ANNUAL REPORT

STUDIES IN THE NATURE AND CONTROL OF SEPSIS IN THERMAL AND COMBINED THERMAL IRRADIATION INJURIES

DA-49-193-MD-2094 Nov. 15, 1965

Responsible Investigator:

William A. Altemeier, et al.

STUDIES IN THE NATURE AND CONTROL OF SEPSIS IN THERMAL AND COMBINED THERMAL-IRRADIATION INJURIES

Annual Report

Responsible Investigator:

William A. Altemeier, M.D., Professor of Surgery and Chairman of the Department of Surgery, University of Cincinnati College of Medicine

Professional Assistants:

Charles M. Barrett, M.D., Professor of Radiological Therapy
Bruce G. MacMillan, M.D., Professor of Surgery
Robert P. Hummel, M.D., Assistant Professor of Surgery
Carl Schowengerdt, M.D., Surgical Resident
James Fidler, M.D., Surgical Resident
E. O. Hill, Ph.D., Assistant Professor of Research Surgery and Microbiology
Vulus R. Dowell, Jr., Ph.D., Instructor in Research Surgery and Microbiology

DA-49-193-MD-2094

November 15, 1965

Work under this contract for the past twelve months has been concerned with the continued exploration of various problems related to infections associated with thermal injuries. The investigations have been both laboratory and clinical and have consisted of the following:

I. A continuing study of the significance of the changing bacterial flora in severe burns.

II. Studies on the influence of the immune response upon morbidity and mortality in experimental and clinical burns.

III. Studies of the effectiveness of early excision on mortality and morbidity of burn patients.

IV. Frequency, nature, and significance of the non-sporulating anaerobic bacteria and the mecahnisms of synergism illustrated by these organisms in polymicrobic infections.

V. Studies of the response and natural course of the burn wound in germ-free animals given a standard burn and maintained in a germ-free environment.

VI. Continuing clinical and laboratory studies of all burn patients treated on the surgical wards of the Cincinnati General Hospital.

I. Significance of the Changing Bacterial Flora in Severe Burns.

A. Epidemiological studies of Staphylococcus aureus UC-18.

Since the Annual Report of January 1, 1965, to July 1, 1965, an additional eleven severely burned patients were monitored by frequent cultures to establish changes in the bacterial flora of the burn wounds during hospitalization. A total of 46 patients have thus been studied during the period January, 1964 to July 1, 1965. As indicated in Table I, the wounds of 43 (93.5%) of these 46 patients became colonized by the Staphylococcus aureus. Staphylococcus aureus UC-18 was the colonizing agent in 38 (82.6%) of this group of patients. Re-examination of the percentage of frequency of the Staphylococcus aureus UC-18 in burn wounds on an annual basis (Table II) indicates a continually increasing incidence of these strains in major burn wounds. Seventeen (90%) of the nineteen patients colonized by Staphylococcus aureus during the first six months of 1965 were colonized by the UC-18 strains. The mechanisms of the evolution of the UC-18 strains are of great interest to us and are under intensive study. This strain continues to be found in various hospitalized patients, but not in the general hospital environment or in unhospitalized patients.

As indicated in Table III, the 145 cultures of Staphylococcus aureus UC-18 isolated from burn wounds continue to demonstrate an unusually high incidence of resistance to many of its antibiotics. Chloramphenicol and novobiocin are the most effective antibiotics in vitro.

Other genera of bacteria isolated in association with Staphylococcus aureus UC-18 are indicated in Table IV. Pseudomonas aeruginosa continues

to occur with the greatest frequency in association with the UC-18 strains, (see also the Annual Report of January 1, 1965) followed by the Aerobacter-Klebsiella group, Proteus sp. and Paracolobactrum sp., respectively. Staphylococcus aureus UC-18 was recovered in pure culture from five specimens, one each from blood, thigh, arm, chest, and neck.

There is no doubt from this and other studies we are doing that the UC-18 Staphylococcus aureus is a new epidemic strain of greater significance in hospital-acquired infections in burns than the type 80,81.

The antibiotic resistance of the gram negative flora are indicated in Tables V, VI, VII, and VIII. The percentages resistant have varied insignificantly from those reported earlier.

Table I.

Staphylococcus aureus UC-18 in Burn Wounds.

(January 1964-June 1965)

Total number patients studied		46	
Number with Staphylococcus aureus		43	(93.5%)
Number with Staphylococcus aureus	UC-18	38	(82.6%)
Number with Staphylococcus aureus	80, 81; 52, 52A, 80, 81	6	(13.0%)*

*Staphylococcus aureus UC-18 replaced 4 of those.

Table II

Annual frequency of Staphylococcus aureus UC-18 in Burn Wounds.
(January 1960 - June 1965)

Year	Number of patients	UC-18	80,81
1960	15	0	2
1961	17	3	0
1962	18	11	0
1963	7	4	0
1964	24	21	5*
1965+	19	17	1
TOTALS	100	56	8

*4 of these were replaced by Staphylococcus aureus UC-18

+ To July 1, 1965

Table III

Antibiotic Resistance of Staphylococcus aureus UC-18
Recovered from Burn Wounds

(Total cultures tested: 145)

	Number Resistant	Per cent Resistant
Penicillin	136	93.8
Streptomycin	142	97.3
Oxytetracycline	118	80.8
Tetracycline	107	73.3
Chlortetracycline	49	33.6
Erythromycin	37	25.3
Neomycin	17	11.7
Bacitracin	15	10.3
Chloramphenicol	5	3.4
Novobiocin	3	2.1

Table IV

Organisms in association with

Staphylococcus aureus UC-18 in burn wounds.

Organisms	Number Cultures
Pseudomonas aeruginosa	60 (26 patients)
Aerobacter-Klebsiella	33 (14 patients)
Proteus sp.	28 (10 patients)
Pseudomonas aeruginosa and *Proteus sp.*	13 (6 patients)
Paracolobactrum sp.	16 (6 patients)
Staphylococcus aureus UC-18 in pure culture	5 (5 patients) (blood 1, thigh 1, arm 1, chest 1, neck 1)

TABLE V

Antibiotic Resistance of Pseudomonas aeruginosa
from Burn Wounds
(Total cultures tested: 204)

	No.	Percent		No.	Percent
Bacitracin	201	98.5	Oxytetracycline	157	76.9
Penicillin	201	98.5	Chloramphenicol	95	46.6
Novobiocin	196	96.1	Neomycin	18	8.8
Chlortetracycline	192	94.1	Colymycin	12	5.9
Tetracycline	179	87.7	Polymyxin	0	0.0

TABLE VI

Antibiotic Resistance of Proteus sp.
from Burn Wounds
(Total cultures tested: 72)

	No.	Percent		No.	Percent
Oxytetracycline	71	98.6	Tetracycline	56	77.8
Polymyxin	68	94.4	Penicillin	36	50.0
Colymycin	68	94.4	Novobiocin	31	43.0
Bacitracin	67	93.0	Chloramphenicol	24	33.3
Chlortetracycline	67	93.0	Neomycin	3	4.2

TABLE VII

Antibiotic Resistance of *Aerobacter-Klebsiella* from Burn Wounds
(Total cultures tested: 99)

	No.	Percent		No.	Percent
Bacitracin	99	100	Colymycin	25	25.25
Penicillin	96	96.9	Tetracycline	22	22.22
Novobiocin	56	56.6	Chloramphenicol	14	14.14
Chlortetracycline	35	35.35	Polymyxin	6	6.06
Oxytetracycline	27	27.27	Neomycin	3	3.03

TABLE VIII

Antibiotic Resistance of *Paracolobactrum sp.* from Burn Wounds
(Total Cultures tested: 52)

	No.	Percent		No.	Percent
Bacitracin	51	98.1	Colymycin	46	88.5
Oxytetracycline	51	98.1	Polymyxin	40	76.9
Penicillin	50	96.2	Chlortetracycline	39	75.0
Tetracycline	49	94.2	Chloramphenicol	5	9.6
Novobiocin	44	84.6	Neomycin	3	5.8

II. Studies on the influence of Immune Response upon Morbidity and Mortality in Experimental and Clinical Burns

Our studies which have been performed on the serum of burn patients in an attempt to demonstrate agglutinin titers to stock strains of Proteus, Pseudomonas aeruginosa and Staphylococcus aureus have been essentially negative. Attempts to correlate the presence of agglutinins in human burn convalescent serum harvested from patients surviving from severe burns complicated by infection have also been negative or inconclusive at best.

During the past year, experiments have been conducted to explore the immune response in experimental animals and to determine any detectable effect of convalescent or normal serum in animals with standardized burns.

A) Continued experimentation with guinea pigs (English Cavies) weighing 400 to 500 grams have been carried out. The first phase of this project was to establish a standard reproducible full thickness burn which would cause death in approximately 50% of the animals at the end of the first week after injury. (See report January 1, 1965.) Individual contact burns of 15 seconds' duration were administered with the specially constructed branding iron measuring 1.25 square inches in surface area. It was determined that 12 burn areas produced with this branding iron at 450°F. for 15 seconds resulted approximately in a 50% mortality at the end of the first week after injury. After burning, the pigs were put in individual cages in an area where the room temperature was maintained between 65 and 75° F. Rockland Guinea Pig Diet with Vitamin C added, greens, and water were placed within the cages for comsumption ad lib.

Because the burn produced at 450° was not consistently full thickness in depth as determined by biopsy, it was decided to inflict burns at 550° F. It was also decided to inject 5 cc. of saline intraperitoneally postburn since in the subsequent experiments all animals would be injected with convalescent serum intraperitoneally in no greater doses than 5 cc. By experimentation on some 300 guinea pigs, it was determined that six branding iron areas approximated a 20% to 25% burn, and when produced at 550° F., produced a mortality of 50% at the end of the first week.

B) Further studies to evaluate the influence of convalescent burn serum in the treatment of these burned guinea pigs have been performed (see previous report January 1, 1965), employing convalescent burn serum obtained by exsanguination from the surviving guinea pigs at the end of two or three weeks after injury. The guinea pigs were divided into five groups of 30 each. Each animal was given the same size and depth burn injury (six areas burned at 550° F. for 15 seconds). Groups of 15 guinea pigs each were injected with convalescent serum at five different time periods: preburn, immediately postburn, four hours postburn, eight hours postburn, and 16 hours postburn. The control group of similarly burned pigs received normal guinea pig serum from unburned animals at the same time intervals. The results of the first experiment seemed to indicate a significantly prolonged survival in the animals who has received convalescent serum 16 hours postburn than that in those animals who had received normal guinea pig serum. No significant difference in survival was evident between convalescent and normal sera in the groups of animals who had received convalescent serum at the other time intervals.

Because of these findings, the experiment was continued and additional animals were treated in a similar manner giving the convalescent serum at the time periods of immediately postburn, 4, 8 and 16 hours postburn. Most of the animals were treated 16 hours postburn in an attempt to obtain a larger number of guinea pigs who had been treated at this time to evaluate any possible protective effect of convalescent serum at this interval after burn injury as suggested by the previous experiments (Table IX).

TABLE IX

GUINEA PIGS SURVIVING BEYOND 7 DAYS

Time Given	Convalescent Serum	Control Serum
24 Hours Preburn	53% (15)	40% (15)
Immediate Postburn	42% (18)	56% (18)
4 Hours Postburn	35% (17)	44% (18)
8 Hours Postburn	42% (18)	24% (17)
16 Hours Postburn	86% (28)	62% (29)

Once again the highest percentage of survival was noted in the guinea pigs who had been treated with convalescent serum 16 hours postburn injury. Eighty-six per cent of the 28 guinea pigs receiving the convalescent serum at 16 hours survived beyond the first 7 days postburn. In the control group, however, there was also a marked improvement, with 62% of the 29 animals surviving beyond 7 days postburn. The burn inflicted was expected to give approximately 50% survival at the end of 7 days following burning. Again, no significant protection was noted from either convalescent serum or normal serum in the immediate postburn, 4 or 8 hour

postburn groups of animals studied. These results are interesting and further experimentation is planned to explore the basis of this protective effect at the 16 hour interval after thermal injury.

