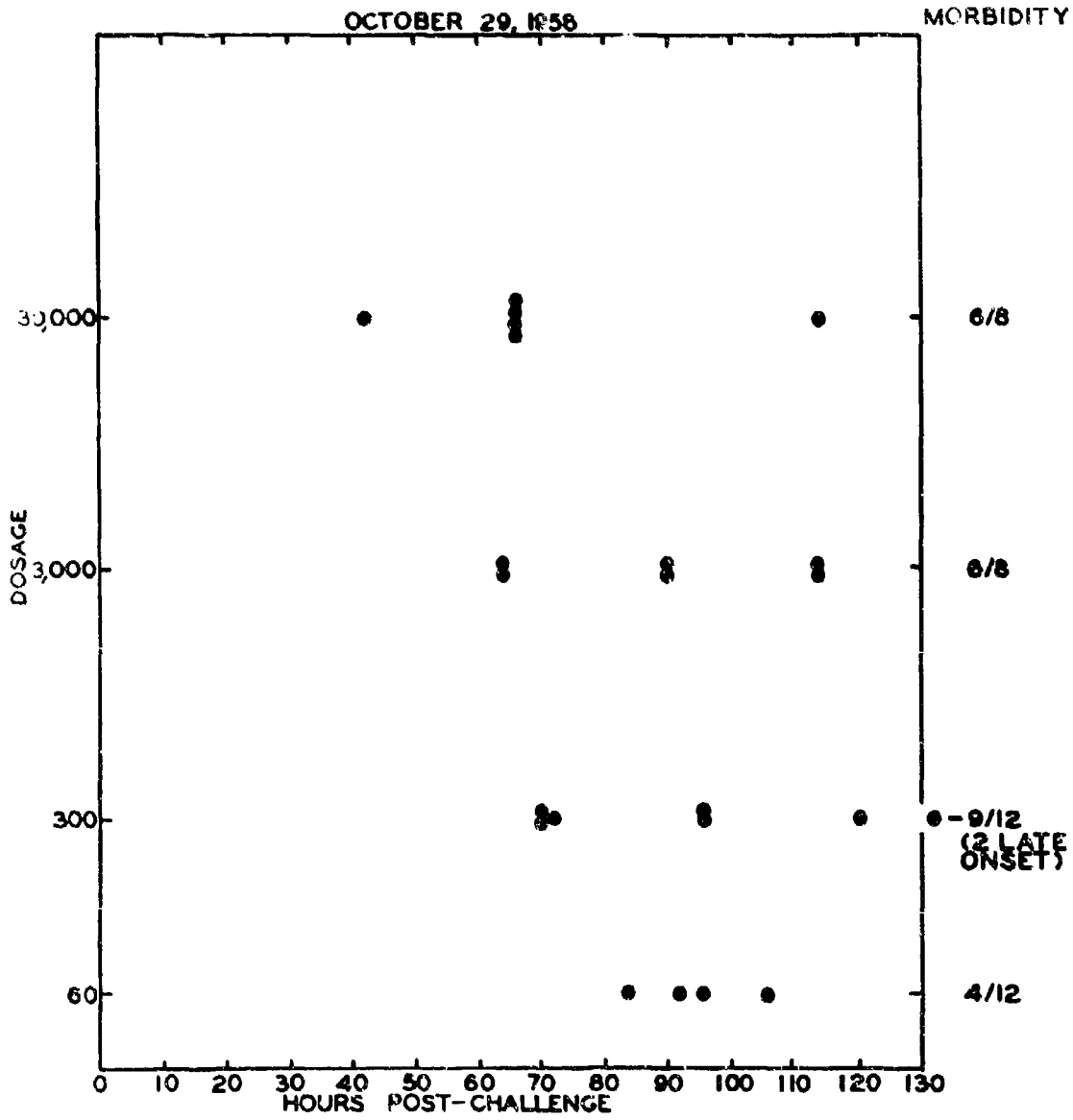
IV. DISCUSSION

This entire run has several puzzling features, and the report has been delayed for this reason. Several instances of late onset of disease occurred at the low dose levels. For example, control animal No. 15 (nominal 300 cells) became ill on day 15 post-exposure, while control animal No. 16 became ill on day 18. Two PV animals (Nos. 61 and 62) at the nominal 60-cell challenge level became ill on days 27 and 31. A PV animal (No. 48) at the 300-cell level became ill on day 23. These late onsets could represent cross-infection, but the animals were handled in a manner identical with that used for the past several years; no cross-infection had been observed previously. It may be noted that animal No. 15, a 300-cell control, had a positive blood culture on day 11, two days prior to fever. Attention is also directed to animal No. 8, a 60-cell control, which never became ill, but by day 19 had developed a low level agglutinin titer. While several alternative explanations can be advanced, none can be validated at this time.

There were apparent "misses" at all dose levels in the control monkeys. Thus, at the nominal 60-cell level, 8 of 12 animals did not develop clinical disease; at the 300 organism level, 3 of 12 did not show clinical disease; at 3,000, the comparable figures are 2 of 8, and at 30,000, 2 of 8. Furthermore, again limiting the discussion to the control animals, over almost a 3-log range of nominal infecting doses there was essentially the same incubation period (Figure 29). Finally, at the maximum "dose" used, 30,000 organisms, there was an average incubation period of 71 hours which differs materially from the incubation period observed in the previous study when animals were given a presumed one-half log higher challenge. In a run on a different exposure device, to be reported later, with a nominal 1,300 organisms challenge, the incubation period was 60 hours, while with 130,000 organisms the incubation period was 40 hours, clearly a dose-dependant relationship.

Prolonged efforts to show any difference in the particular group of animals used in this study compared to those used earlier or later have been

FIGURE 29.
DISTRIBUTION OF INCUBATION PERIODS BY DOSAGE AND HOURS POST-CHALLENGE.



fruitless. The culture of F. tularensis is believed to have had suitable guinea pig virulence. The monkeys were held in two separate laboratories and checked by two different teams, with identical results. One is almost forced to consider that the method of exposure may have produced the disturbing results.

Examination of the exposure records (Table VII) reveals that certain animals, because of the level of anesthesia or for other reasons, had poor respiration rates. The presumed low level exposures are of particular interest. Animal No. 61 falls into this category and did not become ill until day 27; animal No. 38 was likewise noted as having a very poor respiratory rate (the numerical entry is 2 per minute) and is not surprising to find that this was one monkey that did not become infected. Animal No. 16 was noted to have had shallow breathing and he did not develop signs of clinical illness until day 18. Animal No. 45 had a similar notation. He became ill on day 4, and it may or may not be of significance to note that he was one of the two PV animals who received the nominal 300 cell challenge and survived. The other PV vaccinated survivor at this same dose level was No. 57 and his exposure shows the comment "poor breathing".

During this run impinger samples were taken only when certain animals were being exposed. A review of animals exposed when impingers were operating shows the following: at the nominal 60 organism exposure animal No. 2, a control, was not infected, animal No. 8 never developed clinical disease but possibly did develop a late low level agglutinin rise, animal No. 61 (PV) falls into this same group and has already been noted above as not having become ill until day 27, and animal No. 62 (PV) did not become ill until day 31. Continuing at the nominal 300 level; animal No. 18 was exposed at the same time as the impinger was operating and became ill on day 3, animal No. 77, who had received LV, became ill on day 5, while animal No. 48 who had received PV, did not become ill until day 23 post-exposure.

The respiration rates for all animals was recorded. At the low dose exposures several animals not mentioned above had a rate of 22 or less which is definitely below the average recorded. Animal No. 71 with a rate of 14 was a LV who showed no signs of clinical illness, animal No. 58 with a rate of 22 was a PV who did not become ill until day 6. At the 300 challenge level LV animal No. 73 with a rate of 22 never showed signs of clinical illness, while PV monkey No. 41 had an incubation period of 5 days.

In short, of the 27 animals at these two dose levels who showed anomalous results there are 11 known to fall into the categories described above in which there was reason to believe that the dose might have fallen below the desired level. At greater nominal exposure dose levels this discrimination, of course, is not apparent, but one is still left without a suitable explanation for the lack of difference in incubation periods at the varying dose levels.

While it is not possible to account by these methods for all of the odd results seen in this group of animals, the association is sufficient to question the validity of all dose levels. Some monkeys on this run developed

TABLE VII. EXPOSURE RECORDS FOR 60- AND 360-CELL CHALLENGE GROUPS INCLUDING DAY OF FEVER AND DAY OF DEATH

Part A. Nominal 60 organisms/liter

GROUP	ORDER OF EXPOSURE	MONKEY NO.	RESP/MIN.	WEIGHT gm	COMMENTS	POST EXPOSURE HOUR OF FEVER	POST EXPOSURE DAY OF DEATH
Control	34	2	45	2305	Impinger pulled	None	---
	17	3	28	2130		None	---
	24	4	34	2225		None	---
	23	5	48	1915		108	---
	29	6	31	1815		96	25
	12	7	32	1730		84	16
	25	8	46	1560	Impinger pulled	None	---
	27	17	50	2605		92	---
	30	19	30	2565		None	---
	28	21	35	1890		None	---
	32	23	30	1665		None	---
	26	38	2		Very poor respiratory rate	None	---
PV[a]	13	46	40	2350		None	---
	15	49	23	1800		96	9
	Dead	50	--	--	Dead	--	---
	21	58	22	1850		156	---
	10	59	37	2150		144	28
	9	61	24	1950	Shallow breathing. Impinger pulled	27 am[c]	---
	16	62	20	1600	Impinger pulled	31 am[c]	---
LV[b]	18	65	25	4175	Vacuum not pulling properly	120	---
	11	66	26	3885		168	---
	20	67	34	3835		None	---
	14	68	23	3210		None	---
	19	69	33	2755		96	---
	22	70	29	2540		216	---
	32	71	14	2305		None	---
	31	72	35	2480		None	---

a. PV = phenolized vaccine.

b. LV = living-vaccine.

c. Day, and time of day.

TABLE VII. EXPOSURE RECORDS FOR 60- AND 300-CELL CHALLENGE GROUPS INCLUDING DAY OF FEVER AND DAY OF DEATH (Continued)

Part B. Nominal 300 organisms/liter

GROUP	ORDER OF EXPOSURE	MONKEY NO.	RESP/MIN.	WEIGHT gm	COMMENTS	POST EXPOSURE HOUR OF FEVER	POST EXPOSURE DAY OF DEATH
Control	12	9	43	2735		132	10
	5	10	29	2250		120	---
	18	11	25	2095		None	---
	16	12	32	1990		96	12
	23	13	30	1900		96	10
	11	14	33	1810		None	---
	24	15	23	1705		15 am[c]	22
	25	16	18	1470	Shallow breathing.	18 am[c]	31
	27	18	36	2230	Impinger pulled.	70	---
	13	20	31	1950		72	---
	21	22	30	1785		None	---
	26	24	35	1455		70	---
PV[a]	6	41	21	1600		120	13
	4	42	27	1800		132	15
	2	45	37	2050	Shallow breathing.	96	---
	1	48	33	1900	Impinger pulled.	23 am[c]	42
	8	52	25	1500		96	15
	7	54	23	2350		84	11
	3	57	--	1850	Poor breathing.	108	---
LV[b]	15	73	22	4025		None	---
	10	74	57	4065		72	---
	20	75	27	3545		120	---
	14	76	30	2125		None	---
	17	77	25	2655	Impinger pulled.	132	---
	19	78	26	2405		132	30
	9	79	33	2345		None	---
	22	80	27	2105		None	---
			AVERAGE WEIGHT	2371			

a. PV - phenolized vaccine.

b. LV - living-vaccine.

c. Day, and time of day.

tularemia while others did not. Since the degree of exposure must be considered to be unknown the effect of prior vaccination on the outcome cannot be assessed, and the run must largely be discounted insofar as any relationship to challenge dose is concerned.

It is clear that in the animals who developed clinical disease and were not treated that animals who had received the living vaccine prior to challenge were less likely to die than the controls or those receiving the phenolized product. If the nominal 60 and 300 challenge groups are combined and consideration limited to those animals who had incubation periods of less than 10 days, the controls show 5/7 fatalities, the phenolized vaccinates show 6/9 fatalities, while those receiving living vaccine show 1/8 fatalities. The animal in this last group that died had one of the lowest pre-challenge agglutinin titers (1:40).

Some inferences concerning the response to tetracycline are permissable, since all animals treated, did, in fact, have tularemia, although the infecting dose cannot be stated. All animals responded promptly to tetracycline. Relapses or complications after therapy was terminated were seen in three animals, two controls and one FV. No effort was made to retreat these animals.

Finally in groups placed on early therapy there is the suggestion that those previously vaccinated were more likely to have, or to develop, roentgen evidence of disease when compared with controls placed on therapy at the same time.

V. CONCLUSIONS

The calculation of an ID_{50} on this data is not warranted and no conclusions can be reached as to the effectiveness of either vaccine in preventing disease.

In the animals who did become ill there is little difference between the controls and those previously receiving a phenolized vaccine. In contrast, animals previously receiving a viable vaccine had a higher survival rate untreated.

Clinical tularemia in monkeys can be adequately controlled by the early initiation of tetracycline.

STUDIES ON PASTEURELLA TULARENSIS

MODIFICATION BY VIABLE VACCINE IN MACACA MULATTA MONKEYS AEROSOL CHALLENGED WITH 1,300 AND 130,000 CELLS.

(Gochenour, Overholt, Gleiser, Hornick, Hughes, Sills, Byron)

I. INTRODUCTION

This study was undertaken to examine the effects of prior vaccination with a living Pasteurella tularensis vaccine of Macaca mulatta monkeys exposed to an aerosol challenge of P. tularensis (SCHU 4). It differed from the preceding study (60, 300, 3,000, and 30,000 cell challenge[1]) in several ways: (1) the animals were not anesthetized, (2) a different exposure apparatus was employed, (3) a different lot of living vaccine was used, and (4) tetracycline, when given, was administered at 8-hour intervals for 7 or 14 days. Details of each of these will be given in appropriate parts of the report. It was hoped that the short period of therapy would be sufficient for the prevention of relapses in vaccinated animals, and that a differential relapse rate under these circumstances might be used as a measure of the value of a vaccine short of actual disease prevention.

II. METHOD

A. ANIMALS

A total of 72 monkeys were used, divided into challenge groups as shown in Table I.

TABLE I. DISTRIBUTION OF ANIMALS

NOMINAL DOSE	CONTROLS		7-DAY THERAPY		14-DAY THERAPY	
	VACCINE	NO VACCINE	VACCINE	NO VACCINE	VACCINE	NO VACCINE
1,300	6	6	6	6	6	6
130,000	6	6	6	6	6	6

B. VACCINE

The viable P. tularensis vaccine employed was a lyophilized preparation prepared January 17, 1956, and designated as Lot No. 2. Since that time this same lot of vaccine has been extensively used in man and details of the testing in small animals are given under that section[2]. The vaccine was administered to the monkeys by multiple puncture technique 3.5 months prior to challenge. Small local cutaneous lesions developed unaccompanied by any obvious signs of systemic infection.

C. THERAPY

Therapy consisted of oral, unbuffered tetracycline hydrochloride (Lederle), 125 mg every 8 hours for 7 or 14 days, administered by gastric intubation, and was started at the time of the second temperature of 104.0°C or greater; there were four unintentional exceptions to this which will be noted. No attempt was made to re-treat animals showing clinical signs of disease after cessation of the original course of drug.

D. CHALLENGE

Unanesthetized animals were placed in individual boxes with their heads exposed. The head was then placed into a helmet attached to a modified Plummer tube. The characteristics of this equipment are noted in another report.[3] The particle size and the characteristics of the challenge culture were identical to those used earlier. Three animals from different groups were simultaneously exposed for two minutes to an aerosol of streptomycin-sensitive strain of P. tularensis (SCHU 4); the exposure method was repeated until all 72 animals were exposed. The 1,300-cell challenge was conducted on May 15, 1958, and the 130,000-cell challenge on May 16, 1958.

E. STUDIES AND EXAMINATIONS

Rectal temperatures were taken every eight hours; physical examination was made daily. Chest x-rays were obtained three times a week for two weeks, twice a week for two more weeks, and thereafter weekly. X-ray abnormalities were graded as before. C-reactive protein (CRP) determinations were made four times a week during the first week, twice during the second week, and weekly thereafter. Three monkeys from each group were bled for blood culture at 0800 and 1600 hours on days 1, 2, 3, 5, 7, and 9. Blood was also cultured from certain monkeys with fevers on days 14 and 15. Culture methods have been detailed in an earlier report.[4] Tetracycline blood levels (biological assay) were obtained from two monkeys from each therapy group at 16, 24, 48, and 72 hours after initiation of therapy and on days 6, 9, 13, and 16. Serial serum specimens for post-vaccination and post-exposure agglutinin titers were obtained. Autopsies were performed on each animal that died or was sacrificed.

III. RESULTS

Blood culture attempts are shown in Table II, by exact time relative to onset of fever. The various clinical laboratory findings essentially duplicated those previously reported and will not be discussed.

TABLE II. ATTEMPTS TO ISOLATE F. TULARENSIS FROM DUOLITE-TREATED BLOODS OF MONKEYS EXPOSED TO APPROXIMATELY 1,300 AND 130,000 CELLS BY AEROSOL

GROUP	ANIMAL NO.	DAY POST-EXPOSURE 1	2	3	5	7	DAY FIRST FEVER	THERAPY ONSET HOURS	DIFFERENCE BETWEEN FIRST POSITIVE CULTURE & FIRST FEVER HOURS[a]
		Low Dose-1,300 cells. Exposed May 15, 1958							
I Vaccine	T-5	-	-	+	+	D[b]	3 pm	None	0
No Rx	T-14	-	-	+	+	+	2 pm	None	+16[c]
	T-46	-	-	+	+	+	3 pm	None	0
II Vaccine	T-21	-	-	-	-	-	2 pm	+8	No isolation
7-day Rx	T-39	-	-	+	-	-	2 mid	+8	+16
	T-58	-	-	-	-	-	2 pm	+8	No isolation
III Vaccine	T-16	-	-	-	-	-	2 pm	+8	No isolation
14-day Rx	T-38	-	-	-	-	-	2 pm	+8	No isolation
	363	-	-	+	-	-	2 mid	+8	+8
IV No vac-	T-32	-	-	+	+	D	2 mid	None	+8[d]
cine	347	-	-	+	+	+	2 mid	None	+8
No Rx	T-8	-	+	+	+	D	2 mid	None	-16
V No vac-	T-20	-	-	-	-	-	2 pm	+8	No isolation
cine	T-11	-	+	+	-	-	2 pm	+8	-8
7-day Rx	T-29	-	-	+	-	-	2 mid	+8	+8
VI No vac-	T-22	-	+	+	-	-	2 pm	+8	-8
cine	358	-	-	+	+	-	3 pm	0	-8
14-day Rx	362	-	-	+	-	-	3 am	+8	0
		High Dose-130,000 cells. Exposed May 16, 1958							
VII Vaccine	T-18	-	+	+	D	D	2 mid	None	-16
No Rx	352	-	+	+	+	D	2 am	None	+8
	403	-	+	+	D	D	2 am	None	0
VIII Vaccine	T-1	-	-	-	-	-	1 mid	+8	No isolation[d]
7-day Rx	T-17	-	-	-	-	-	1 pm	+8	No isolation
	T-19	-	-	-	-	-	1 pm	+8	No isolation
IX Vaccine	T-9	-	-	-	-	-	1 am	+16	No isolation
14-day Rx	371	-	-	-	-	-	1 pm	+8	No isolation
	392	-	-	-	-	-	1 mid	+8	No isolation
X No vac-	T-7	-	-	+	+	D	1 pm	None	+48[d]
cine	T-26	-	+	+	+	D	1 mid	None	+16
No Rx	354	-	+	+	D	D	2 am	None	0
XI No vac-	360	-	-	-	-	-	1 mid	+8	No isolation
cine	356	-	+	+	-	-	2 am	+8	0
7-day Rx	373	-	+	+	-	-	1 mid	+8	+8[d]
XII No vac-	T-41	-	+	-	-	-	1 mid	+8	+16[d]
cine	T-45	-	+	-	-	-	1 mid	+8	+8
14-day Rx	T-47	-	+	-	-	-	2 am	+8	+8

a. Minus sign (-) indicates culture preceded first fever; plus sign (+) indicates culture followed first fever.

b. D indicates animal dead.

c. Preceding sample not obtained.

d. Preceding sample contaminated.

A. 1,300 ORGANISM DOSE GROUP

1. Morbidity and Mortality

Morbidity and mortality rates for non-vaccinates (NV) and vaccinates (V) are shown in Table III. One vaccinate failed to become ill and is known not to have been exposed due to a mechanical error.

TABLE III. MORBIDITY AND MORTALITY 1,300-CELL GROUP

GROUP		MORBIDITY		MORTALITY	
		No.	%	No.	%
Controls	NV[a]	6	100	6	100
	V	5	83	5	100
7-Day Therapy	NV	6	100	1[b]	17
	V	6	100	0	0
14-Day Therapy	NV	6	100	1[c]	17
	V	6	100	0	0

a. NV - non-vaccinated. V - vaccinated.

b. Died day 37 - tularemia.

c. Died day 25 - tularemia.

2. Disease Course

Temperatures, x-ray status, and blood culture findings are graphically presented in Figures 1 - 6. The incubation period averaged 60 hours with a range of 58 to 76 hours. There was no difference between vaccinates and non-vaccinates. Physical examination, in untreated controls and vaccinates at the onset of fever revealed anorexia and lethargy. Pulmonary rales developed shortly thereafter. Severe dehydration and hypothermia usually preceded death. All untreated vaccinates and non-vaccinates died within 6 to 9 days with the average time between onset of fever and death being 4.2 days with a range of 2.5 to 6.0 days. In contrast, the non-vaccinates and vaccinates receiving tetracycline became afebrile on an average of 20 hours after onset of therapy (range: 8 to 48 hours) and only a brief period of anorexia and lethargy was observed.

All but two of the infected animals developed abnormal chest roentgenograms, usually within 24 hours after onset of fever; the two exceptions were from the non-vaccinated, 7-day therapy group (Figure 3). In the untreated controls and vaccinates the chest films demonstrated rapid progression of miliary to diffuse multiple bronchopneumonia. In contrast, the 7- and 14-day therapy non-vaccinates and vaccinates, with similar initial lesions regressed under therapy. A difference was noted between the 7- and 14-day therapy groups in terms of extent of clearing at the completion of tetracycline, i.e., in 7 of 12 animals receiving 7 days of tetracycline (three non-vaccinates and four vaccinates) the x-ray lesions had not completely cleared when therapy was stopped;

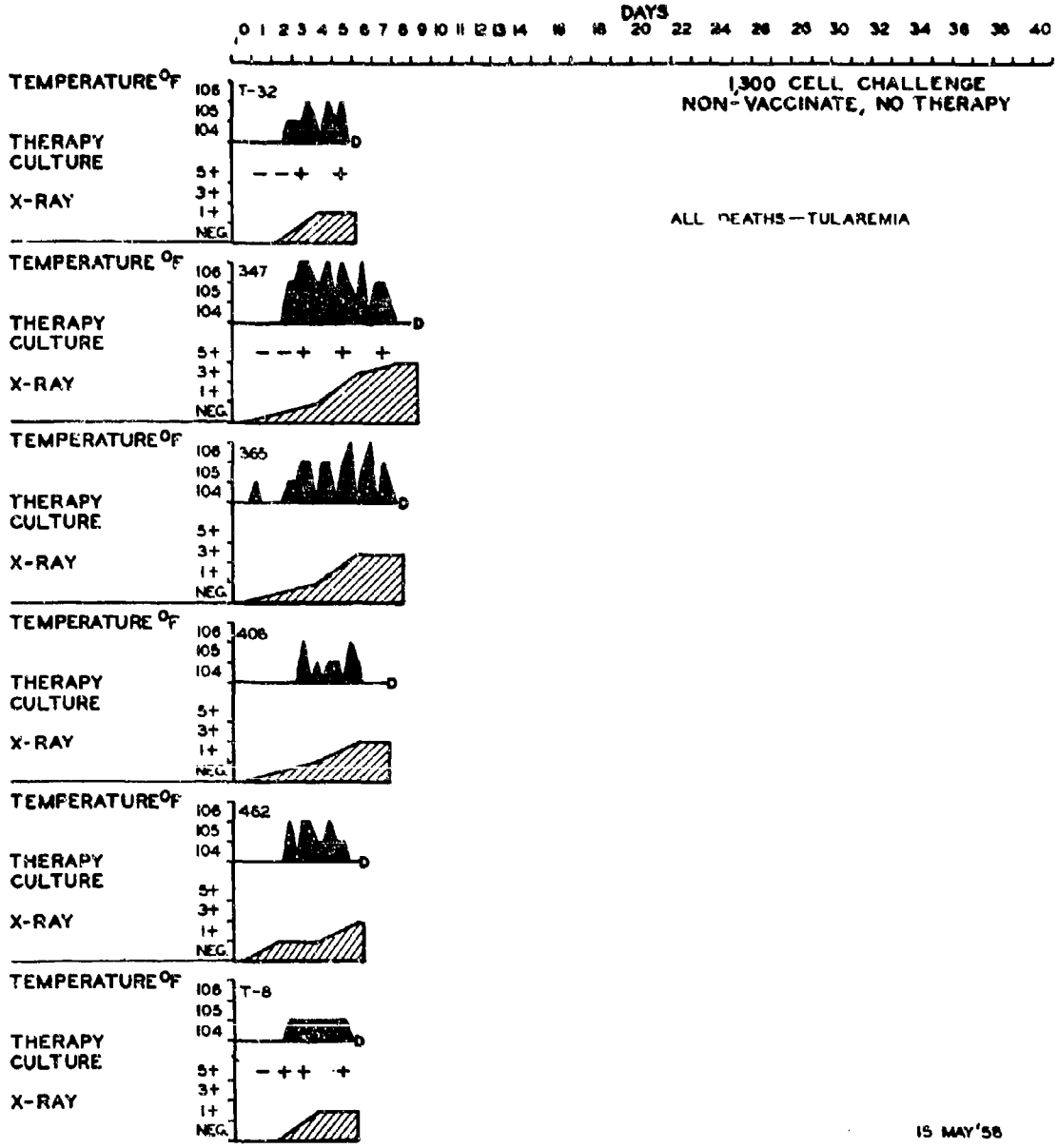


FIGURE 1. TEMPERATURES, BLOOD CULTURES, & X-RAY IN CONTROLS (1300 CELL CHALLENGE)

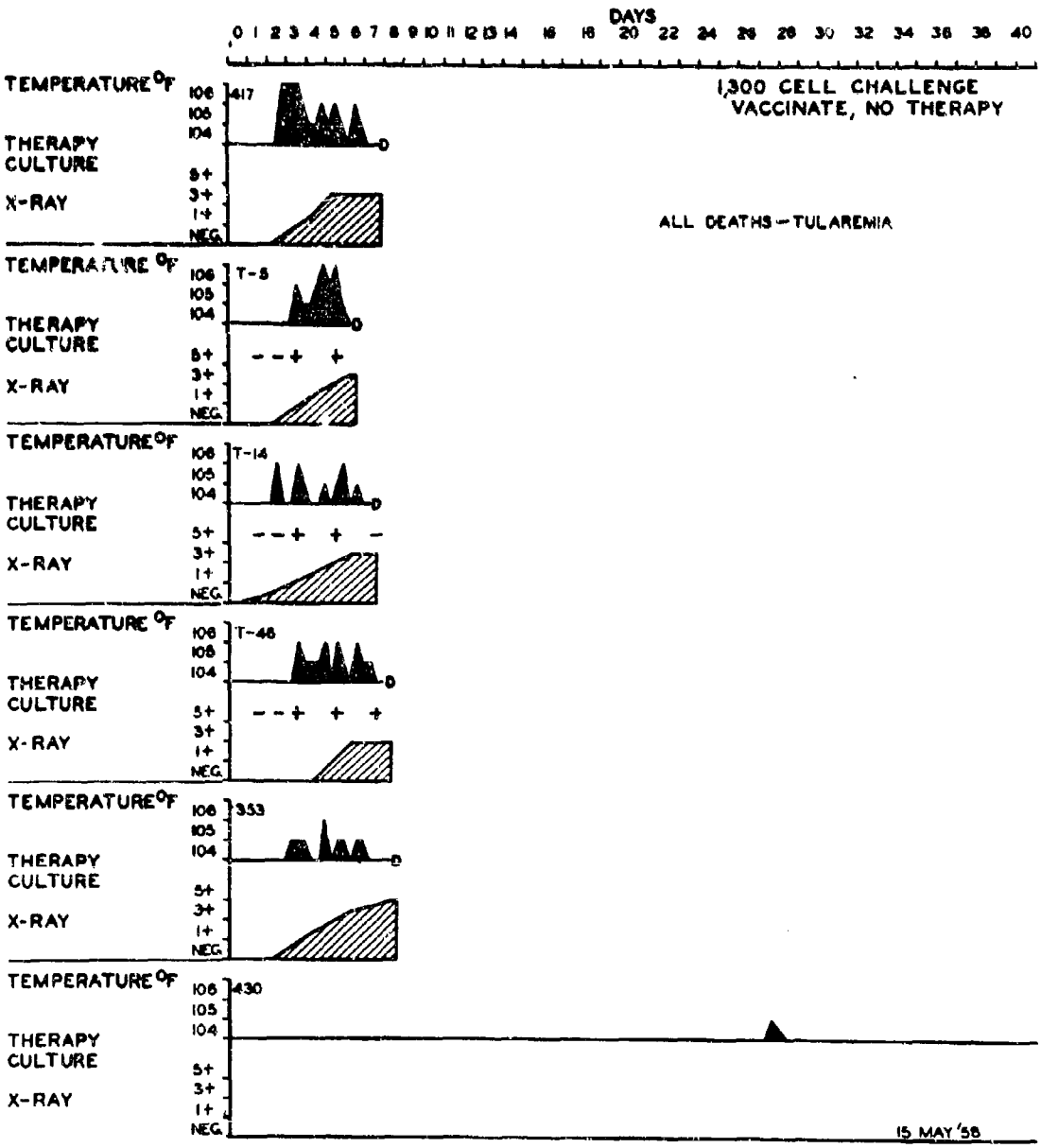


FIGURE 2. TEMPERATURES, BLOOD CULTURES, & X-RAY STATUS IN VACCINATES – NO THERAPY (1300 CELL CHALLENGE)

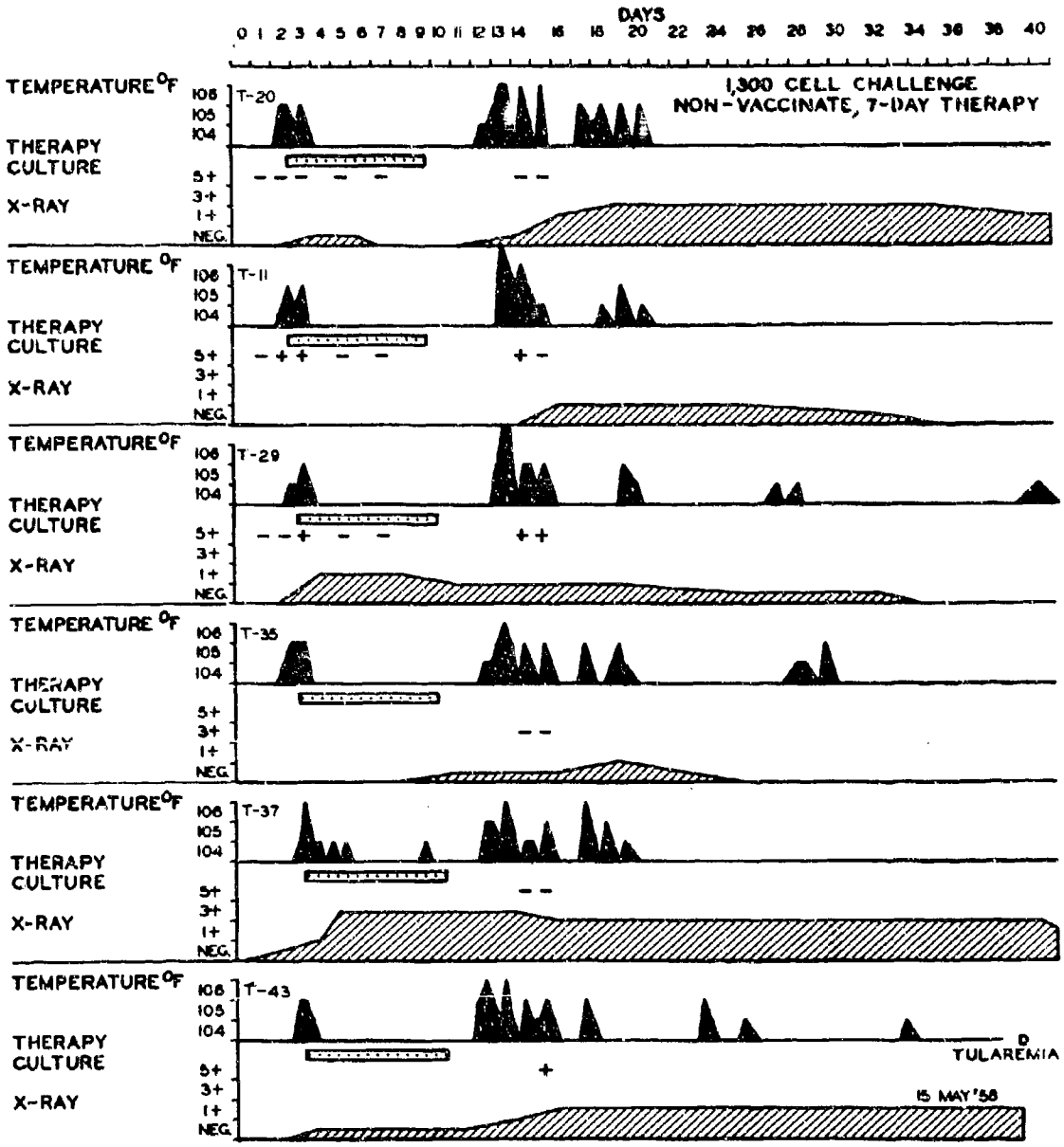


FIGURE 3. TEMPERATURES, BLOOD CULTURES, & X-RAY STATUS IN NON-VACCINATES — 7-DAY THERAPY (1300 CELL CHALLENGE)