Since the technic of experimentation with germfree and gnotobiotic animals has been developed in our Research Surgical Bacteriology Laboratory, the prophylactic and therapeutic effect of convalescent serum will be investigated further in germfree and mono-contaminated animals.

III. Studies of the Effectiveness of Early Excision on Mortality and Morbidity of Burn Patients.

Regardless of the extent of the full thickness burn, the wound often cannot be prepared for grafting by conventional methods for periods less than approximately 20 to 30 days, and skin grafting often cannot be completed before the 60th postburn day in many instances. In consideration of this prolonged period of morbidity, we have continued our studies to explore the value and limitations of early excision of small localized confluent areas of full thickness burn on the day of injury or the 2nd postburn day. In the patients who have been treated successfully by early excision, the period of postburn morbidity has been markedly shortened, and the period of hospitalization has been decreased by approximately 60 per cent.

As a result of the favorable results obtained with surgical excision of smaller areas of full thickness burn in these and in previous patients, it was decided to apply this method of treatment to patients sustaining full thickness burns involving approximately one fourth to one half of the total body surface. All confluent areas of full thickness burn involving at least 25 per cent of the total body surface were excised by sharp dissection down to the underlying fascia or muscle in an effort to obtain a higher percentage of graft take. These excisional procedures were done in one or two stages, and between the 2nd and 5th postburn days. Autogenous and/or homografts were applied to the wounds either immediately or 48 hours after excision of the full thickness burns. If grafting was not done immediately, the wound was covered with a single layer of fine mesh gauze covered by a compression dressing. In a

similar manner the wounds were covered with a compressive occlusive dressing following the application of skin graft.

The extent of the area excised was determined largely by the patient's ability to discriminate sharp pin prick, this procedure being repeated at least three times during the first 24 hour postburn period. Adjacent areas of questionable deep thermal burn were included in the excision when possible to insure primary wound healing when autogenous grafts and/or homografts were applied. The interval of 48 hours between excision and grafting in some of the patients was chosen in an effort to obtain hemostasis prior to grafting. Areas of remaining normal skin were utilized as donor sites for autografts at the time of the initial grafting procedure. Homografts were used to complete the initial skin coverage. When both autografts and homografts of skin were applied, they were not intermixed but placed in separate areas. Fresh homograft skin has been used only occasionally, stored homograft skin being relied upon for this type of temporary wound closure. The average period of protection afforded by the homograft skin has been approximately four weeks.

During the past year, 16 patients were treated with excision of areas of full thickness burn. Four of these patients fell into the extensively burned category, and the remaining 12 patients had small areas of full thickness burns.

In the four patients with extensive burns, the mean age was 20 years; and the mean area of total body surface burns was 63 per cent. The mean area of full thickness burn was 54 per cent. The areas excised in these patients ranged from 18 per cent to 40 per cent, with the mean area being

29 per cent. These procedures were initiated on the initial to the third postburn day, with the mean being the second postburn day. Mean requirement for whole blood during these procedures was 1067 cc. per procedure (1600 cc. per patient). Closure of the excised wounds was by autografts utilizing sheets in one patient, postage stamps in a second, and microdermagrafts in the remaining two. There were three deaths in this group of four patients. The causes of death in the cases were: Pseudomonas septicemia in one occuring on the twelfth day, cardiac arrest in one on the third day, and pulmonary infarction in the third on the third postburn day. The surviving patient was completely covered with autogenous skin on the 32nd postburn day, and was discharged from the hospital on the 59th postburn day.

As a result of the high mortality encountered with excision of large areas of burn, this method of treatment has been largely abandoned as a means of treatment for patients with extensive full thickness burns. However, very favorable results have been obtained in patients with smaller burns. In the group of 12 patients with smaller areas of full thickness burn, which were treated by total excision, the mean age wa 15 years. The area of full thickness burn excised varied from 1 to 10 per cent. The mean day of excision was the third postburn day. Autografting was performed in all patients on the day of excision. The mean day of complete autogenous skin coverage was the 20th postburn day and the mean period of hospitalization was 40 days. As indicated above, there were no deaths in this group of patients.

The clinical data on these two groups of patients who were by early excision of the burn wound is summarized in Tables X and XI.

TABLE X

EARLY EXCISION OF SMALLER AREAS OF FULL THICKNESS BURN

PATIENT	AGE	SEX	EXTENT OF BURN TOTAL	EXTENT OF BURN 3°	SITE OF BURN	EXCISION EXTENT (%)	EXCISION PBD	EXCISION BLOOD (cc)	GRAFTING (PBD)	TYPE & % INITIAL COVERAGE	COMPLETE COVERAGE (PBD)	DEATH CAUSE	DEATH PBD
K. G.	5	M	4	4	Right lower leg	4	0	0	-	Auto 100	16	---	--
K. R.	3	F	10	10	Abdomen	10	3	250	3	Auto 100	10	---	--
L. R.	20	F	6	4	Left hand and left knee	4	2	0	2	Auto 100	16	---	--
P. B.	16	M	8	8	Left hand, right hand, left foot	8	4	0	6	Auto 100	20	---	--
L. Y.	4	M	3	3	Right posterior popliteal area	3	0	0	0	Auto 100	14	---	--
I. L.	1	F	2	2	Buttock	2	0	0	0	Auto 100	14	---	--
D. S.	10	M	3	1.5	Right upper arm	1.5	3	0	3	Auto 100	11	---	--
B. K.	8	M	13	9	Both thighs, right wrist	9	7	0	9	Auto 100	41	---	--
J. M.	2	M	1	1	Dorsum of left 2-3-4-fingers	1	1	0	1	Auto 100	12	---	--
H. R.	17	M	2	1	Right upper arm	1	3	0	3	Auto 100	9	---	--
L. B.	43	F	1	1	Right anterior chest	1	11	0	-	Primary closure	26	---	--
C. D.	45	M	9	6	Left arm and axilla	6	4	1500	6	Auto 100	64	---	--
12	15		5	4		4	3		3	Auto 100	20		

TABLE XI

EARLY EXCISION OF EXTENSIVE AREAS OF FULL THICKNESS BURN

PATIENT	AGE	SEX	EXTENT OF BURN TOTAL	EXTENT OF BURN 3°	SITE OF BURN	EXCISION EXTENT (%)	EXCISION PBD	EXCISION BLOOD (cc)	GRAFTING (PBD)	TYPE & % INITIAL COVERAGE	COMPLETE COVERAGE (PBD)	DEATH CAUSE	DEATH PBD
P. J.	5	F	40	32	Upper arms and posterior chest	25	3	800	8	Auto 80 Homo 0	--		12
					Buttocks and thighs	15	5	2000	-	-- -		Ps.septicemia (12th day)	
R. A.	45	M	90	70	Right leg	16	2	500	2	Auto 100 (Micro)	--		3
					Left leg	18	3	1500	3	Auto 100 (Micro)		Cardiac arrest (3rd day)	
R. B.	25	M	90	90	Right leg	18	0	1000	0	Auto 100 (Micro)	--		3
												Pulmonary infarct (3rd day)	
A. S.	4	F	30	25	Ant. trunk, post. trunk & shoulders	25	2	600	2	Auto 100 Homo 0	32	--	-
4	20		63	54	Individual Procedures	20	3	1067	3	Auto 95 Homo 0	32		6
					Individual patient	29	2	1600	3				

Patients with extensive areas of full thickness burns treated by early primary excision between February 1, 1962 and October 15, 1965.

IV. The frequency, nature, and significance of the non-sporulating anaerobic bacteria in burn sepsis and studies of the machanisms of synergism demonstrated by these organisms in polymicrobic infections.

A. Continuation of studies on the incidence of non-sporulating anaerobes in surgical infections.

Use of cultural methods described in the Annual Report of November 15, 1962 and published elsewhere (Dowell, Hill, and Altemeier, 1962, 1964, 1965) has been continued in studying a total of 472 specimens from patients on the Surgical Services of the Cincinnati General and the C. R. Holmes Hospitals. The percentage frequency of all of the micro-organisms isolated are listed in Table XII. Of the anaerobes, Peptostreptococcus sp. and Sphaerophorus sp. continue to occur with the greatest frequency (each with a frequency of 18.6%) followed by Bacteroides melaninogenicus (17.4%), and Bacteroides sp. (13.3%).

The organisms most frequently isolated in association with these non-sporulating anaerobes are other non-sporulating anaerobes (see Annual Report, January 1, 1965) followed by the aerobic flora commonly found in the intestinal tract.

Incidence of Recovery of Aerobic and Anaerobic Organisms from Surgical Infections

Aerobes

Species	Number	% Incidence
Aerobacter aerogenes	3	.63
Aerobacter sp.	1	.21
Aerobacter-Klebsiella	70	14.83
Alcaligenes sp.	2	.42
Bacillus sp.	57	12.07
Bacterium anitratum	2	.42
Candida albicans	8	1.69
Candida sp.	3	.63
Corynebacterium sp.	38	8.05
Diplococcus pneumoniae	1	.21
Escherichia coli	35	7.41
Escherichia frundii	1	.21
Escherichia intermedia	2	.42
Escherichia sp.	82	17.37
Flavobacterium sp.	1	.21
Gaffkya sp.	14	2.96
Haemophilus influenzae	2	.21
Klebsiella sp.	21	4.44
Micrococcus sp.	27	5.72
Mycoplasma sp.	4	.84
Neisseria sp.	8	1.69
Paracolobactrum:		
aerogenoides	3	.63
coliforme	8	1.69
intermedium	4	.84
Pseudomonas		
aeruginosa	40	8.47
flourscens	2	.42
species	7	1.48
Proteus:		
inconstans	2	.42
mirabilis	41	8.68
morganii	6	1.27
rettgeri	1	.21
species	5	1.05
vulgaris	8	1.69
Staphylococcus:		
aureus	91	19.27
epidermidis	84	17.79
species (coag. neg.)	22	4.66
Streptococcus:		
Alpha-hem.	71	15.04
Beta-hem.	19	4.02
Non-hem.	29	6.14
faecalis	33	6.99
lactus	15	3.17
species	1	.21
Trichoplyton tonsurans	1	.21
Unidentified organisms	7	1.48
Yeast	25	5.29

Table XII
(B)
Incidence of Recovery of Aerobic and Anaerobic Organisms from Surgical Infections

Anaerobes Species	Number	% Incidence
Actinomyces bovis	1	.21
Actinomyces sp.	3	.63
Bacteroides:		
corrodens	1	.21
melaninogenicus	82	17.37
sperpens	1	.21
species	63	13.34
Clostridium:		
bifermentans	2	.42
butyricum	1	.21
fallax	1	.21
histolyticum	2	.42
paraputurficum	1	.21
perfrengens	17	3.60
septicum	1	.21
sphenoides	1	.21
species	8	1.69
sporogenes	1	.21
tertium	1	.21
Catenabacterium sp.	3	.63
Corynebacterium:		
granulosum	1	.21
parvum	1	.21
species	25	5.29
Dialister sp.	1	.21
Fusobacterium sp.	10	2.11
Peptococcus sp.	40	8.47
Peptostreptococcus sp.	88	18.64
Ramibacterium	5	1.05
Sarcina sp.	1	.21
Sphaerophorus sp.	88	18.64
Microaerophilic Strept.	2	.42
Veillonella sp.	1	.21
Unidentified Gram pos. rod	4	.84
Total Number of Specimens (A and B)	472	
Total Number of Specimens Sterile by Culture (A and B)	79	16.73
Total Number of Specimens Positive by Culture (A and B)	393	83.26

IV. B. Continued studies on the mechanisms of synergism between organisms isolated from polymicrobic infections.

The preliminary studies reported in the Annual Report of January, 1965, have been extended to include the baseline studies on Sphaerophorus necrophorus 2263, data of which is reported below. In addition, similar studies have been initiated with the same strains of Pseudomonas aeruginosa employed in our gnotobiotic program. It is hoped that such basic information will enable us to stimulate in continuous culture the polymicrobic flora found in infections and thereby to study of the mechanisms of synergism demonstrated by such mixtures. Answers may be established for such questions as, "why do burned gnotobiotic rats, contaminated with Pseudomonas aeruginosa and Sphaerophorus necrophorus demonstrate 100% mortality whereas rats contaminated with either organism alone do not?"

Comparison of the growth of S. necrophorus 2263 in filtered spent medium with that obtained in fresh medium. The results from the stationary and continuous culture experiments on S. necrophorus 2263 reported in the Annual Report January 1965 suggested that the transition of the bacilli to spheroplast-like bodies was initiated in some manner by changes in the growth menstrum. The two most likely possibilities to account for an environmental change which would trigger the morphogenesis appeared to be (1) the accumulation of a metabolic produce (s) from the growth menstrum. Experiments were designed to explore these possibilities.

In the first experiment of this series, the cultural characteristics of S. necrophorus 2263 grown in filtered spent medium and in fresh thioglycolate medium were compared. The filtered spent medium was obtained by milipore filtration (filter porosity = 0.45 mu) of steady-state culture grown at a dilution rate of 0.05 hr $^{-1}$. The spent medium and fresh basal thioglycolate broth were tubed aseptically in 10 ml quantities in sterile Klett tubes plugged with cotton. Glucose was added aseptically to a concentration of 0.0012 M in broth media from a sterile 36% glucose solution. The media were inoculated with 0.1 ml of a steady-state culture (dilution rate = 0.05 hr $^{-1}$) consisting primarily of large spherical bodies. The cultures were incubated at 37C in a waterbath under stationary conditions and examined at intervals for turbidity and cellular morphology over a period of 26 hours. The pH of each culture was measured at the beginning of the experiment and at the final sampling period.

The growth curves and sketches of the cellular morphologies of the

cultures are presented in Fig. 1. The final turbidity values of the two cultures were the same at the end of 26 hours. The morphological development of both cultures was essentially the same, with the exception that the changes in spent medium were slower due to the extended lag period before growth was initiated. The large bodies "germinated" and produced similar basophilic bacilli in both media within 1 hour at 37°C, but the lag period before exponential growth was 4 hours in spent medium as compared to 1 hour in fresh medium. The pH values are shown in Table XIII.

Comparison of results of this experiment with data from earlier batch culture experiments (Dowell, 1962) revealed some interesting relationships between turbidity, pH, oxidation-reduction (O-R) potential and changes in morphology. These data are compared graphically in Fig. 2. Notice particularly the rapid drop in O-R potential and the gradual decrease in pH during the period of "germination" of the spheroplast-type bodies to basophilic rods (0 hours through 1 hour) and the diauxie type growth response following the formation of basophilic-type large bodies (3 hours through 10 hours).

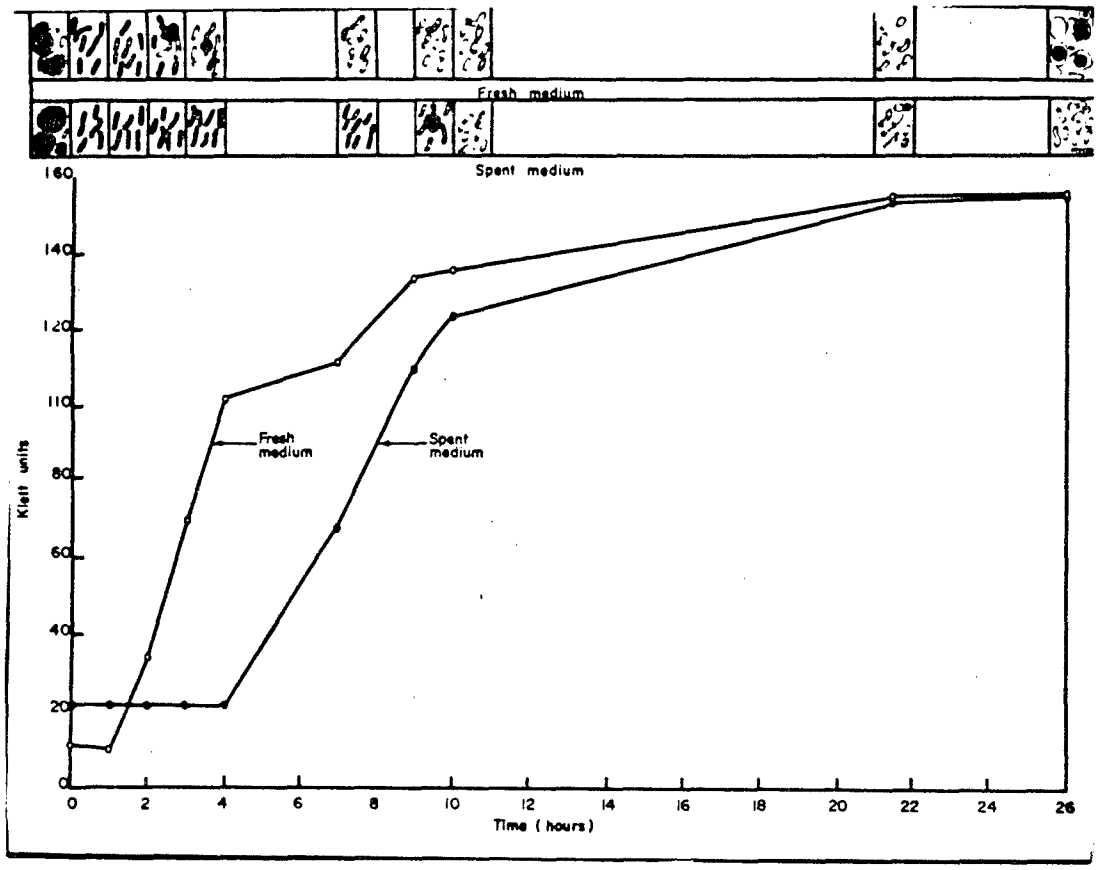

Fig. 1. Turbidity and cellular morphology of S. necrophorus 2263 in spent medium and fresh medium. Glucose was added to a concentration of 0.0012 M in both media. Turbidity was measured at 540 mu in a Klett-Summerson photoelectric colorimeter. The cells were sketched from preparations stained with Loeffler's methylene blue.

Table XIII

Initial and final pH values of stationary cultures of S. necrophorus 2263 grown in fresh thioglycolate broth and spent medium from a steady-state culture (dilution rate = 0.05 hr^{-1}) containing 0.0012 M glucose.

	Spent Medium	Fresh Medium
Initial pH (0 hr)	6.97	7.38
Final pH (26 hr)	6.00	6.30

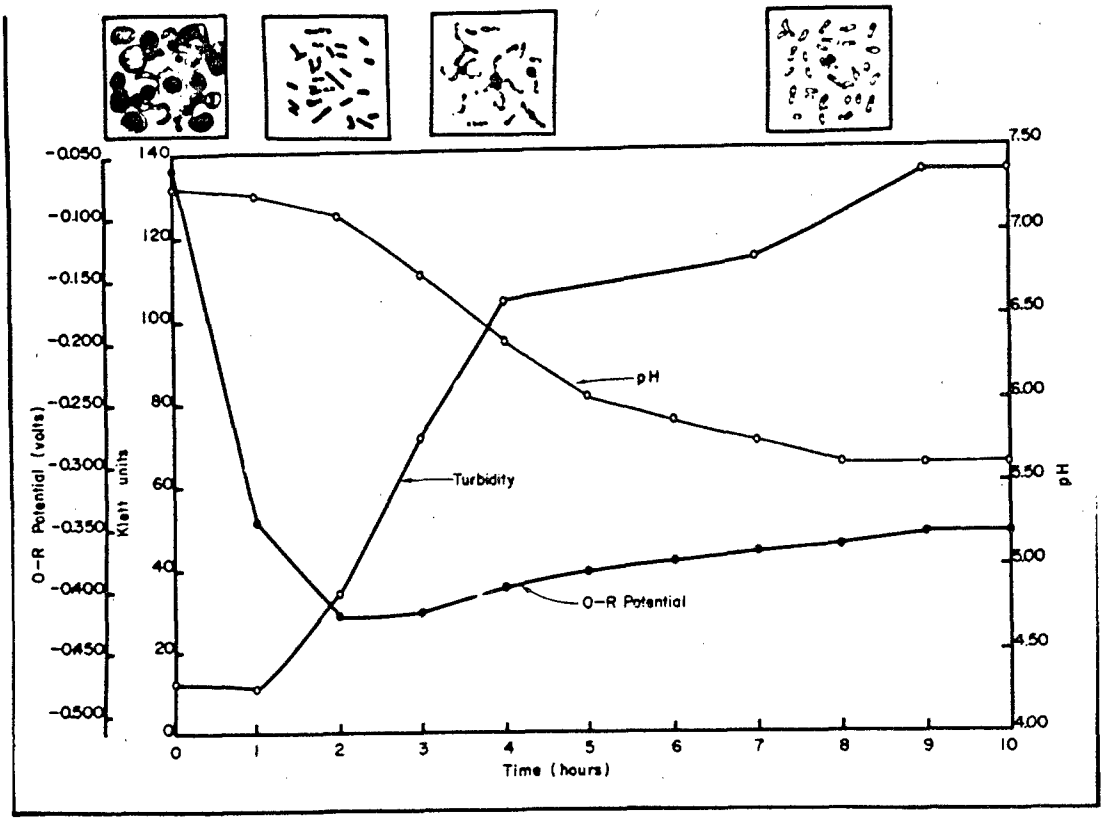

Fig 2. Relationships between turbidity, pH, O-R potential and changes in morphology in stationary cultures of S. necrophorus 2263. The data represented are from 2 different experiments. O-R Potential and pH values are from a culture grown in Brewer's thioglycolate broth (Dowell, 1962.) The turbidity values are from a culture grown in thioglycolate broth containing 0.0012 M glucose. The sketches of the morphology of the organism were made from preparations stained with Loeffler's methylene blue stain.

The change in O-R potential was quite dramatic during the first hour, dropping from minus 0.094 volts at 0 hour to minus 0.364 volts at 1 hour. During this period the pH decreased gradually from 7.32 at 0 hour to 7.20 at 1 hour, a change of only 12 pH units. Exponential growth started at 1 hour and plateaued at the end of 4 hours. The O-R potential dropped to a low of minus 0.419 volts at 2 hours 15 minutes and rose gradually to minus 0.354 volts at 9 hours. It is interesting that the development of the basophilic bodies occurred just before the plateau in growth and the O-R potential began to rise after the large bodies were formed. Only pale safety-pin type rods were present in the culture between 15 hours and 10 hours. On the basis of these observations, it was decided to compare changes in turbidity, pH, O-R potential, glucose utilization, and morphology of S. necrophorus 2263 in stationary cultures grown in thioglycolate broth containing growth limiting and adequate concentrations of glucose.