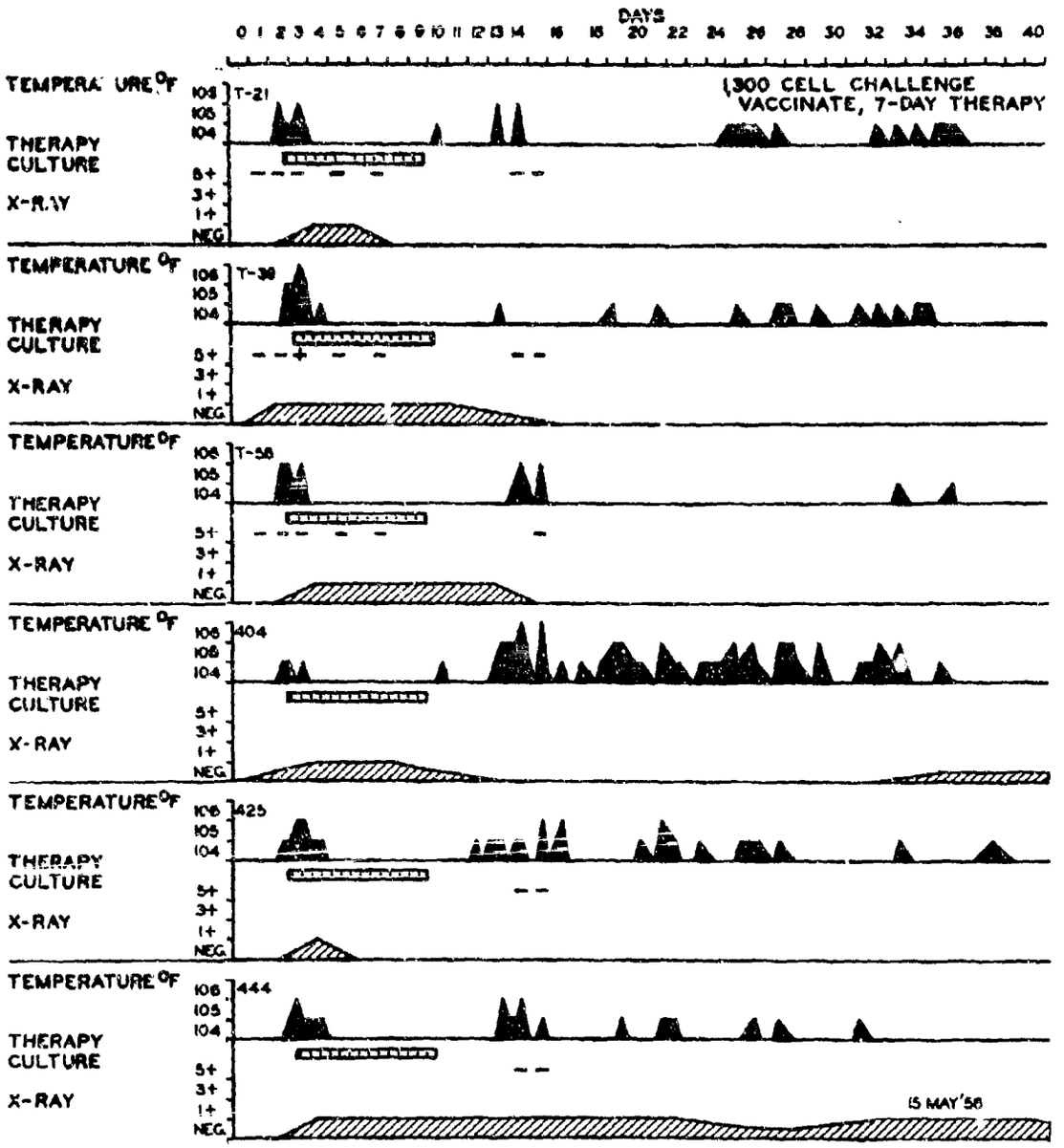


FIGURE 4. TEMPERATURES, BLOOD CULTURES, & X-RAY STATUS IN VACCINATES — 7-DAY-THERAPY
(1300 CELL CHALLENGE)

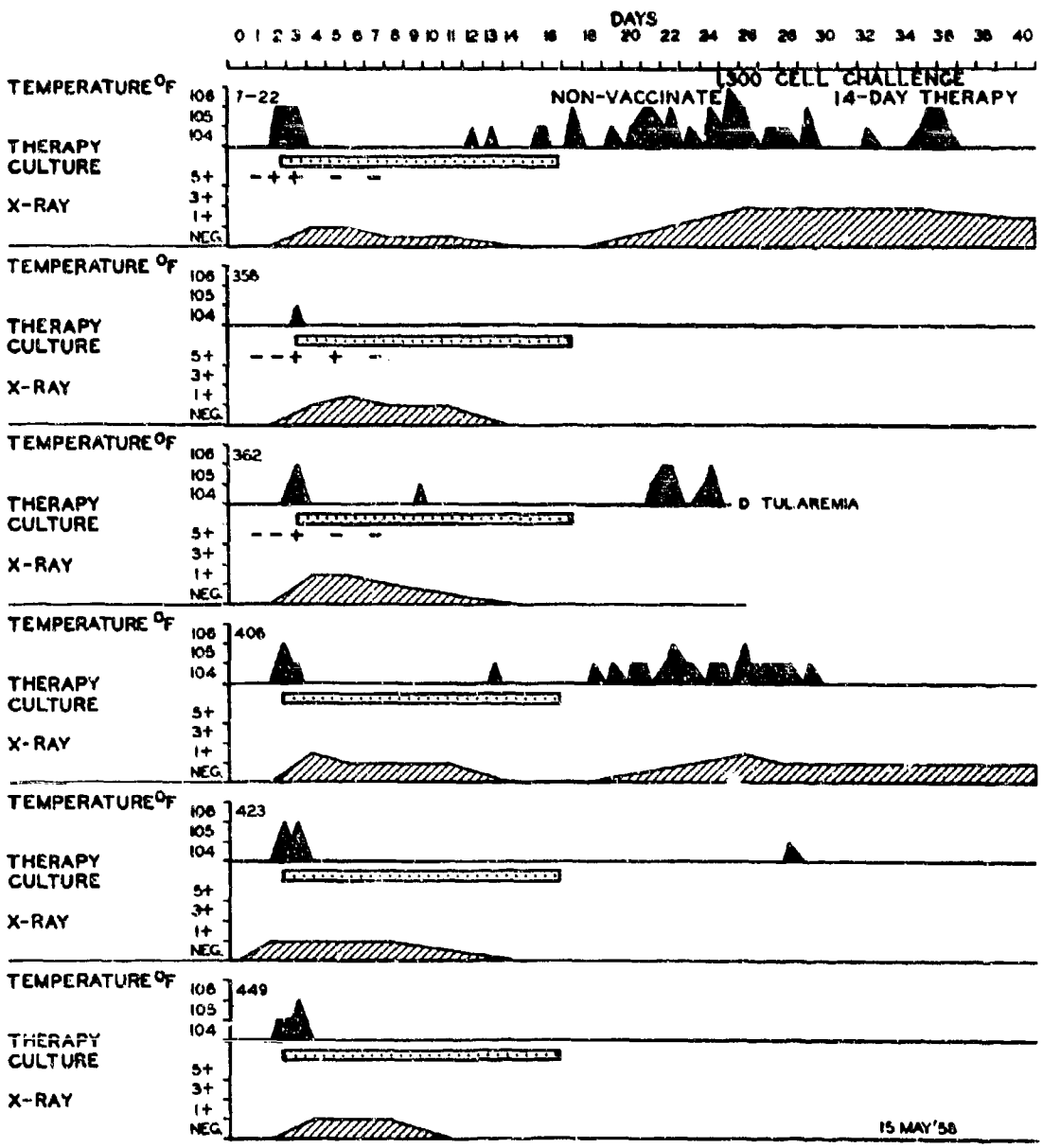


FIGURE 5. TEMPERATURES, BLOOD CULTURES, & X-RAY STATUS IN NON-VACCINATES - 14-DAY-THERAPY (1300 CELL CHALLENGE)

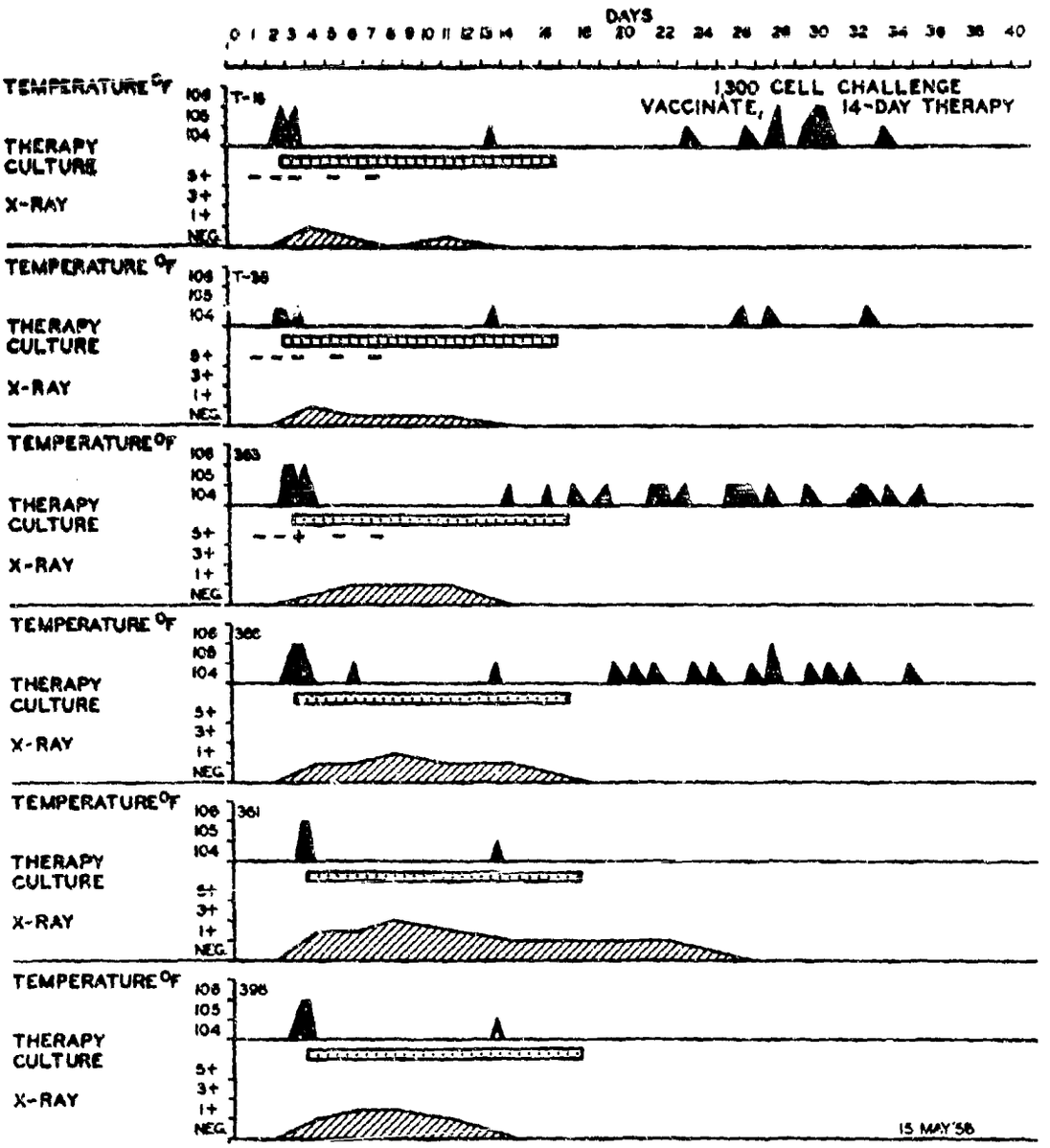


FIGURE 6. TEMPERATURES, BLOOD CULTURES, & X-RAY STATUS IN VACCINATES—14—DAY—THERAPY
(1300 CELL CHALLENGE)

whereas only one of 12 animals treated for 14 days had an abnormal chest film. Chest films were negative in this vaccinated monkey a week after completion of therapy.

Blood cultures in the untreated controls and vaccinates were positive during the first 24 hours of disease in the three animals tested from each group and persisted to death. In the therapy groups positive cultures were also obtained from 5 of 6 non-vaccinated and 2 of 6 vaccinates during the initial phases of and preceding illness. The organism could not be isolated from the blood from these animals after 24 to 48 hours of tetracycline therapy.

3. Disease Course Following Therapy

In the non-vaccinated 7-day therapy group all animals showed an elevation of temperature approximately four days after the drug was stopped. The individual temperature records in the post-therapy period are shown in Figure 7. There was reappearance, worsening, or persistence of chest x-ray abnormalities and several blood cultures were obtained. Although most of these animals did not appear particularly ill, the pattern is routinely that of a worsening of infection with one death on day 37.

In the vaccinates with 7 days of therapy the picture was somewhat modified. Temperature elevations were less evident, x-ray changes, less prominent, if present at all, and in the five animals examined at this presumed critical period, blood cultures were negative.

In the 14-day therapy group, 3 of 6 non-vaccinates relapsed within 1 to 4 days. One died after four days of fever and was found to have extensive tularemia. No contributory cause of death was apparent. Antibiotic blood levels were not obtained. The remaining two recovered after a 10- to 14- day febrile illness. Each had reappearance of abnormal chest roentgenograms, which slowly cleared over the next two months. The temperature pattern after completion of 14 days of treatment in the vaccinated animals is difficult to evaluate. If meaningful at all, it is certainly low grade; more importantly, the chest x-rays remained normal and there were no deaths.

In animal No. 358, non-vaccinated-14-day therapy, tetracycline administration was begun coincident with the initial fever, at which time the blood culture was positive. Although a second positive blood culture was obtained, this animal had no more fever and no relapse (Figure 5).

B. 130,000-ORGANISM DOSE GROUP

1. Morbidity and Mortality

Morbidity and mortality rates are shown in Table IV.

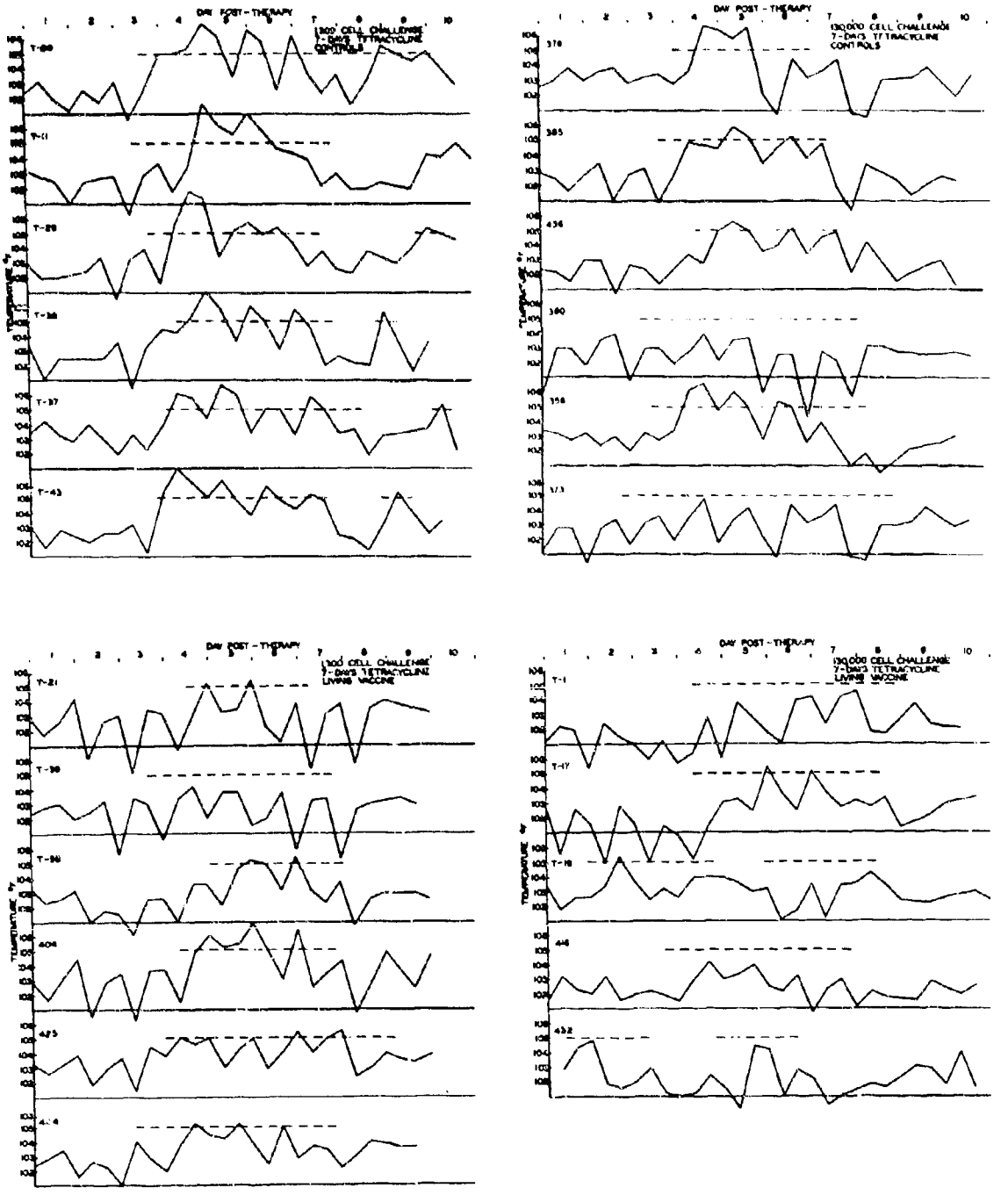


FIGURE 7 TEMPERATURES OF CONTROLS AND VACCINATES AFTER 7 DAYS OF THERAPY

TABLE IV. MORBIDITY AND MORTALITY 130,000 CELL GROUP

		MORBIDITY		MORTALITY	
		No.	%	No.	%
Controls	NV[a]	6	100	6	100
	V	6	100	6	100
7-Day Therapy	NV	6	100	1[b]	17
	V	6[c]	100	1[d]	20
14-Day Therapy	NV	6	100	0	0
	V[e]	6	100	0	0

a. NV - non-vaccinated. V - vaccinated.

b. Moribund and sacrificed day 27.

c. One animal aspirated tetracycline and died 2nd day. This animal is not included in tularemia mortality figures.

d. Therapy begun 32 hours after initial fever. Died day 67 of tularemia.

e. Therapy delayed for 16 hours in two animals - T-9 and 438.

2. Disease Course

Temperature, x-ray status, and blood culture findings are graphically presented in Figures 8 - 13. The incubation period averaged 40 hours (range: 28 to 60), in contrast to 60 hours in the 1,300 cell challenge group. Physical findings in untreated controls and vaccinates were similar to those found in the 1,300-cell challenge groups. Pulmonary rales were heard shortly after onset of fever, and severe dehydration with hypothermia preceded death. All of the animals in the untreated groups died 4 to 5 days post challenge. The average time between onset of fever and death was 2.5 days, with a range of 1.5 to 3.5 days. In contrast, treated non-vaccinates and vaccinates became afebrile in an average of 32 hours (range: 8 to 64); however, approximately one-half of the animals in each group had an occasional temperature elevation during tetracycline therapy.

All but one, a non-vaccinate, had abnormal chest roentgenograms within 24 to 48 hours of onset of fever. In the untreated controls and vaccinates the initial pulmonary abnormalities were more diffuse than in the lower dose challenge groups. In all treated animals, as in the 1,300-cell challenge group, the progression of pulmonary involvement was sharply curtailed. Animals treated for 14 days usually had normal chest films at completion of therapy (Figures 12 and 13); this was the exception following 7 days of therapy (Figures 10 and 11).

Blood cultures in the untreated controls and vaccinates were positive during the first 24 to 48 hours of disease (3 monkeys from each group). Cultures were negative after 24 to 48 hours of therapy.

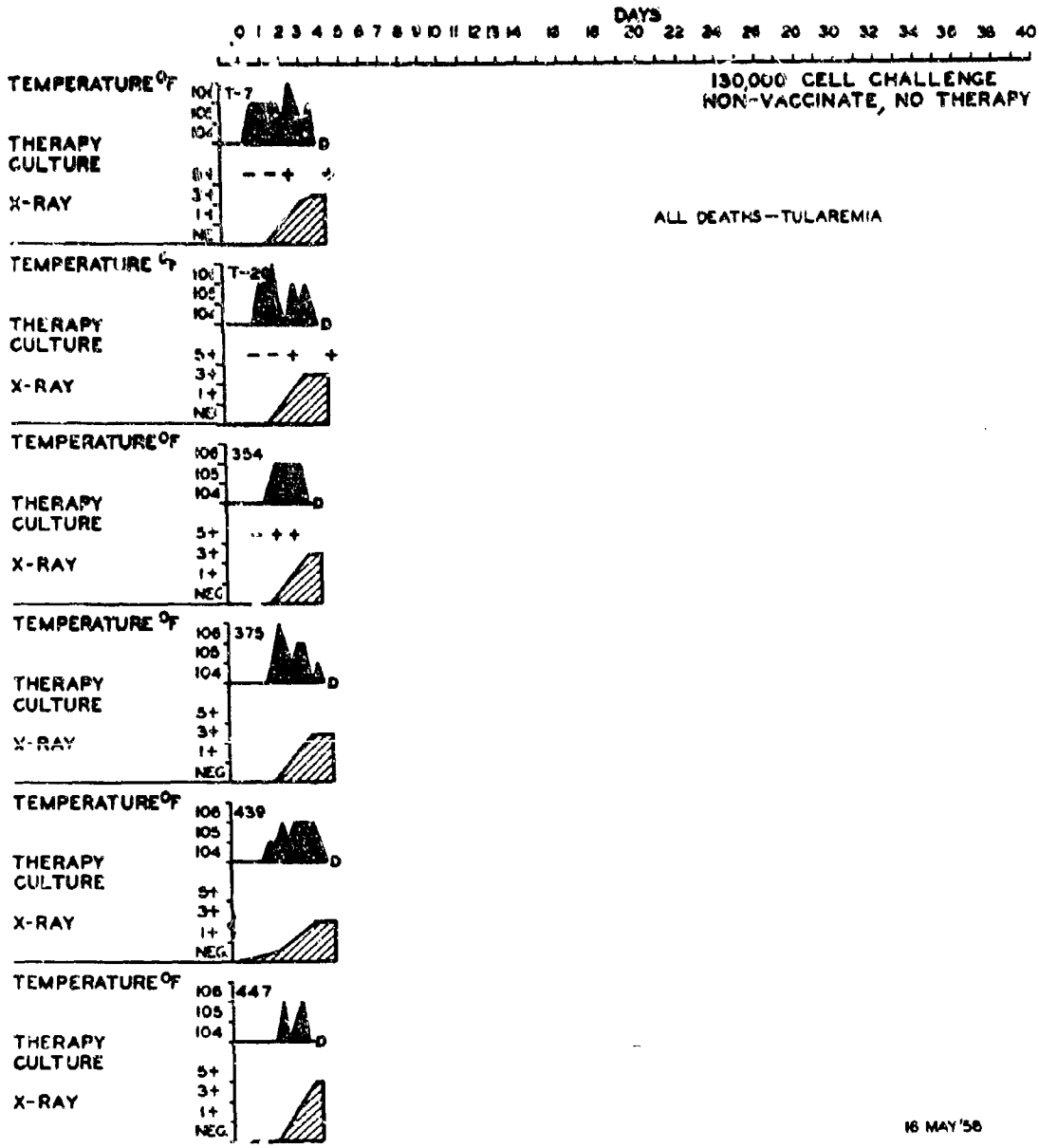


FIGURE 8. TEMPERATURES, BLOOD CULTURES, & X-RAY STATUS IN CONTROLS (130,000 CELL CHALLENGE)

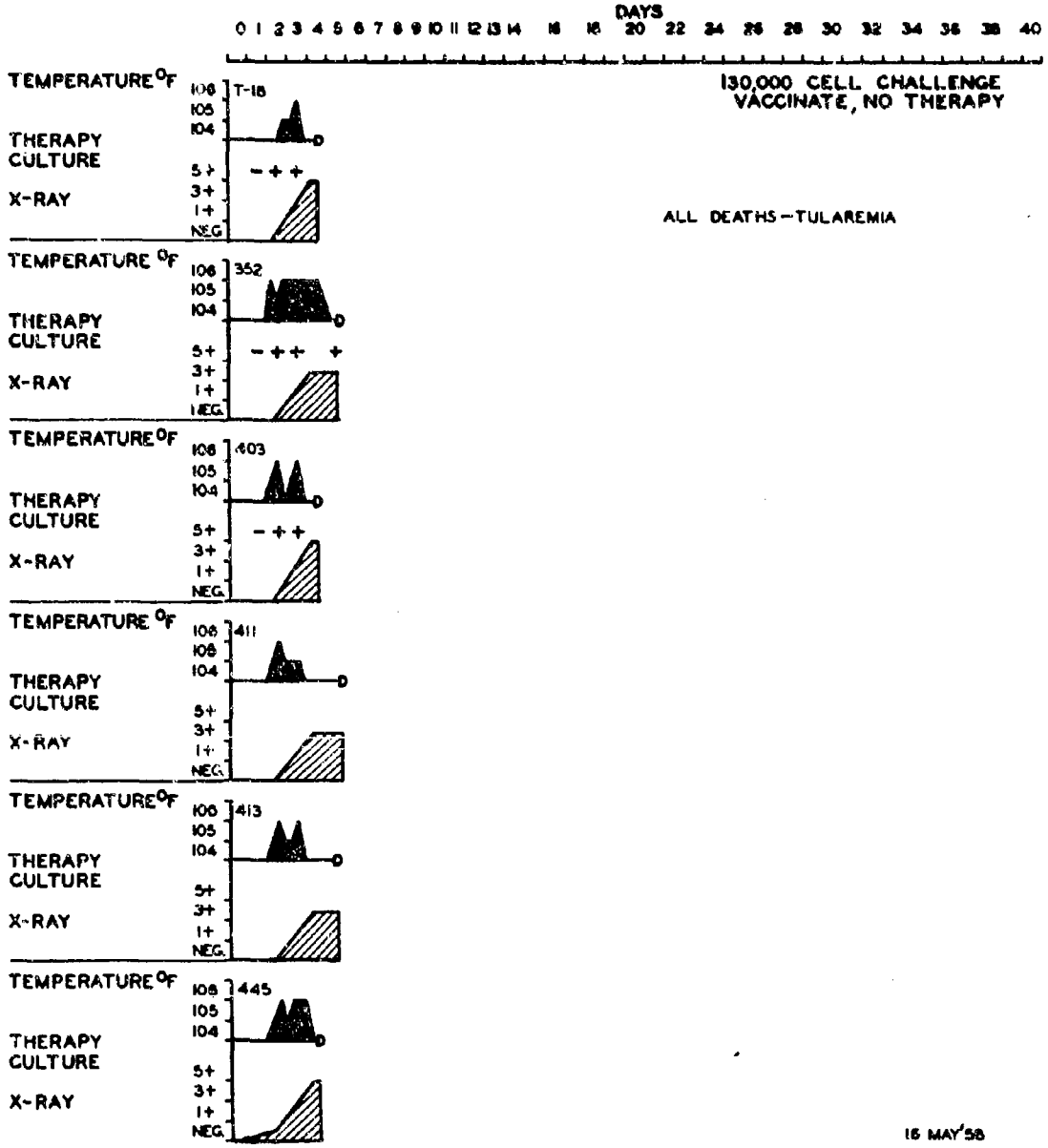


FIGURE 9. TEMPERATURES, BLOOD CULTURES, & X-RAY STATUS IN VACCINATES – NO THERAPY
(130,000 CELL CHALLENGE)

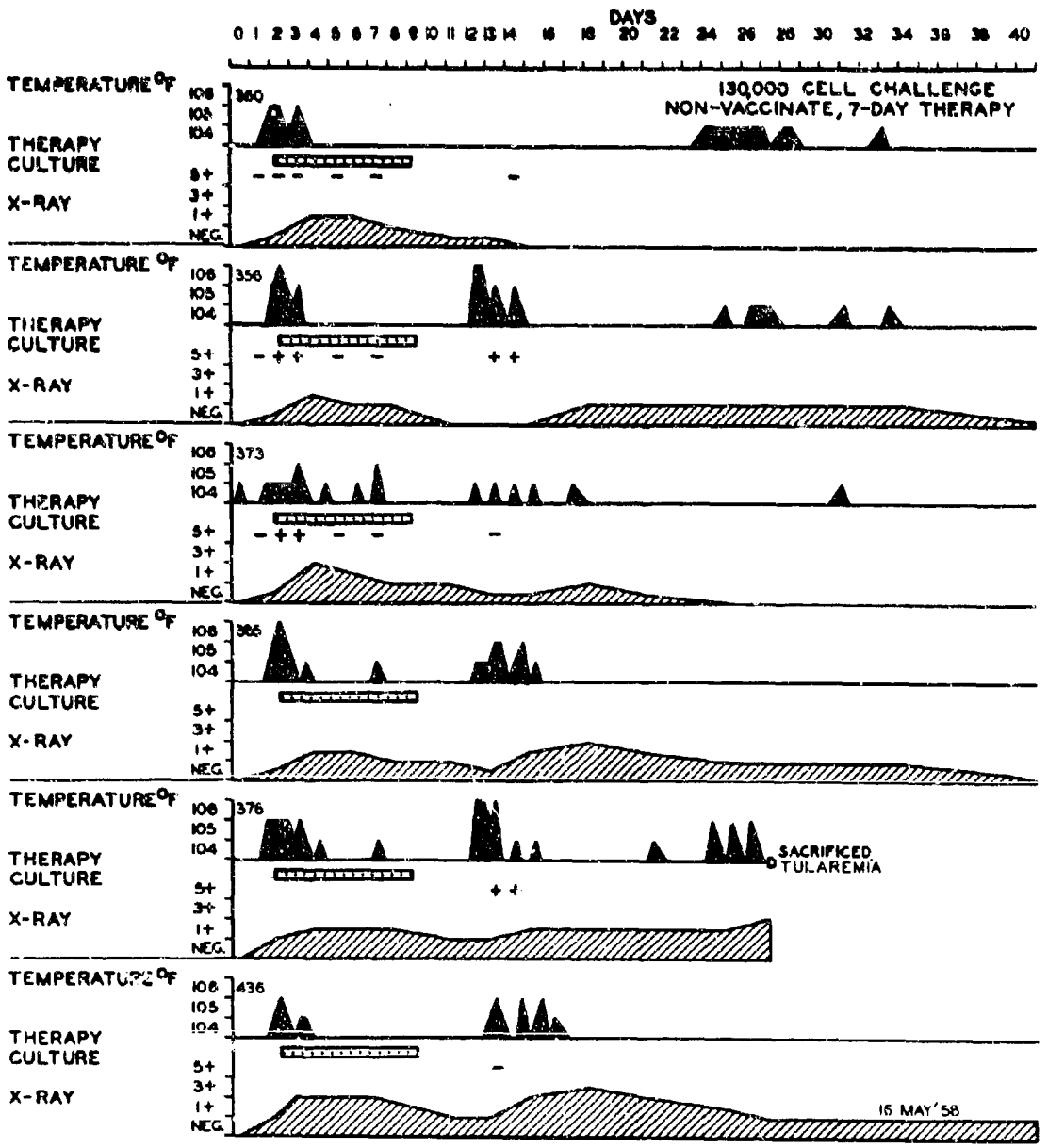


FIGURE 10. TEMPERATURES, BLOOD CULTURES, & X-RAY STATUS IN NON-VACCINATES - 7-DAY-THERAPY (130,000 CELL CHALLENGE)

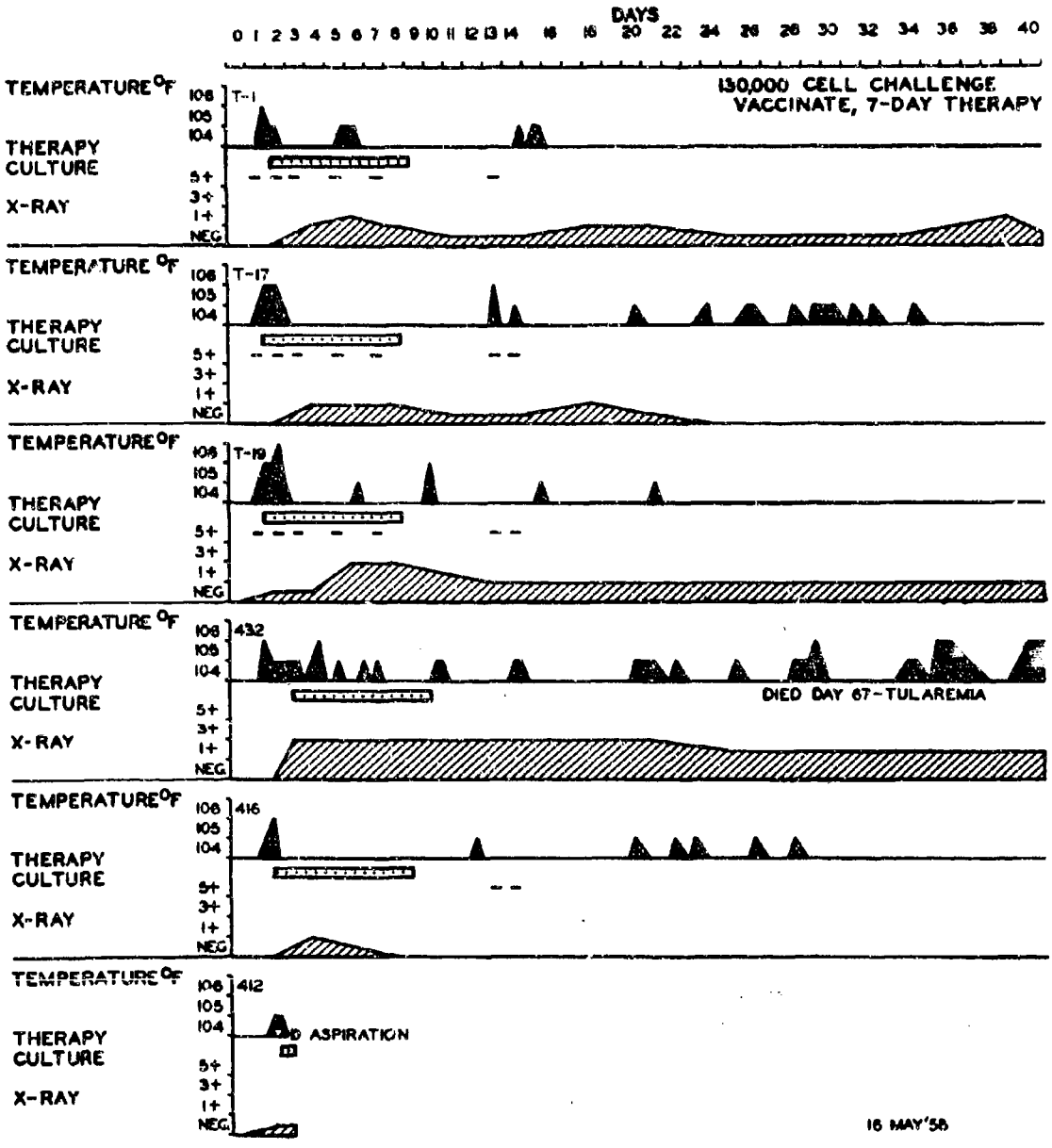


FIGURE 11. TEMPERATURES, BLOOD CULTURES, & X-RAY STATUS IN VACCINATES — 7-DAY-THERAPY
(130,000 CELL CHALLENGE)