In these experiments, S. necrophorus 2263 was cultivated in a culture vessel of the type shown in Fig. 3 at 37C in a waterbath under stationary conditions. Mixing was effected with a Teflon coated magnetic stirring bar. The cultures were inoculated and sampled with sterile luer lok syringes by means of a stainless steel 3-way stopcock (B-D) attached to a length of 4 mm glass tubing which was mounted in a port of the vessel with a one hole rubber stopper. The culture vessel containing 500 ml of basal thioglycolate broth was autoclaved at 121°C for 15 minutes. Glucose was added from a sterile 10% solution after the medium was autoclaved. Following the addition of glucose, electrodes

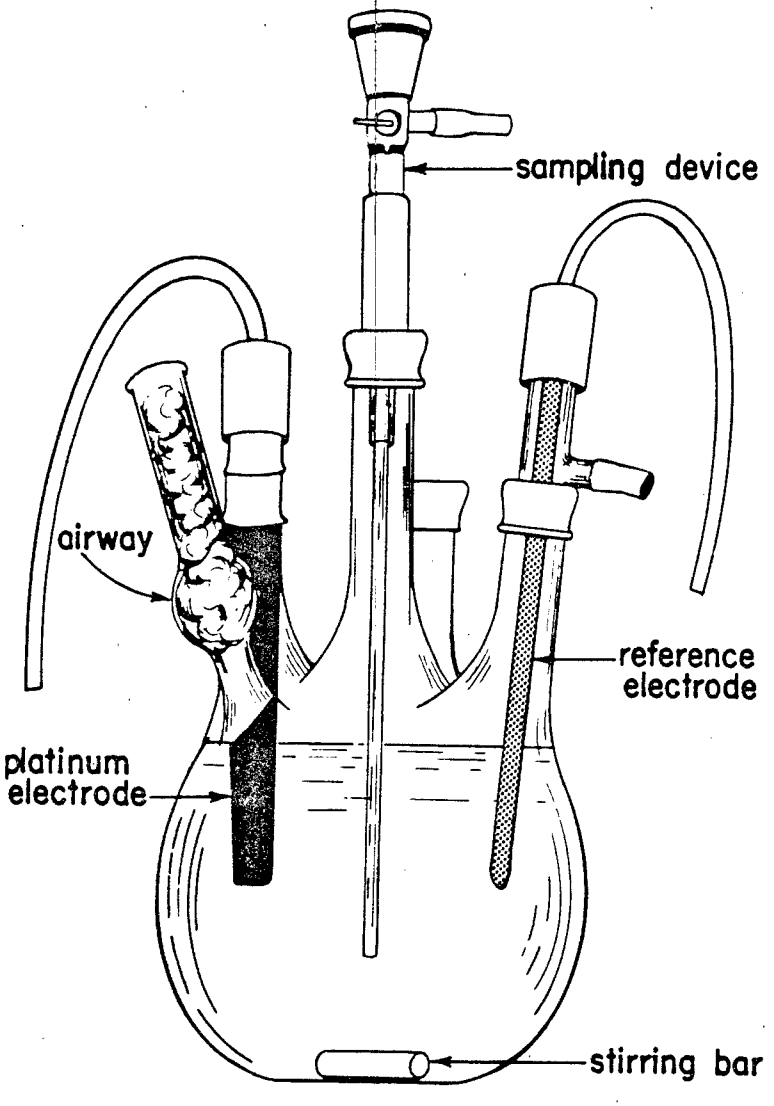

Fig. 3 Sketch of culture vessel employed for cultivation of S. necrophorus 2263 in stationary culture.

for measuring O-R potential were mounted in the culture vessel and the sterility of the arrangement was checked for 24 to 48 hours at 37C. The medium was inoculated with 5 ml of a 72 hour Brewer's thioglycolate broth culture of S. necrophorus 2263 and the culture was then checked at hourly intervals for O-R potential, pH, turbidity, residual glucose, and cellular morphology. These parameters were studied in cultures grown in thioglycolate broth containing two concentrations of glucose (0.0047 M and 0.053 M).

Correlation of turbidity, pH, O-R potential, glucose utilization, and morphological changes of a stationary culture of S. necrophorus 2263 grown in thioglycolate broth containing 0.0047 M glucose. The overall relationships between turbidity, O-R potential, pH, and changes in morphology of S. necrophorus 2263 grown in thioglycolate medium containing 0.0047 M glucose (Table XIV, Fig. 4) were quite similar to those observed in the data presented earlier in Fig. 2. After a lag period of 3 hours, there was a definite diauxie growth response in which the turbidity of the culture increased rapidly for 4 hours to 120 Klett units, plateaued for 1 hour, and then increased to a maximum turbidity of 150 Klett units at 11 hours.

No residual glucose was detected in the culture fluid after 6 hours incubation (Table XIV, Fig. 4). This culture also showed a rapid decrease in O-R potential and only a slight decrease in pH during the period of germination from spheroplast-type bodies to basophilic rods (0 through 2 hours). The O-R potential began to rise after the basophilic bodies were produced at 6 hours and leveled off after 10 hours incubation. Notice also that the pH of the culture increased slightly after the large bodies were formed (Fig. 4). Copious quantities of gas were produced by the culture in the exponential growth phase (3 hours through 7 hours) and little gas was observed between 7 hours and 13 hours incubation (Table XIV). These data suggested that the secondary growth response of the culture following the formation of the basophilic bodies might be due to an alternate metabolic pathway which was inactive in the presence of glucose. To investigate this possibility, the culture was next grown in

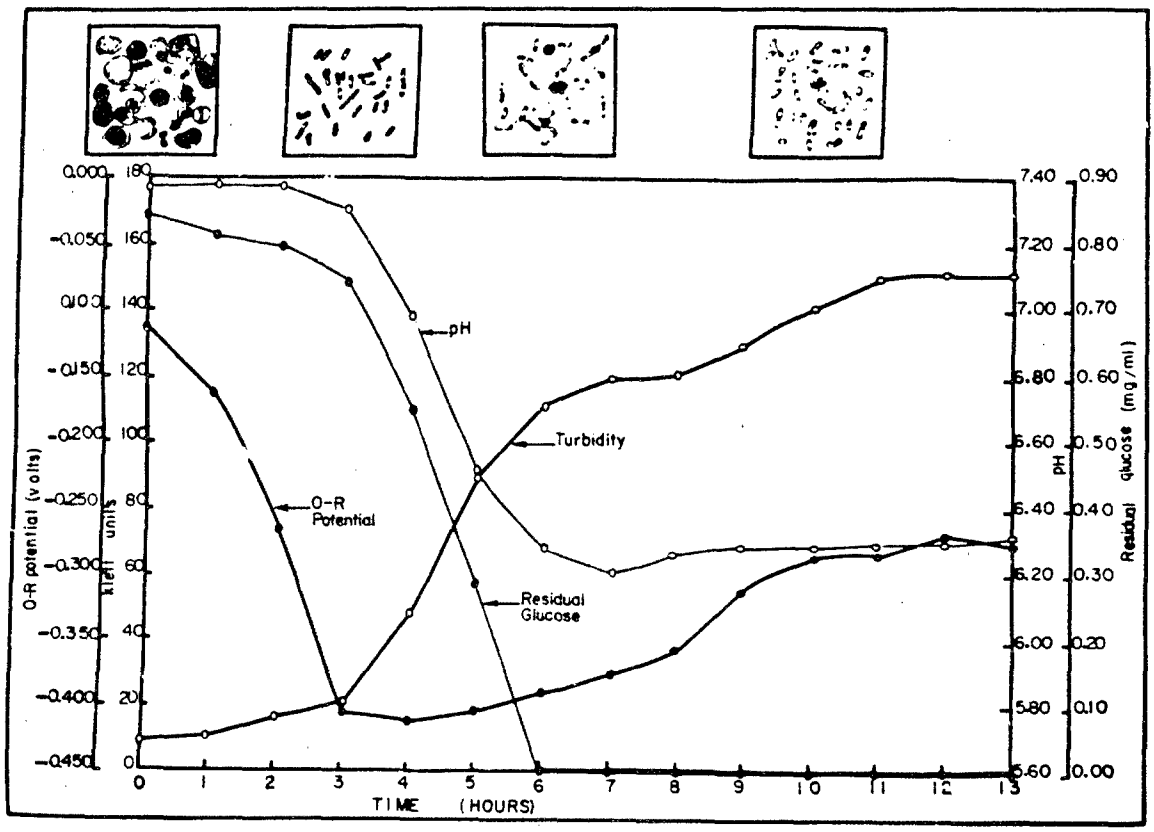

Fig. 4 Turbidity, pH, O-R potential, residual glucose and morphological changes of S. necrophorus 2263 grown in Thioglycolate broth containing 0.0047 M (0.84 mg/ml) glucose at 37 C under stationary conditions.

Table XIV Turbidity, pH, O-R potential and residual glucose values of a stationary culture of S. necrophorus 2263 grown in thioglycolate broth containing 0.0047 M (0.84 mg/ml) glucose at 37C

Incubation time (hr)	Turbidity (klett units)	pH	O-R potential (volts)	Residual glucose (mg/ml)
0	11	7.39	- 0.119	0.84
1	11	7.38	- 0.167	0.81
2	15	7.38	- 0.267	0.80
3*	20	7.30	- 0.402	0.76
4	47	6.97	- 0.417	0.56
5	88	6.52	- 0.404	0.28
6	110	6.30	- 0.392	0.05
7+	120	6.22	- 0.374	"
8	123	6.26	- 0.354	"
9	130	6.28	- 0.334	"
10	141	6.29	- 0.284	"
11	150	6.32	- 0.284	"
12	150	6.30	- 0.269	"
13	150	6.34	- 0.282	"

* Rapid evolution of gas was observed after 3 hr.

+ Very little gas was observed between 7 hr. and 13 hr.

thioglycolate broth containing a concentration of glucose (0.053 M) which was not growth limiting and checked for changes in turbidity, pH, O-R potential, glucose utilization, and morphology.

Turbidity, pH, O-R potential, residual glucose, and morphological changes of S. necrophorus 2263 grown in thioglycolate broth containing 0.053 M glucose under stationary conditions. In this experiment, S. necrophorus 2263 was cultivated in a medium containing a concentration of glucose (0.053 M) which was not growth limiting. The cultural conditions employed were the same as for the culture in 0.0047 M glucose medium with the exception that the 0.053 M glucose medium was incubated at 37C for 72 hours before inoculation instead of 24 hours for the 0.0047 M glucose medium. The extended period of incubation probably allowed excessive aeration of the medium and could possibly account for the positive O-R potential (+ 0.106 volts) at 0 hour and the long lag period (8 hours) before exponential growth was initiated (Table XV, Fig. 5).

With the exception of the extended lag period, the changes in turbidity, pH, O-R potential, glucose utilisation, and morphology in 0.053 M glucose medium were quite similar to those in 0.0047 M glucose meduum. The spheroplast-like bodies in the inoculum (from a 72 hour stationary Brewer's thioglycolate culture) germinated within 1 hour and produced small, dark, evenly stained basophilic bacilli. The basophilic bacilli were present in the culture for 8 hours and then small bacilli with light centers and dark basophilic ends appeared. The large basophilic bodies were produced in the period between 13 hours and 14 hours which was just before the plateau in growth occurred (Fig. 5).

Table XV. Turbidity, pH, O-R potential and residual glucose values of a stationary culture of S. necrophorus 2263 grown in thioglycolate broth containing 0.053 M (9.6 mg/ml) glucose at 37C

Incubation time (hr)	Turbidity (klett units)	pH	O-R potential (volts)	Residual glucose (mg/ml)
0	16	7.28	+ 0.106	9.6
1	16	7.25	+ 0.096	9.4
2	16	7.26	+ 0.091	9.6
3	16	7.27	+ 0.084	9.4
4	15	7.24	+ 0.069	9.5
5	11	7.22	+ 0.057	9.5
6	15	7.11	+ 0.034	9.5
7	9	7.20	- 0.113	9.5
8	18	7.12	- 0.284	9.5
9	27	7.00	- 0.316	8.8
10	63	6.70	- 0.368	8.0
11	112	6.22	- 0.404	8.0
12	150	6.18	- 0.394	8.0
13	180	5.88	- 0.384	7.2
14	196	5.80	- 0.324	6.8
15	199	5.75	- 0.289	6.6
16	220	5.75	- 0.289	6.6
17	230	5.75	- 0.284	6.4
18	230	5.75	- 0.284	6.0
25	230	5.50	- 0.259	4.8
50	270	5.40	- 0.139	Not determined

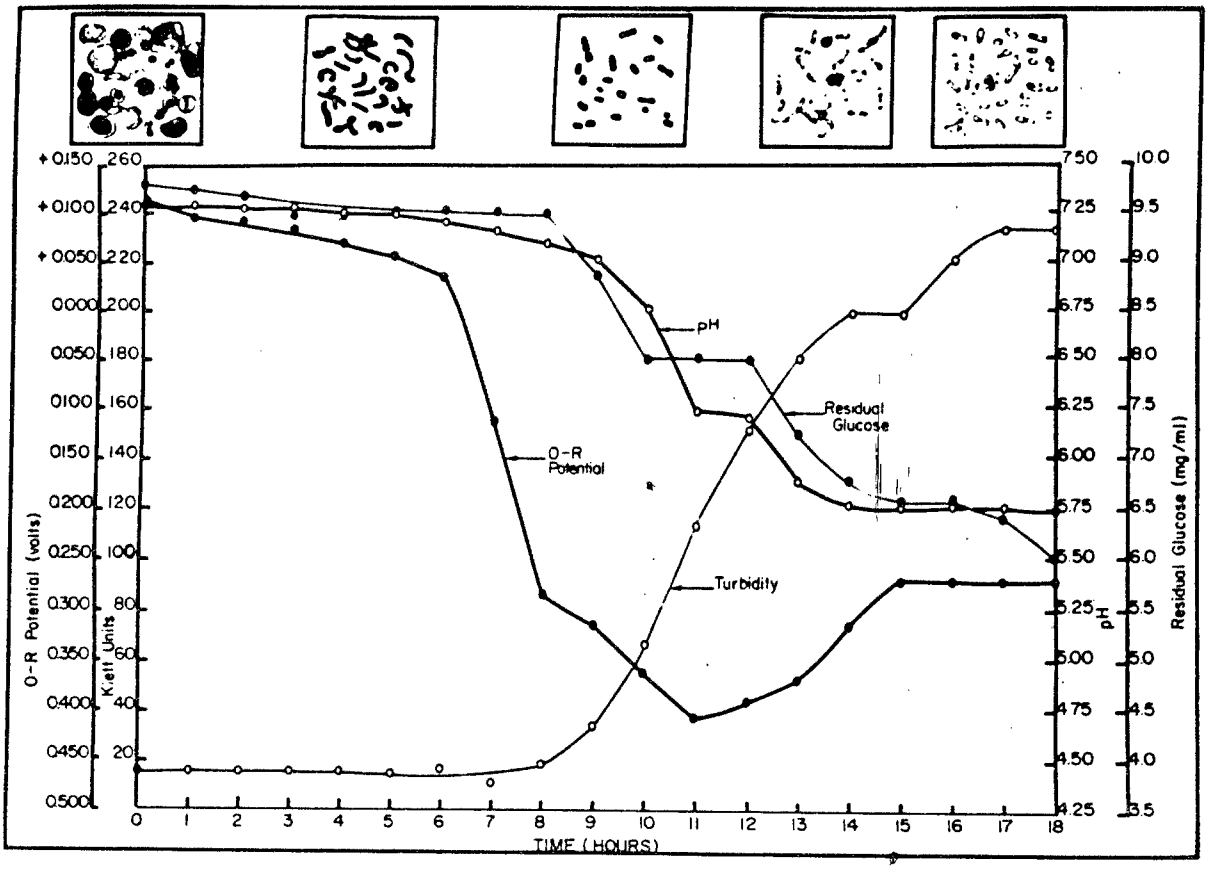

Fig. 5. Turbidity, pH, O-R potential, residual glucose and morphological changes of S. necrophorus 2263 grown in thioglycolate broth containing 0.053 M (9.6 mg/ml) glucose at 37C under stationary conditions.

Small pale safety-pin type bacilli predominated between 15 hours and 18 hours and spheroplast-type bodies were present after 25 hours of incubation.

The changes in the O-R potential of the culture were particularly interesting in the 8 hours before the initiation of growth (Table XV, Fig. 5). Starting with a value of + 0.106 volts at 0 hours, the O-R potential gradually decreased to a value of + 0.034 volts at 6 hours and then rapidly dropped to minus 0.284 volts at the end of 8 hours. During the 8 hour period there was little change in either the residual glucose or pH values. The data suggested that the changes in O-R potential of S. necrophorus 2263 cultures before the initiation of growth were not dependant upon the utilization of glucose. It is also interesting that the residual glucose and pH curves plateaued in the period of growth immediately preceeding the formation of the basophilic-type large bodies (Fig. 5). It was shown that the secondary growth response of S. necrophorus 2263 was not dependant upon the exhaustion of glucose from the medium since there was a similar diauxie growth response in a medium containing excess glucose to that produced in a medium containing a growth limiting concentration of glucose (compare Fig. 4. and Fig. 5).

Changes in turbidity, pH, O-R potential, residual reducing sugars, and morphology of a culture of the spheroplast-type large bodies of S. necrophorus 2263 in glucose free thioglycolate medium under stationary conditions. The results from previous experiments suggested that the change in O-R potential accompanying the germination of the spheroplast-

type large bodies of S. necrophorus 2263 was not dependant upon the utilization of glucose (Fig. 4, Fig. 5). To further investigate this situation it was decided to study the changes in turbidity, pH, O-R potential, residual reducing sugars, and morphology of a culture of the spheroplast-type bodies of S. necrophorus 2263 in glucose-free-thioglycolate broth under stationary conditions.

The changes of the various parameters of the culture were studied in a culture cultivated in a culture vessel of the type illustrated in Fig. 3, in a waterbath at 37C. The cultural techniques and sampling procedures were the same as those employed with the previous stationary cultures except that the culture fluid of the various samples were assayed for residual total reducing sugars by the Folin-Malmros (1929) procedure and the glucose oxidase procedure was not used.

Five hundred ml of thioglycolate broth without glucose were inoculated with 10 ml of a steady-state culture of S. necrophorus 2263 grown at a dilution rate of 0.07 hr^{-1} (Turbidity = 84 Klett units). At this dilution rate, the culture consisted primarily of large spheroplast-type bodies, with and without filamentous protrusions; some small spherical bodies and small "safety-pin type bacilli (Fig. 6). The changes in turbidity, pH, O-R potential, residual reducing sugars, and morphology were observed at hourly intervals for 8 hours in thioglycolate medium without glucose and then glucose was added to a concentration of 1% and the changes in the various parameters were observed for an additional period of 17 hours. After a short lag of 1 hour, the culture grew exponentially for 4 hours, reached a turbidity of 90 Klett units, and then stopped growing

(Fig. 6). Growth was immediately resumed following the addition of glucose (at 8 hours 20 minutes) and continued to a final turbidity of 208 Klett units at the end of 25 hours (Table XVI, Fig. 6). The changes in morphology of the culture were quite similar to those observed in thioglycolate broth with glucose (Fig. 6). Large basophilic bodies appeared after 3 hours incubation and persisted in that culture until after the addition of glucose. Only pale, "safety-pin" type bacilli were observed between 9 hours and 11 hours. Numerous rosette shaped masses of the "safety-pin" type rods held together with a central mass staining red with Giemsa stain were observed in the 2 hour sample (Fig. 6). These structures appeared to have been formed by the fusion of several cells. Similar structures were observed in other stationary cultures of S. necrophorus 2263 preceeding the formation of spheroplast-type large bodies (Dowell, 1962).

The changes in the O-R potential of the culture in a medium without glucose were very similar to those observed in a culture grown in a medium containing glucose (Table XVI, Fig. 6). The behavior of the pH of the culture without glucose was also quite similar to that observed in a medium with glucose except that the pH leveled off at 5 hours in the medium without glucose when growth stopped (Fig. 6). Notice that the quantity of residual reducing sugars in the culture fluid increased during the "germination" period (0 through 1 hour) and then decreased as the turbidity of the culture increased (Table XVI, Fig 7). The uninoculated medium contained 0.380 mg of reducing sugars per ml. The data suggested that reducing sugars were either released from the large bodies

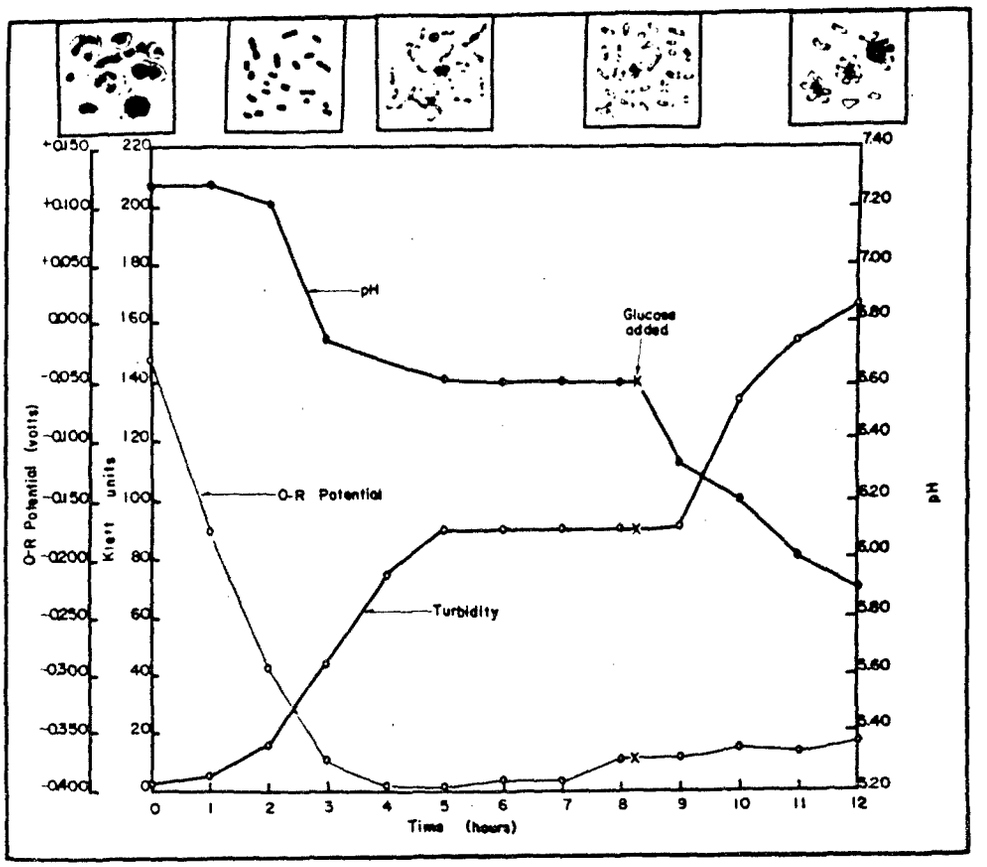

Fig. 6. Turbidity, pH, O-R potential and morphological changes of the spheroplast-type bodies of S. necrophorus 2263 in thioglycolate medium with and without glucose. Sketches of the morphology of the organism were prepared from preparations stained with Loeffler's methylene blue.

Table XVI. Turbidity, pH, O-R potential, and residual total reducing sugars in a culture of spheroplast-type bodies of S. necrophorus 2263 in thioglycolate broth with and without glucose.