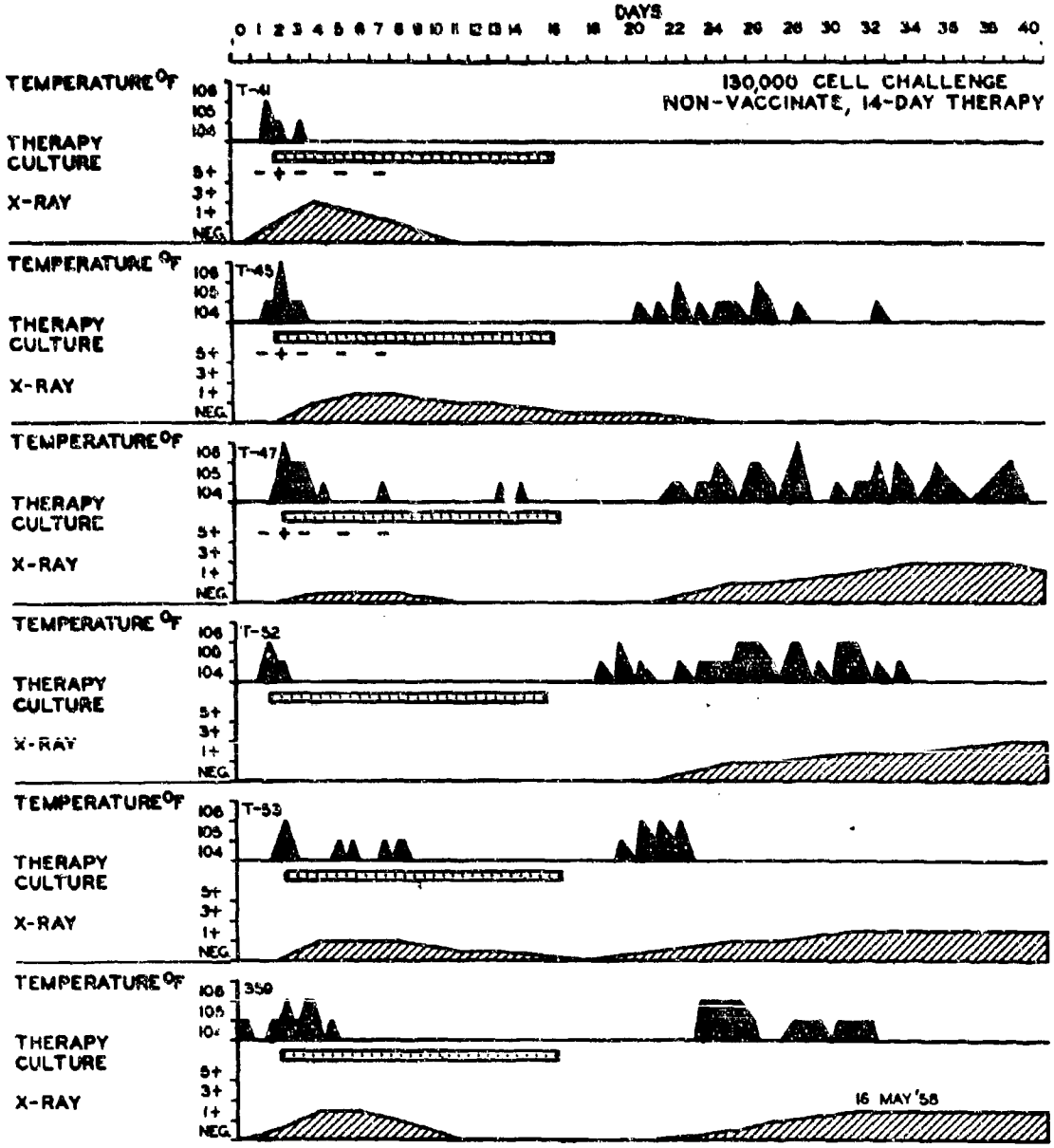


FIGURE 12. TEMPERATURES, BLOOD CULTURES, & X-RAY STATUS IN NON-VACCINATES-14-DAY-THERAPY (130,000 CELL CHALLENGE)

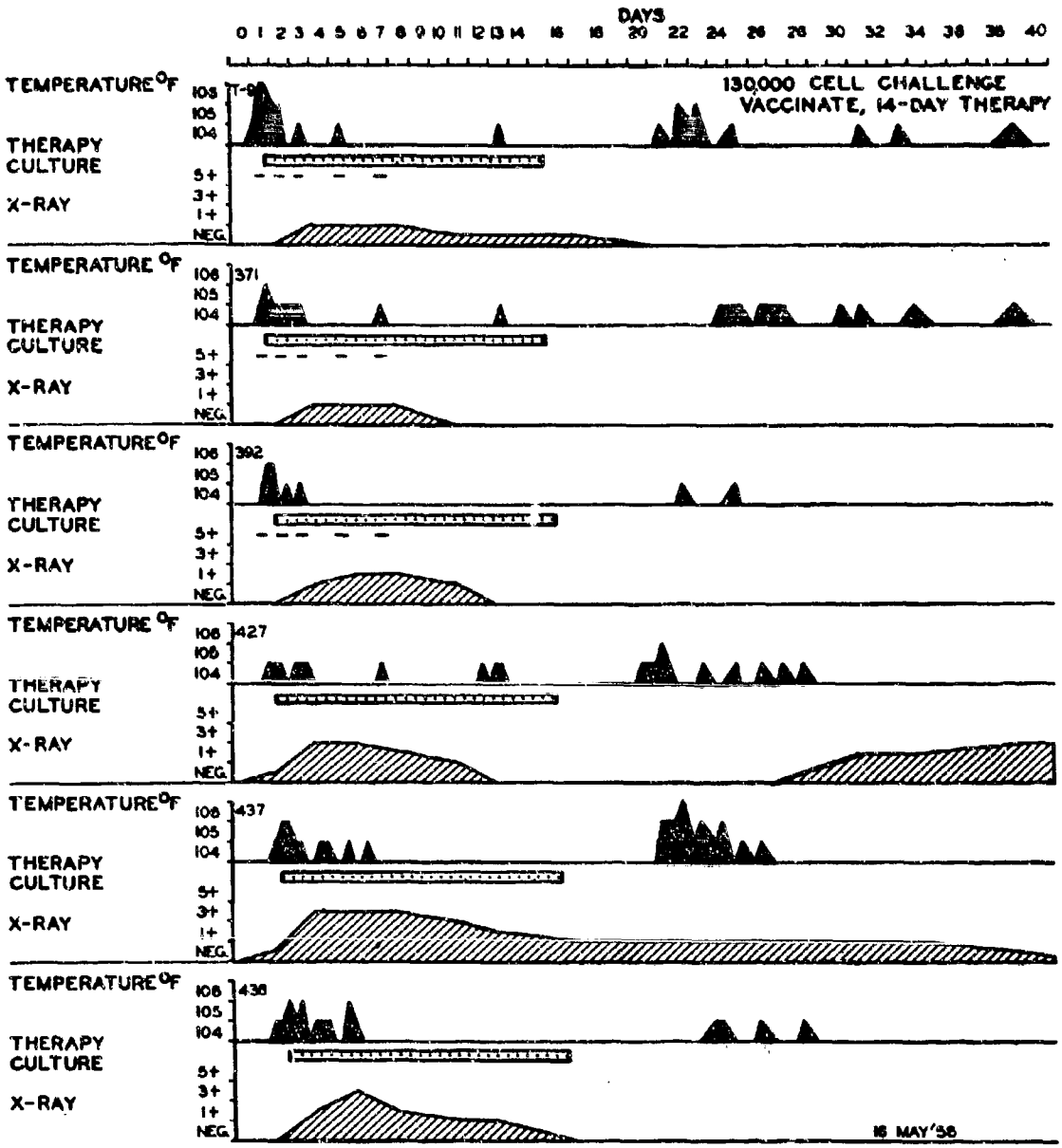


FIGURE 13. TEMPERATURES, BLOOD CULTURES, & X-RAY STATUS IN VACCINATES—14—DAY—THERAPY
(130,000 CELL CHALLENGE)

3. Disease Course following Therapy

Temperature patterns after seven days therapy are shown in Figure 7. Of those receiving 7 days of therapy, positive blood cultures were obtained only in the non-vaccinates, and a more marked febrile response on cessation of therapy is evident when compared to the vaccinates; there was no apparent difference in the chest x-ray findings in the two groups. One animal (No. 376) in the non-vaccinate, 7-day therapy group became moribund and was sacrificed on day 27. One animal (No. 432) in the comparable vaccinated group had a 6-week febrile illness and died on day 67 following an episode of diarrhea. At autopsy there were no gross lesions seen in the lungs of these two animals while the hilar nodes contained focal lesions from which P. tularensis was recovered. By mistake there had been a delay in initiation of therapy in the latter animal, the drug not having been started until 32 hours after onset of fever.

In the 14-day therapy groups there were no deaths. There was a recurrence of prolonged fever and roentgen positivity in four of the non-vaccinates and in two vaccinates after therapy was stopped.

C. GROSS AUTOPSY FINDINGS

Monkey No. 412, expiring (by drug aspiration) soon after the initial febrile response is of interest because of the minimal changes seen in the lungs. This animal belonged to the high dose vaccinated group and had received only one previous dose of drug. Histologically there were multiple small focal areas of bronchopneumonia. Positive cultures were obtained from both spleen and liver.

All 24 of the untreated animals died 4 to 9 days post-challenge with tularemia. Grossly, there appeared to be somewhat less evident involvement of the hilar and mediastinal nodes in the high-dose animals. There was no obvious difference between vaccinates and non-vaccinates at the two dose levels.

Among the 48 treated animals four died of tularemia. In two low-dose animals only some of the lobes of the lungs were involved; definite hilar node involvement was seen. In the high-dose animal sacrificed on day 27 all lobes were involved and the hilar nodes grossly were little changed. In animal No. 432, high-dose group, dying on day 67, the lungs showed little gross change; the hilar nodes were prominent.

A non-specific and fulminating diarrhea (Shigella) caused the death of seven animals between 30 and 80 days post-challenge; four of these animals had minimal residual lesions of tularemia. Twenty-seven animals were sacrificed 80 to 133 days post-challenge; only six had gross evidence of tularemia. The extent and type of late, gross, residual tularemia lesions did not differ between the groups. Histologic examinations are not completed. Nine animals are still alive.

D. TETRACYCLINE BLOOD LEVELS

Tetracycline blood level determinations are tabulated in Table VI. At 16 hours after onset of therapy (125 mg of tetracycline hydrochloride every 8 hours orally) the average blood level was 1.6 µg%; at 24 hours, 3.1 µg%; at 48 hours,

2.3 µg%; at 72 hours, 2.4 µg%; and at 6 days 2.3 µg%. In those receiving 14 days of tetracycline, the average levels at 9 days were 3.7 µg%, and at 13 days 3.8 µg%.

TABLE VI. TETRACYCLINE BLOOD LEVELS (BIO-ASSAY)
(125 mg tetracycline HCl given orally every 8 hours)

GROUP	MONKEY NO.	TETRACYCLINE BLOOD LEVELS (µg/ml) After Therapy Started							AVERAGE
		Hours				Days			
		16	24	48	72	6	9	13	
7-DAY THERAPY									
1,300 cells									
CONTROL	T-37	<0.5[a]	2.1	1.4	5.6	<0.5			2.0
	T-43	3.0	3.9	3.0	3.9	0.5			2.9
VACCINE	425	2.1	1.2	2.5	1.4	2.5			1.9
	444	1.4	ND[b]	<0.5	1.8	1.4			1.3
130,000 cells									
CONTROL	436	1.4	2.5	2.5	2.5	1.4			2.1
VACCINE	416	1.2	3.9	3.6	3.0	2.5			2.8
	432	0.5	1.4	1.2	0.5	2.1			1.1
14-DAY THERAPY									
1,300 cells									
CONTROL	449	2.5	3.0	3.0	1.4	3.9	4.7	4.7	3.3
	423	1.8	3.0	1.8	1.0	3.0	2.5	2.5	2.2
VACCINE	398	1.2	3.6	3.6	3.0	3.0	5.6	3.0	3.3
	361	2.5	4.7	3.6	3.6	3.0	4.7	5.6	3.9
130,000 cells									
CONTROL	359	1.2	1.8	2.1	1.2	1.2	3.0	ND	1.7
	T-53	1.2	3.9	2.5	2.5	1.4	2.5	3.0	2.4
VACCINE	438	2.5	>5.6	1.4	2.1	2.1	3.0	5.6	3.2
	437	<0.5	ND	ND	ND	ND	ND	2.1	---
AVERAGE		1.6	3.1	2.3	2.4	2.0	3.7	3.8	

a. <0.5 used as 0.5 in averages.

b. ND indicates no data.

The average tetracycline blood level at 16 hours was lower than expected; it will be noted that three animals were very low (<1.0 µg/ml) at this time (T-37, 432 and 437); for example, T-37, a low-dose, 7 day therapy control, showed the most extensive x-ray changes in this group; No. 432, a high-dose, 7-day therapy vaccinate, was unintentionally given therapy late; and No. 437, a high-dose, 14-day therapy vaccinate, was not followed for additional levels but his clinical course was one of the poorest short of death. This animal died of tularemia on day 67.

IV. DISCUSSION

Examination of the incubation periods at the two dose levels shows a clear dose dependence (Figure 14). This is in contrast to observations made and reported in an earlier study[1]. Although not plotted on the scatter diagram the incubation periods of the vaccinated animals were essentially identical to the controls.

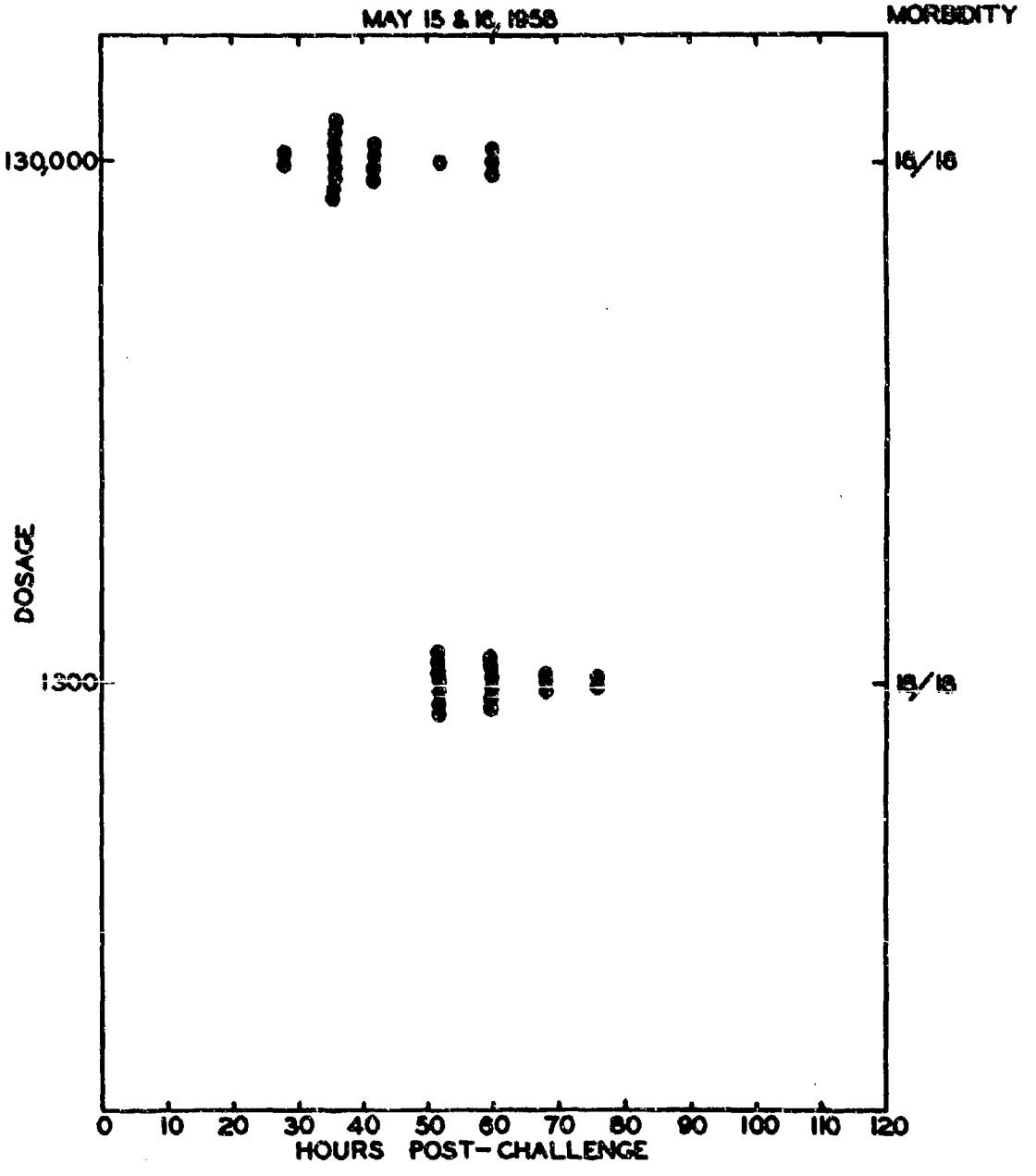
All of the untreated animals died and the clinical courses in the vaccinates closely paralleled those seen in controls. This, too, is in contrast with earlier studies wherein it was noted that animals receiving the viable vaccine survived a respiratory challenge with P. tularensis (SCHU 4). There are too many variables to permit an explanation. The vaccine employed may have lost "virulence" in the interval between use in the two studies, but a point believed to be of more importance lies in the difference in the challenges. Reasons have been advanced to indicate that the inhaled respiratory dose in the earlier study[1] may have been much smaller than the nominal figures given by the impinger counts. In any event the present protocol fails to show any evidence of vaccine protection, insofar as fatality rate, incubation periods, and time to death may be concerned. In the animals examined the same is true for time of blood culture positivity.

There is again a suggestion that the pathogenesis differs with large and small challenge doses. For example, in Table II showing time of blood culture positivity with respect to onset of fever, it may be seen that positive blood cultures were frequent prior to onset of fever in the low challenge dose animals but often lagged until after fever was established in the high dose animals. Monkey No. 412 dying, as a result of drug aspiration shortly after the onset of fever following a high dose challenge, showed the primary findings to be limited to the lungs in the form of numerous small bronchopneumonic patches with only minimal lesions in the mediastinal nodes. Roentgen examination also shows more marked and diffuse involvement in the high dose animals and all the findings are in consonance with a period of primary proliferation of P. tularensis in the lung, followed by a septicemic phase.

The primary response to drug administration was excellent in all groups, and if the single animal (No. 432) in which therapy was delayed is discounted, there is no apparent difference among the eight treated groups with regard to the initial response to drugs.

The four groups of animals receiving seven days of therapy generally showed some evidence of recrudescence of fever within 4 to 5 days after the drug was stopped (Figure 13). This finding closely parallels the finding in man treated in a similar manner[5]. Fever was the only clinical sign of disease noted during the post-7-day therapy period. Using this criterion alone the less severe "relapses" were seen in the high-dose vaccinates, and the more severe in the lose-dose controls. Both vaccinated groups seemed to fare better than the non-vaccinated. During this "relapse" period positive blood cultures were obtained from non-vaccinates, and none, from the vaccinates.

FIGURE 14.
DISTRIBUTION OF INCUBATION PERIODS BY DOSAGE AND HOURS POST-CHALLENGE (NON VACCINATED)



With 14 days of therapy this difference between vaccinates and non-vaccinates, if it be in fact a difference, is less obvious. Possibly the vaccinates have slightly modified post-therapy courses as indicated by fever, less recurrence of positive roentgen films of the lung, and no fatalities. The findings may be interpreted as showing some effect of prior vaccination in modifying the course of illness under conditions in which the vaccine alone does not prevent death in any of the challenged animals. It would appear that to test this hypothesis a therapy course of less than seven days would be required combined with a larger challenge dose.

V. CONCLUSIONS

1. Under the challenge conditions used the incubation period of respiratory-induced tularemia in monkeys varied inversely with the size of the challenge dose. The time between exposure and death varied in a similar manner.

2. The clinical response to the broad spectrum antibiotic tetracycline was identical at the challenge levels of 1,300 and 130,000 cells.

3. With either challenge level seven days of drug therapy was sufficient to prevent death in most animals.

4. Prior vaccination with a viable P. tularensis vaccine did not prevent death of challenged monkeys or in any way alter the clinical course in untreated animals. Following courses of therapy the vaccinated animals perhaps showed less evidence of recrudescence of disease than did the unvaccinated animals.

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1. Army Medical Service Activities Report, USAMU (9906.05), "Studies on Pasteurella tularensis, Living Vaccine and Tetracycline Therapy Evaluation in Macaca mulatta." (Overholt, Eigelsbach, Hornick, Hughes), January, 1959.

2. Ibid, "Studies on Pasteurella tularensis, Evaluation of a Living Vaccine for Tularemia." (Hornick and Vosti), January, 1959.

3. Ibid, "General Reports, Characteristics of Aerosol Exposure Equipment." (Gochenour), January, 1959.

4. Ibid, "Studies on Pasteurella tularensis, Effect of Duolite Treatment on Growth Promoting Properties of Blood and Plasma." (Ward, Tresselt and Gaspar), January, 1959.

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STUDIES ON PASTEURELLA TULARENSIS

INOCULATION OF EMBRYONATED EGGS
(Yager)

I. INTRODUCTION

Studies of Pasteurella tularensis in embryonated eggs were undertaken to ascertain whether (1) chick embryo inoculation (yolk sac) offered any advantage as a diagnostic method for tularemia, (2) streptomycin-resistance modified the response, (3) there was any evidence of streptomycin-dependence, and (4) titration with a living-vaccine might have value in vaccine assay.

Embryonated hen eggs were inoculated with varying numbers of P. tularensis of three different strains: streptomycin-sensitive, streptomycin-resistant, and living-vaccine. The characteristics of these strains have been described elsewhere.

II. MATERIALS AND METHODS

All inoculations were made via the yolk sac route using embryonated hen eggs (CE) of various ages. Appropriate dilutions were made in gelatin-saline solution; 0.2 ml amounts were inoculated as promptly after dilution as possible. Control counts were obtained by culture of aliquots of each dilution on glucose cysteine blood agar (GCBA) and checked by mouse inoculation.

The virulent strains of P. tularensis were 24-hour cultures from GCBA slants. The living-vaccine strain had been lyophilized January 17, 1958; it was used immediately following rehydration.

The eggs were candled twice daily, following inoculation; films were made from eggs found non-viable, stained, and examined, to eliminate contaminants.

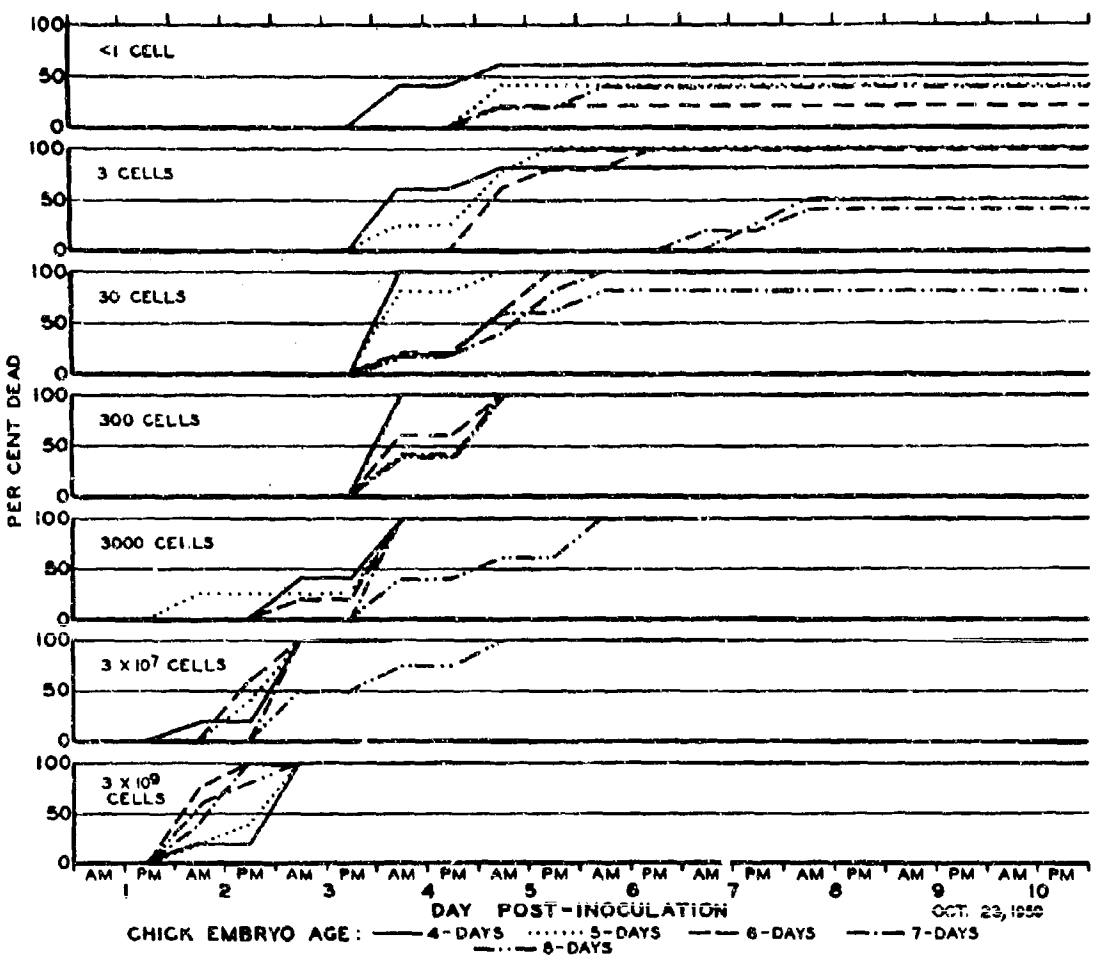
III. RESULTS

A. STREPTOMYCIN-RESISTANT STRAIN

Embryonated eggs (CE), 4, 5, 6, 7, and 8 days old, were inoculated via the yolk sac route in groups of five each with doses varying from one to 3 billion cells; the results are shown in Figure 1. With the largest inoculums all embryos were dead by the morning of the third day. A distinct delay in death was evident in the 8-day CE when 3×10^7 and 3×10^3 organisms were inoculated. When 300 cells were inoculated all 4- and 5-day embryos were dead by the morning of the third day, while the 6-, 7- and 8-day CE survived until the morning of the fifth day. At the 30-cell level the 100 per cent death time of the 4-day CE was four days compared to six days for the 7-day CE; there was one survivor from the 8-day CE group at the 30-cell challange level.

Using the same streptomycin-resistant strain the effect of adding streptomycin was examined in 5-day CE. The drug was added at the level of 250 µg/ml of inoculum. At all titration levels, differences to time of death of embryos with and without added streptomycin were insignificant. The actual values show a minimal increase in survival time of those CE given streptomycin.

FIGURE 1. PATHOGENICITY OF P. TULARENSIS (STREPTOMYCIN-RESISTANT) IN CHICK EMBRYOS OF VARIOUS AGES



B. LIVING-VACCINE STRAIN

Various dilutions of a rehydrated, vaccine strain of P. tularensis were inoculated into 6-day CE (10 at each dilution). The number of cells per egg ranged from 2 to 21,000, based upon plate counts. The 2-cell nominal dose killed 7 of 10 embryos; as shown in Figure 2 the time to death at higher dilutions was a function of the size of the dose. Mice were inoculated with aliquots of these dilutions; at eight days in the 2,000-cell group 2 of 30 were dead, while with 20,000 cells 6 of 30 were dead.

C. STREPTOMYCIN-SENSITIVE STRAIN

In another titration, streptomycin-sensitive and living-vaccine strains were inoculated into groups of 10- to 11-day CE (no difference was observed between these two ages) at doses ranging from 3 to 11,000 cells. Five CE were used at each challenge level for the streptomycin-sensitive strain and 10 at each level for the vaccine strain. The results are shown in Figure 3. All CE inoculated with 11,000 cells of the streptomycin-sensitive strain were dead on the morning of the sixth day at which time CE inoculated with an equal number of attenuated organisms were just beginning to die. By the seventh day, the maximum kill from all doses had been obtained with the virulent strain. Some CE survived at the 110-cell level, and below, with both strains. Aliquots of the vaccine strain were inoculated into white mice; with a dose of 1,750 organisms 1 of 30 mice died, while with 17,500 cells 5 of 30 died.

IV. DISCUSSION

Within the scope of this study, the age of the chick embryo at time of yolk sac inoculation influences the outcome with all strains of P. tularensis examined. The "break point" appears to be between eight and ten days; older CE may survive challenge with the living-vaccine strain. With younger CE the time to death is a function of the size of the inoculum.

The time to death of embryos is such that CE inoculation does not appear to offer any advantages over direct culture or guinea pig inoculation as a diagnostic procedure. No effort was made to determine the presence of organisms prior to death.

Based on time to death of CE there is no evidence that the streptomycin-resistant strain has any streptomycin-dependence.

Regardless of age of CE or dose level, when comparable numbers of cells are injected via the yolk sac the virulent strain kills more rapidly than does the vaccine strain. It would seem that CE inoculation might be useful in the study of attenu ation for the development of vaccine strains.

Following inoculation with the attenuated strain, the prolonged time to death of older CE, if death does ensue, should provide a useful method for checking the presence of contaminants in vaccine preparations.

FIGURE 2. PATHOGENICITY OF VACCINE STRAIN OF P. TULARENSIS IN 6-DAY CHICK EMBRYOS

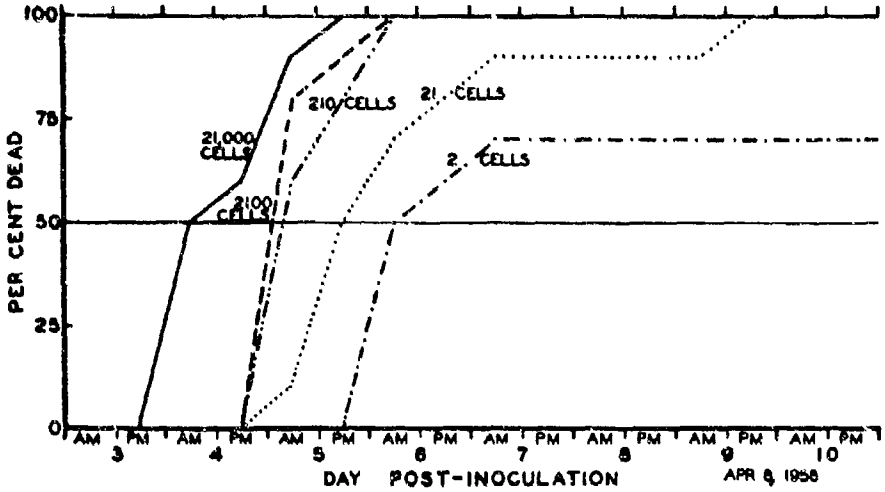
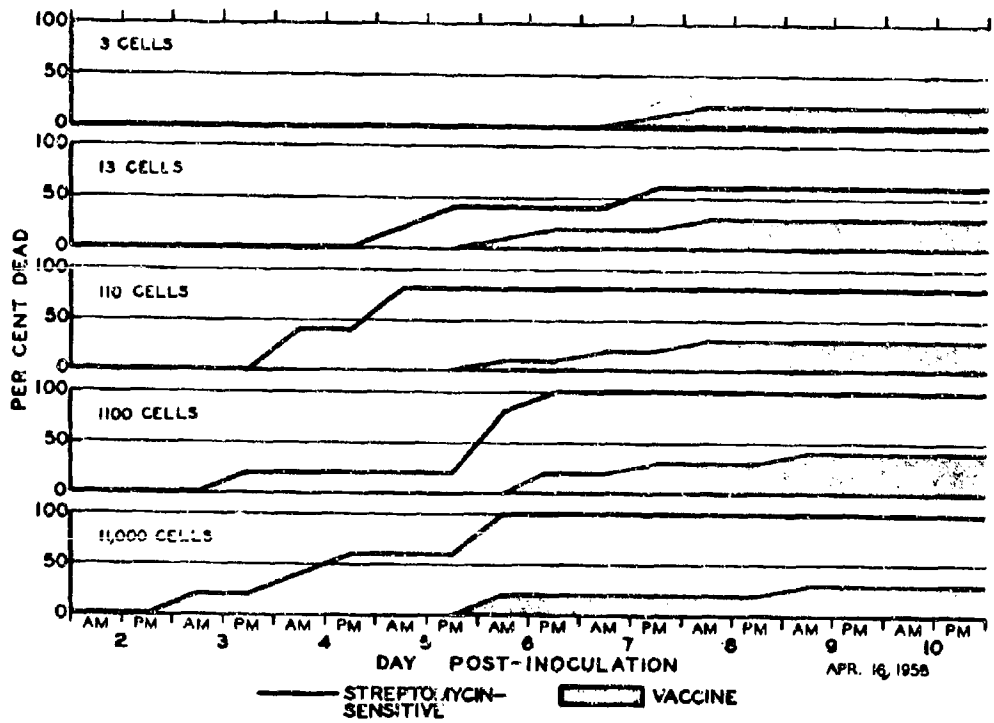


FIGURE 3. COMPARATIVE PATHOGENICITY OF STREPTOMYCIN-SENSITIVE AND VACCINE STRAINS OF P. TULARENSIS IN 10-TO 11-DAY CHICK EMBRYOS



V. CONCLUSIONS

The response obtained following inoculation via the yolk sac of various strains of *P. tularensis* in embryonated eggs has been described. The method has no apparent advantages as a clinical diagnostic test, but may be useful in the assay of a live vaccine or in the detection of contaminants in vaccine assays.

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STUDIES ON PASTEURELLA TULARENSIS

EVALUATION OF A LIVING VACCINE FOR TULAREMIA
(Hornick, Vosti)

I. INTRODUCTION

Russian investigators have been working on the development of attenuated strains of Pasteurella tularensis as vaccines for a number of years and have obtained several strains which seem to fulfill their requirements.[1/] These have been extensively used in the vaccination of laboratory workers and in a large number of people in endemic areas of the Soviet Union. Investigations in laboratory animals by Medical Unit personnel will be presented in other parts of the present annual report. The purpose of this report is to present serial data on the vaccination site, regional or systemic reactions, serological response, and the results of skin testing in a group of volunteers vaccinated with an attenuated strain of P. tularensis.

II. MATERIALS AND METHODS

The vaccine used in this study was prepared by Eigelsbach[2/] from an "immunogenic" variant recovered from an ampule of Russian "viable tularemia vaccine". After adjustment to a count of approximately 10^9 organisms per ml it had been lyophilized and held for several months. Upon rehydration plate dilution counts indicated a similar level of viable organisms. Mouse virulence levels of two ampules at rehydration gave the following results (Yager):

Ampule A		Ampule B	
Dose	Deaths/Total	Dose	Deaths/Total
20,000	6/30	17,500	5/30
2,000	2/30	1,750	1/30

After a preliminary trial in two volunteers, a group of 22 young white male volunteers with no known history of previous infection with P. tularensis were selected. On April 16, 1958, they were vaccinated alternating between the right and left arms by the following procedure: the upper arm was cleansed with ether in a manner similar to the preparation done prior to smallpox vaccination. One hundred and fifty needle punctures were made over an area approximately 10 mm in diameter with just enough pressure to raise a slight serosanguineous response. To this site 1 to 2 drops of vaccine (1 billion cells/ml lyophilized vaccine, Ampule B, rehydrated with distilled water) were applied with the aid of a capillary and bulb instrument similar to that used for smallpox vaccination. The site of vaccination was allowed to air dry; no dressing or cover other than normal clothing was worn. These men were seen daily for two weeks, twice daily for the next two weeks and at approximately monthly intervals thereafter. They were questioned at these times as to the possibility of systemic or local reactions. Their temperatures were recorded and inspection of the vaccination area was also made for possible regional adenopathy. The vaccination sites of two randomly chosen individuals were studied serially by photography. At appropriate intervals blood was drawn for agglutination studies and for paper electrophoresis of serum proteins. The agglutination titers (against P. tularensis SCHU 4 killed organisms) were read at the last level of positivity and 1+ end points reported.

III. RESULTS

Observation revealed erythema averaging 10.1 mm at 24 hours. At one month the average diameter was 7.8 mm but of a much fainter color. Induration was never marked and was usually gone by the first week. Figure 1 shows selected views of a vaccination site. Vesicular pustular lesions were noted in 8 of 22 (36%) of the patients and usually occurred in the second week after vaccination; their presence was usually noted for a week or less. All vaccination sites were fading or had disappeared in one month.

Regional adenopathy was observed in 16 of 22 patients. Its onset was noted in 25 per cent of these by the second day and in 50 per cent by the first week. Adenopathy, when observed, was usually minimal, varying from shotty to 0.5 to 1 cm nodes; in only one patient, who also had the largest vaccination site measuring 15 to 17 mm in diameter, a 3 to 4 cm node was found. There were no systemic reactions.

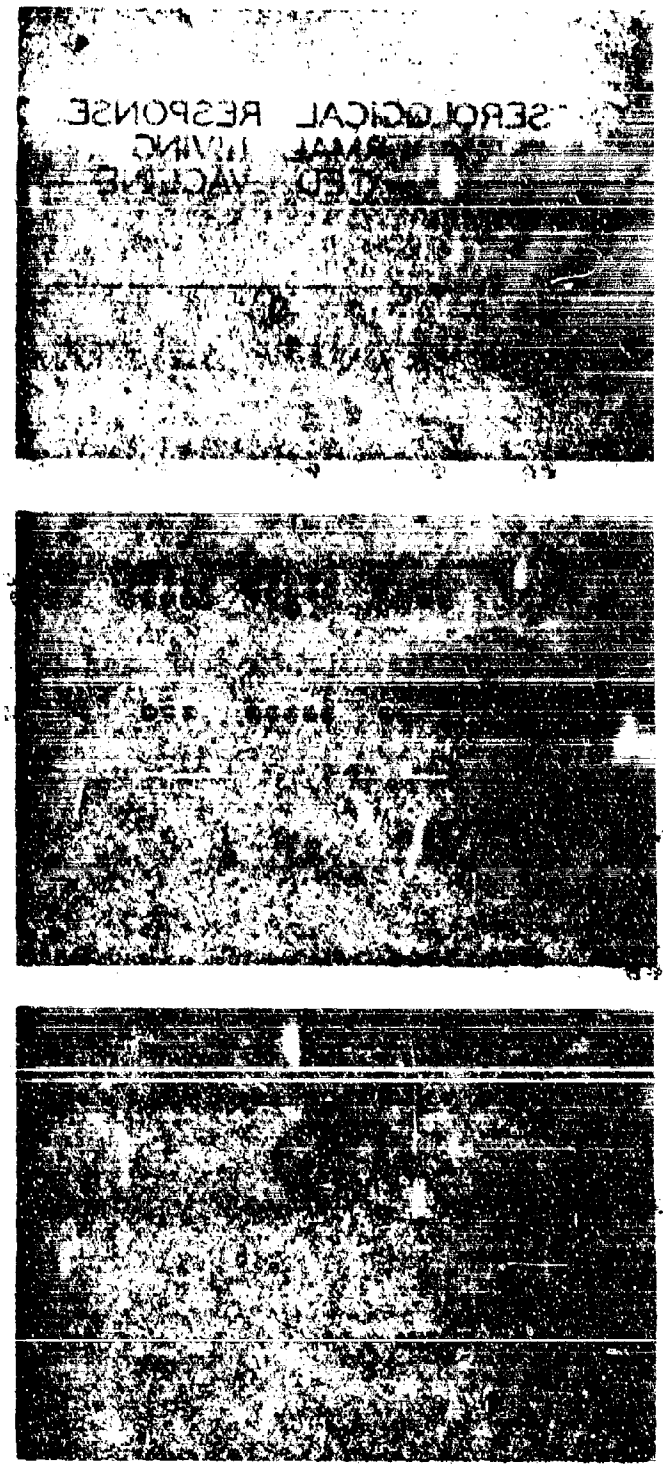
Base-line serological studies revealed that 16 of the 22 patients had negative tularemia agglutinin titers while six had positive titers, three at 1:10 and three at 1:20 (Figure 2). All patients showed an 8-fold rise in titer by the sixth week. It may also be seen that the serological response reached a plateau between the fourth and sixth week post-vaccination and that this plateau persisted to date (19th week) with a slight decline at the 31st week. The degree of serological response could not be correlated with the size of the vaccination site.

Skin testing at the fifth week revealed 15 of 22 patients to have a positive test (0.1 ml of tularemia skin test material injected intradermally in the flexor surface of the forearm. Read as positive if erythema and edema 1 cm in diameter or greater were present at 48 hours). There was no correlation between the positivity of the skin test and the degree of response in the agglutinin titer. Eleven of the 16 patients with adenopathy also had positive skin tests while four with positive skin tests had no regional adenopathy.

Complete evaluation of the serial serum protein electrophoretic fractions has not been obtained but a superficial survey would suggest that there are no marked changes observed by this technique.

IV. CONCLUSION

Vaccination with an attenuated strain of *P. tularensis* did not produce significant systemic reactions and regional adenopathy was minimal. The serological response is similar to that obtained following infection with a virulent strain and persists at a high level for at least 31 weeks.



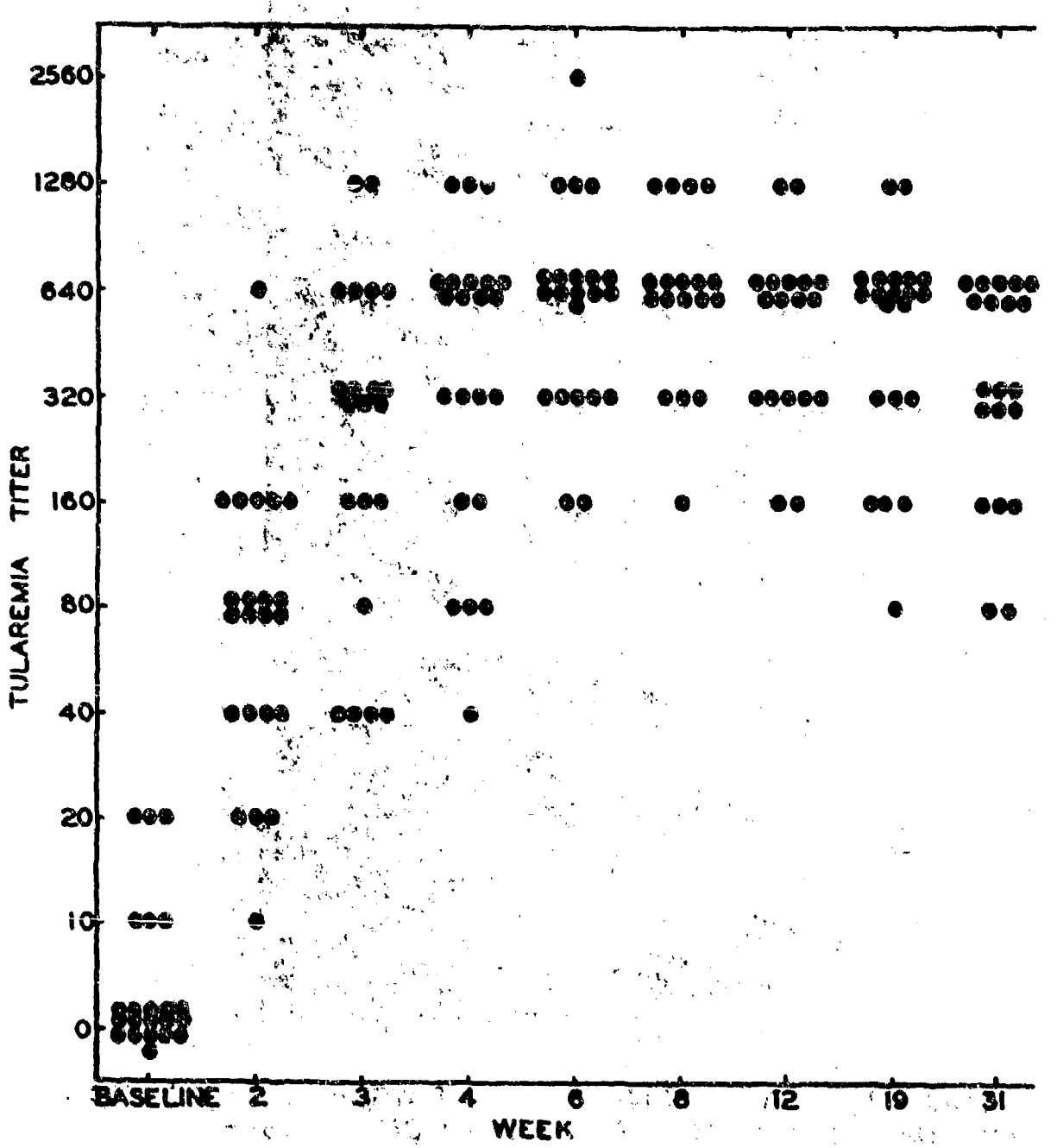
(A)

(B)

(C)

FIGURE 1. VACCINATION SITE (A) 2 DAYS POST-VACCINATION, (B) 16 DAYS POST-VACCINATION, (C) 29 DAYS POST-VACCINATION.

FIGURE 2. SEROLOGICAL RESPONSE TO INTRADERMAL LIVING ATTENUATED VACCINE



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STUDIES ON PASTEURELLA TULARENSIS
EFFECT OF DUOLITE TREATMENT ON GROWTH PROMOTING PROPERTIES OF BLOOD AND PLASMA
(Ward, Tresselt, Gaspar)

I. INTRODUCTION

Work of Huddleson reported in 1957[1/] indicated that treatment of certain blood specimens with cationic exchange resins, particularly Duolite C-3$^{(H+)}$, markedly enhanced their ability to support the growth of Brucella organisms. The conclusion reached by Huddleson on the basis of this work was "That the growth of brucella cells in a mixture of culture medium (peptone type) and blood is not due to the growth-promoting constituents in the medium but to the presence of an agent or agents that inactivate the bacterial growth-inhibiting factor usually present in normal blood. The blood then becomes an excellent culture medium." He presented data which demonstrate beyond doubt that treatment of blood from the three species studied (cow, horse, and man) with sufficient cationic exchange resin in the hydrogen form to lower the pH of the specimen to 6.0 to 6.2 does produce an excellent culture medium which promotes rapid growth of small numbers of organisms of test strains of all species of Brucella. Further, if procedures described are carried out in a closed container (vaccine bottle with rubber diaphragm stopper), the artificial addition of carbon dioxide (CO_2) for the growth of CO_2-dependant strains of Brucella abortus was found to become unnecessary.

In view of the facts that Pasteurella tularensis (1) in certain respects, resembles closely organisms of the Brucella group and (2) is difficult to isolate consistently from the blood stream of known infected animals and man, particularly early in disease, it occurred to us that attempts to adapt similar methods to studies with this organism might be fruitful. It was also hoped that investigations into the basic mechanisms involved in the tremendous enhancement of growth of small inocula might give useful information necessary for development of selective media for the isolation of P. tularensis from clinical specimens, other than blood, normally containing other flora which rapidly overgrow this organism when present methods are used.

For purposes of discussion, the work done to date may be conveniently divided into three sections:

Part I. Attempts to repeat Huddleson's observations with Duolite-treated blood and plasma, using P. tularensis as the test organism;

Part II. Attempts to adapt the Huddleson technique to blood culture work with experimental animals and man;

Part III. Biochemical studies on factors involved in the enhancement of growth of small inocula in resin-treated blood and plasma.

II. PART I

ATTEMPTS TO REPEAT HUDDLESON'S OBSERVATIONS WITH DUOLITE-TREATED BLOOD AND PLASMA USING P. TULARENSIS AS THE TEST ORGANISM

A. MATERIALS AND METHODS

1. Bacterial suspension for inoculum

The organism used in all experiments was the streptomycin-sensitive SCHU strain of P. tularensis. A heavy culture was grown for 24 hours at 37°C on plates of glucose-cystine blood agar (GCBA) prepared with Bacto cystine heart agar base (Difco) and 5 per cent defibrinated sheep blood. A concentrated bacterial suspension was made by emulsifying the growth from one or more of these plates in 2 to 5 ml amounts of tryptose-saline (0.1 per cent Bacto tryptose in 0.5 per cent saline, adjusted to pH 7.2 to 7.4). This suspension was adjusted to approximate a predetermined density as measured by the Bausch and Lomb Spectrotronic 20 colorimeter. Light transmittance of the suspensions was determined at four different wave lengths for the initial calibration and in preparation of subsequent suspensions. The adjusted suspension was subsequently diluted serially to that concentration which would give the desired number of organisms in 0.2 ml, the volume used as inoculum for all tests. All density adjustments and dilutions were made with the tryptose-saline solution. A total inoculum of 50 to 100 cells was used in all experiments and in two sets of tests, inocula of 5 to 10 cells were also employed.

2. Preparation of test and control blood and plasma samples

Duolite C-3$^{(H+)}$, manufactured by Chemical Process Company, Redwood City, California, was used. This is a phenolic matrix with methylene sulfonic acid as the functional group. It was washed and titrated for each batch of blood or plasma exactly as prescribed by Huddleson[17]:

> "The resin granules are added to several volumes of distilled water in a glass beaker and stirred. When the large particles have settled, pour off the turbid supernatant. Repeat, adding distilled water, stirring and decanting until the supernatant is clear.
>
> "Pour washed granules into a Buchner funnel containing No. 1 filter paper, or a coarse fritted glass filter. Remove surplus water by suction.
>
> "Spread resin granules on paper. Dry for 24 hours at 37°C. Store in a closed bottle."

For each experiment, the resin granules are titrated with blood or plasma in order to determine the amount that should be used to obtain a pH of 6.0 to 6.2.

"Titration. Weigh out 0.3, 0.4, and 0.5 grams of the granules. Add each amount to a 50 ml. glass bottle. To each add 2 ml. of a 5 per cent solution of sodium citrate and 10 ml. of unclotted blood. Mix the added agents well. At the end of two hours at room temperature pour the mixture into a glass beaker and read the pH (glass electrodes). The pH recorded will be near or the same as that recorded after 3-6 days incubation in a closed (rubber stopper) bottle containing the same agents. It is not necessary to conduct the titration aseptically."

After titration, the determined amounts of Duolite were weighed out and placed in 60-ml vaccine bottles to which were added 2.0 ml of a 5 per cent sodium citrate solution (referred to hereafter as citrate). After being plugged with cotton, the bottles were autoclaved at 121°C for 20 minutes and allowed to cool to room temperature. The cotton plugs were then replaced with sterile rubber diaphragm vaccine stoppers.

Using a syringe and needle, 10 ml aliquots of test bloods were introduced into the bottles and the contents mixed thoroughly by rotation.

In one early experiment two types of control bottles were prepared: one with 10 ml of blood plus 2 ml of 5 per cent citrate without Duolite and the other with 12 ml (to make total volumes comparable) of defibrinated blood without either Duolite or citrate. In subsequent experiments only the second control bottle was used, since 5 per cent citrate alone in blood inhibited the growth of P. tularensis.

In several experiments plasma obtained by centrifugation of defibrinated blood was substituted for whole blood. The procedures used for preparation and testing were the same in both cases.

In all cases, the blood specimens were used within 72 hours of collection and samples from individual animals were pooled before titration.

3. Test Procedures

Test and control bottles were each inoculated with 0.2 ml aliquots of the test suspension. The initial dosage of organisms is expressed as the number per milliliter of culture fluid, i.e., 6 organisms in 12 ml of culture fluid is expressed as 0.5 organisms/ml. Similar aliquots of the suspension were plated on four or more plates of GCBA to determine the actual number of cells used for inoculum.

The bottles were incubated in an Amino-Dubnoff shaker incubator equipped with a water bath adjusted to 37°C. During the incubation period the shaker was operated at 125 cycles per minute. All plates and those duplicate bottles in experiments designed to compare shaker with static cultures were incubated in a water-jacketed incubator at 37°C. Sufficient duplications of test and control bottles were prepared to allow for removal of samples for examination at the 12, 24, 48, and 72 hour time periods.

The method of examination was as follows:

Four aliquots of 0.2 ml each were removed from each bottle with a sterile syringe and needle and inoculated on to plates of GCBA. This inoculum was spread over the entire surface of the plates with the conventional type of glass spreading rod. If, on the basis of previous experience, confluent growth was expected, serial dilutions of the material were made in tryptose-saline and 0.2 ml aliquots of the dilutions were similarly plated. These plates were incubated as described above for 72 hours before making final counts to determine the extent of bacterial multiplication.

B. RESULTS

A summary of results of all experiments to determine the effect of Duolite treatment on the growth of P. tularensis in rabbit, sheep, and human blood is given in Table I.

TABLE I. EFFECT OF DUOLITE ON THE GROWTH OF P. TULARENSIS IN SHEEP, RABBIT, AND HUMAN BLOODS

INCUBATION TIME hours	MEAN RECOVERY org/ml of culture fluid							
	Sheep Blood (3 trials)		Rabbit Blood (5 trials)				Human Blood (1 trial)	
	w/o Duolite	w/ Duolite	w/o Duolite		w/Duolite		w/o Duolite	w/ Duolite
	5.7/ml[a]	5.7/ml	5.7/ml	0.5/ml	5.7/ml	0.5/ml	5.7/ml	5.7/ml
12[b]	< 1	5	43	20	87	< 1	ND[c]	ND
24	38	25	530	3	80,000	13,000	60	11,000
48	8	500	1,600	6	4.0×10^9	2.1×10^9	1,600	2.5×10^6
72	510	50,000	100,000	7	5.7×10^9	5.3×10^9	12,000	1.0×10^8

a. These figures represent the average number of organisms per milliliter of culture fluids used as inocula in replicate experiments. The extremes observed with the larger inocula were 4.2 and 6.6/ml and in the two experiments with smaller inocula, the control counts were identical 0.5/ml.

b. Counts at 12 hours were made for sheep blood only once. Counts for rabbit blood are the average of 3 experiments.

c. ND indicates no data.

The recoveries given in the table are the arithmetic means of average plate counts obtained in three separate trials in the case of sheep blood, five trials with rabbit blood and a single experience with human blood. Included in the table also are the results obtained when the inoculum size was reduced by one log (from 5.7/ml to 0.5/ml) in two experiments with rabbit blood only. Table II presents the same data, with the elimination of the results with the smaller inoculum, rearranged to give a direct comparison of the effect of blood of the three species on growth of P. tularensis before and after treatment with Duolite.

TABLE II. COMPARISON OF MEAN RECOVERIES[a] OF P. TULARENSIS IN BLOODS FROM DIFFERENT SPECIES AT VARIOUS INCUBATION TIMES
MEAN INOCULUM: 5.7 org/ml of culture medium

TEST BLOOD	DUOLITE	MEAN RECOVERY org/ml of culture fluid			
		TIME OF INCUBATION hours			
		12	24	48	72
Sheep	None	< 1	38	8	510
Rabbit		43	530	1,600	100,000
Human		ND[b]	60	1,600	12,000
Sheep	Present	< 5	25	500	50,000
Rabbit		87	80,000	4×10^9	5.7×10^9
Human		ND	11,000	2.5×10^6	1×10^8

a. Recoveries in number of organisms per ml of medium expressed as the arithmetic mean of available mean counts.

b. ND indicates No Data.

It is readily seen from these tables that both rabbit and human blood are superior to that of the sheep for growth of P. tularensis and that the Duolite treatment markedly enhances the growth of the organism in blood of all three species.

Of special interest, are the data in Table I dealing with results of the two experiments in which the standard inoculum used in previous tests was reduced 10-fold. There was evidence of continued multiplication of the organisms in untreated rabbit bloods inoculated with approximately 70 organisms. However, when the initial number of cells was reduced to approximately 6 organisms in 12 ml blood, there appeared to be some multiplication within the first 12 hours but none thereafter. In Duolite-

treated rabbit blood examined in parallel, approximately the same counts were obtained after the first 12 hours regardless of size of inoculum.

In the work with sheep and rabbit bloods where the results of three to five replicate experiments are available, remarkably little variation from one experiment to another was observed, despite the use of different batches of blood and the experimental error inherent in procedures involving sampling and counting. For these experiments, it was not feasible to plate at identical time intervals; consequently, those results given for 24, 48, and 72 hours varied as much as two to four hours either way from experiment to experiment. While the generation time of F. tularensis in Duolite-treated blood cannot be accurately calculated from available data, in the period between 24 and 48 hours it appears to be between 30 to 60 minutes. Consequently, counts made during this period would be expected to vary as much as one log or more in any two to four hour period.

The foregoing results were all obtained under the conditions selected initially as standard procedure: i.e., using whole defibrinated blood, with and without Duolite treatment, prepared in vaccine bottles with rubber diaphragm closures and incubated on a shaker with constant temperature water bath.

Several variations of this procedure were added to occasional experiments as time permitted:

(1) A comparison of static with shaker incubation conditions for treated and untreated bloods. (Table III)

TABLE III. COMPARISON OF STATIC VS SHAKER INCUBATION USING DUOLITE-TREATED RABBIT BLOOD
INOCULUM: 0.5 org/ml

INCUBATION CONDITION	RECOVERY org/ml of culture fluid AT INDICATED TIME INTERVALS		
	24 hours	48 hours	72 hours
Static (water jacketed incubation 37°C)	$> 1.5 \times 10^4$	2.5×10^8	2.5×10^8
Shaker (Amino-Dubnoff 37°C water bath 125 strokes/min)	$> 1.5 \times 10^4$	4.2×10^9	5.7×10^9

(2) A comparison of the growth promoting properties of whole blood with plasma, with and without Duolite treatment. (Table IV)

(3) A study of the effect of removal of the Duolite after initial treatment but prior to inoculation with the test organism. (Table IV)

In each case, experience was limited to a single trial.

It appears that counts obtained in shake cultures of Duolite-treated rabbit blood are about one log higher than those obtained from similar preparations incubated under static conditions. (Table III)

The data in Table IV are of theoretical, as well as practical, interest.

TABLE IV. COMPARISON OF RABBIT BLOOD AND RABBIT PLASMA WITH DUOLITE PRESENT AND DUOLITE REMOVED AFTER TREATMENT
INOCULUM: 0.5 org/ml Shaker incubated

MEDIUM	RECOVERY org/ml of culture fluid AT INDICATED TIME INTERVALS		
	24 hours	48 hours	72 hours
Blood + Duolite	$> 1.5 \times 10^4$	4.2×10^9	5.7×10^9
Blood - treated but Duolite removed	$> 1.5 \times 10^4$	$> 5.0 \times 10^7$	5.8×10^9
Plasma + Duolite	5	< 5	< 5
Plasma - treated but Duolite removed	1.7×10^3	1.5×10^7	1.9×10^9

There is apparently no real difference in counts obtained in Duolite-treated rabbit blood under the two experimental conditions; however, it would appear that the Duolite must be removed from the plasma prior to inoculation and incubation if one is to achieve enhancement of growth of P. tularensis in this medium. In this connection it is of interest to note that the amount of Duolite required to adjust the pH of 10 ml of rabbit plasma to the desired range of 6.0 to 6.2 is almost exactly one-half the amount required for the same volume of whole blood. No satisfactory explanation for this observation can be given at the present time, although it seems possible that the buffering action of erythrocytes might be involved. The complete reversal of the stimulatory effects of Duolite on growth of P. tularensis in pretreated plasma, observed when the resin was left in the plasma during the incubation period came as a surprise. It had previously been observed by Huddleson, and also by us, that an equilibrium in pH is reached in Duolite-treated whole blood in a maximum of two hours and that no further changes occurred in periods of observations up to five days in uninoculated bottles. It was assumed that the same thing would be true of plasma and no pH determinations were made after the 2-hour period chosen for initial titrations; consequently, we do not know whether the inhibitory effect of the presence of Duolite in the plasma during the incubation period was one of slowly, developing changes

in pH or of some other factor.

C. CONCLUSIONS

1. Duolite treatment markedly enhances the growth of P. tularensis in rabbit, sheep, and human bloods.

2. Duolite treated rabbit and human blood are superior to sheep blood for growth of P. tularensis.

3. Based on only one experiment, shaker culture is superior to static culture.

4. Based on only one experiment, plasma treated with Duolite but with the Duolite removed before inoculation supports growth of P. tularensis in a comparable fashion to Duolite treated whole blood.

III, PART II

ATTEMPTS TO ADAPT THE HUDDLESON TECHNIQUE TO BLOOD CULTURE WORK WITH EXPERIMENTAL ANIMALS AND MAN

Experience to date has been limited to a single study using bloods of experimentally infected monkeys.

A. MATERIALS AND METHODS

Blood specimens were collected from 36 monkeys selected from a total of 72 animals challenged with P. tularensis by the respiratory route on May 15 and 16, 1958, and reported in detail elsewhere. Half of the entire group had received 0.5 ml of a viable P. tularensis vaccine three months before challenge. Therapy consisted of 7-day or 14-day courses of tetracycline 0.6 gm daily in four equally divided doses, started on the second temperature above 104.0°F. Each of the 36 animals was bled twice daily at 0800 and 1600 hours on days 1, 2, 3, 5, and 7 post-challenge. Two milliliters of blood were collected at each bleeding and inoculated directly into 15 ml vaccine bottles with rubber diaphragm stoppers. These bottles had been previously prepared with appropriate amounts of Duolite-citrate mixture, as determined by titrations carried out on pooled monkey blood by procedures described in Part I.

All specimens were incubated for 72 hours in a water-jacketed incubator at 37°C; two 0.4 ml-aliquots from each bottle were then plated on GCBA. These plates, in turn, were also incubated as before for 72 hours before making final counts.

B. RESULTS

The results of these isolation attempts are presented in Table V. Animals in Groups I through VI received a challenge dose of approximately 1,300 cells and those in Groups VII through XII, approximately 130,000 cells.

As can be seen, P. tularensis was recovered from 24 of 36 animals. With only one exception (No. T-7, high dose, Group X) the first isolation from each positive animal was recovered from a blood specimen drawn within the first 24 hours of clinical illness as determined by onset of fever. In the low dose group, four isolations were obtained from specimens drawn 8 to 16 hours before the appearance of the first fever spike, all in non-vaccinates. In contrast only one isolation was made prior to the onset of fever in the high dose group. It is perhaps also of interest that only 8 of 18 vaccinated animals yielded positive cultures, whereas 16 of 18 of the non-vaccinates had detectable bacteremia.

C. CONCLUSIONS

Using this method positive blood cultures were obtained with considerable frequency in the late incubation or early febrile phases of monkey tularemia. The possible meaning of such findings is discussed elsewhere.

TABLE V. ATTEMPTS TO ISOLATE P. TULARENSIS FROM DUOLITE-TREATED BLOODS OF MONKEYS EXPOSED TO APPROXIMATELY 1300 AND 130,000 CELLS BY AEROSOL

GROUP	ANIMAL NO.	DAY POST-EXPOSURE 1	2	3	5	7	DAY FIRST FEVER	THERAPY ONSET HOURS[a]	DIFFERENCE BETWEEN FIRST POSITIVE CULTURE & FIRST FEVER HOURS[a]
		Low Dose-1300 cells. Exposed May 15, 1958							
I Vaccine	T-5	-	-	+	+	D[b]	3 pm	None	0
No Rx	T-14	-	-	+	+	+	2 pm	None	+16[c]
	T-46	-	-	+	+	+	3 pm	None	0
II Vaccine	T-21	-	-	-	-	-	2 pm	+8	No isolation
7-day Rx	T-39	-	-	+	-	-	2 mid	+8	+16[d]
	T-58	-	-	-	-	-	2 pm	+8	No isolation
III Vac-	T-16	-	-	-	-	-	2 pm	+8	No isolation
cine	T-38	-	-	-	-	-	2 pm	+8	No isolation
14-day Rx	363	-	-	+	-	-	2 mid	+8	+8
IV No vac-	T-32	-	-	+	+	D	2 mid	None	+8[d]
cine	347	-	-	+	+	+	2 mid	None	+8
No Rx	T-8	-	+	+	+	D	2 mid	None	-16
V No vac-	T-20	-	-	-	-	-	2 pm	+8	No isolation
cine	T-11	-	+	+	-	-	2 pm	+8	-8
7-day Rx	T-29	-	-	+	-	-	2 mid	+8	+8
VI No vac-	T-22	-	+	+	-	-	2 pm	+8	-8
cine	358	-	-	+	+	-	3 pm	0	-8
14-day Rx	362	-	-	+	-	-	3 am	+8	0
		High Dose - 130,000 cells. Exposed May 16, 1958							
VII Vac-	T-18	-	+	+	D	D	2 mid	None	-16
cine	352	-	+	+	+	D	2 am	None	+8
No Rx	403	-	+	+	D	D	2 am	None	0
VIII Vac-	T-1	-	-	-	-	-	1 mid	+8	No isolation[d]
cine	T-17	-	-	-	-	-	1 pm	+8	No isolation
7-day Rx	T-19	-	-	-	-	-	1 pm	+8	No isolation
IX Vac-	T-9	-	-	-	-	-	1 am	+16	No isolation
cine	371	-	-	-	-	-	1 pm	+8	No isolation
14-day Rx	392	-	-	-	-	-	1 mid	+8	No isolation
X No vac-	T-7	-	-	+	+	D	1 pm	None	+48[d]
cine	T-26	-	+	+	+	D	1 mid	None	+16
No Rx	354	-	+	+	D	D	2 am	None	0
XI No vac-	360	-	-	-	-	-	1 mid	+8	No isolation
cine	356	-	+	+	-	-	2 am	+8	0
7-day Rx	373	-	+	+	-	-	1 mid	+8	+8[d]
XII No vac-	T-41	-	+	-	-	-	1 mid	+8	+16[d]
cine	T-45	-	+	-	-	-	1 mid	+8	+8
14-day Rx	T-47	-	+	-	-	-	2 am	+8	+8

a. Minus sign (-) indicates culture preceded first fever; plus sign (+) indicates culture followed first fever.

b. D indicates animal dead.

c. Preceding sample not obtained.

d. Preceding sample contaminated.

IV, PART III

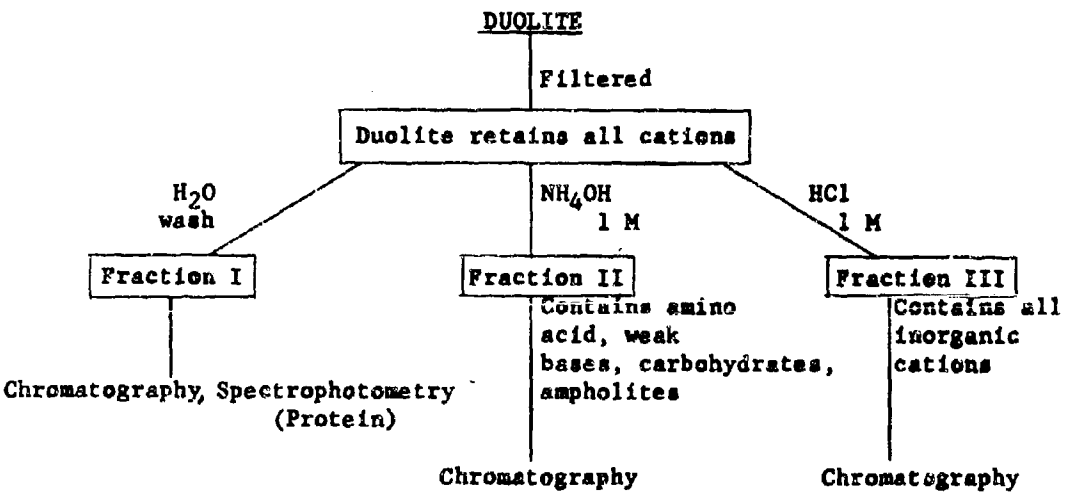
BIOCHEMICAL STUDIES ON FACTORS INVOLVED IN THE ENHANCEMENT OF GROWTH OF SMALL INOCULA OF P. TULARENSIS BY RESIN-TREATED BLOOD AND PLASMA

A. MATERIAL AND METHODS

Some information about the properties of Duolite C-3$^{(H+)}$ was found in the literature. This phenolic methylene sulfonic resin has a capacity of 3.25 meq/gm and exchanges hydrogen ions for cations in solution, thereby lowering the pH of the material with which it is mixed. Elemental analysis of Duolite C-3$^{(H+)}$ indicated trace amounts of silver, thorium, and cadmium.

The procedure for treatment of test samples was the Huddleson[1] method described earlier. After treatment, the Duolite was filtered from the blood or plasma and fractionated according to the procedure shown in the following diagram (Figure 1) to determine what substances might have been removed from the specimen by the resin.

Figure 1. The Fractionation of Duolite C-3 after Treatment of Blood or Plasma Sample



The three fractions were analyzed as follows:

Fraction I: paper electrophoresis[2], spectrophotometry, and paper chromatography[3] in search of protein components.