Time (hr)	O-R potential (volts)	Turbidity (klett units)	pH	Total Reducing sugars (mg/ml)	Remarks
0	-0.031	3	7.28	0.490	
1	-0.179	5	7.28	0.875	
2	-0.291	15	7.22	0.530	
3	-0.378	47	6.73	0.530	
4	-0.396	74	not measured	0.420	
5	-0.399	90	6.61	0.440	
6	-0.386	90	6.60	0.440	
7	-0.391	90	6.60	0.440	
8	-0.374	90	6.60	0.440	
8 hr 20 min.					glucose added
9	-0.372	102	6.32	not measured	
10	-0.366	135	6.20	"	
11	-0.368	155	6.00	"	
12	-0.342	166	5.90	"	
23	-0.222	200	5.48	"	
24	-0.192	202	5.46	"	
25	-0.230	208	5.40	"	

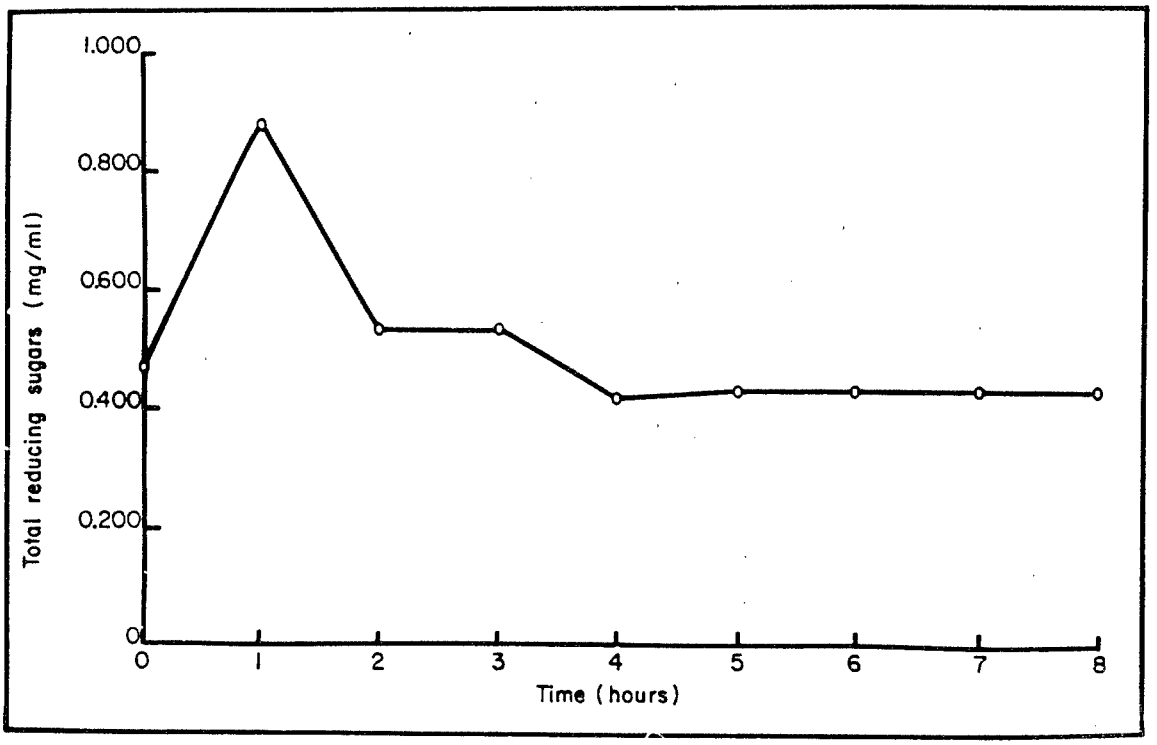

Fig. 7. Changes in residual total reducing sugars in the culture fluid of a culture of the spheroplast-type bodies of S. necrophorus 2263 in thioglycolate broth without glucose. Total reducing sugars were determined by the method of Folin and Malmros 1929 on the supernatant fluid following centrifugation of the culture samples at 2000 X g for 15 minutes, employing glucose as a standard.

during the "germinati - " period or they were synthesized by the culture during this period. In the next experiment the change in turbidity, pH, O-R potential and morphology of a stationary culture of S. necrophorus 2263 in glucose-free medium starting with the rod form of the organism from a steady-state culture were studied.

Changes in turbidity, pH, O-R potential, residual reducing sugars and morphology of a stationary culture of S. necrophorus 2263 in thioglycolate broth with and without glucose inoculated with bacilli from a steady-state culture grown at a dilution rate of 0.42 hr^{-1}. This experiment was conducted in the same manner as the experiment just described except that the medium was boiled for 10 minutes and cooled to 37C just prior to inoculation with the culture. This resulted in a lower O-R potential (minus 0.272 volts) at 0 hour than that in the other experiment (minus 0.031 volts).

The growth response of the bacilli in thioglycolate broth without glucose (Fig. 8) was quite different from that of the spheroplast-type bodies (Fig. 6). The bacilli showed a diauxie type growth response similar to that exhibited by the culture of S. necrophorus 2263 in thioglycolate broth containing glucose (Fig. 4, Fig. 5). The culture contained dark basophilic rods at 1 hour, rods with pale centers and dark ends at 2 hours, and basophilic type large bodies appeared at 4 hours. Pale "safety-pin" type bacilli predominated between 6 hours and 11 hours (Fig. 8). The pH of the culture decreased rapidly during the first 3 hours of incubation and then changed very little until after glucose was added to the culture (Table XVII, Fig. 8).

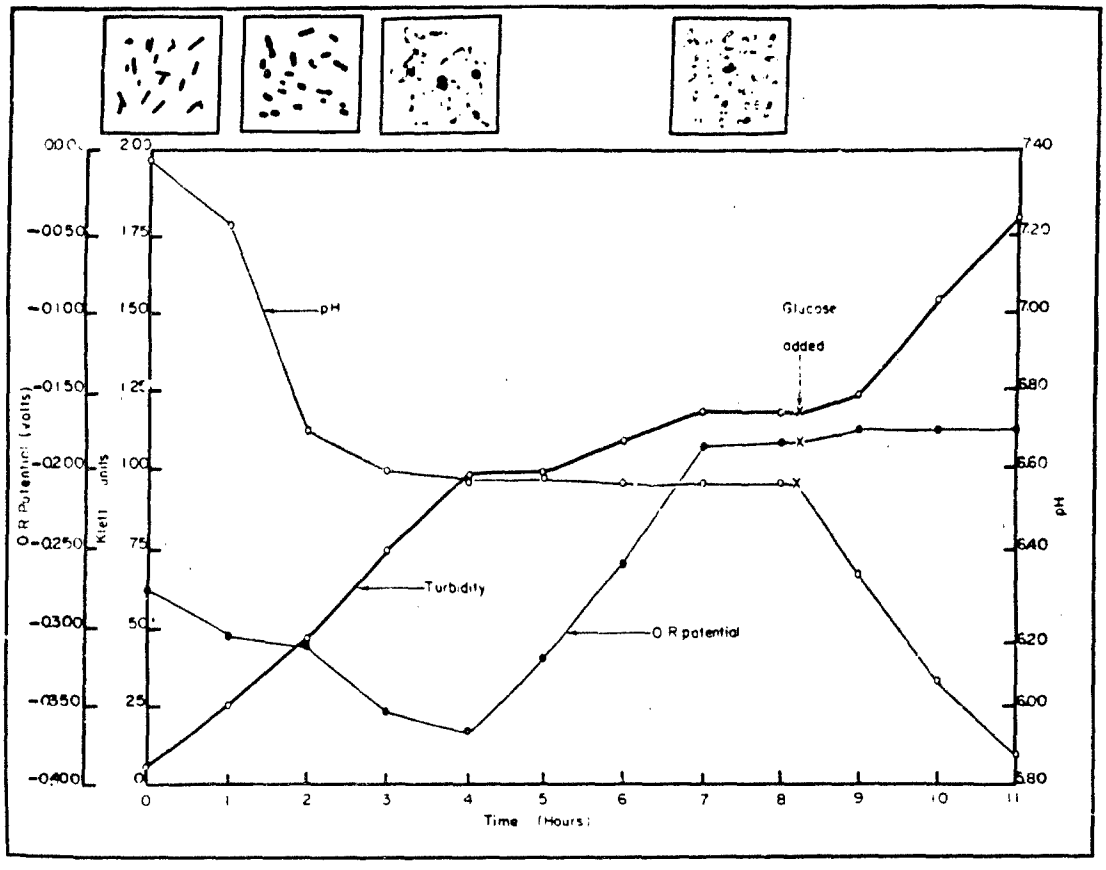

Fig. 8. Changes in turbidity, pH, O-R potential, and morphology of a stationary culture of S. necrophorus 2263 in thioglycolate broth with and without glucose inoculated with bacilli from a stady-state culture grown at a dilution rate of 0.42 hr^{-1}.

Table XVII. Turbidity, pH, O-R potential and total reducing sugars of a stationary culture of S. necrophorus 2263 in thioglycolate broth with and without glucose inoculated with bacilli from a steady-state culture grown at a dilution rate of 0.42 hr^{-1}.

Time (hr)	O-R potential (volts)	pH	Turbidity (klett units)	Residual Total reducing sugars mg/ml
0	- 0.272	7.39	12	0.268
1	- 0.302	7.23	25	0.236
2	- 0.309	6.70	45	0.268
3	- 0.353	6.60	75	0.236
4	- 0.369	6.59	98	0.259
5	- 0.314	6.59	98	0.236
6	- 0.256	6.55	108	0.236
7	- 0.192	6.56	116	0.236
8	- 0.190	6.55	115	0.247
* 8 hr 15 min.				
9	- 0.174	6.34	122	not measured
10	- 0.174	6.08	158	" "
11	- 0.174	5.88	182	" "

* Glucose added to a concentration of 1%.

Although the O-R potential of the culture of the bacilli in thioglycolate medium without glucose at 0 hour (Table XVII, Fig. 8) was considerably lower than the initial O-R potential of the spheroplast-type bodies in thioglycolate broth without glucose (Table XVI, Fig. 6) the overall changes in O-R potential of the two cultures were quite comparable. Notice the rapid rise in the O-R potential following the formation of the large basophilic bodies and the plateau in the turbidity of the culture after 4 hours incubation (Fig. 8). The changes in the residual reducing sugar content of the culture of the bacilli in the medium without glucose (Table XVII, Fig. 9) were quite different to those in the culture of the spheroplast-type bodies in thioglycolate broth without glucose (Table XVI, Fig. 9). The uninoculated medium in this experiment contained 0.412 mg of reducing sugars per ml. Following inoculation with the bacilli, the content of residual reducing sugars dropped to 0.268 mg per ml and varied between 0.236 mg per ml and 0.268 mg per ml until glucose was added to the culture (Table XVII, Fig. 9).

An analysis by the manufacturer (Baltimore Biological Laboratories) showed that phytone (BBL) has a high total carbohydrate content (37%) and it was calculated from this data that the basal thioglycolate broth without glucose used in these experiments contained approximately 1 mg of total carbohydrate per ml of medium. The major component of thioglycolate medium (trypticase, BBL) contained no carbohydrate according to the manufacturer's analysis.

Changes in turbidity and morphology of stationary cultures of S. necrophorus 2263 in basal thioglycolate broth with and without phytone.

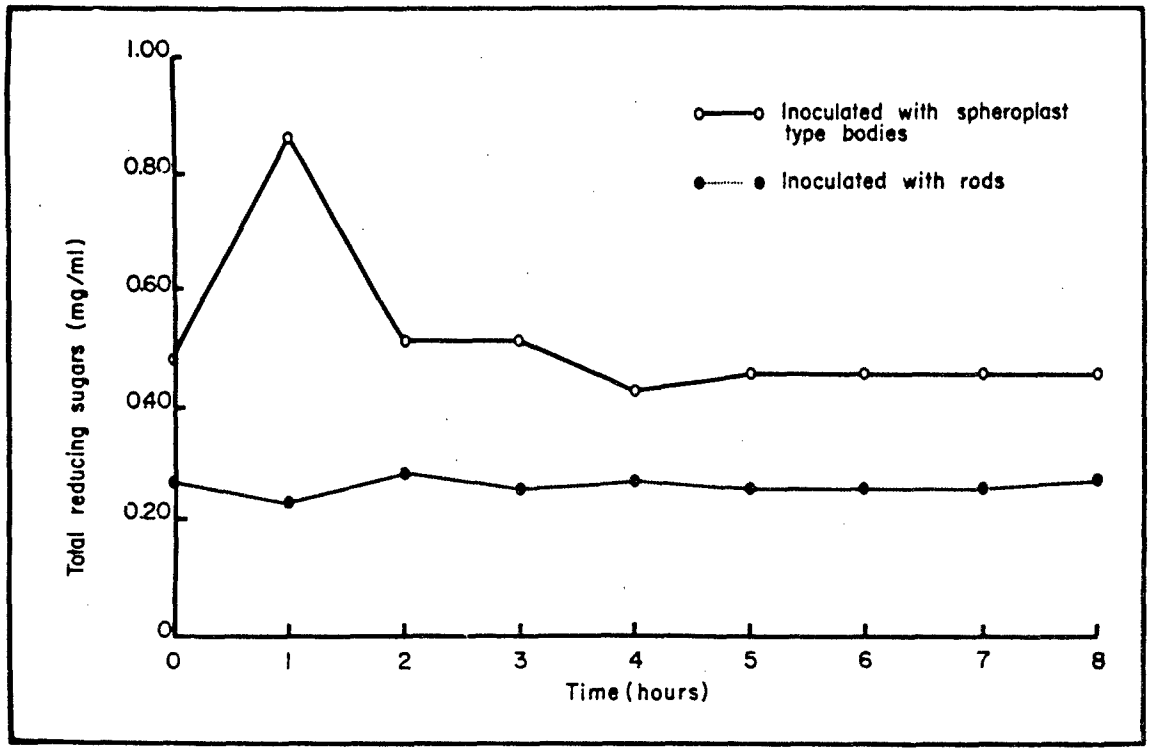

Fig. 9. Residual total reducing sugar content of stationary cultures of S. necrophorus 2263 in thioglycolate broth without glucose. One culture was inoculated with a steady-state culture of the spheroplast-like bodies grown at a dilution rate of 0.07 hr^{-1} and the other culture was inoculated with a steady-state culture of bacilli grown at a dilution rate of 0.42 hr^{-1}.

Thioglycolate broth without glucose (pH 7.4) and thioglycolate broth without glucose or phytone (pH 7.4) were prepared and dispensed in 10 ml quantities into Klett tubes, plugged with cotton, and autoclaved at 121C for 15 minutes. Tubes of each medium were inoculated with 0.2 ml of a steady-state culture of the spheroplast-type bodies (dilution rate, 0.07 hr^{-1}) and incubated at 37C for 18 hours. The turbidity and cellular morphology of the cultures were checked at 0 hour and after 18 hours. The turbidity and cellular morphology of the cultures were checked at 0 hour and after 18 hours incubation. The results are summarized in Table XVIII.

The turbidity after 18 hours incubation was much lower in the medium without glucose and phytone (24 Klett units) than in the medium with only glucose deleted (110 Klett units). The large bodies "germinated" and formed basophilic bacilli in the medium without phytone but apparently no further morphological development occurred.

In a separate experiment, glucose was added to a concentration of 1% in the thioglycolate medium minus phytone and inoculated with 0.2 ml of a steady-state culture of the spheroplast-type bodies (dilution rate, 0.07 hr^{-1}) per 10 ml of medium. In this case, the culture reached a turbidity of 154 Klett units after 18 hours at 37C and the culture was composed chiefly of "safety-pin" type bacilli and a rare spheroplast-type body.

The requirement of energy for further development of steady-state cultures of S. necrophorus 2263 incubated under stationary conditions. The purpose of this experiment was to determine whether the morphology and turbidity of steady-state cultures of S. necrophorus 2263 grown in thioglycolate broth with glucose as the growth limiting factor, remained

Table XVIII. Turbidity and morphology of stationary cultures of S. necrophorus 2263 in thioglycolate broth without glucose and thioglycolate broth with glucose and phytone deleted.

Medium	Turbidity in Klett units 0 hr	18 hr
minus glucose	6 units	110 units
minus glucose & phytone	6 units	24 units

Medium	Morphology in Gram stained smears 0 hr	18 hr
minus glucose	Spheroplast-type bodies with and without filaments	Small slim bacilli with metachromatic staining
minus glucose & phytone	Spheroplast-type bodies with and without filaments	Small, plump evenly stained, basophilic bacilli

the same or changed when incubated under stationary conditions. Samples of the steady-state cultures were removed from the continuous culture vessel and 10 ml were added aseptically to each of two sterile cotton plugged Klett tubes. Glucose was added to the culture in one of the tubes, to a concentration of 1% and the cultures were then incubated for 24 hours at 37C under stationary conditions. The turbidity and morphology of the cultures were checked at 0 hour and after 24 hours incubation. The results from two steady-state cultures (grown at dilution rates of 0.07 hr^{-1} and 0.42 hr^{-1}) studied in this manner are shown in Table XIX and Table XX.

These data showed that no changes in turbidity or morphology occurred when steady-state cultures of S. necrophorus 2263 were incubated under stationary conditions unless glucose (the energy source) was added. It was also shown that a steady-state culture of spheroplast-type bodies (dilution rate, 0.07 hr^{-1}) to which glucose was added and incubated under stationary conditions increased in turbidity but retained the same morphology after 24 hours incubation. On the other hand, a steady-state culture of bacilli (dilution rate 0.42 hr^{-1}) with glucose added and incubated under stationary metachromatic staining bacilli to larger, pale, "safety-pin" type bacilli and spheroplast-type bodies (Table XIX, Table XX).

The technique described in the above experiment was also used to determine the ability of S. necrophorus 2263 to utilize other substrates as a source of energy. Employing a steady-state culture grown at a dilution rate of 0.08 hr^{-1} (consisting of spheroplast-type bodies with

Table XIX. Turbidity of steady-state cultures of S. necrophorus 2263 with and without glucose under stationary conditions.

Dilution rate of the continuous culture	Turbidity under stationary conditions (Klett units)	
	0 hr	24 hr
($0.07\ hr^{-1}$)		
1% glucose	84	177
		87
no glucose	87	87
($0.42\ hr^{-1}$)		
1% glucose	115	197
no glucose	115	114

Table XX. Morphology of steady-state cultures of S. necrophorus 2263 with and without glucose under stationary conditions.

Dilution rate of the continuous culture	Morphology under stationary conditions	
	0 hr	24 hr
($0.07\ hr^{-1}$) 1% glucose	Spheroplast-type bodies	spheroplast-type bodies
no glucose	spheroplast-type bodies	spheroplast-type bodies
($0.42\ hr^{-1}$) 1% glucose	slim rods with metachromatic staining	large pale "safety pin" type rods and a rare spheroplast-type body
no glucose	slim rods with metachromatic staining	slim rods with metachromatic staining

and without filaments, long pale filamentous structures and pale safety-pin type bacilli) a definite increase in turbidity of 20 to 50 Klett units was demonstrated with the addition singly of sodium pyruvate, sodium lactate, and sodium citrate to a concentration of 0.1% and no growth response was observed with the addition of sodium acetate to 0.33% or with calcium pantothenate to a concentration of 10 mcg per ml. None of the compounds mentioned induced any significant changes in the morphology of the organisms under these conditions.

Morphological changes of S. necrophorus 2263 in dialysis sacs implanted in the abdominal cavity of a rabbit. The purpose of this experiment was to determine if S. necrophorus 2263 exhibited similar morphological changes in vivo to those observed in vitro. Sacs four inches in length were prepared from 5/8 inch diameter (inflated) dialysis tubing with a pore size of 4.8 mu (Arthur H. Thomas Co). A diaphragm-type rubber stopper was used to close one end of the tubing and the other end was tied in a knot. A six inch length of 0.022 inch (I.D., polyethylene tubing) (B.D., PX-022) was mounted in the rubber stopper to serve as an inoculating and sampling tube. The assembled sacs were immersed in 0.85% saline solution and autoclaved at 121C for 15 minutes.

Two sacs were surgically implanted in the abdominal cavity of a rabbit. The polyethylene tubes were passed through the flank of the rabbit to the outside through the bore of a 16 guage needle. Sterile, blunt, 23 guage needles were inserted into the polyethylene tubes and the needles were fitted with sterile MacQuigg adapters (B.D.). Five ml of a 24 hour Brewer's thioglycolate broth culture of S. necrophorus 2263

(consisting of spheroplast-type bodies and small, pale "safety-pin" type bicilli) were added to each sac with a syringe. Samples of the cultures were removed from the sacs at intervals and the morphology of the organisms were observed in Grams, Loeffler's methylene blue, and Giemsa stained preparations. In addition, each sample was checked for purity on blood agar medium incubated aerobically and anaerobically.

The morphological development of the cultures in the two sacs was quite similar at first, consisting of pale, "safety-pin" type rods after 18 hours and a mixture of spheroplast-type bodies, pale "safety-pin" type rods and extremely small granular forms at 45 hours. However, the morphology of the organisms in the two sacs was quite different in the next sample (six days). At six days, one of the sacs contained spheroplast-type bodies, pale "safety-pin" rods, and granular forms as observed in the 45 hour samples, but the other culture was composed chiefly of small, slim, gram negative, evenly stained, basophilic bacilli and a rare large, gram positive rod. The gram positive rod was isolated and identified as an aerobic, spore forming Bacillus species. The sacs were sampled again at ten days and the morphology of the two cultures was essentially the same as observed in the six day samples.

At this time(ten days), the other sac was deliberately inoculated with 2 ml of a 12 hour brain heart infusion broth culture of the Bacillus species. Two days later, the contents of the sacs were sampled and it was found that both sacs contained a mixture of slim, gram negative, basophilic rods: large gram positive bacilli; and rare gram negative, basophilic-type large body. No spheroplast-type bodies or safety-pin rods were

observed in either culture.

A similar change in morphology was induced in S. necrophorus in an in vitro culture when the Bacillus species was added. A 48 hour Brewer's thioglycolate broth culture (consisting of spheroplast-type bodies and pale, "safety-pin" bacilli) was inoculated with 1 ml of a 12 hour brain heart infusion broth culture of the Bacillus species and after 24 hours incubation at 37C, the culture contained slim, gram-negative rods; similar to those in the sac cultures in vivo and large, gram-positive rods.

A rabbit was injected intradermally at different sites with 0.2 ml of pure cultures (24 hour cultures grown in Brewer's thioglycolate broth at 37C) of the Bacillus species and S. necrophorus 2263 and each of the mixed cultures (grown in vivo). Five days after injection, distinct nodular, dermal abscesses (2 to 3 mm in diameter) were present in the sites injected with the mixed cultures; a smaller abscess (0.5 to 1 mm in diameter) was produced by S. necrophorus 2263; and no abscess was detected where the bacillus species was injected.

Reversion of spheroplast-type bodies of S. necrophorus 2263 to bacilli by the addition of DAP to a continuous culture. Lenderburn and St. Clair (1958) succeeded in the isolation of auxotrophic mutants of Escherichia coli which required diaminopimelic acid (DAP). In an osmotically suitable medium not containing DAP, the mutants grew as L colonies but reverted to the bacillary form in a medium containing DAP. They proposed that L colonies are colonies of protoplasts whose aberrant mode of growth is conditioned by the loss of the cell wall and the failure of normal cell division. They also proposed that a partial or complete defect of the cell wall may be brought

about either by external inhibition (as with penicillin) or by internal genetic blocks affecting any of various aspects of cell wall formation.

S. necrophorus 2263 produced bacilli with apparently normal cell walls in the early stages of growth in stationary cultures or at high dilution rates in continuous cultures bu exhibited spheroplast-type bodies with defective cell walls in later stages of growth in stationary cultures or at low dilution rates in continuous cultures. The data from the various experiments performed with the organism in stationary and continuous cultures in vitro and the in vivo experiment suggested that the transition of the bacilli to large bodies was in some manner due to the inhibition of normal cell wall formation by changes in the environment. The inhibition of cell wall formation was alleviated when S. necrophorus 2263 was grown in the presence of a Bacillus species in a mixed culture. It seemed possible that the cell wall defect might be due to the inhibition of DAP synthesis and/or incorporation into cell wall material by the environmental changes. It was decided to determine if the addition of DAP to a steady-state culture of the spheroplast-type bodies would repair the metabolic defect and allow the formation of normal cell walls.