Fraction II: paper chromatography for amino acids[4], weak bases[5], carbohydrates[6], and ninhydrin positive areas other than amino acids[4].

Fraction III: paper chromatography[7] for inorganic ions.

The sodium (Na^+) form of Duolite C-3 was prepared by mixing the hydrogen (H^+) form with saturated sodium chloride followed by water to wash out the excess salt and hydrochloric acid.

Hydrogen gas was generated with zinc and hydrochloric acid in the conventional manner.

Direct current was obtained from a RECO power supply used for paper electrophoresis.

The pH and Eh measurements were made with a Beckman Model G pH meter. Platinum electrodes were made by sealing a ½ inch length of 20 gauge platinum wire in a 3-inch piece of 4 mm-glass tubing. Contact was made by a drop of mercury. These electrodes were standardized against the Beckman platinum electrode #281. Eh curves were obtained using an apparatus diagrammatically shown in Figure 2. Six test cells were placed in an improvised water bath shaker and connected to the potassium chloride (KCl) manifold and distributor switch. The pH of only one cell was determined because only one glass electrode was available.

The cell used to provide electrical control of the Eh is diagrammed in Figure 3.

Sheep plasma was used throughout this study except where otherwise designated.

The streptomycin-sensitive strain of P. tularensis was used throughout. Heavy suspensions of the organism were prepared from GCBA plates as described in Part I.

For the purposes of these studies growth is defined as increased oxygen consumption as measured by the Warburg respirometer. Data obtained from two experiments in which bacterial counts were made on the preparations in the Warburg flasks at several time intervals and also a comparison of the results of work with the Warburg with those described in Part I, where definitive bacteriological work was done, indicate that the basic assumption in this definition of growth is valid.

Test procedures for Warburg determinations were as follows: 2 ml of plasma or whole blood were placed in a test flask and 1 ml of tryptose-saline, containing approximately one to three billion cells of P. tularensis, was introduced into the side arm. The center well of each flask contained 0.2 ml of a 5 per cent solution of potassium hydroxide with a filter paper fan. The flasks were equilibrated for 15 to 20 minutes before adding the inoculum from the side arm. Zero time was designated as 10 minutes after addition of the organisms.

B. RESULTS AND DISCUSSION

1. Attempts to identify an inhibiting factor by chemical methods

Huddleson and others suggested that Duolite exerts its effect on the blood by removing in some, as yet undetermined, manner an inhibitory factor

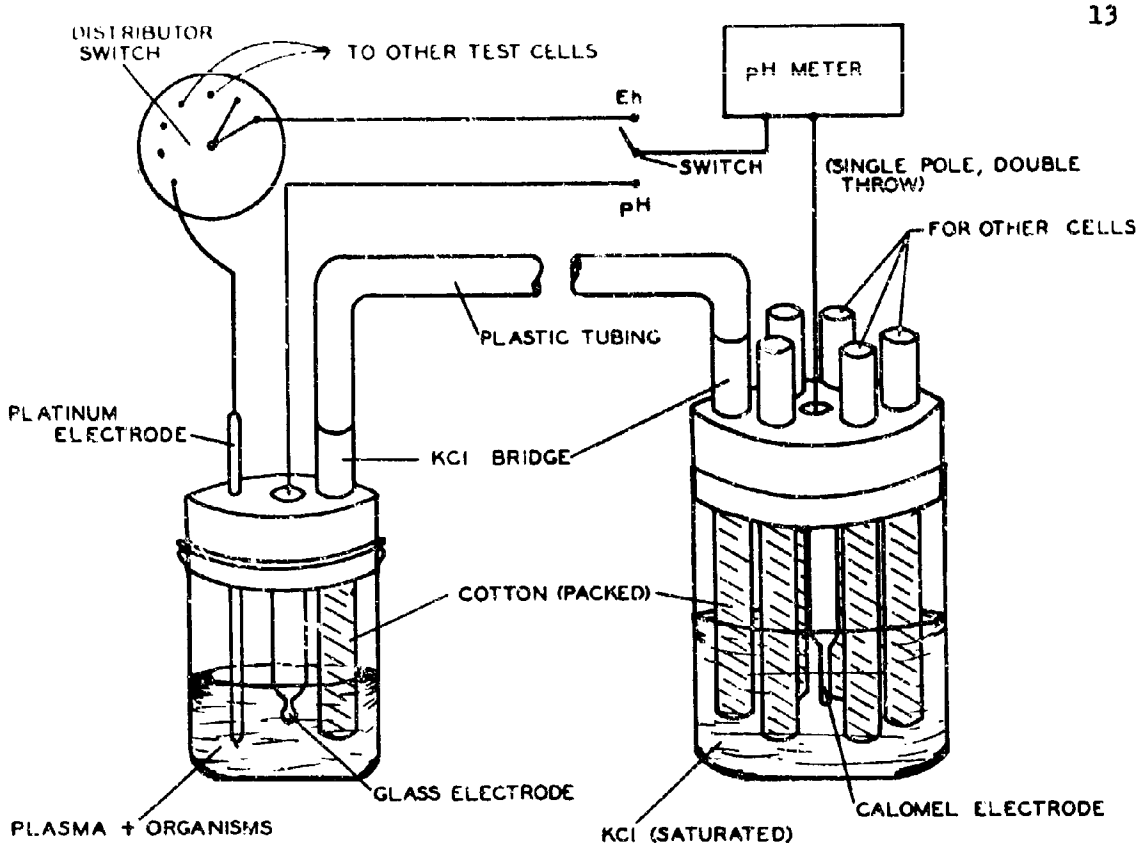


FIGURE 2. APPARATUS FOR pH & Eh DETERMINATIONS (DIAGRAM).

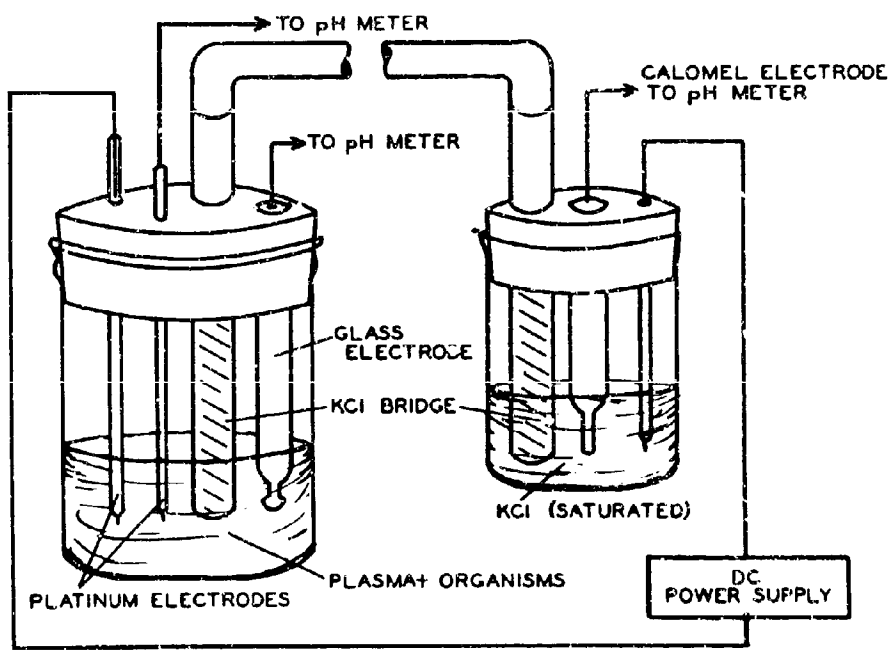


FIGURE 3. APPARATUS FOR ELECTRICAL CONTROL OF Eh (DIAGRAM).

TABLE VI. OXYGEN CONSUMPTION OF P. TULARENSIS IN VARIOUS MEDIA
(Sheep Blood or Plasma Used as Base)

EXPERIMENT	Test Sample	µl O_2 Consumed in 3-4 hours
I	Whole blood	13.5
	Plasma	18.0
	Whole blood + Duolite	23.0
	Plasma + Duolite	22.8
II	Plasma	15.1
	Plasma + Duolite	49.3
	Plasma with Duolite removed	51.1
III [a]	Plasma	15.1
	Plasma pH 6.0 with HCl	33.2
	Plasma pH 6.0 with Duolite	41.1
IV	Whole blood	19.9
	Whole blood + H_2 gas	21.3
	Plasma	4.0
	Plasma + H_2 gas	15.8
V [a]	Whole blood	28.2
	Whole blood pH 6.0 (HCl)	31.3
	Whole blood pH 6.0 (HCl) + H_2 gas	32.2
	Whole blood + Duolite	32.4
	Plasma	15.8
	Plasma pH 6.0 (HCl)	22.0
	Plasma pH 6.0 (HCl) + H_2 gas	36.9
	Plasma + Duolite	28.3
VI	Plasma	14.2
	Plasma + Duolite H+ (pH 6.0-titration)	29.0
	Plasma + Duolite Na+ (pH 6.0 HCl)	13.3
VII	Aged plasma	11.3
	Aged plasma + Duolite H+	11.5
	Aged plasma + Duolite Na+	11.7
VIII [b]	Normal plasma (pH unadjusted)	14.2
	Plasma + Duolite C-3 H+	30.0
	Plasma + Duolite C-3 Na+ (HCl)	23.5
	Plasma + Duolite C-20 H+	29.0
	Plasma + Duolite C-20 Na+ (HCl)	13.3
	Plasma + Amberlite IR120 H+	33.5
	Plasma + Amberlite IR120 Na+ (HCl)	17.4
IX	Normal rabbit blood	24.0
	Normal sheep blood	11.0
	Rabbit blood + Duolite	134.0
	Sheep blood + Duolite	50.0

a. In these experiments Duolite was removed from blood or plasma before inoculation.

b. In this experiment the pH of all media with resin treatment was adjusted to pH 6.0.

normally present in blood. Fractionation of the Duolite was tried in an attempt to identify or isolate this inhibitory substance. Fraction I, the water wash, was found to contain hemoglobin as identified by spectrum analysis. This material was apparently only absorbed by the Duolite since it could be removed easily by pulverizing the porous resin granules and washing with water. Fraction II, when chromatographed, did not yield any known ninhydrin areas. The one spot observed near the origin was presumed to be an artifact resulting from the ammonium hydroxide treatment. Fraction III was found to contain iron, calcium, copper, and magnesium. Iron was identified by four specific color reagents. The other metals were in trace amounts and could be identified only by their location on chromatograms visualized by ultraviolet light. The total inorganic material adsorbed was less than 0.1 per cent of the amount present in the test sample. The three fractions were concentrated, neutralized, and added back to Duolite-treated blood from which they were obtained. Growth in these test samples was entirely comparable to that obtained in Duolite-treated blood. If a growth-inhibiting factor was adsorbed by the Duolite, it could not be demonstrated by the methods tried.

Paper electrophoresis failed to show any significant difference between normal plasma and Duolite-treated plasma. A protein or protein complex did not appear to be involved in the growth enhancement.

2. Results of Warburg determinations

While the information obtained from oxygen consumption determinations does not give accurate information as to variations in bacterial count, such determinations do indicate rates of early enzymatic activity in the test mixtures. Despite the fact that the inoculum used was extremely large as compared to that used in the bacteriological studies reported in Part I, it was felt that perhaps the information gained by these measurements might be helpful in determining the nature of the enzymatic or environmental adjustment the organism found it necessary to make in the so-called "lag" phase before it could proceed into the logarithmic phase of its growth. It was also hoped that by comparing the effects of various changes in the medium, using oxygen consumption determinations with large inocula on the one hand and the extent of multiplication of small inocula as determined by actual bacterial counts on the other, we might obtain information which would allow us to use (within certain recognized limitations) Warburg determinations as a screening procedure in planning future bacteriological studies. The advantage of such a screening procedure in terms of total number of man-hours saved is obvious. It should be emphasized that even with all else equal, the Warburg data gave information only about changes occurring in the first four to six hours after transfer of the inoculum to the test mixture and did not necessarily represent what happened in cultures on prolonged incubation.

A number of experiments were conducted employing the Warburg apparatus and variations in the medium presented for growth of P. tularensis. The results expressed in terms of oxygen consumption are shown in Table VI. The following general observations seem indicated from these data:

(1) Growth of the organism is enhanced by Duolite treatment of plasma as well as whole blood, if both are adjusted to pH 6.0 to 6.2. (Smaller amounts of Duolite are required to adjust the pH of plasma to this point.) (2) The enhancement accomplished by Duolite treatment remains unchanged upon removal of the resin by filtration or centrifugation after the titration procedure. (3) Plasma adjusted to pH 6.0 with hydrochloric acid was superior to normal plasma but not as good as Duolite-treated plasma at the same pH. (4) Plasma through which hydrogen gas was bubbled was superior to normal untreated plasma. (5) Plasma adjusted to pH 6.0 with hydrochloric acid followed by hydrogen gas treatment gave results equal to those obtained by Duolite treatment. (6) Results obtained with blood or plasma treated with the Na^+ form of the resin were, in general, no different from those obtained with the untreated media. (7) Duolite treatment of aged (6 to 9 weeks old) blood or plasma did not result in growth enhancement. (8) Treatment with other cationic resins in the H^+ and Na^+ forms produced effects on growth similar to comparable forms of Duolite C-3. (9) Normal and Duolite-treated rabbit blood were superior to similar preparations of sheep blood.

Although some of the difference in total oxygen consumption over the time period observed may not be great enough to justify per se the above conclusions, most of the experiments listed in the table have been repeated and the same general trends in results observed. It is of interest to note that where comparisons are available (points 1, 2, & 9 - See Tables I, II, III & IV) the data obtained with bacteriological methods, using small inocula and prolonged incubation are in complete agreement with those obtained with the respirometer with large numbers of organisms and studied for only a few hours.

From these observations and the results of the Duolite fractionation it was concluded that the Duolite was contributing to the medium rather than combining with or adsorbing some inhibitory factor in the blood. Since the Na^+ form was not as effective as the H^+ form of the resins studied, it was concluded that the H^+ was the active factor. In addition to lowering the pH of blood, the H^+ might alter the Eh or oxidation-reduction (O-R) potential also. Since molecular hydrogen similarly effects changes in Eh, this was assumed to be the probable explanation of the beneficial action of hydrogen gas on plasma at pH 6.0. (See Exp. IV - Table VI)

The passage of a direct current through a solution also alters its Eh. Application of 5 to 10 volts of direct current to blood resulted in a rapid shift of Eh to the reducing side. Organisms inoculated into blood treated in this manner, however, rapidly readjust the O-R potential. To maintain a particular Eh reading in media with growing organisms, therefore, additional voltage must be applied intermittently every few minutes. In one experiment in which it was attempted to control the Eh at a point well on the reducing side, a definite increase in turbidity of the culture could be visualized within a few hours, whereas the control culture required overnight incubation for similar evidence of growth. Because of the many technical difficulties encountered in this type of experiment no conclusions could be drawn from its results until more refined methods of control of Eh by electrical means can be developed.

There are numerous data in the literature detailing the effect of Eh on bacterial growth. Some workers have shown that a drop in Eh to the reducing side occurs during the "lag" phase of bacterial growth and that multiplication of the organisms could not be demonstrated until a specific Eh was attained. There are reports also which indicate that the value of the particular Eh needed before initiation of the logarithmic growth phase varies with different species of organisms.

C. CONCLUSION

Changes in O-R potentials developed during incubation of P. tularensis in several media have been determined, namely, in normal guinea pig plasma, normal sheep plasma, Duolite-treated sheep plasma with and without removal of the resin after treatment, and sheep plasma adjusted to pH 6.0 with hydrochloric acid.

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AN ANALYSIS OF 42 CASES OF LABORATORY-ACQUIRED TULAREMIA

(Overholt, Tigertt, Kadull, Ward, Charkes, Rene, Salzman, Stephens)

I. INTRODUCTION

Since 1912, when McCoy and Chapin[1,2] first described Pasteurella tularensis, the hazard of infection to the laboratory worker has been well recognized. Few individuals escape illness if they continue to work with the organism. Over a 24-month period starting in August 1956, 34 cases of laboratory-acquired tularemia were admitted to the Medical Service. An additional eight non-hospitalized cases were detected. The clinical and laboratory course was documented according to a protocol initiated at the onset of this study. There were three primary objectives: (1) to evaluate the clinical and laboratory manifestations of the disease and attempt to establish criteria to achieve an earlier diagnosis; (2) to assess the efficacy of phenolized[3] and/or acetone-extracted[4] tularemia vaccine in the prevention and/or modification of the disease; and (3) to determine the effectiveness of tetracycline as a therapeutic agent. The majority of these infections resulted from a streptomycin-resistant strain of P. tularensis.

II. METHODS

Symptoms and signs were noted and graded daily as to severity. Rectal temperatures and vital signs were obtained every four hours. Supportive therapy consisted of bed rest, as desired by the patient, and codeine or Demerol (Winthrop Laboratories, New York) for the more severe headache and myalgia; antipyretics were intentionally withheld. Isolation procedures were not practiced. Prior to the initiation of therapy each case was evaluated as to the severity of illness and graded as mild, moderate, or severe. The illness was considered as mild if the symptom-complex permitted the patient to work throughout the day but afternoon fatigue, chilliness, slight fever and malaise were noted; as moderate if the patient was unable to work and bed rest for part of the day was desired; and as severe if the patient was compelled to remain in bed throughout the day.

Routine blood cell count, sedimentation rate (Wintrobe), C-reactive protein (Schieffelin and Co. reagents), and chest roentgenogram were obtained two to three times weekly during hospitalization. Fasting gastric aspirates were obtained for the first two or three days following admission in 29 of the 31 hospitalized non-ulceroglandular cases. From 30 of these patients pharyngeal washings were obtained, using 15 ml of nutrient broth as a gargle. Initially, morning and evening pharyngeal washings were collected for the first three consecutive hospital days, but as the study progressed, the afternoon specimen was discontinued. Following institution of therapy, gastric and pharyngeal specimens were studied as often as practicable. In 14 patients a mildly productive cough permitted evaluation of sputum. Isolation attempts were also made by bronchial lavage in three patients and nasal swabs in seven. Blood cultures were tried in 17 patients and discontinued when it became apparent that they were generally unrewarding. Material from local skin lesions in three patients with ulceroglandular disease was cultured prior to, and after,

the onset of therapy. Pleural fluid from three patients was available for isolation attempts.

Tularemia agglutinin titers were determined at least weekly during the early phase of disease and on each follow-up visit. As a part of another study by one of us (N.D.C.), serial serum hemagglutinin levels were obtained in 17 of 34 patients. Tularemia skin tests were performed shortly after admission and usually weekly thereafter until positive. Each patient returned for weekly follow-up evaluations during the first month after discharge from the hospital, monthly for the next three months and then every third month to complete a year. These evaluations included interval history, physical examination, complete blood count, sedimentation rate, C-reactive protein (CRP), agglutinin titer and chest film.

Isolation attempts for P. tularensis from pharyngeal, gastric, sputum, bronchial lavage, and pleural fluid specimens were performed in the following manner: two 300 to 400 gm male guinea pigs received 2 ml-aliquots each, 1 ml subcutaneously and 1 ml intraperitoneally. An additional 0.5 to 2 ml aliquot was plated on two to four glucose-cystine-blood (GCB) agar[5] plates, with and without streptomycin (100 µg/ml of medium). Blood specimens were examined by injection of 4 ml of a heparinized specimen into guinea pigs as described above and by culturing 5 to 10 ml in a diphasic medium which contained GCB agar and GCB broth. The bottles were observed daily for 30 days. The guinea pigs were observed daily for temperature elevation, roughing of the fur, and hunching of the back. In a sick animal, fever usually was noted 2 to 5 days post-inoculation. Deaths occurred 3 to 7 days after inoculation. At autopsy, an infected animal had excessive, thick, clear peritoneal fluid and a thickened greater omentum. There was a mucopurulent exudate around the spleen and liver. In guinea pigs surviving one week the liver and spleen were greatly enlarged and a fine granular infiltrate was readily noted. A preliminary diagnosis was attempted by smearing a loop of exudate and staining by Wayson[6] and Gram methods. The latter stain may demonstrate Gram-negative cocco-bacilli compatible with the morphological characteristics of P. tularensis. However, organisms were difficult or impossible to visualize against the pink background of the exudate. Wayson stain was superior since the organisms stained a dark blue against a pink background. Regardless of the results from smears, exudate from the surface of the spleen and liver was cultured on GCB agar with and without 100 µg of streptomycin per milliliter of medium. Growth occurred on both plates if the organism was streptomycin-resistant but only on plates without streptomycin if the organism was sensitive to this antibiotic. Grey, translucent, 1 to 2 mm colonies were apparent at 48 to 72 hours; a smear revealed a Gram-negative cocco-bacillus. The organism was identified by a slide agglutination technique using specific antiserum. Swabs of the nasal mucosa or skin ulcer were similarly cultured. At least one isolate from every patient was examined for sensitivity to discs impregnated with 5 and 30 µg of chloromycetin, tetracycline, oxytetracycline and chlortetracycline. In addition, sensitivity to tetracycline was further evaluated by determining growth on serial plates containing GCBA with 1 through 5 µg of tetracycline per milliliter of medium. In those instances where the organism did not grow in the presence of 100 µg of streptomycin per milliliter of medium, the sensitivity to 10 µgm was determined.

Two strains of P. tularensis were responsible for the cases of tularemia in this study. The parent strain is a virulent culture (SCHU) originally isolated by Foshay from a human ulcer in 1941. In 1951, Eigelsbach, Braun, and Herring[7] while correlating colonial morphology, pathogenicity, and immunological properties of this strain, isolated distinctly different, colonial types from a supposedly pure stock culture. From these types a variant (SCHU S4) was picked which gave rise to a stable homogeneous subculture with high virulence for mice, guinea pigs, monkeys, and rabbits. The streptomycin-resistant mutant (SCHU S5) was derived from SCHU S4. Subsequent evaluation has indicated a retention of resistance to streptomycin as well as the virulence characteristics of SCHU S4.

Skin tests were performed with 0.1 ml of vaccine diluted 1:1000 in physiological saline, injected intradermally into the flexor surface of the forearm. A skin test was positive if 10 mm or more of erythema and edema were apparent at 48 hours.

The agglutinin titer was performed in the following manner: to the first of 6 tubes, 0.9 ml of physiological saline was added and 0.5 ml to the remaining 5 tubes. One-tenth milliliter of test serum was added to the first tube, mixed with the saline aliquot and 0.5 ml transferred to the second tube. This then was mixed and serial dilutions were carried out, discarding 0.5 ml of serum-saline mixture from the last tube. The antigen, a formalin-killed preparation of the SCHU S4 strain (concentration of 3×10^9 organisms/ml), was then added in 0.5 ml aliquots to each of the serum-saline dilutions. After shaking to insure proper mixing, the tubes were centrifuged for seven minutes at 2000 rpm and immediately read for visible agglutination. The highest serum dilution capable of agglutinating the antigen was taken as the end-point and read as follows: 4^+, coarse flocculation with slightly cloudy supernatant; 3^+, 75 per cent coarse flocculation with slightly cloudy supernatant; 2^+, 50 per cent coarse flocculation with moderately cloudy supernatant; 1^+, definite fine flocculation with moderately cloudy supernatant; plus-minus, minor degree of flocculation with cloudy supernatant; negative, cloudy or opaque solution with no flocculation. The serum dilutions were recorded as 1:10, 1:20, 1:40, etc. The highest serum dilution in which there was one plus or greater flocculation was the reported titer.

The hemagglutination test was performed after the method of Alexander, Wright and Baldwin[8] and Wright and Feinberg[9]. Essentially, this method utilizes type O blood cells sensitized to the polysaccharide extraction (with liquid phenol) of acetone-killed P. tularensis. Five-tenths milliliter of a 0.5 per cent sensitized-erythrocyte suspension was added to each tube containing 0.5 ml of serial two-tube dilutions of patient's serum at room temperature, and the settling pattern observed two hours later. The titer of a serum was recorded as the greatest dilution giving partial or complete hemagglutination. An attempt was made in 14 patients to detect the cellular polysaccharide of P. tularensis in various body fluids and excretions utilizing the hemagglutination-inhibition method. This test was performed by adding 0.25 ml of specimen (pharyngeal washing, gastric washing, sputum, or urine) to 0.25 ml of human serum having a high tularemia agglutinin titer. After over-night incubation at room temperature, 0.5 ml of a 0.5 per cent suspension of erythrocytes

sensitized with P. tularensis polysaccharide was added, the titer noted and compared with a control set of tubes containing saline instead of a body fluid.

III. MATERIAL

All but one of the 42 patients were under the age of 45 with a range of 21 to 65 years. There were 41 males, seven of whom were negroes, and one female, a negress. With the exceptions of a patient who had "chronic brucellosis" and another with chronic sinusitis, all were in excellent health prior to the onset of tularemia. None had a history of naturally-occurring tularemia prior to the initial vaccination series. Immunization procedures had consisted of three subcutaneous injections of phenolized or acetone-extracted tularemia vaccine, 0.25 ml on the first day and 0.5 ml daily on the next two days. Routine booster series, consisting of 0.25 ml and 0.5 ml for two consecutive days, were usually given six months to one year following this procedure except in the occasional patient who had a positive skin test at the time for the booster series. As may be seen in Tables I and II the initial vaccination series was accomplished from one month to as long as 11 years prior to the onset of illness in the 42 cases. Thirty-two of the group had received one to seven booster series, with approximately 90 per cent receiving four or less. Fourteen of these 32 became ill less than six months after their last booster. The remaining 10 had received an initial series only, ranging from one month to as long as five and a half years prior to infection. Seven of the 10 became ill less than six months after immunization. Thus, at least one-half of the cases had either their initial or booster series less than six months prior to illness. All but two patients were known to have had agglutinating antibodies at some time before infection; in 80 per cent this titer had been determined within six months preceding the illness.

Occupational activities resulted in frequent potential respiratory exposure to P. tularensis in most of these individuals. In 21 of the 42 patients, incidents, such as breakage of seeded petri plates, leaks in hoods, etc., occurred. Such instances permitted an estimation of the incubation periods. In 17 the presumed incubation period was from 3 to 6 days, with the remaining four between 7 and 12 days.

Of the 34 hospitalized patients, three had ulceroglandular disease, 14 typhoidal disease, and 17 a typhoidal form with pulmonary involvement. In neither of the latter two categories were skin lesions present and they differed only in that the pulmonic group had chest x-ray abnormalities. The eight non-hospitalized cases arbitrarily were classified as typhoidal.

A. CLINICAL OBSERVATIONS IN HOSPITALIZED PATIENTS

A grippal symptom-complex, of varying severity, consisting of feverishness, headache, chilliness, diaphoresis, malaise, and anorexia was seen in the 34 hospitalized patients. On the basis of subjective criteria described earlier, the illness was considered mild in 19, moderate in 9, severe in 6. (Table I) This division was further emphasized when the day of hospital admission was compared to the day of illness: all six of the severely ill patients were admitted within the first week of illness, whereas over one-half of the mild to moderately ill patients were admitted after the first week. This delay in hospitalization is attributed to the mildness of the illness so that the patient

TABLE I. SUMMARY OF 34 HOSPITALIZED CASES OF TULAREMIA WITH RESPECT TO SEVERITY, VACCINE ADMINISTRATION, AND AGGLUTININ TITER (1956-1958)

CASE NO.	YEAR	SEVERITY a/	VACCINE ADMINISTRATION: Initial Series Month b/	Booster: No.	Booster: Month b/	TULAREMIA AGGLUTININ TITER day b/: Pre-Infection level 1:	Pre-Infection level Day	First Obtained During Illness 1:	First Obtained During Illness Day	4-fold Rise Day	Maximum 1:	Maximum Day
ULCEROGLANDULAR CASES												
1	1956	Mild	-62	4	-27	320	-125	320	2	None	320	44
2	1957	Mild	-72½	1	-61	0	-91	10	11	22	1280	39
3	1958	Mild	-41	2	-5	80	-41	640	10	30	1280	30
TYPHOIDAL CASES WITH PULMONARY INVOLVEMENT												
4	1956	Mild	-66	1	-54	40	-75	80	8	26	2560	40
5	1956	Mild	-8	1	-3	20	-3	40	3	23	1280	23
6	1956	Mod..	-65	0	-65 c/	80	-101	80	4	30	2560	46
7	1956	Mild	-5	0	-5 c/	80	-24	320	3	6	2560	9
8	1957	Mild	-91	5	-33	160	-61	160	10	None	1280	31
9	1957	Mild	-37	1	-26	80	-60	40	2	29	640	29
10	1957	Mild	-40	3	-<1	80	-12	80	6	34	1280	34
11	1957	Mod.	-103	6	-6	20	-187	40	5	23	2560	43
12	1957	Mod.	-56	1	-4	160	-150	160	7	18	2560	18
13	1957	Mild	-50	2	-1	40	-58	40	6	34	1280	49
14	1956	Sev.	-14	1	-6	320	-28	1280	6	None	1280	6
	1957	Mod.	-36	1	-29	160	-197	160	1	None	1280	60
15	1957	Mild	-36	3	-2	40	-54	40	3	19	1280	28
16	1958	Mod.	-43	1	-11	160	-53	40	3	30	640	30
17	1958	Sev.	-61	2	-3	40	-131	80	2	25	2560	31
18	1958	Sev.	-81	3	-49	10	-106	0	1	11	2560	28
19	1958	Mild	-127	7	-9	320	-72	160	7	23	1280	23
20	1958	Mod.	-13	1	-7	40	-62	40	3	23	1280	37
TYPHOIDAL CASES WITHOUT PULMONARY INVOLVEMENT												
21	1956	Mod.	-26	1	-18	80	-4	80	9	33	1280	33
22	1956	Mild	-44	0	-44 c/	40	-103	80	8	19	1280	19
23	1956	Mild	-16	1	-14	160	-9	80	5	19	2560	34
24	1956	Mild	-28	1	-21	40	-187	40	1	27	1280	30
25	1957	Mild	-48	0	-48 c/	40	-3	20	1	20	2560	27
26	1957	Sev.	-5	0	-5 c/	20	-17	40	3	30	1280	33
27	1957	Mod.	-62½	1	-51	0	-53	10	5	23	640	31
28	1957	Sev.	-53	2	-½	20	-19	320	2	None	640	29
29	1957	Mild	-59	0	-59 c/	40	-43	40	10	21	2560	40
30	1957	Sev.	-5	0	-5 c/	0	-143	20	2	31	1280	38
31	1958	Mod.	-41	1	-39½	20	-129	10	5	12	1280	22
32	1958	Sev.	-95	5	-1½	160	-7	80	2	None	640	60
33	1958	Mild	-48½	1	-40	10	-13	10	3	19	1280	36
34	1958	Mild	-14	1	-5½	20	-199	10	4	25	320	25

a. See text for definition.

b. Time is given in months or days relative to Day 1 of disease. Minus (-) sign means interval before onset.

c. No booster given, time is that of initial series.

TABLE II. SUMMARIES OF 8 NON-HOSPITALIZED CASES OF TYPHOIDAL TULAREMIA WITH RESPECT TO VACCINE ADMINISTRATION, SYMPTOMS, AND AGGLUTININ TITER (1956-1958)

CASE NO.	YEAR	VACCINE ADMINISTRATION			SYMPTOMS		TULAREMIA AGGLUTININ TITER			
		Initial series month[a]	Booster No.	Booster Month[a]	Type	Duration days	Base Line 1:	Base Line Day[a]	8-fold Rise 1:	8-fold Rise Day[a]
35	1957	-1	0	-1[b]	Chills, night sweats, malaise, anorexia, fatigue.	5 - 6	20	-30	2560	76
36	1958	-80	4	-4	Headache, lethargy, chilliness, anorexia.	9 - 10	80	-150 & 7	1280	38
37	1958	-54	3	-5	Slight fever, chilliness, dry cough, malaise.	6	20	-180 & 3	2560	36
38	1958	-3	0	-3[b]	URI, cough, headache, sore throat	10	40	-12	1280	11 & 30
39	1957	-5	0	-5[b]	Mild URI.	3	80	-4	1280	18
40	1957	-84	3	-10	Frontal headache, cough low-grade fever, malaise.	14[c]	20	-150	1280	40
41	1957	-5	0	-5[b]	Headache, anorexia, nasal stuffiness	2 - 3	80	-60 & 1	2560	25
42	1957	-17	1	-11	Chills, slight fever, sore throat, slight cough, headache, back ache	6	40	-60	1280 2560	2 6

a. Time is given in months or days relative to presumed Day 1 of disease: Minus (-) sign means interval before onset.

b. No booster given, time is that of initial series.

c. Therapy, consisting of 0.5 gm streptomycin, given on days 1, 13, and 14 of disease.

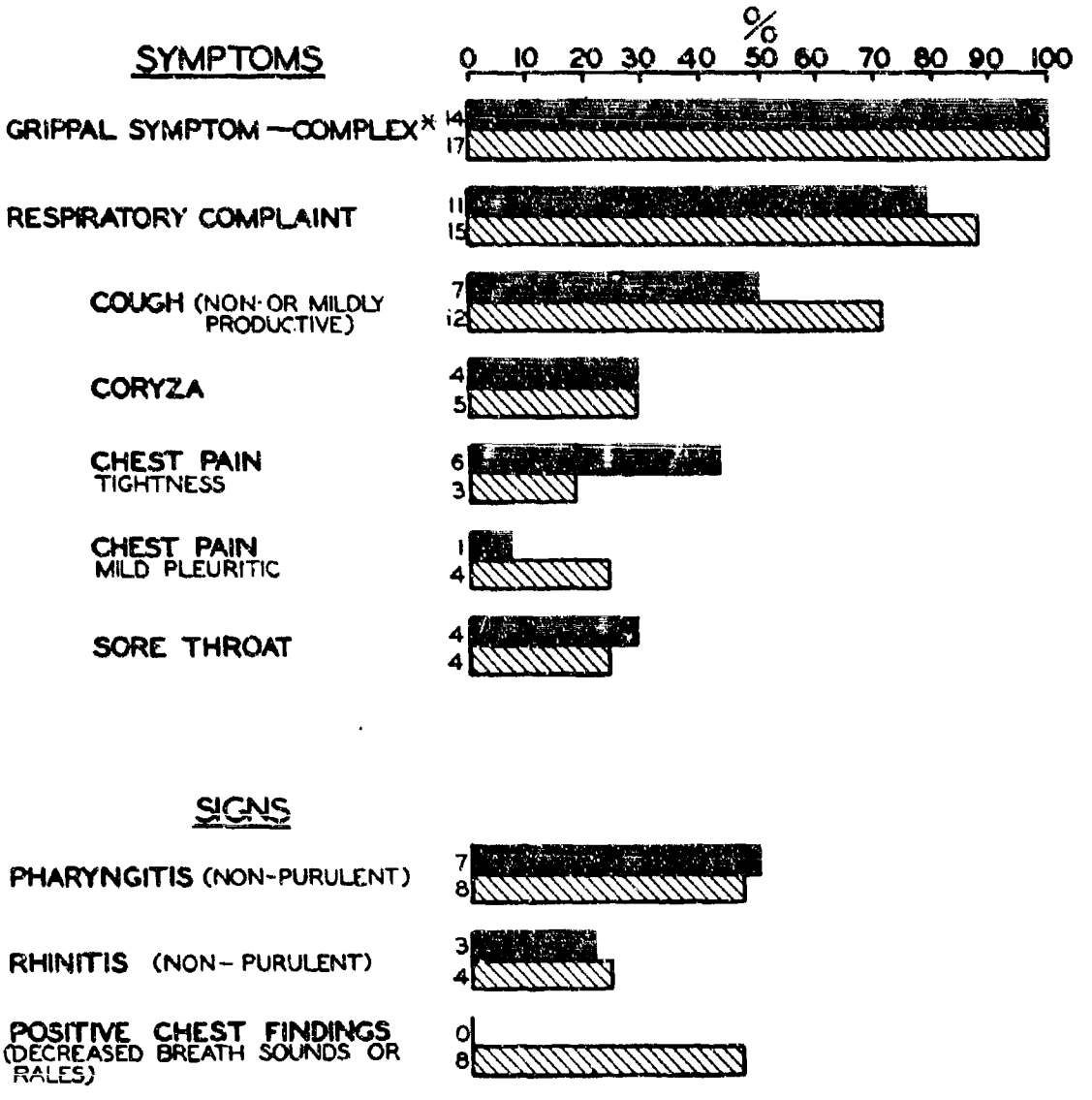
did not seek medical attention until persistence of symptoms became disturbing. Of equal importance was the inability of the physician to differentiate the symptoms of tularemia from a common respiratory or grippal state. A concurrent influenza epidemic (described in detail elsewhere) complicated recognition; during its course one-half of our group became ill with tularemia. In the absence of acute symptoms, it was not practicable to admit all grippal cases and such patients were followed closely in the out-patient clinic. Individuals were admitted as suspect tularemia if there was persistence of grippal symptoms and fever, with or without abnormal sedimentation rate and CRP, or if x-ray evidence of pulmonary involvement was found.

The high incidence of respiratory symptoms was striking in the 31 non-ulceroglandular hospitalized cases. All but four had at least one of the following: cough, coryza, sore throat, or chest distress accompanying or appearing shortly after the onset of grippal symptoms (Figure 1). These respiratory symptoms were usually mild and included a dry to slightly productive cough, minimal nasal stuffiness, a raw feeling of the throat, and vague substernal tightness. Symptoms of a severe upper respiratory infection, such as marked nasal stuffiness, severe rhinorrhea, substernal soreness or cough, were not seen. With the exception of more frequent pleuritic chest pain and cough in the patients with radiographic evidence of pulmonary involvement, there was no difference in the incidence, severity, or type of respiratory symptoms between the typhoidal and typhoidal-pulmonic groups. Furthermore, the presence or absence of x-ray evidence of pulmonary disease could not be related to the severity of the grippal state or fever, with the exception of the one critically ill patient who had extensive pulmonary involvement.

The maximum rectal temperature usually occurred in the evening hours, ranging from 101.0 to 105.8°F, and generally paralleled the severity of illness. In about one-half of the 34 patients the fever did not exceed 103.0°F. Positive physical findings (Figure 1) in the non-ulceroglandular cases were usually limited to the respiratory tract. Non-purulent pharyngitis and rhinitis were common. Chest findings were limited to the x-ray-positive group and consisted of signs of pleural fluid in four patients and of fine to coarse rales in four patients with bronchopneumonia. With the exception of transient splenomegaly in one patient and persistent submandibular adenopathy in another, there were no other physical findings.

In the three ulceroglandular cases, symptoms were limited to a mild grippal state and regional lymph node enlargement, pain and tenderness. In two individuals the skin lesion consisted of a 3 to 4 mm painless, erythematous papule which rapidly progressed to ulceration. The lesion was located in one patient on the dorsal aspect of the right wrist at the margin where the protective rubber glove ended and, in another patient, under the nail tip of the left third finger. The third patient had a perionychia of the left thumb with two 1-cm satellite ulcers, one on the lower one-third and the other on the upper one-third of the medial aspect of the forearm. In all instances there was probable local trauma or a preceding lesion at the time of contamination of the skin site. Tender, non-fluctuant, 1- to 2-cm epitrochlear and axillary nodes promptly appeared in all three individuals.

FIGURE 1. PRINCIPAL SYMPTOMS AND SIGNS IN 31 HOSPITALIZED CASES OF TYPHOIDAL TULAREMIA.



*GRIPPAL SYMPTOMS-COMPLEX = VARIOUS COMBINATIONS OF FEVER, CHILLINESS, HEADACHE, DIAPHORESIS, MALAISE, FATIGUE, MYALGIA, ARTHRALGIA, BACKACHE, ANOREXIA.

NO ROENTGEN CHANGES (14)
ROENTGEN CHANGES (17)

B. LABORATORY OBSERVATIONS IN HOSPITALIZED PATIENTS

Serial chest roentgenograms were negative in the three ulceroglandular and positive in 55 per cent (17) of the 31 typhoidal cases. In these 17 patients a pneumonic infiltrate was noted in 15, hilar adenopathy in 9, pleural effusion in 4, and perihilar linear streaking in one. The pneumonic lesion had a distinctive appearance; in 13 of the 15 there was an oval 2 to 8 cm density with indistinct borders (Figure 2a), being multiple in only three patients. There was no predisposition to any one lobe but in five the infiltrate was juxta-hilar, merging with the hilar shadows. In one of the 15 patients (Case 18) lobar consolidation was observed (Figure 2b). The remaining case had a diffuse bronchopneumonia of one lobe. The availability of multiple pre-illness chest films permitted a critical evaluation of hilar shadows. The moderate, unilateral hilar enlargement was always associated with other abnormalities, occurring with a pneumonic infiltrate in 8 patients and perihilar streaking with pleural effusion in the remaining case. Pleural effusion was seen as an isolated occurrence in but one patient.

The initial white blood cell count exceeded 14,000/cu mm in only two of the 34 patients. The differential count usually demonstrated a "shift to the left" of the neutrophils and a few atypical lymphocytes. The sedimentation rate (corrected sedimentation rate used throughout) and the CRP were found to be abnormal shortly after the onset of illness and paralleled one another during the acute phase. In 16 of the 34 cases examinations were made during the first 3 to 4 days of illness and were abnormal that early in the disease.

A 4-fold or greater rise in the specific agglutinin titer was usually apparent by the third week of illness. An 8-fold (considered diagnostic) rise was noted in three patients within the first two weeks and in 25 between the 3rd to the 5th weeks from the onset of illness. The remaining six patients did not develop 8-fold rises, the diagnosis being confirmed by isolation of the organism. This latter group had pre-infection titers of 1:160 to 1:320; one mild ulceroglandular case had no rise in agglutinin titer, the other five had 2- to 6- fold titer rises. In contrast the pre-infection titer, in the 28 patients in whom there was at least an 8-fold rise in titer, ranged from 0 to 1:80 (Table I).

Elevation of hemagglutinin and agglutinin titers were detectable simultaneously on 17 patients when tests were performed on serial specimens. However, in the sera of ten patients there were 8-fold rises in the hemagglutinins 7 to 10 days earlier than in the agglutinating antibodies. The peak level was reached simultaneously in 6 of the 7 remaining cases. In one instance there was no rise in the hemagglutinin or agglutinin titer.

The cellular polysaccharide of P. tularensis was not identified in the 33 pharyngeal, 10 gastric, 4 sputum, and 18 urine specimens obtained during the acute phase of illness from 14 patients.

Skin tests were performed during illness in 29 of 34 patients. Twelve patients had negative skin tests when first seen (4 during the first week, 5 during the second week, and 3 in the third and fourth week) which converted to

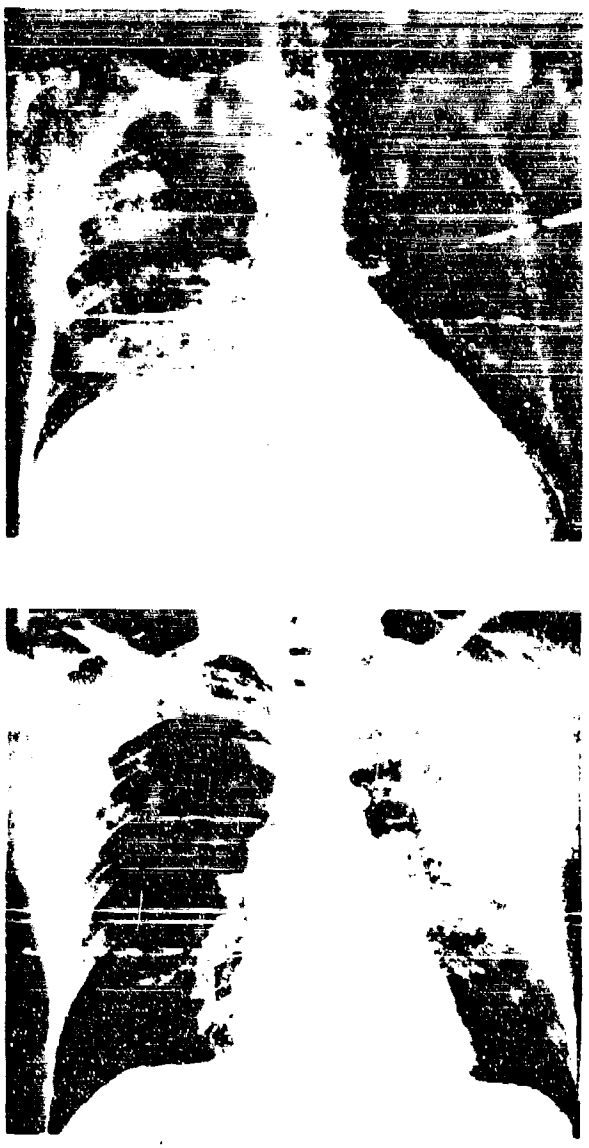


FIGURE 2. CHEST X- RAYS.
A. 6TH DAY OF ILLNESS, SHOWING A 7 CM OVAL INFILTRATE IN THE RIGHT UPPER LOBE.
B. CASE 18, 6TH DAY OF ILLNESS, SHOWING EXTENSIVE BRONCHOPNEUMONIA INVOLVING THE LEFT UPPER LOBE WITH BEGINNING CONSOLATION AND ASSOCIATED LEFT HILAR ENLARGEMENT

positive. The remaining 17 had positive skin tests shortly after admission. Thus in approximately 40 per cent of the cases the skin test was not helpful in early diagnosis.

Table III shows the status of isolation attempts from gastric, pharyngeal, and sputum specimens.

TABLE III. STATUS OF ISOLATION ATTEMPTS[a/] FROM GASTRIC, PHARYNGEAL, AND SPUTUM SPECIMENS IN 30 TYPHOIDAL TULAREMIA CASES

SPECIMEN	CASES BY INITIAL WEEK TRIED						TOTAL
	First		Second		Third		
	No. Positive / No. Tried	%	No. Positive / No. Tried	%	No. Positive / No. Tried	%	No. Positive / No. Tried
Gastric	14/17	82	6/10	60	1/2	50	21/29
Pharyngeal	12/18	67	4/10	40	0/2	0	16/30
Sputum	6/7	86	5/6	83	1/1	100	12/14

a. Attempts by guinea pig inoculation and/or culture on GCBA medium.

Fasting morning gastric aspirates yielded P. tularensis in 70 per cent of 29 typhoidal cases. In 17 patients the first gastric specimen was positive, with the second attempt adding only four additional cases. Further gastric specimens were negative if the first two attempts were unsuccessful. Pharyngeal washings were positive in 16 of 30 patients. The initial specimen yielded the best results, being positive in 75 per cent of the cases. Only two patients, positive by this method had negative gastric washings. In 23 patients where gastric and/or pharyngeal specimens were positive, 21 had positive gastric washings, whereas only 15 had positive pharyngeal washings. Sputum was positive for P. tularensis in 12 of 14 patients; three of these did not have x-ray evidence of pulmonary involvement. Eleven of the 12 with positive sputa also had positive pharyngeal and/or gastric washings.

Initial attempts to isolate the organism from gastric, pharyngeal, and sputum specimens were made in 18, 10, and 2 patients during the first, second, and third week of disease respectively. Miscellaneous specimens included bronchial lavage, which were positive in 2 of 3 patients, and nasal swabs, positive in 1 of 7; these positive isolations were from patients who also had positive isolates from the gastric and/or pharyngeal washings. In the five patients from whom no organisms were isolated, the diagnosis was established by the subsequent 8-fold or greater rise in agglutinin titer in the convalescent phase sera. In summary, the organism was isolated from 25 of 30 patients with typhoidal tularemia, utilizing gastric, pharyngeal and/or sputum samples. The organism was isolated from the three ulceroglandular cases by culturing the

exudate from the local lesion. In spite of culture and guinea pig inoculation of blood from 17 patients, the organism was recovered from the blood of only the critically ill case.

At least one isolate from each patient was examined for antibiotic sensitivity. All were sensitive to 5 μg of chlortetracycline, oxytetracycline, tetracycline and chloromycetin. In addition, bacteriostasis of each isolate was demonstrated on GCB agar containing as little as 1 μg of tetracycline per milliliter of medium. In 22 of the 28 patients, (25 typhoidal, 3 ulceroglandular) the isolates grew readily in the presence of 100 μg of streptomycin. The strain was sensitive to streptomycin in the remaining 6 patients. In every instance the in vitro evaluation of the isolate agreed with the sensitivity status of the organism to which the patient had been exposed prior to infection.

Thirty-two of the 34 hospitalized patients were treated (Table IV); all received bacteriostatic drugs with two receiving streptomycin as well. Oral tetracycline in four divided doses (every 6 hours) was given to 27 patients: 2 gm daily to 26, 1 gm daily to one. Sixteen of these 27 cases received an initial one gram "loading dose." Three patients were given other bacteriostatic drugs: one, 2 gm daily of chlortetracycline (Case 24); one, 2 gm daily for 10 days of chloromycetin (Case 22) followed 18 days later by 2 gm daily (10 days) of Albamycin (Upjohn Co., Kalamazoo, Mich.); and one (Case 18), 1.5 gm daily for 5 days of intravenous oxytetracycline, followed by 3 gm daily of oral tetracycline for 16 days (total 21 days). Two patients were given tetracycline and streptomycin (2 gm daily) for 10 days (Cases 1 and 25). A summary of therapy administration is given in Table V.

TABLE V. DURATION OF THERAPY IN 32 PATIENTS

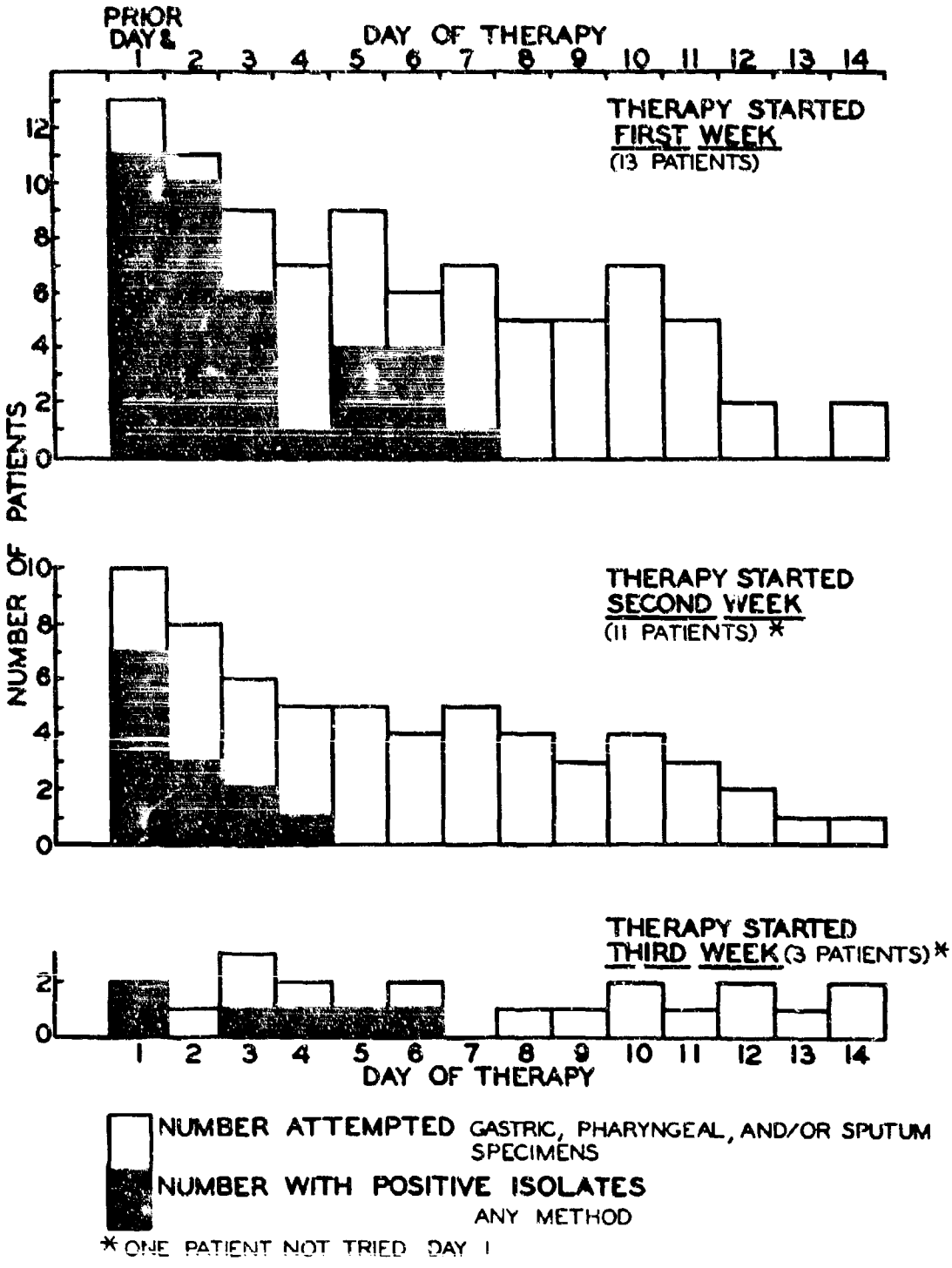
THERAPY	NUMBER OF PATIENTS					
	Duration of therapy days					
	6-8	10-11	13-14	16	21	TOTAL
Tetracycline	5	14	6	1	1	27
Chlortetracycline	1	0	0	0	0	1
Chloromycetin	0	1	0	0	0	1
Oxytetracycline (IV) and Tetracycline	0	0	0	0	1	1
Tetracycline and Streptomycin	0	2	0	0	0	2
TOTAL	6	17	6	1	2	32

TABLE IV. RESPONSE TO ANTIBIOTIC TREATMENT IN 34 HOSPITALIZED CASES OF TULAREMIA INCLUDING SENSITIVITY TO STREPTOMYCIN

SEVERITY [a]	CASE NO.	BROAD SPECTRUM THERAPY Initiation day [b]	L.D. [c]	Duration days	FEVER [d] POST-THERAPY days	STREPTOMYCIN SENSITIVITY OF ISOLATE AND OTHER COMMENTS
ULCERO-GLANDULAR						
Mild	2	12	-	14	2	Resistant
	1	5 [e]	-	9	3	Resistant
	3	9	-	14	5	Resistant
TYPHOIDAL WITH PULMONARY INVOLVEMENT						
Mild	7	None	-	None	4 [f]	Resistant
	5	10	-	10	0	No isolation
	4	15	-	10	1	Resistant
	10	15	-	13	1	Sensitive
	9	4	+	16	2	Resistant
	8	9	+	14	2	Resistant
	19	10	+	11	3	No isolation
	15	17	+	11	3	Sensitive
	13	10	+	21	11	Sensitive
Moderate	6	7	-	7	1	Resistant
		None	-	None	17 [f]	Untreated relapse
	16	6	+	10	2	Resistant
	14	9	+	10	2	Sensitive (Reinfection)
		6 [e]	-	11	2	Resistant (1st Infection)
	20	5	+	11	3	Sensitive
		30	-	13	3	Treated relapse
	12	9	+	14	4	Resistant
	11	9	+	8	5	Resistant
Severe	17	3	+	7	2	Sensitive
		25	-	13	2	Treated relapse
	18	6	+	21	16	Resistant
TYPHOIDAL WITHOUT PULMONARY INVOLVEMENT						
Mild	33	None	-	None	3 [f]	No isolation
	23	9	-	7	1	Resistant
	34	2	+	14	2	Resistant
	29	14	+	10	4	Resistant
	25	20 [e]	-	27	4	No isolation
	24	14	-	7	6	No isolation
	22	12	-	10	7	Resistant
Moderate	31	5	+	11	2	Resistant
	27	6	+	10	2	Resistant
	21	10	-	10	2	No attempts to isolate
Severe	26	6	-	10	1	Resistant
		31	-	15	5	Treated relapse
	28	2	-	6	2	Resistant
	32	3	+	11	2	Resistant
	30	6	+	11	2	Resistant

a. See text for definition.
b. Day of disease.
c. L.D. = 1 to 2 gm "loading" dose of antibiotic.
d. Fever = any rectal temperature greater than 100.0° F.
e. Broad spectrum therapy with added streptomycin.
f. Total days of fever.

FIGURE 3. STATUS OF ISOLATION ATTEMPTS IN 27 TYPHOIDAL TULAREMIA PATIENTS WITH RESPECT TO BEGINNING AND DAY OF BACTERIOSTATIC DRUG THERAPY



* ONE PATIENT NOT TRIED DAY 1

Therapy was instituted during the first, second and third week of illness in 14, 14, and 4 patients respectively.

In all cases there was a dramatic fall of fever within the first 24 hours with only four patients having rectal temperatures exceeding 101.0°F after 48 hours. The mean duration of any rectal temperature elevation above 100.0°F (Table IV) following onset of therapy was 3.3 days with a median of 2 days. There was a corresponding improvement in symptoms within the first 48 hours. The response was equally rapid in the six cases categorized as being severely ill and in 10 patients who did not receive the one gram "loading dose" of tetracycline. Only two patients had a low grade fever exceeding one week after initiation of drug. One of these individuals was mildly ill but had a persistent pleural effusion (Case 13); the other was a critically ill patient with lobar pneumonia (Case 18). With these two exceptions, all patients were essentially asymptomatic in less than one week after initiation of therapy. The majority of patients demonstrated a fall in the sedimentation rate and a return to normal of the CRP during or within two weeks following completion of drug therapy. Likewise, the lung lesions began to improve during the first week of treatment, thereafter slowly resolving and clearing by the fourth to the sixth week. In the three ulceroglandular cases the skin lesions began to improve during the first week of treatment and had healed by the second to third week. The lymph nodes regressed to the point of being non-tender and shotty at 3 to 4 weeks.

The recovery rate of F. tularensis following the initiation of bacteriostatic drug therapy in the typhoidal tularemia cases is demonstrated in Figure 3. It should be noted that the organism was regularly isolated from sputa, pharyngeal washings, and/or gastric washings throughout the first, but not the second, week of treatment, irrespective of whether therapy was started during the first, second, or third week of illness.

Only three patients had complicated clinical courses during their initial treatment. One individual on the 12th day of illness developed a large submandibular lymph node which was eventually biopsied (Case 22). Another patient had a slowly clearing pleural effusion with residual pleural thickening in spite of 21 days of tetracycline therapy (Case 13). The third patient, who was critically ill, responded promptly to therapy but required a two month convalescence (Case 18).

Four patients had an excellent initial response to tetracycline, followed by a recrudescence of disease one to two weeks after cessation of therapy (Figures 4 and 5). In each instance the symptoms were less severe than during the initial illness. Sedimentation rate and CRP were again abnormal.

Case 26 with typhoidal tularemia relapsed 7 days after a 10-day course of tetracycline started on the 6th day of disease.

Case 17 with typhoidal-pulmonic disease relapsed 14 days after completion of 7 days of tetracycline begun on the 3rd day of illness. Therapy was initiated on the 2nd day of relapse and attempts to isolate the organism from the pharyngeal and gastric washings were unsuccessful.

FIGURE 4. SUMMARIES OF TWO RELAPSE CASES OF TYPHOIDAL TULAREMIA. CASE 6. WITH PULMONARY INVOLVEMENT, UNTREATED DURING RELAPSE. CASE 17. WITH PULMONARY INVOLVEMENT, TREATED DURING RELAPSE.

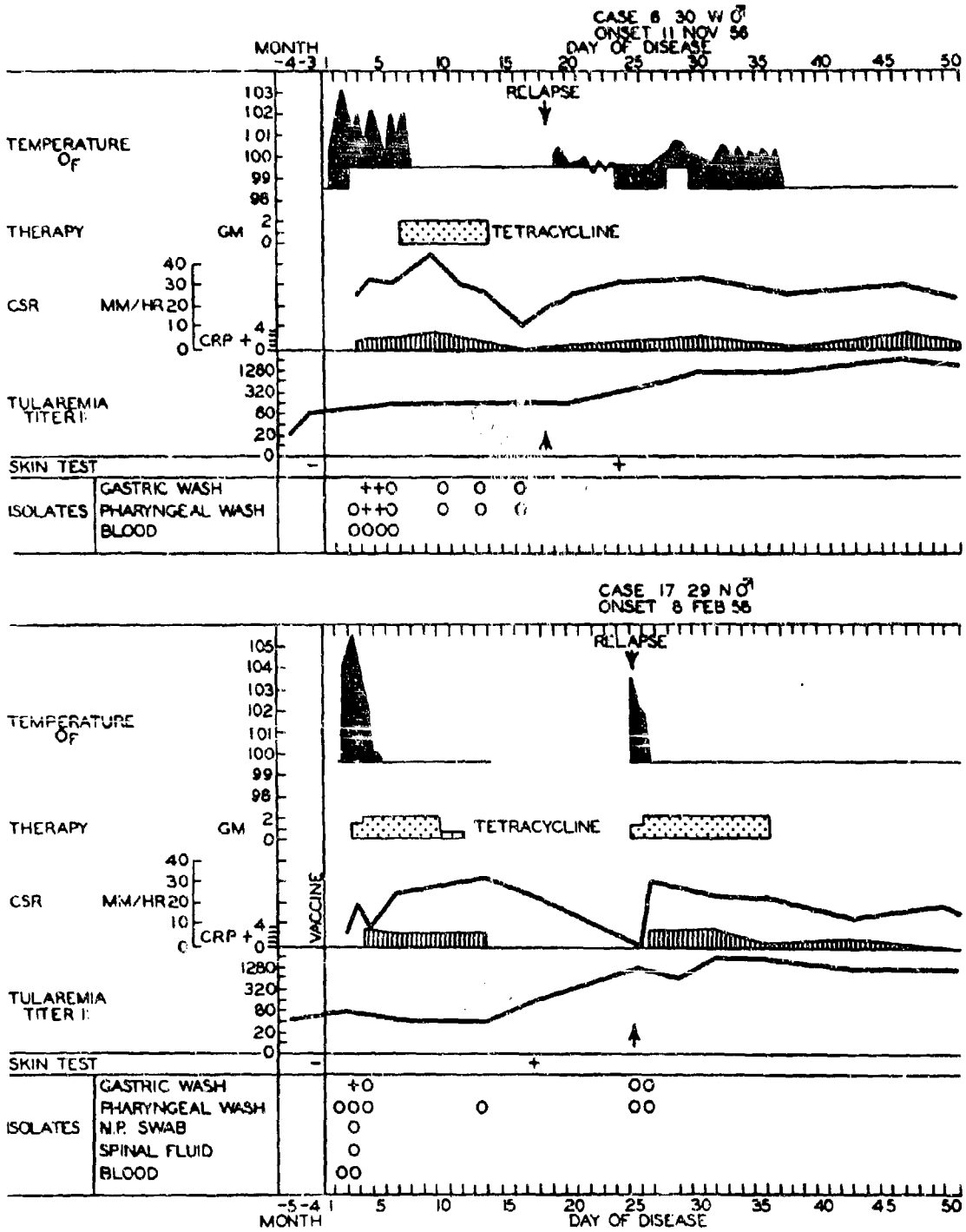
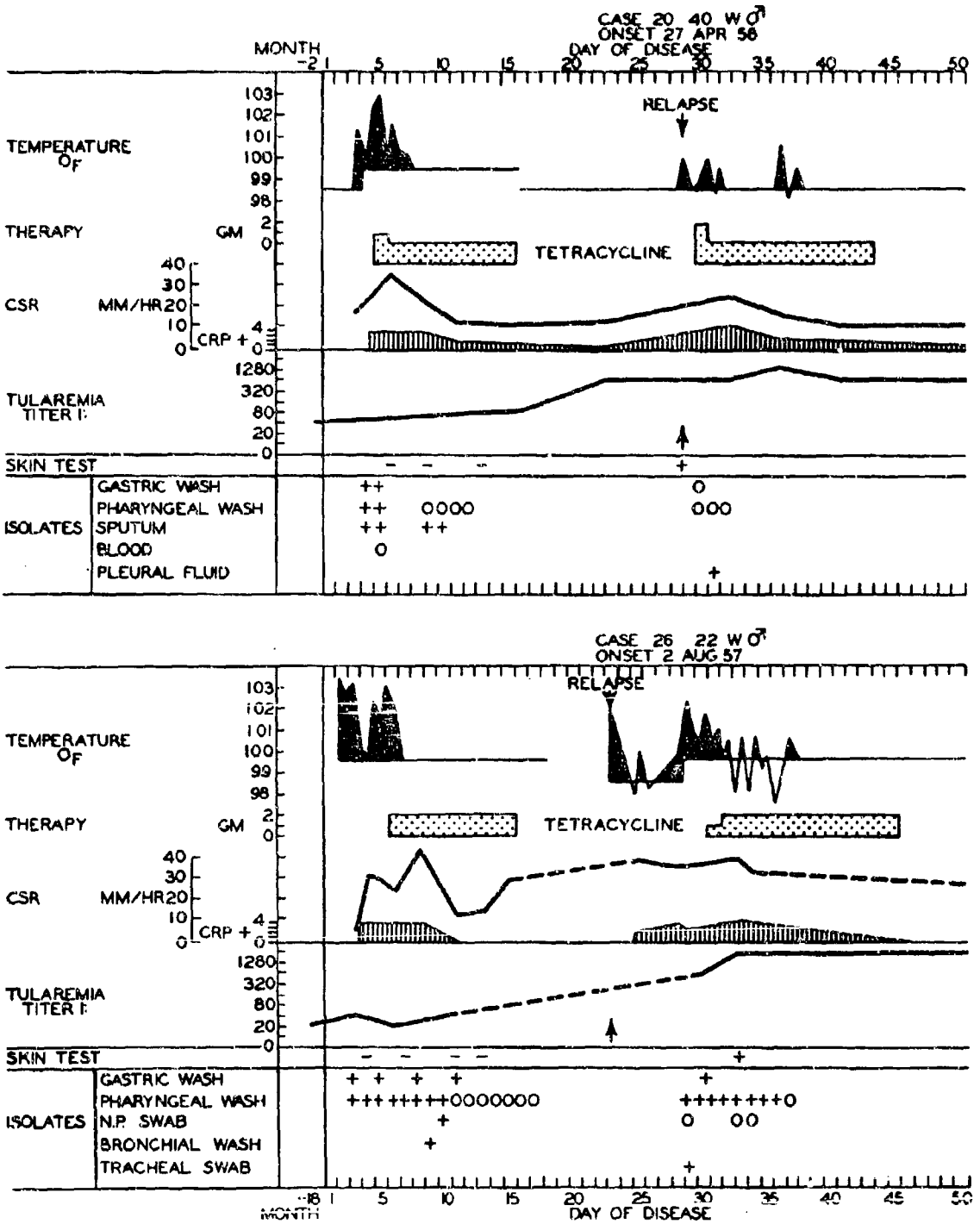


FIGURE 5. SUMMARIES OF TWO RELAPSE CASES OF TYPHOIDAL TULAREMIA. CASE 20. WITH PULMONARY INVOLVEMENT, TREATED DURING RELAPSE. CASE 26. WITH PULMONARY INVOLVEMENT, TREATED DURING RELAPSE.



Case 20 with typhoidal-pulmonic disease had a relapse of grippal symptoms and pleuritic chest pain 14 days after completing 11 days of tetracycline instituted on the 5th day of disease.

In each instance, a 2 gm daily oral dose of tetracycline, given for 10 to 14 days, promptly controlled the disease.

Case 6 with typhoidal-pulmonic disease relapsed 7 days after completion of a week of tetracycline started on the 7th day of illness. Unfortunately no attempt was made to isolate the organism during this presumed relapse, nor was additional therapy given.

The interrelationship of the week broad spectrum therapy was started, duration of therapy, and relapse incidence is demonstrated in Table V.

Thirteen of the 28 treated patients with typhoidal tularemia, were given bacteriostatic antibiotics within the first 7 days of their illness; the four relapses occurred in this group. In contrast, there were no recurrences in the 15 individuals who were treated with a similar dosage and duration after the first week of illness. Despite the absence of frank relapse in this latter group, five asymptomatic patients (Table VI) had rises in sedimentation rate and/or CRP occurring at about the same time as the previously described clinical relapses, i.e., 1 to 3 weeks after therapy (Figure 6).

TABLE VI. RELAPSES AND "SUBCLINICAL"[a] REBOUNDS IN 30 CASES OF TULAREMIA[b] WITH RESPECT TO TIME OF INITIATION AND DURATION OF BROAD SPECTRUM THERAPY

DURATION OF THERAPY days	NUMBER OF CASES					
	Therapy started first week	Clinical Relapses	"Subclinical" Rebounds	Therapy started second or third week	Clinical Relapses	"Subclinical" Rebounds
6 - 8	3	2	0	3	0	2
10 - 11	7	2	1	7	0	0
13 - 14	1	0	0	5	0	2
> 14	2	0	1	2	0	1
Total No.	13	4	2	17	0	5

a. See text for definition.

b. Includes 28 typhoidal and 2 ulceroglandular cases.

This "subclinical" rebound also occurred in two patients in whom therapy had been initiated during the first week of illness.

Two hospitalized cases did not receive specific therapy. One patient (Case 7) was hospitalized on the fourth day of an illness characterized by low-grade fever and mild grippal symptoms. He was asymptomatic the following day. The other patient (Case 33) complained of frontal headache, low back pain, weakness, moderate cough, and occasional shaking chills of one weeks duration. On the first hospital day his temperature reached 104.0°F; on the following day he was afebrile and asymptomatic. Chest films were normal and attempts to isolate the organism were unsuccessful. A positive skin test and a subsequent rise in tularemia agglutinating antibody from 1:10 to 1:1280 confirmed the diagnosis.

C. NON-HOSPITALIZED PATIENTS (Table II)

It has become a routine procedure to obtain follow-up tularemia agglutinin titers and skin tests after non-specific respiratory or grippal illness that may occur in a laboratory worker. Three cases (Nos. 35, 36, 37) were identified in this manner. Each had had mild grippal and upper respiratory symptoms lasting for 7 to 10 days. Convalescent sera demonstrated an 8-fold rise in the tularemia agglutinin titers within four to six weeks and the skin test converted to positive. The remaining five patients were not seen during their illness. One individual (Case 38) reported a potential exposure; blood was drawn and a tularemia antibody titer was reported as 1:40. At a follow-up evaluation 23 days later the titer was 1:1280; and the skin test had converted to positive. Interval history indicated a mild grippal and respiratory illness of approximately ten days duration which occurred 12 days following exposure. A fifth patient (Case 39) was identified by his participation in a vaccine evaluation study in which monthly agglutinin titers were obtained. Five months following the initial vaccine series his titer was known to be 1:80. One month later it was noted to be 1:1280 and the skin test was positive. An interval history revealed a mild respiratory illness of three days duration occurring 18 days before. Another individual (Case 40) was seen by his local physician and treated for possible sinusitis and bronchitis. He received 400,000 units of penicillin and 0.5 gm of streptomycin on the first day of illness. There was temporary improvement but similar therapy on the 13th and 14th days of disease were necessary before he became asymptomatic. Such a response should be anticipated since this individual worked with a streptomycin-sensitive strain of P. tularensis. Five months before this illness his titer was 1:20. There had been no intervening booster series or serial tularemia agglutinin titers. Forty days after onset of his mild bronchitis, the tularemia titer was 1:1280 and his skin test converted to positive. He was the only patient of the non-hospitalized group to receive any therapy. In the remaining two patients (Cases 41 and 42) a high titer and a positive skin test were noted at the time of evaluation for booster vaccination. Anamnesis revealed a mild respiratory and grippal symptom complex from three to seven days in duration which had occurred during the preceding month and could have represented a mild episode of typhoidal tularemia. Thus the eight non-hospitalized cases had illnesses characterized by mild grippal symptoms, occasional slight evening fever, slight cough, minimal sore throat, and nasal stuffiness varying in duration from three days to as long as two weeks.

FIGURE 6.
"SUBCLINICAL" REBOUND AS DEMONSTRATED BY CORRECTED SEDIMENTATION RATE IN 7 PATIENTS
THERAPY – TETRACYCLINE 2 GM/DAY EXCEPT IN CASE 10 (1 GM/DAY)

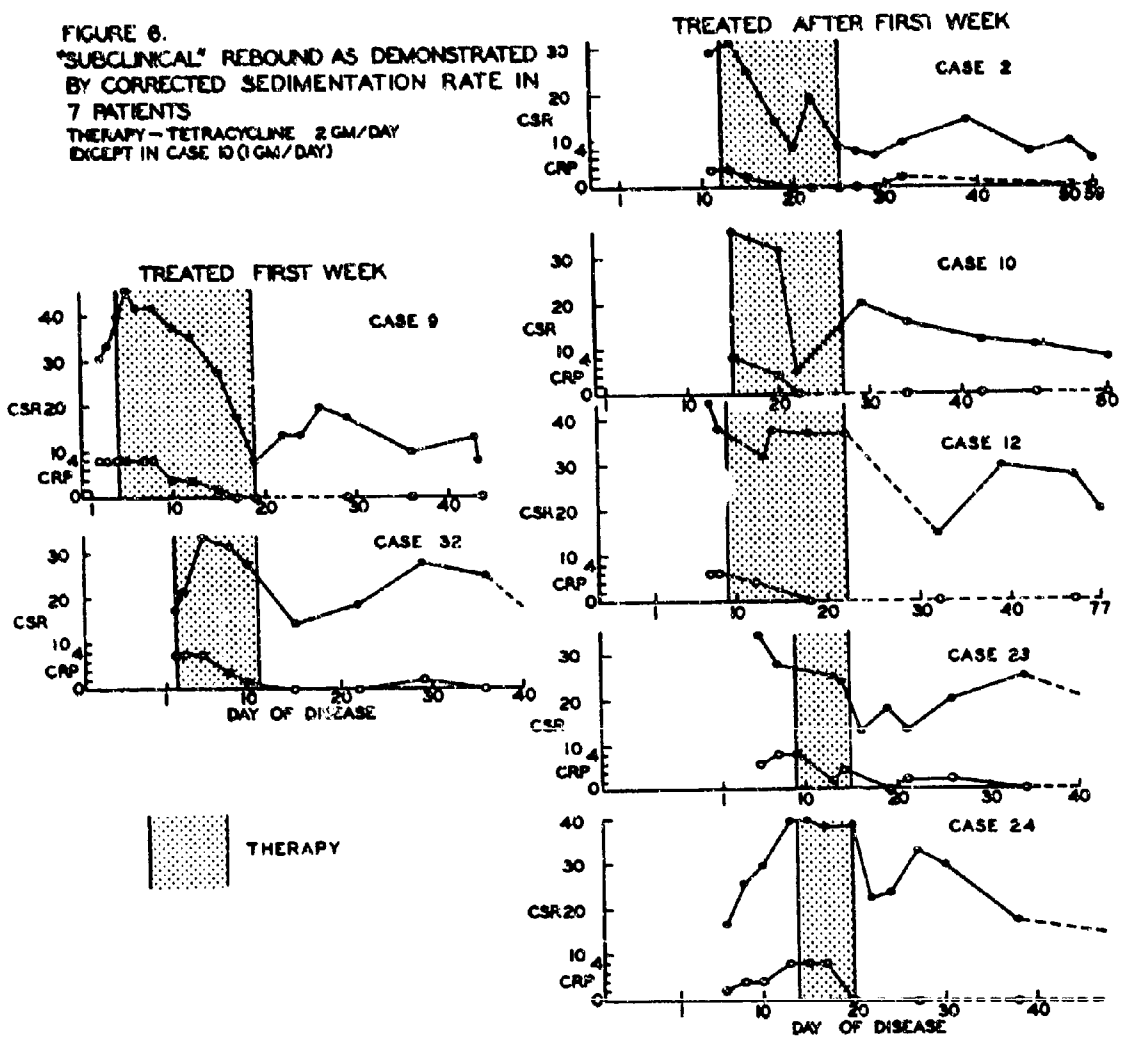
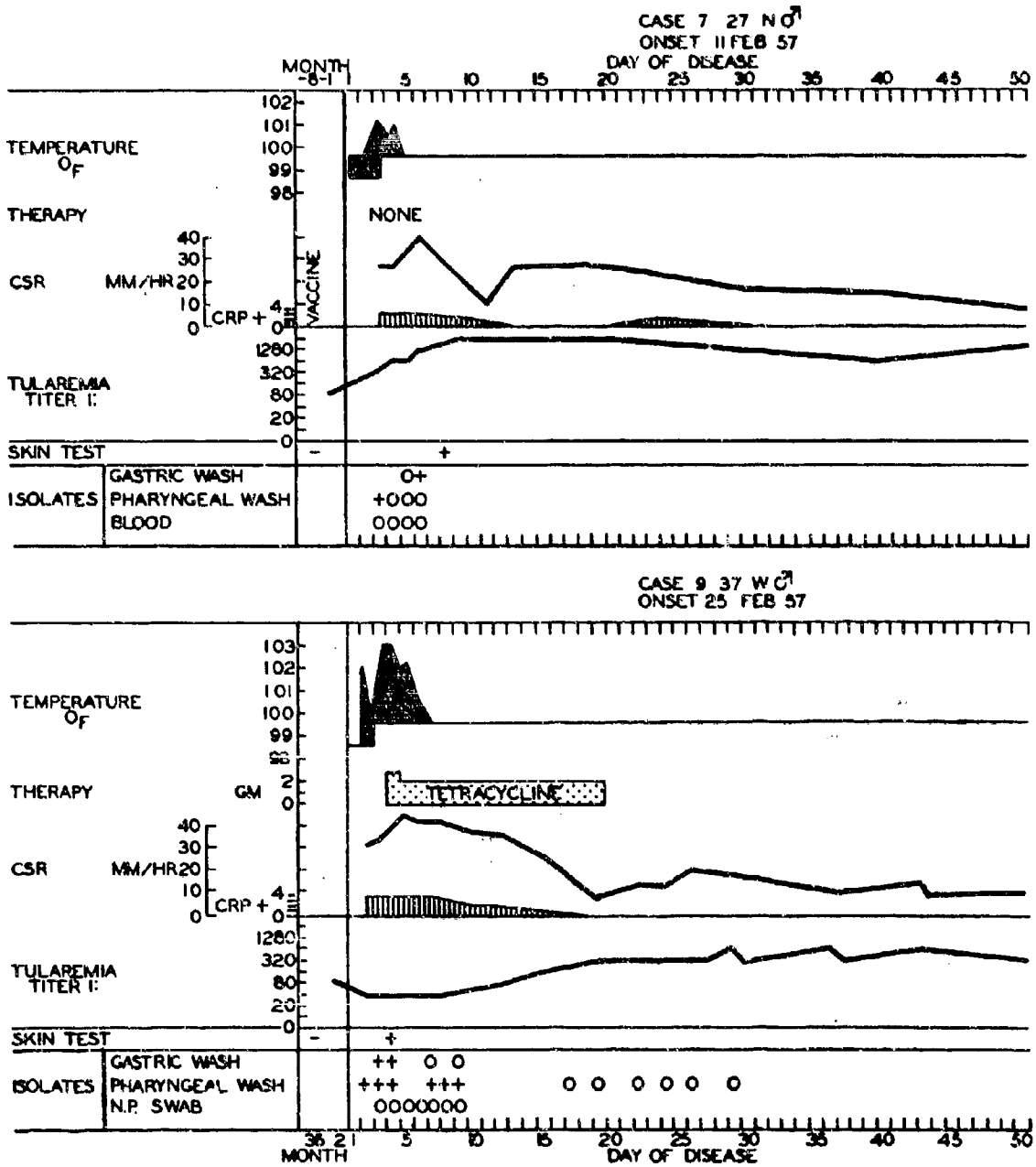


FIGURE 7 SUMMARIES OF TWO CASES OF TYPHOIDAL TULAREMIA WITH PULMONARY INVOLVEMENT. CASE 7. MILDLY ILL, UNTREATED. CASE 9. MODERATELY ILL., TREATED.



D. FOLLOW-UP EXAMINATION

Approximately three-fourths of the 42 patients have been followed for at least six months after their illness. Thirty-nine of the group have remained asymptomatic; three typhoidal cases have had residual complaints. One individual (Case 13) in whom therapy was started on the 10th day of illness continued to have a low-grade fever for the first 11 days of a 21-day course of tetracycline. The multiple ovate bronchopneumonic lesions promptly subsided, but the pleural reaction failed to clear completely until eight months later. This patient's only complaint was dull intermittent right lower posterior chest pain. A stag-horn calculus of the right kidney, which existed prior to tularemia infection, was removed seven months after discharge from the hospital and the vague lower chest pain subsided. The remaining two typhoidal cases had clinical relapses following excellent responses to initial therapy. One individual (Case 26) promptly responded to the second course of tetracycline therapy. Nevertheless, he continued to complain of easy fatigability and generalized myalgia on damp days in spite of the absence of subjective physical findings, normal temperature, normal sedimentation rate, and negative CRP over the succeeding nine month follow-up period. The remaining patient (Case 6) did not receive antibiotic therapy during his relapse. He complained of easy fatigability, substernal chest tightness, generalized arthralgias, and noted an occasional 1 to 2° evening temperature rise. In addition, the sedimentation rate and CRP remained abnormal. The symptoms and signs of low-grade illness spontaneously subsided after six months; during the past year he has enjoyed good health. One of the 17 patients with x-ray evidence of pulmonary involvement has residual changes: a small linear area of fibrosis in the area of previous lobar pneumonia is still present eight months later (Case 18).

E. CASE REPORTS

Case 7 (Figure 7)

This 27 year old negro was admitted November 14, 1956. Three days earlier he noted intermittent chilliness, slight feverishness, and a daily oral temperature ranging from 100.0 to 101.0°F. Physical examination revealed a robust male in no distress. Physical findings were limited to a few crepitant rales over the mid-portion of the left posterior lung field and a rectal temperature of 101.0°F. On the 5th day of illness he spontaneously became afebrile and asymptomatic with the chest rales clearing by the end of the first week of observation. Admission laboratory data included a white blood cell count of 10,000/cu mm with a normal differential, a sedimentation rate of 26 mm/hour, and a 3+ CRP. Blood cultures on the 3rd through the 6th day of illness were negative. A streptomycin-resistant strain of P. tularensis was isolated on direct culture and by guinea pig inoculation from the pharyngeal washing of the 3rd day of illness. A gastric washing obtained on the 6th day of disease was positive (the patient was afebrile and asymptomatic). Pharyngeal and gastric washings were negative on the 16th day of illness. Admission chest film revealed a prominent left hilar area and a juxta-hilar 4 by 5 cm bronchopneumonic patch involving the left upper lobe. These abnormalities regressed within the first week and thereafter slowly cleared, being no longer evident by the 30th day of illness. Because of the mildness and prompt remission of symptoms no specific

therapy was instituted. The CRP was negative by the 13th day while the sedimentation rate reached normal by the 39th day of illness.

Five months prior to illness the patient received an initial vaccine series for tularemia. There were no intervening booster series; the agglutinin titer 25 days before illness was 1:80. The titer rose to 1:1280 and 1:2560 by the 6th and 9th days of illness, respectively; one year later it was still 1:640. The skin test on the 5th hospital day was positive. Throughout a 12-month follow-up he has remained well.

COMMENT

This case demonstrates how minimal and self-limited the symptoms may be in laboratory-acquired tularemia. The hilar lymph node enlargement and juxta-hilar bronchopneumonia occurred in the absence of severe illness or respiratory symptoms. P. tularensis was isolated from pharyngeal and gastric washings during the first week of disease. Eight non-hospitalized patients and one other ward patient also had as mild illnesses. No correlation with the number of previous tularemia vaccinations and severity of disease was evident. This patient exemplifies another unusual feature: the 8-fold rise in titer appeared in the first week of illness; this early rise was observed in only one other equally mild case. The remaining 40 patients failed to have such rapid rises in agglutinin titer, although many were only mildly ill.

Case 9 (Figure 7)

This 37 year old white male was admitted February 27, 1957. There had been no recognized exposure although 4 days before onset of symptoms he had cleaned a potentially contaminated laboratory. He had received an initial vaccine series about three years prior to illness. There was one intervening booster, 26 months before illness. Two months before infection the tularemia agglutinin titer was 1:80.

Two days prior to admission the patient noted mild feverishness, chilliness, intermittent sweats, moderate fatigue, generalized myalgias, anorexia, and frontal headache. By the 3rd day of symptoms there was mild nasal stuffiness and an irritating non-productive cough. A dull anterior left chest pain became apparent by the 6th day of illness.

Physical examination revealed a well-nourished white male who appeared to be only mildly ill. Rectal temperature was 103.0°F and there was injection of the nasal and pharyngeal mucosa. Admission laboratory data included a white blood cell count of 10,000/cu mm with 72 per cent neutrophils and 16 per cent bands, a sedimentation rate of 34 mm/hour, and a 4+ CRP. Admission chest film revealed a 1-cm oval, juxta-hilar, bronchopneumonic patch in the left mid-lung field which fused with the prominent hilar area. Blood cultures of the 3rd, 4th, 5th, and 8th days of illness were negative. Pharyngeal washings of the 1st through the 3rd hospital days were positive on culture and guinea pig inoculation for a streptomycin-resistant strain of P. tularensis. Gastric washings of days 2 and 3 were positive for a similar isolate by guinea pig inoculation. Material from nasal swabs was cultured throughout the same period but was negative.

a skin test performed on the 4th day of illness was read as positive 48 hours later.

Oral tetracycline was started on the 2nd hospital day, 2 gm "loading dose" plus 2 gm daily in four divided doses for the next 16 days. Within 36 hours he was afebrile, fully ambulatory, and complained only of slight nasal stuffiness, vague left subpectoral chest pain, and a dry non-productive cough. These symptoms disappeared within the ensuing week.

The pharyngeal washings remained positive for P. tularensis from the 2nd through the 7th days of treatment, but were negative thereafter. Forty-eight hours after starting tetracycline, the bronchopneumonic patch had enlarged to 2 to 3 cm; thereafter it slowly resolved and was no longer evident by the 43rd day of illness. The sedimentation rate and CRP returned to normal by the 14th day of therapy. Although the patient remained asymptomatic seven days following completion of therapy the sedimentation rate was found to be 20 mm/hour. It spontaneously returned to normal by the 36th day of illness. The tularemia agglutinin titer rose during the second week and reached a diagnostic level of 1:640 by the 29th day. In a 12-month follow-up period he has remained well.

COMMENT

This case is representative of the majority of typhoidal-pulmonic disease seen in this series. In addition to grippal symptoms there were dry irritating cough, vague chest ache, and nasal stuffiness. The x-ray revealed a left hilar enlargement and a juxta-hilar oval bronchopneumonic patch. All but two of the 17 cases with pulmonary involvement had similar pneumonic infiltrates. The positive skin test was helpful in suggesting tularemia and a specific diagnosis was possible 72 hours after admission by culture of gastric and pharyngeal washings. As was usually the case, the "diagnostic rise" in the agglutinin titer did not occur until the 4th week.

The prompt loss of fever and symptoms and improvement of the abnormal chest film, sedimentation rate, and CRP were characteristic responses to tetracycline therapy. The organism could regularly be isolated during the first week of drug treatment. The rise of the sedimentation rate 7 days after completion of tetracycline and in the absence of symptoms was considered a "subclinical rebound" (Figure 3). This occurred at the same time-interval following completion of therapy as did other clinical relapses, i.e., 1 to 3 weeks.

Case 14 (Figure 8)

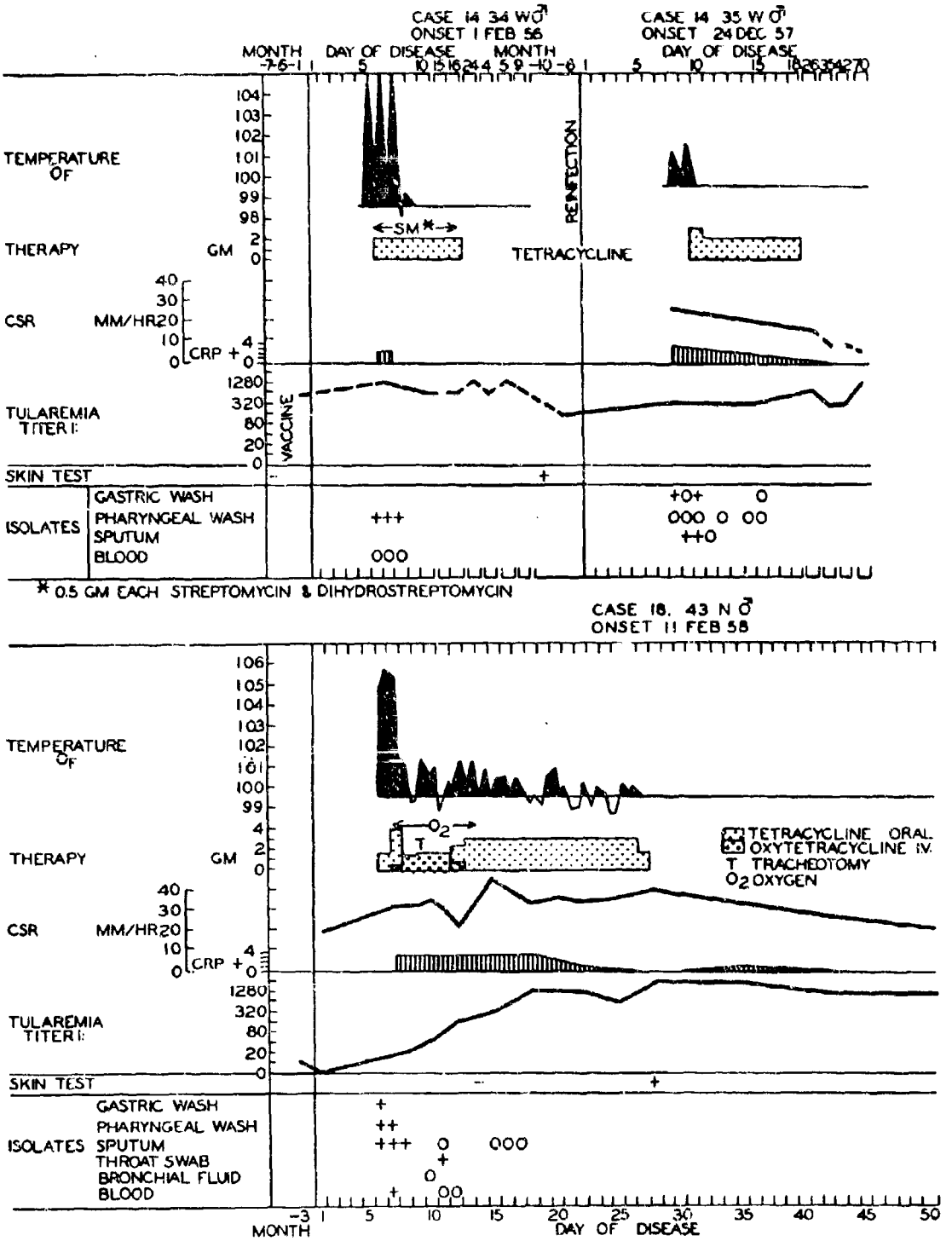
This 34 year old white male was initially admitted February 5, 1956. His first attack of tularemia occurred prior to this study. This patient received an initial vaccine series 14 months prior to his first infection, with one intervening booster six months before his first illness.

Four days before this first admission, he noted the onset of generalized malaise, frontal headache, raw throat, nasal stuffiness, dry non-productive cough,

FIGURE 8. SUMMARIES OF TWO CASES OF TYPHOIDAL TULAREMIA WITH PULMONARY INVOLVEMENT.

CASE 14. SEVERELY ILL, FIRST INFECTION, TREATED. MODERATELY ILL, REINFECTION, TREATED.

CASE 18. CRITICALLY ILL, TREATED.



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intermittent shaking chills, drenching sweats, and feverishness. Physical examination revealed a moderately toxic appearing male with an oral temperature of 103.0°F. There were diminished breath sounds and inspiratory rhonchi over the posterior left lower lung field. Admission laboratory studies revealed a white blood cell count of 10,300/cu mm with normal differential and a sedimentation rate of 5 mm/hour. A chest film revealed a 3 by 2 cm oval density lying in the third anterior interspace of the left lung merging with the hilar shadows. Bloods on culture and guinea pig inoculation were negative for P. tularensis on the 2nd, 3rd, and 4th hospital days. Pharyngeal washings on these same dates were positive for the streptomycin-resistant strain.

Therapy was instituted on the 2nd hospital day and consisted of 2 gm of oral tetracycline and 1 gm of intramuscular streptomycin daily for 11 days. During the first 48 hours of treatment the oral temperature fluctuated between 100.0 and 105.0°F; there were alternating severe chills and sweats. At each temperature spike aspirin was given. After 72 hours he was afebrile and essentially asymptomatic with the exception of dull, left chest pain and a mild non-productive cough; these symptoms subsided by the completion of therapy. The pulmonic lesions regressed and there was only a slight residual infiltrate by the 24th day of illness. There were no further films during the acute phase but the chest film nine months later was normal. The tularemia agglutinin titer 27 days prior to illness was 1:640 and varied between this level and 1:1280 throughout the illness. The tularemia skin test one year later was positive.

In the intervening 18 months prior to reinfection he enjoyed good health. He was admitted the second time on December 31, 1957; seven days earlier he had noted intermittent feverishness, chilliness, night sweats, increasing fatigue, generalized myalgia, mild frontal headache, non-productive cough, and diffuse substernal chest soreness. Physical examination revealed a well-developed, well-nourished male who appeared mildly ill. The only physical findings were a rectal temperature of 101.0°F and a few crepitant rales in the left mid-axillary line at the level of the angle of the scapula. Admission laboratory data showed a white blood count of 13,000/cu mm with normal differential, sedimentation rate, 26 mm/hour, and CRP of 4+. A chest film revealed a 2-cm oval infiltrate with indistinct borders lying in the mid-portion of the left lower lung field. This infiltrate was slightly lateral to the previous area of involvement. An admission gastric washing was positive for the streptomycin-sensitive strain of P. tularensis. The organism was isolated in a similar manner from small amounts of sputum obtained on the 2nd and 3rd hospital days. However, the pharyngeal washings of the 1st through the 6th hospital days were negative.

Oral tetracycline, 2 gm daily, was started on the 2nd hospital day and continued for 10 days. The grippal symptoms and fever subsided within 48 hours; the dry cough and vague chest distress disappeared after 5 days. Sedimentation rate and CRP were normal by the 28th day; the pulmonary infiltrate had cleared by the 42nd day of illness. The tularemia agglutinin titer six months prior to reinfection was 1:160. During the 2nd through the 6th week of illness the titer fluctuated between 1:320 and 1:640 and reached a peak level of 1:1280 on the 70th day. Throughout a 9-month follow-up the patient has remained well.

COMMENT

During each illness, upper and lower respiratory tract symptoms were prevalent; bronchopneumonia involving the left lower lobe was noted. During the first episode the symptoms were moderately severe and incapacitating. It is probable that the use of aspirin at the peaks of fever contributed to the disability by producing wide swings in the temperature with alternating severe chills and sweats. This has not been observed since the use of antipyretics was discontinued.

This was the only instance of recognized reinfection among our patients though all have returned to the laboratory and worked with P. tularensis. It should be noted that the initial disease was caused by a streptomycin-resistant organism whereas reinfection was caused by a streptomycin-sensitive strain; both infections occurred with high pre-illness agglutinin titers. Tetracycline was effective and the use of streptomycin during the initial illness was unwarranted.

Case 18 (Figure 8)

This 43 year old colored male was admitted February 16, 1958. He had received an initial vaccine series seven years earlier. The last of three subsequent boosters had been given approximately four years prior to illness; as late as October, 1957 the agglutinin titer was 1:10. There had been no recognized exposure. Five days prior to admission the patient noted the sudden onset of chilliness, generalized aching, nasal stuffiness, and retrobulbar pain. In the Out-patient Department three hours later the physical examination was normal with the exception of an oral temperature of 99.6°F. The white blood cell count was 14,900/cu mm with 75 per cent polymorphonuclear leukocytes and the sedimentation rate was 19 mm/hour; the tularemia agglutinin titer was negative. Symptomatic therapy was prescribed for the mild upper respiratory illness and the patient was advised to return should the symptoms persist or progress in severity. For the next five days there were increasingly severe sweats, fever, sore throat, productive cough, nausea, and vomiting. By the 5th day of illness, fatigue and weakness confined him to bed. Dyspnea, blood-tinged sputum, and moderate diarrhea were now also present and an occasional oral temperature taken at home varied between 104.0 and 106.0°F.

He again sought medical attention on the evening of the 6th day of illness. Physical examination revealed a well-developed, well-nourished, oreinted male who appeared apprehensive, dyspneic, and acutely ill. Rectal temperature was 105.0°F, the pulse rate was 130, regular and full, and the blood pressure was 130/70. There was moderate edema of the nasal mucosa and diffuse hyperemia of the pharynx. Dullness to percussion, crepitant rales, and a pleural friction rub were apparent over the upper two-thirds of the left chest but the right chest was clear. The abdomen was not distended, no masses were palpable, and peristalsis was normal on auscultation.

Admission laboratory data showed a white blood cell count of 8,800/cu mm with 87 per cent polymorphonuclear leukocytes of which 70 per cent were bands, a sedimentation rate of 32 mm/hour, a CRP of 4^+, and a normal urinalysis except

for a 2+ albuminuria. A sickle cell preparation was negative. The chest film (Figure 2b) revealed a diffuse, mottled infiltrate in the left upper lobe, left hilar prominence and an early reticulated infiltrate in the right cardiophrenic angle. Lateral films of the chest revealed the apical posterior segment of the left upper lobe to be principally involved.

The severe respiratory illness, normal white blood count and diffuse, multiple pulmonary infiltrates with hilar adenopathy prompted the diagnosis of "probable tularemia." One hour after admission a one gram "loading dose" of oral tetracycline was given, followed by 0.5 gm every six hours. Within 24 hours the peak rectal temperature of 106.0°F had fallen to 101.8°F. However, during this period the respiratory rate rose to 60 per minute with accompanying cyanosis and tracheal rattle. The cough was non-productive; there were wet rales throughout both lung fields indicating an extension of the disease process. Chest films on the 7th day of illness indicated multiple 0.5-cm lesions throughout the right and left lung fields. In addition, there was abdominal distention with progressive diarrhea. Consequently, the oral tetracycline was replaced with intravenous oxytetracycline in a dose of 1.5 gm daily for the next five days. Supportive therapy consisted of intravenous fluids and electrolytes, oxygen by tent, and intermittent Isuprel HCl (Winthrop Laboratories) by nebulization along with intravenous aminophylline in an attempt to decrease the respiratory distress. In spite of the control of fever and absence of vascular collapse, the patient's condition remained critical. On the 3rd hospital day paraldehyde was necessary to control the delirium. At this time the shallow and rapid respirations had increased to 70 per minute, heart rate was 150, the cough was still non-productive, cyanosis continued and the thick bloody bronchial secretion could not be aspirated by suction.

A tracheotomy was performed on the 4th hospital day to reduce dead air space and permit more direct aspiration of bronchial secretions. This allowed the intermittent removal of thick tenacious yellow and bloody secretions. Within the next 48 hour the respiratory rate decreased to 40 per minute, the tracheal rattle cleared, and the rales throughout both lung fields decreased. By the 6th hospital day intravenous fluids were no longer necessary; oral tetracycline in a dose of 3 gm daily was begun and continued for the remainder of the 21-day course of broad spectrum therapy. By the 13th hospital day the patient was afebrile, the respiratory rate was normal, and there was only a slight non-productive cough. The following day he complained of mild headache and low back ache; rectal temperature was 101.6°F. Because of the presence of several influenza cases on the ward, a pharyngeal washing was obtained and Type-A-Japan-57 was recovered. This virus was not recovered from the pharyngeal washings obtained on the day of admission. Within three days the mild grippal symptoms subsided. At the completion of drug therapy the patient's only complaints were moderate fatigue, slight dyspnea on exertion, and mild right ankle edema.

Thirty-six hours after admission the laboratory reported the isolation of the streptomycin-resistant strain of *P. tularensis* from the sputum. Blood cultures on the second hospital day were also positive, as were the gastric and pharyngeal washings. The organism was readily isolated from the sputum on the first two days of therapy but could not be isolated on the 6th day. However,

material taken directly from the trachea was positive on guinea pig inoculation as late as the 6th day of drug therapy. The organism could not be isolated from the sputum during the 2nd week of treatment. A skin test was negative on the 14th day of illness but positive on the 28th day. The tularemia agglutinin titer rose during the 2nd week of illness to 1:160 and reached 1:1280 by the 3rd week.

During the second month of convalescence his strength returned, the exertional dyspnea subsided and he regained the 25 pounds lost during the acute phase of illness. The CRP was negative by the 25th day of illness but the sedimentation rate remained elevated for an additional month. At a 9-month follow-up evaluation the patient was asymptomatic with the exception of mild right ankle edema due to phlebitis following intravenous oxytetracycline therapy. Serial chest films revealed only a fine linear strand in the area of previous pneumonitis.

COMMENT

This was the only critically ill patient of this group. The severe grippal and respiratory symptoms, delirium, abdominal distention, and diarrhea are characteristic of the fatal forms of untreated, naturally-occurring typhoidal tularemia. This case provided the only occasion when the organism was isolated from the blood. This was consistent with prior experience, i.e., positive blood cultures have been obtained only in the fulminating and terminal phase of illness.

Broad spectrum therapy dramatically controlled fever; however, the extensive pulmonary involvement with resulting respiratory insufficiency remained a threat to the patient's survival. The tracheotomy on the 4th day of hospitalization appeared to turn the tide. The pulmonary infiltrate began to resolve within the first week of therapy and thereafter slowly cleared; a minimal residual fibrotic strand was all that remained nine months later. It was possible to isolate the organism throughout the first week of therapy. The skin test was not helpful in early diagnosis since it was negative in the first two weeks of illness and the agglutinating antibodies were not at a "diagnostic" level until the third week.

Case 20 (Figure 5)

This 40 year old white male was admitted April 30, 1958. Six days earlier he had inoculated guinea pigs with P. tularensis, SCHU S4 strain. Three days later he noted mild malaise, feverishness, occasional sweats, nasal stuffiness, a slightly productive cough, and a dull, aching, left lower chest pain accentuated by breathing. An initial vaccination series had been given approximately a year prior to illness followed by a booster series six months later. Two months prior to illness the tularemia agglutinin titer was 1:40.

Physical examination revealed an obese, white male who appeared to be mildly ill with a rectal temperature of 100.2°F. Admission laboratory data included a white blood cell count of 9,750/cu mm with a normal differential, a sedimentation rate of 21 mm/hour, and a 4+ CRP. A linear radiodensity was noted in the left lower lung field compatible with a small area of plate-like

atelectasis. On the 2nd hospital day the temperature reached 104.0°F; he appeared more toxic and complained of pleuritic pain in the left chest and substernal tightness.

Oral tetracycline in a dosage of 2 gm daily was begun on the 3rd hospital day and continued for 11 days. Within 48 hours he was afebrile, and by 96 hours asymptomatic. On the 2nd day of therapy the chest film revealed the maximum extent of involvement. In addition to the atelectasis, there was a small left pleural effusion and two oval 1 by 2 cm bronchopneumonic patches lying juxta-hilar in the left 3rd and 4th anterior interspaces. The admission gastric, pharyngeal, and sputum specimens were reported as positive for the streptomycin-sensitive strain. The sputum remained positive through the 6th day of drug therapy. The skin test was negative on the 5th through the 13th days of illness but was positive on the 30th day. The agglutinin titer was 1:80 on the 16th day of illness but rose to 1:640 and 1:1280 by the 23rd and 37th days respectively. The x-ray abnormalities, elevated sedimentation rate and CRP returned to normal at the completion of 11 days of tetracycline.

Except for mild fatigue the patient was asymptomatic until 14 days after completion of therapy, or 29 days from onset of illness. He again noted low grade fever, "sticking" left lower chest pain, and a dry cough. Readmission white blood cell count and differential were normal but the sedimentation rate was 24 mm/hour and CRP was 4+. Chest films revealed a moderate left pleural effusion and two areas of plate-like atelectasis in the left lower lobe. Gastric and pharyngeal washings were negative on culture and guinea pig inoculation for P. tularensis. Thoracentesis was performed on the 4th day of relapse with removal of 650 ml of a turbid, straw-colored fluid. Specific gravity was 1.019, total protein 4.2 gm per cent and white blood cell count of 31,850/cu mm, predominately polymorphonuclear leukocytes. This fluid was positive on culture and guinea pig inoculation for the streptomycin-sensitive strain of P. tularensis.

On the 3rd day of relapse tetracycline therapy was instituted using a 1 gm "loading dose" and 2 gm daily thereafter for 14 days. Forty-eight hours later the patient was afebrile. However, dull left lower chest pain, fatigue, and a non-productive cough persisted throughout the first week of the second course of treatment. The pleural effusion cleared; the abnormal sedimentation rate and CRP returned to normal by the completion of the 14-day treatment. He has remained well during a five month follow-up period.

COMMENT

Upper and lower respiratory tract symptoms were prominent in this case. The presumptive diagnosis of tularemia and prompt institution of therapy were based on the grippal and respiratory symptoms, with a normal white blood cell count, an elevated sedimentation rate and CRP, and bronchopneumonia in an individual who worked with P. tularensis. The skin test was negative as late as the 13th day of illness and the agglutinin titer did not reach a "diagnostic" level until the 23rd day. However, the streptomycin-sensitive organism was isolated from the gastric, pharyngeal, and sputum samples of the 4th and 5th day of illness. The sputum remained positive as late as the 6th day of therapy.

Though the clinical and laboratory evidence of disease cleared with the 11-day course of tetracycline, a relapse occurred 14 days later. The symptoms were less severe but the pleural effusion reoccurred; P. tularensis was recovered from the pleural fluid. The isolate obtained during relapse retained

its *in vitro* sensitivity to tetracycline and a second course of this drug was effective. It should be noted that the relapse occurred in spite of a positive skin test and a high agglutinin titer.

Case 22

This 25 year old negro male was admitted October 16, 1956. He had received an initial vaccine series approximately 45 months earlier; there were no intervening booster series. The agglutinin titer three months before infection was 1:40. There had been no recognized exposure but he had worked with the SCHU S5 strain of *P. tularensis* on only one day, four days prior to onset of symptoms. Eight days before admission he noted night sweats, evening fever, moderate anorexia, and mild frontal headache. Though the symptoms did not increase in severity and he continued to work daily, their persistence prompted his admission.

Physical examination revealed a well-nourished male who did not appear ill. The oral temperature was 100.4°F. Admission laboratory data showed a white blood cell count of 8,520/cu mm with normal differential, a sedimentation rate of 26 mm/hour, CRP of 3+, and a normal chest film. During the first three days of observation the patient complained of moderate night sweats, anorexia, slight frontal headache, and low back ache. The oral temperature ranged between 101.0 and 102.0°F. On the 4th hospital day, the 12th day of illness, a painless swelling developed just anterior and overlying the angle of the right mandible. This mass measured 4 by 4 cm; it was round, firm, non-tender, easily movable submental node. Dental and mandible films as well as daily examination of the oral cavity were normal.

Two grams daily of oral chloromycetin were started on the 4th hospital day and continued for 10 days. Within 48 hours the patient's mild symptoms subsided and the temperature fell to 99.0 to 100.0°F where it persisted for the next six weeks. The mass on the right mandible remained unchanged and the elevated sedimentation rate and CRP persisted in spite of therapy.

Pharyngeal washings on the 1st and 2nd hospital day were negative for *P. tularensis* on guinea pig inoculation and culture, but a gastric washing obtained the 3rd hospital day was positive for streptomycin-resistant strain. The admission skin test was positive and tularemia agglutinin titer rose to 1:640 by the 7th and 1:1280 by the 11th hospital days.

The mass remained unchanged and he was transferred to another hospital for an excisional biopsy on the 36th day of illness. At surgery it appeared to be lymphoid tissue, firmly adherent to the adjacent tissue but did not involve the bone. Removal by dissection was not possible; it was therefore removed by curettage. The biopsy site rapidly healed. No organisms were cultured from the biopsy specimens; microscopic examination demonstrated areas of necrosis surrounded by epithelioid cells and a few giant cells with marked infiltration by round cells, plasma cells, and macrophages. A diagnosis of an inflammatory granulomatous reaction compatible with tularemia was made.

Following surgery the patient felt well, but a low-grade evening fever persisted; consequently a 10-day course of 2 gm daily of oral Albamycin was started on the 6th post-surgical day with the temperature subsiding to normal within 24 hours. However, on the 7th day of therapy he developed moderate fever and a pruritic, erythematous, macular rash which cleared three days after

discontinuing the antibiotic. One month later the sedimentation rate and CRP were normal. The patient has remained well throughout a one year follow-up period. The skin test has remained positive and the tularemia agglutinin titer persists at 1:1280.

COMMENT

The history of potential exposure to the organism on only the 4th day before onset of illness permitted an estimation of the incubation period. The absence of any respiratory symptoms was noted in only four of the 39 typhoidal cases. Nevertheless, the organism was obtained from gastric washings. The mild but persistent symptoms were consistent with the type of illness seen in the majority of our cases.

The appearance of the lymphoid mass over the right mandible on the 12th day of illness was an unusual feature. Chloromycetin promptly controlled the patient's mild symptoms but the low grade fever and mass persisted. This has been noted also following the late use of antibiotics in naturally-occurring ulceroglandular disease. Failure to isolate the organism from the involved node in this patient was to be expected; even without treatment the recovery of P. tularensis from local nodes is rare after the first month of illness.

More commonly, cervical lymph node involvement is associated with pharyngotonsillar or the angiose form of disease. This form of tularemia is seen in the pediatric age group and commonly follows infection by ingestion. In this case, no oral lesion was apparent. Nevertheless, direct extension from the oral cavity seems probable.

Case 26 (Figure 5)

This 22 year old white male was admitted to the hospital on August 2, 1957. He had received an initial vaccine series approximately five months prior to onset of illness; two weeks before infection the tularemia agglutinin titer was 1:20. The day prior to admission the patient noted a severe pounding, bilateral, temporal headache with anorexia, and insomnia. The following day nasal stuffiness, moderate sore throat, severe myalgias of the lower back and calf muscles, sweats, fever, and weakness compelled him to remain in bed. These symptoms appeared three days after a recognized laboratory accident. A co-worker became ill five days after this exposure with a typhoidal form of tularemia.

Physical examination revealed a young white male who appeared moderately ill. The physical findings were limited to a rectal temperature of 103.6°F, pulse 100, and an injected pharynx and nasal mucosa. Admission laboratory data included a white blood cell count of 7,800/cu mm, 74 per cent neutrophils (19 per cent bands), sedimentation rate of 4 mm/hour, and a 4^{+} CRP. The following day the sedimentation rate had risen to 30 mm/hour. Serial chest films throughout the two-week period of hospitalization were normal. The tularemia skin test was negative from the 4th through the 13th day of illness, but was positive when next tested on day 33. The streptomycin-resistant strain of P. tularensis was isolated from gastric and pharyngeal washings of the 2nd hospital day. On the 2nd hospital day and without specific therapy the temperature spontaneously fell to near normal only to spike to 103.0°F on the following day.

Oral tetracycline in a dose of 2 gm daily was started on the 5th hospital day and continued for 10 days. Within 24 hours the patient was afebrile, the severe grippal symptoms had subsided, and after 5 days the nasal stuffiness and moderate sore throat had cleared. At the completion of therapy the CRP was normal and the sedimentation rate was falling. It was possible to isolate the organism from pharyngeal washings throughout the first five days of therapy and from gastric aspirates on days 3 and 6 of therapy. Two other specimens were positive, a bronchial lavage on the first day and a nasopharyngeal swab on the second day of treatment.

Seven days after completion of tetracycline and while on convalescent leave, he noted the return of general malaise, calf and back aches, mild sore throat, and feverishness. He was readmitted on the 7th day of relapse. Physical examination revealed a mildly ill individual with tenderness over the anterior cervical and submandibular chains of lymph nodes and with a granular, inflamed, posterior pharynx. The white blood cell count was 12,700/cu mm with 68 per cent neutrophils, 31 per cent lymphocytes of which 50 per cent were atypical, sedimentation rate, 36 mm/hour, and CRP 4^{+}. Serial chest films were normal.

The differential diagnosis rested between a relapse of tularemia and infectious mononucleosis. Subsequent serial heterophile tests were normal; pharyngeal and gastric washings were again positive for the streptomycin-resistant strain of P. tularensis. The report of these positive isolations prompted a second 2-week course of oral tetracycline (2 gm daily). Symptoms subsided by the 5th day of treatment; nevertheless it was possible to isolate the organism from the pharyngeal washings as late as the 6th therapy day. At the completion of therapy the CRP had again returned to normal, but the sedimentation rate was still 28 mm/hour. The sedimentation rate dropped to normal range by the fifth month.

The agglutinin titer remained at a 1:40 level throughout the second week of illness but by the 25th day, 3 days after relapse, it was 1:640 and reached the peak level of 1:1280 by the 33rd day. During one year follow-up the sedimentation rate, CRP, chest film and physical examination have remained normal. However, the patient has complained of mild generalized muscular and joint aches on damp days, which have been effectively controlled with aspirin.

COMMENT

This is a case of moderately severe typhoidal tularemia. He and his associate became ill on the 3rd and 5th days respectively following an accident which could have been expected to create an aerosol containing P. tularensis. In addition to the severe grippal symptoms, nasal stuffiness and sore throat were prominent complaints. In our patients these symptoms were common and occurred with or without roentgen evidence of pulmonary changes. The injected and slightly granular appearance of the posterior pharynx was noted in approximately half of the patients. The finding of 5 to 15 atypical lymphocytes during the acute phase of tularemia is not uncommon. There was the usual prompt response to tetracycline during the initial and relapse episodes, yet it was possible to isolate the organism on both occasions through the 6th day of therapy.

Of the four relapses observed in this series of patients, all had in common the following: return of symptoms 7 to 14 days after completion of at least one week of tetracycline started during the first week of disease, milder symptoms than initially, and a prompt response when retreated with tetracycline. In this case, the relapse occurred seven days after completion of tetracycline in a dosage of 2 gm daily for 10 days.

IV. DISCUSSION

The American literature clearly describes the etiology, epidemiology, clinical course and pathology of naturally-occurring tularemia[10,11,12,13,14,15,16,17,18]. In this country, as a result of contact with lower animals (particularly the rabbit), through dressing of carcasses, or by insect bites (principally the tick), approximately 90 per cent of the cases are of the ulceroglandular variety. In contrast, laboratory-acquired disease is principally of the typhoidal-pulmonic form. Thirty-nine of the 42 cases in this series belong in this latter category.

Circumstantial evidence points to the respiratory tract as the portal of entry. The potential exposure to aerosol particles of P. tularensis as a result of laboratory procedures is a constant hazard. The high incidence of illness in the absence of recognized exposure, and the occasional case following remote contact also implicate this route of infection.

In keeping with the previously reported naturally-occurring and laboratory-acquired typhoidal cases the majority of our patients noted respiratory symptoms such as coryza, pharyngitis, substernal tightness, and cough at the onset of illness without regard to the chest x-ray status. It is apparent that the conventional classification of typhoidal vs typhoidal with pulmonary involvement does not connote a difference in pathogenesis but merely the extent of respiratory involvement.

The wide range of severity of illness in our group of patients added to the problem of recognition of infection. Based on symptoms alone it was impossible to differentiate the milder cases from the common cold. In the more ill patient, Asian influenza, infectious mononucleosis, psittacosis, Q fever, "atypical pneumonia," typhoid fever, brucellosis, and tuberculosis were a few of the diseases warranting consideration. There was nothing specific for tularemia about the respiratory or grippal symptoms and signs. The elevated sedimentation rate and CRP as well as the normal to mildly elevated white blood cell count early in the course of illness were characteristic but not diagnostic of tularemia. The agglutinin titer rise during the 2nd to 3rd week and the 8-fold or greater rise thereafter supplied confirmatory information but was not useful in early recognition of the disease. Hemagglutinin titers, which sometimes showed a diagnostic rise as much as a week earlier than agglutinin titers, had similar limitations. Attempts to demonstrate a capsular polysaccharide in pharyngeal, gastric, sputum, and urine specimens utilizing a hemagglutination inhibition technique were unrewarding.

Foshay[19] has reported a 92 per cent incidence of positive skin tests during the first seven days of naturally-occurring tularemia. However, about 40 per cent of our hospitalized patients had negative skin tests at the end of the first week of illness. The skin test had the usual diagnostic limitations.

The admission chest films were abnormal in 17 of 31 typhoidal cases. This 55 per cent incidence of pulmonary involvement compares favorably with the reported 50 to 77 per cent in naturally-occurring typhoidal tularemia[20,21,22,23,24,25,26]. Bibbs and Berland[26] attempted to discriminate different types of pulmonary involvement in the ulceroglandular and typhoidal forms of disease; subsequent reports have failed to substantiate their observations. From 16 cases, eight of whom were of the typhoidal variety, Ivie[24] noted an oval area of infiltration in nine and hilar adenopathy in five patients. Ten of the cases had some degree of pleural effusion which usually occurred late in the course of illness. Dennis and Boudreau[25] reported 14 cases of pleuropulmonary tularemia, seven of whom were not ulceroglandular in origin. In addition to the findings of Ivie, these observers emphasized the frequently widespread and bilateral pulmonary involvement. Thirteen of our 15 patients with pneumonia had a 2- to 8-cm oval infiltrate. There was attending hilar adenopathy in eight and a pleural effusion in two of the patients with pneumonia. Another patient had hilar adenopathy, perihilar streaking, and pleural effusion. Pleural effusion was observed as the only abnormality in one case. These observations are in agreement with those of Ivie and Dennis and Boudreau. Though not diagnostic, the presence of an oval pneumonic patch or pleural effusion associated with hilar adenopathy occurring during a febrile illness should strongly suggest the possibility of tularemia.

Because of the danger of laboratory infections, few hospital laboratories have made serious efforts to recover the organism in typhoidal tularemia. In endemic areas the conventional approach has been to consider the posibility of typhoidal tularemia in any severe atypical pneumonia or febrile illness not responding to penicillin. In such instances a 2- to 3-day course of streptomycin results in prompt clinical improvement and has been recommended as a therapeutic test, the diagnosis being confirmed by the appearance of agglutinating antibodies during the 2nd week of illness with subsequent rise between the 4th and 6th weeks[27]. Since the advent and widespread general use of broad spectrum antibiotics and their effectiveness in controlling tularemia, it is probable that the typhoidal form of disease is often unsuspected.

The desire to establish an earlier definitive diagnosis prompted our efforts to isolate the organism. Utilizing the gastric and pharyngeal washings, and sputum when available, the organism was isolated in 25 of 30 typhoidal cases and as late as the third week of untreated illness. Nineteen of these isolates were resistant to streptomycin, the remaining 6 were sensitive. Sputa and gastric washings were superior to pharyngeal washings. A definitive diagnosis was usually made within 48 to 72 hours after admission when the strain could be cultured. The percentage of positive cultures was materially increased by the simple maneuver of inoculating several culture plates from each specimen. For the streptomycin-resistant organism, when the quantity of material inoculated into animals and onto culture plates was approximately the same, the results were identical by either method. The method of culture of the streptomycin-sensitive organism from pharyngeal washings is not yet satisfactory. In those patients

in whom the culture was negative, but guinea pig inoculation was positive, specific identification was not possible on the average until the 5th day following admission (range 4 to 7 days). Various methods of shortening the time required for a laboratory diagnosis are under study and will be reported elsewhere.

It is well known that P. tularensis can be isolated from cases with pulmonary involvement from the sputum and/or pleural fluid, utilizing guinea pig inoculation and less often by culture on GCB media[20,21,23]. In untreated disease the organism has been isolated from the sputum of a pneumonic case as long as 49 days and from pleural fluid as late as four months[27]. On the contrary, it has not been generally appreciated that the organism could be isolated from the sputa of patients without pulmonary involvement as was possible in three of our cases. Johnson[28] reported isolations on the 17th to the 30th day of disease from sputa inoculated into white mice. This typhoidal illness occurred in a 12 year old girl following playing with rabbits, and was characterized by fever, subacute pharyngitis, and a normal chest x-ray examination. Larson[29] described a similar experience in a 10 year old girl with fever, sore throat, and normal chest x-ray following rabbit exposure. It was possible to isolate the organism from mice inoculated with sputum from the 15th day of illness. Gastric and pharyngeal washings have not been successfully exploited previously as sources of diagnostic material. It is worthy of note that these specimens from our series could be positive whether chest x-ray evidence of pulmonary involvement was present or not.

In vitro studies demonstrate that streptomycin is bacteriostatic for the parent strain of P. tularensis at a concentration of less than 0.4 µg/ml and bactericidal at 1.0 µg/ml[30]; however, the resistant strain isolated from our patients was not sensitive to 10 or 100 µg/ml. Hunt[23] demonstrated the bactericidal property of streptomycin in patients with pneumonic tularemia by collecting samples of sputum or pleural fluid prior to and five days after therapy. The initial specimens were positive utilizing white mice; post-treatment specimens were negative. In contrast, the broad spectrum antibiotics in vitro are bacteriostatic for P. tularensis. Growth of the isolates from each of our patients was inhibited by concentrations of 1 µg/ml of tetracycline. This difference in drug action probably accounts for the continued isolations of P. tularensis from the naso-pharynx of the patients for several days after the initiation of therapy with broad spectrum antibiotics. Similar observations have been made in other diseases.

The bacteriostatic action of these drugs has been demonstrated in experimental animals. Gochenour[31] challenged guinea pigs with P. tularensis SCHU-S4 by the intraperitoneal route and initiated prophylaxis with parenteral tetracycline 24 hours later. Two schedules were used; in one the drug was given every six hours, in the other every 12 hours. The total daily dose of drug was the same in the two groups. The animals treated at 12-hour intervals became febrile almost as soon as the untreated controls, while the animals treated four times a day did not become febrile as long as the drug was continued.

The work of McCrumb et al[32] in induced ulceroglandular tularemia in volunteers has emphasized the difference between the prophylactic and therapeutic action of streptomycin and the broad spectrum drug chloromycetin. These investigators inoculated volunteers intradermally with 400 to 10,000 P. tularensis cells (Rector strain). In the six controls a reddish papule appeared within 24 to 48 hours. By the third post-inoculation day the local lesion had enlarged to 1 to 2 cm, and there was moderate axillary adenopathy, fever, and mild constitutional symptoms. At this point the disease was promptly controlled with either five days of chloromycetin or streptomycin. Daily therapy consisted of 2 gm of streptomycin or 3 gm of chloromycetin in four and two patients respectively. Both patients who received chloromycetin experienced febrile relapses, whereas the streptomycin-treated patients remained well.

Seventeen volunteers were similarly challenged[32], and one hour later antibiotics were started. Nine of the ten patients who received streptomycin for 5 days failed to develop disease or agglutinating antibodies. Overt disease followed the cessation of therapy in only one individual, a 200 pound male who received a total of 3 gm of streptomycin over a 5-day period. In contrast, a 5-day course of chloromycetin failed to prevent disease in five of seven patients but did prolong the incubation period; ulceroglandular disease then developed which was responsive to a second course of this antibiotic.

These observations are in agreement with the results following streptomycin or broad spectrum drug administration in naturally-occurring tularemia. The earlier antibiotics are started during the illness, the shorter the active phase of disease. However, Johnson et al[33], Berson and Harwell[34], Taylor[35] and Overholt and Summers[36] have emphasized that antibiotics are less effective upon low-grade fever and lymphadenopathy in the common ulceroglandular disease when started after the first two weeks of infection. Liquifaction and subsequent drainage of the lesion may still occur.

The recommended dosage for streptomycin or dihydrostreptomycin is 0.5 to 2.0 gm daily for 5 to 10 days depending upon the severity of illness[23,33,34,35,36,37,38,39,40,41,42]. Relapses have not been reported following such therapy. The reported clinical experience with the tetracycline group of drugs and chloromycetin has been less extensive[41,42,43,44,45,46,47,48,49,50,51]. Their effectiveness in controlling the acute phase of illness has been comparable to that of streptomycin, e.g., precipitous drop in fever and control of symptoms within 24 to 48 hours. Corwin and Stubbs[41] pointed out that chlortetracycline was effective in securing remissions but unlike streptomycin was not curative. They described eight patients who completed a dose schedule ranging from 8 gm in 4 days to 12 gm in 6 days; seven returned to the hospital within 5 to 9 days with a relapse of symptoms; however, these authors failed to indicate on which day of disease therapy was started. Wimberley[51] reported a case of ulceroglandular disease which relapsed following treatment with chlortetracycline; therapy was instituted on the second day of illness and continued for four days. The dose schedule consisted of 1 gm initially and 1.5 gm daily thereafter. Three days later the symptoms returned but were promptly controlled with a 9-day course of 2 gm daily. Symptoms again returned within two days but were controlled with one day of chlortetracycline. Parker et al[45] observed two relapses among six patients treated with chloromycetin. The therapy schedule consisted of a

"loading" dose of 2 to 3.5 gm and 3 gm daily thereafter. One of the cases was a laboratory worker who had the typhoidal form of disease with pulmonary involvement. Therapy was instituted on the 6th day of illness and continued for 5.3 days. Four days later fever and symptoms returned. A 10-gm course of chloromycetin promptly controlled the symptoms but two days later the symptoms returned. A third course of 18 gm for seven days brought the illness under control. The other relapse occurred in a patient with ulceroglandular tularemia. Therapy was started on the second day of illness and continued for five days. Three days later his symptoms returned but were promptly controlled with 3 gm of chloromycetin over the next 4.5 days.

In our patients, the rapid decline in fever, control of symptoms within 24 to 48 hours, and the prompt regression of abnormal sedimentation rate, CRP and chest x-ray after the onset of broad spectrum drug therapy were markedly uniform. The response was equally dramatic in the more serious cases. However, four relapses were noted among the 13 typhoidal cases who were treated within the first week of illness. Exacerbation of the disease was not observed among the 17 patients treated after the first week of infection and who received antibiotics for a comparable period. Time of initiation of therapy may not be the sole factor. Patients treated in the second or third week of illness in general had milder symptoms.

The broad spectrum group of drugs fails to free the host of P. tularensis. If therapy is started during the initial week of illness, before there is a period of adequate antigenic stimulus, relapses may occur after 7 to 10 days of treatment; a high relapse rate is expected if less than 7 days of broad spectrum therapy is given. However, after approximately a week of untreated illness, or in other words "antigenic" experience, the host's immune mechanism is stimulated to curtail bacterial growth once the 7 to 10 day course of bacteriostatic drug is completed. Nevertheless, five of 17 of the patients who were so treated had a "subclinical rebound" (rise in sedimentation rate). This occurred at about the same time as did the clinical relapse, i.e. 1 to 3 weeks following competion of broad spectrum therapy. This renewed bacterial growth apparently is held in check by the host's partially mature immune status. During the second week of illness a single 10-day course of treatment appears sufficient. As yet there is no proved therapeutic regimen which will prevent relapses if broad spectrum antibiotics are begun during the first week of illness. Perhaps a 14-day or longer course will be sufficient. Smadel et al[52] have prevented scrub typhus relapses when bacteriostatic drug therapy was started within the first week of illness by interrupted therapy. It is possible that relapses can be prevented by a similar approach in tularemia. The problem is now under study in animals and man.

The phenolized and acetone-extracted vaccines do not prevent infection. The non-viable vaccine has failed to protect white mice from subsequent infection with minimal doses of a virulent strain[3]. Rigelsbach[53] has extended these studies to monkeys and was unable to demonstrate any significant immunity following the use of this vaccine. There are over 200 reported laboratory infections in vaccinated personnel. Foshay et al[3] have collected data on the incidence, severity, and duration of disease in 2,145 vaccinated persons, consisting primarily of hunters, meat handlers, butchers, farmers, farm wives,

and laboratory workers. Because of the unknown size of the control group and the lack of data on the relative risk of exposure it was not possible for these investigators to demonstrate conclusively the effectiveness of the vaccine as a disease preventive. Similarly, Kadull et al[4] in an uncontrolled study cited a 14 per cent disease rate among 163 vaccinated persons at Fort Detrick and suggested that the vaccine could be responsible for this relatively low infection rate. Evaluating vaccine prophylaxis in laboratory personnel is complicated when it is appreciated that the clinical features of mild typhoidal disease are similar to a common cold and case recognition is extremely difficult. Foshay et al[3] in naturally-occurring, and Kadull et al[4] and Van Metre and Kadull[54] in laboratory-acquired untreated tularemia have reported modifications of severity and of duration of disease in vaccinated personnel. In addition, they described individuals with unexplained diagnostic rises in agglutinin titers. Whether such cases were truly subclinical or similar to the mildly ill, eight non-hospitalized and two untreated hospitalized, patients of our series is a moot question. Asymptomatic infection in the unvaccinated individual has not been documented with the virulent strain common to North America.

In our experience typhoidal tularemia in the immunized laboratory worker differs in severity of manifestations from the reported naturally-occurring disease. In contrast to naturally-occurring typhoidal tularemia where the majority of patients were quite ill, only 15 per cent of our group of patients were considered to be severely ill in spite of the fact that more than one-half were admitted after the first week of illness. However, the causative strain, conditions of exposure, time of onset of therapy, and type of therapy are not comparable. Furthermore, it should be recognized that the high index of suspicion of disease and system of medical investigation employed in the present study has permitted an appreciation of the wide range of severity of the typhoidal form of disease. Therefore, it is impossible to correlate the comparatively mild disease seen in these patients with prior vaccination.

No relationship could be established between the severity of illness on admission and the number of booster series or the time interval from the last booster. An individual who had received his initial series four years earlier with no subsequent boosters was as likely to have a mild disease as an individual who had received his booster within the last six months, and all but two patients had low grade tularemia agglutinin titers prior to illness.

Recently Saslaw[55] has defined more clearly the role of a phenolized vaccine in the prevention or modification of disease in a controlled volunteer study. The minimal infecting dose of SCHU S4 strain of P. tularensis via the intracutaneous route was employed in three groups of men: 1) non-vaccinated, 2) vaccinated and challenged three weeks later, and 3) vaccinated, followed by a booster six months later, and challenged three weeks after booster. All challenged subjects developed local lesions and 11 of 12 non-vaccinated men had associated fever and constitutional symptoms. In contrast only 3 of 14 vaccinated men and 2 of 5 vaccinated-plus-booster men had constitutional symptoms. Infection was not prevented but prior vaccination modified the severity of the disease resulting from this low challenge intracutaneous dose. Similar studies with typhoidal disease have not yet been reported.

Killed vaccine preparations have been reported to be ineffective in preventing tularemia in Russia[56]. In the past decade a live attenuated vaccine has been extensively used. It is stated that previously immunized individuals have a high degree of protection during epidemics, that laboratory infections no longer occur, and that vaccination during epidemics sharply curtails the new case rate[56,57]. Russian morbidity and mortality rates in untreated naturally-occurring tularemia are not comparable to the American experience and it is generally agreed that the Continental strains are less virulent than American strains[58]. In Russian, inapparent infection, a milder illness, and a mortality rate of less than 1 per cent are in contrast to the disease seen in this country. Whether a viable vaccine would be effective as a prophylactic agent against tularemia in this country is unknown.

It is widely accepted that one attack of tularemia confers permanent immunity, However, there are at least eight well documented re-infection ulceroglandular cases in this country[35,59,60]. Taylor[35] reported two patients with naturally-occurring ulceroglandular tularemia who returned, a month and 18 months later, with second distinct, acute attacks of ulceroglandular tularemia. Both of these cases had been treated with antibiotics within the first week of initial illness. The remaining six re-infections were laboratory-acquired, mild ulceroglandular disease which appeared from a few months to as long as several years after their initial untreated illness. One re-infection (Case 14) occurred among our 42 patients. On both occasions this patient had bacteriologically proven typhoidal disease with pulmonary involvement. In the previously cited investigation of Saslaw[55], eight volunteers were rechallenged six months after their initial illness. These individuals had received streptomycin as soon as the initial disease became apparent; nevertheless all developed agglutinins. Following the second challenge, local lesions developed in all eight patients, but only two had constitutional symptoms. These observations suggest that mild re-infection ulceroglandular or typhoidal disease may occur more often than is appreciated in endemic areas. How often a "winter" cold or slowly healing cut in rabbit hunters represents re-infection is unknown. It is apparent that immunity is relative; following re-exposure, and depending upon the interplay between the [illegible] immunity, challenge dosage, and virulence of the organism, illness in [illegible] form may occur.

V. CONCLUSION

Of the 42 vaccinated laboratory personnel who acquired tularemia, 39 resulted from probable aerosol exposure. The resulting typhoidal disease was characterized by non-specific grippal and respiratory symptoms of varying severity; the majority of patients were considered to be only mildly to moderately ill. The phenolized and acetone-extracted vaccines were ineffective in preventing clinical disease but could have been responsible for the milder form of disease.

Among the hospitalized patients, 17 (55 per cent) of typhoidal cases had abnormal chest roentgenograms. These abnormalities included single or multiple, ovoid bronchopneumonic infiltrates, pleural effusion, hilar adenopathy, or combinations thereof. Such findings should prompt the consideration of tularemia

in the differential diagnosis. Agglutinin and hemagglutinin serum levels were not useful in the early recognition of disease. However, the etiologic agent, F. tularensis, was regularly isolated from sputum, when available (12 of 14 patients), and frequently from gastric (21 of 29 patients) and pharyngeal (16 of 30 patients) washings as late as the third week of untreated illness.

Approximately three-fourths of the 34 hospitalized cases of this report resulted from a strain resistant to streptomycin, which has not been reported in naturally-occurring disease. Two of the 34 hospitalized and 7 of 8 non-hospitalized patients with minimal symptoms did not receive antibiotics. Thirty-two of the hospitalized group were treated with broad spectrum antibiotics without regard for the streptomycin sensitivity of the isolates: 27 with tetracycline alone, one with tetracycline followed by intravenous oxytetracycline, two with tetracycline and streptomycin, one with chloromycetin and subsequent albamycin, and one with chlortetracycline. Broad spectrum drugs in a dosage of 2 gm daily for 7 to 14 days promptly controlled the acute phase of disease. F. tularensis could be isolated from sputa, pharyngeal washings or gastric washings throughout the first week of therapy. Among 13 of the patients treated only with broad spectrum antibiotics which were started during the first week of disease, four suffered relapses whereas with similar therapy instituted after the first week of disease, no relapses occurred in 17 patients. It is apparent that this group of drugs are bacteriostatic and fail to eradicate the organism from the host. When such therapy is started early in the illness the host's immune status often is incapable of preventing relapses. Retreatment during relapse with the same antibiotic is effective.

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STUDIES ON PASTEURELLA TULARENSIS

EFFECT OF CORTISONE ON LATENCY OR CHRONICITY IN MONKEYS SURVIVING RESPIRATORY CHALLENGE WITH P. TULARENSIS

(Hornick, Hughes)

I. INTRODUCTION

In certain infections the administration of steroids may cause latent disease to become evident. An effort was made to determine if this was true in monkeys which had recovered from, or survived, respiratory tularemia.

II. MATERIALS AND METHODS

Twelve Macaca mulatta monkeys surviving from various tularemia experiments were subjected to daily steroid administration. Their past history and status at the time this study was begun is shown in Table I. Cortisone acetate

TABLE I. PAST HISTORY AND STATUS OF 12 MONKEYS IN CORTISONE STUDY

ORIGINAL CHALLENGE DOSE	PREVIOUS GROUP	MONKEY NO.	PREVIOUS THERAPY & DURATION (days)	CORTISONE STARTED DAY POST-CHALLENGE	DURATION (days)
3,000	Control	28[a]	Tetracycline (14)	146	32
	Control	32	None--no disease	146	28
	Living vaccine	85	Tetracycline (14)	146	28
	Phenolized vaccine	43	Tetracycline (14)	146	28
		63	Tetracycline (14)	146	28
30,000	Control	37	None--no disease	146	28
	Control	40	None--no disease	146	28
	Living vaccine	89	Tetracycline (14)	146	28
	Living vaccine	96	Tetracycline (14)	146	28
100,000	Living vaccine	11	None	174	32
	Living vaccine	132	None	174	32
500	Control	1A[b]	None	395	32

a. Proven chronic tularemia.

b. Surviving control with biopsy-proven cutaneous tularemic abscesses.

(Merck), 25 mg/ml, was given in daily 50 mg doses to four animals for 32 days. The remaining eight monkeys received this same dose of drug for 28 days; however, during the last 11 days of treatment they were used for other studies. Blood cultures were obtained three times a week for the first two weeks and once in the fourth week. Blood was withdrawn from the femoral vein or artery; 1 ml was injected intraperitoneally immediately into each of two guinea pigs and an additional portion was used for routine blood studies.

Weekly weights and chest x-rays were obtained. Physical examinations were done daily as were temperature recordings. Bacteriological examinations were done at time of autopsy on both the monkeys and guinea pigs when indicated grossly.

III. RESULTS

Facial edema appeared first after nine days of cortisone and eventually was present in all test animals. Hirsutism was noted during the second week of treatment. Ten of the animals developed increased amount and thickness of the facial hair. One monkey developed scrotal edema after 22 days of cortisone. Three had flushing of the face. The average weight of the group rose slightly in the first week, i.e., 75 gm, but declined steadily thereafter. There was no apparent effect on the temperatures. Nine of the animals developed small, shotty, axillary lymph nodes, some appearing after two days of treatment. There was no obvious change in the irascible behavior of the monkeys.

The usual changes seen in the peripheral blood following steroid therapy were demonstrated (Figure 1). There was an early mild, transient leukocytosis, a development of a relative neutrophilia secondary to an actual lymphocytopenia and a decrease in the eosinophils. The sedimentation rates and the C-reactive proteins rose and remained elevated throughout the treatment period.

Even though two of the monkeys had proven chronic tularemia prior to cortisone treatment, no positive blood cultures were obtained from these or any of the other cortisone treated monkeys.

The animal with chronic cutaneous tularemia showed no change in the lesions during cortisone administration. No monkey died during this period. The adrenal glands in these animals, at sacrifice, were grossly diminished from normal size. There were no gross visible infective processes, with the exception of two animals: No. 28 had a small splenic abscess positive on culture for *P. tularensis*; No. 1A had splenic scars and residual cutaneous abscesses.

IV. CONCLUSIONS

The dosage of cortisone used in this study caused clinical and laboratory aberration typical of hypersteroidism. There was no evidence that this was sufficient to break down tularemic foci. Thus, whether the remaining monkeys, which had been treated for their acute infection with antibiotics, had any viable organisms persisting undetected could not be determined by this study.

FIGURE 1. EFFECT OF CORTISONE ON WHITE BLOOD COUNT, SEDIMENTATION RATE AND C-REACTIVE PROTEIN.