Diaminopimelic acid was added to a concentration of 20 mcg per ml (from a 0.1% solution autoclaved at 121C for 15 minutes) to a steady-state culture of the spheroplast-type bodies of S. necrophorus 2263 growing at a dilution rate of 0.07 hr^{-1}). At the same time, DAP was added to a concentration of 20 mcg per ml to 2 liters of medium in the reservoir. After the medium containing DAP was consumed (5 days) medium without DAP was added to the reservoir and the culture was observed for an

additional 10 days at the same dilution rate (0.07 hr^{-1}). The morphology of the organisms in Gram's and Giemsa stained preparations and the turbidity of the culture with and without DAP were checked at intervals. The results are illustrated in Fig. 24.

Within 24 hours after the addition of DAP, the turbidity of the culture increased from 86 Klett units to 125 Klett units. Concomitantly with the increase in turbidity, the morphology of the organisms changed from spheroplast-type bodies with and without filaments to small, slim, evenly stained, basophilic rods and very small, coccoid, basophilic rods. The basophilic bacilli were maintained in the steady-state culture until after the addition of the DAP-free medium to the reservoir (10 days). The basophilic rods were then gradually replaced, first with basophilic-type bodies, then "safety-pin" type rods and finally with spheroplast-type bodies, (15 days). The turbidity of the culture remained between 120 and 125 Klett units until the DAP-free medium was added and then gradually decreased to 81 Klett units and leveled off at 84 Klett units when the spheroplast-type bodies reappeared (Fig. 10).

Effects of lowering the temperature of incubation from 37C on a steady-state culture of the spheroplast-type bodies of S. necrophorus 2263. Preliminary studies on the nutritional requirements of S. necrophorus 2263 showed that the organism probably requires pantothenic acid for growth. Moderate growth (90 to 100 Klett units at 540 mu) was obtained after 48 hours at 37°C in a medium containing 0.5% vitamin-free casamino acids (Difco); 0.2% L tryptophan; 0.025% L cystine; 0.05% sodium thioglycolate; 0.25% solium chloride (pH 7.4) with 10 mcg of calcium pantothenate added per ml but no growth was obtained in the same medium without calcium pantothenate.

Lichstein and Begue (1960) found that certain strains of Saccharomyces cerevisiae required pantothenic acid when cultured at 38° C

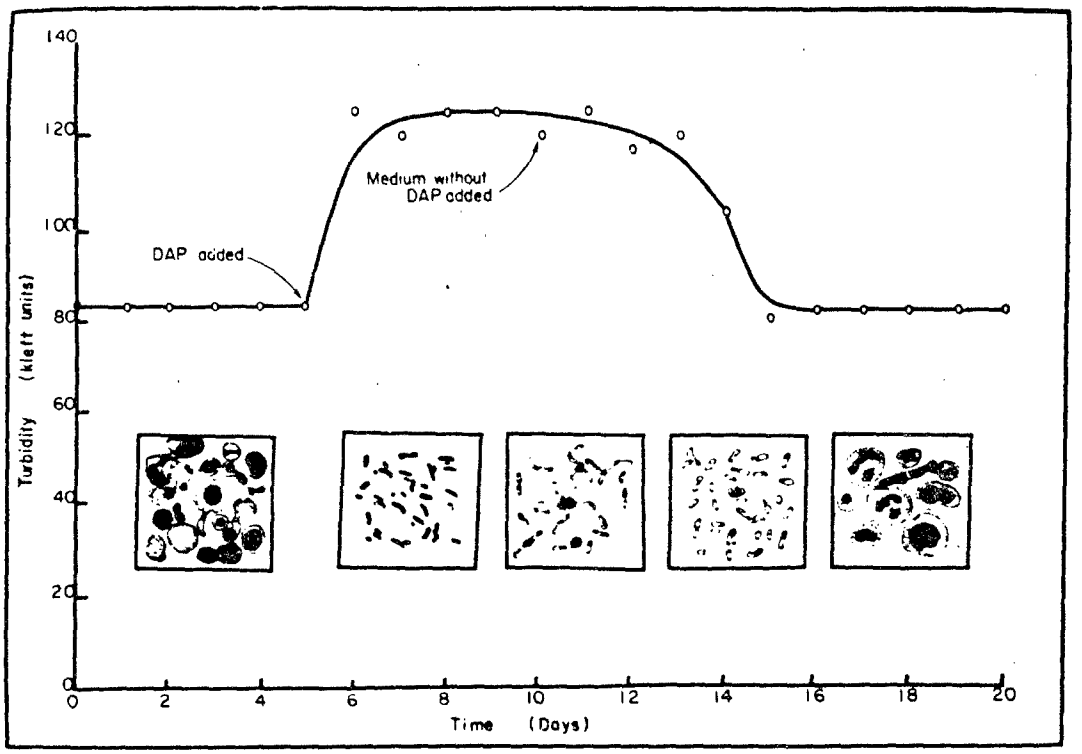

Fig. 10. Morphology and turbidity of a steady-state culture of S. necrophorus 2263 grown at a dilution rate of 0.007 hr^{-1} in thioglycolate broth containing 0.002 M glucose, with and without diaminopimelic acid (20 mc. per ml). Sketches of the morphology of the organisms were made from preparations stained with Gram's and Giemsa Stains.

V. Studies of the Response and Natural Course of the Burn Wound in Germ-free Animals Given a Standard Burn and Maintained in a Germ-free Environment.

Continued experimentation has been performed with germ-free animals obtained from the laboratory animal facilities of Ohio State University, Columbus, Ohio, under the Direction of Dr. ... D. Henthorne, VDM. These studies were begun because of the great number and variety of contaminating bacteria which complicated the task of detecting the agglutinin and precipitin levels against each isolate in normally contaminated laboratory animals. It has also been impossible to determine which, if any, of the bacterial invaders was the cause of death. Experimentation with germ-free animals to obtain base line values and determine the response of the animals to thermal injury in a germ-free environment has been reported in our earlier report of January 1, 1965. Comparison of this data to that obtained with normal Sprague-Dawley rats that were placed into the germ-free environment burned and followed throughout the experiment in completely germ-free surroundings have also been reported.

Despite the extensive bacterial contamination present on the burned wounds on the normal rats, the mortality rate was approximately the same as in the germ-free group (2 of 14), with one late death again due to evisceration of the intestinal contents through a complete full thickness burn of the animal's back. Biopsy studies in this group revealed full-thickness burns with bacteria in some sections. Autopsy results were essentially negative as they were in the germ-free group.

A) Monocontamination of Germ-free Burned Animals

Because of the great variety and numbers of bacterial isolates present on the burn wounds of normal laboratory animals even when they are studied and housed in a germ-free environment, immunologic studies become difficult to evaluate. In an attempt to obtain data that could be better analyzed, a series of experiments were performed in which germ-free colonies of Sprague-Dawley rats were contaminated with a single bacterial strain.

Selective contamination of germ-free rats prior to burning would seem to be an excellent method of studying the effect of bacterial infection and perhaps differentiating them from a non-bacterial toxemia. Three separate groups of rats were studied. These groups were contaminated with a single strain of Staphylococcus aureus (phage type UC-18), Ps. aeruginosa, and Sphaerophorus necrophorus respectively. The Staphylococcus aureus and Ps. aeruginosa were selected because they are frequently the causes of serious infection in burns, and the S. necrophorus as a relatively non-pathogenic bacteria. Two cc. of an 18 hour old culture of the contaminating organism were placed in 58 cc. of the animals' drinking water. Forty-eight hours later quantitative stool cultures indicated the organisms had become established in the intestinal tract in high numbers.

Four days after selective contamination the rats were burned for ten seconds in boiling water as previously described (Fig. 11). Cultures of the burn wounds and of tissue under the burn eschar taken at the time of biopsy, all grew the same single strain of contaminating bacteria which had been placed in the drinking water. No other organisms were encountered

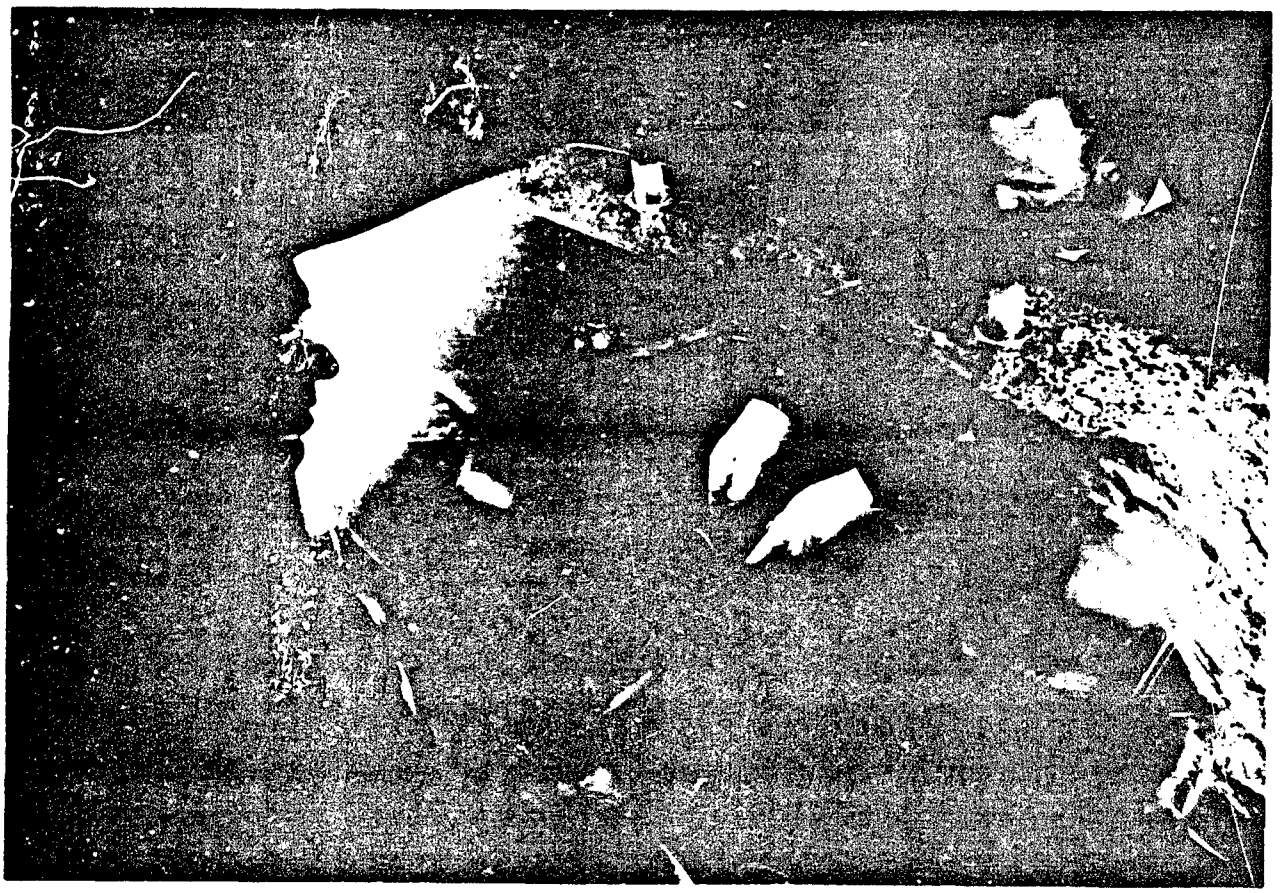

Fig. 11. Overall view of burning an anesthetized germ-free rat inside the isolator.

during any of the experiments performed. All other studies, including Hgb., WBC, weight, temperature and biopsies were performed as previously described.

Ps. aeruginosa (12 rats) was first employed as a monocontaminating organism. The rats thus contaminated demonstrated a more precipitous drop in Hct. on the second day following the burn than had the germ-free rats, but there was a gradual recovery until postburn day 21 when a second unexplained drop in Hct. averaging some 10 per cent occurred (Fig. 12). The White Blood Cell Count was very similar to that in the germ-free burned animals, except that a larger rise on the first and second postburn days occurred. This rise was followed by a drop on the fourth day but began to rise on the seventh day gradually returning toward normal similar to the experience with germ-free rats (Fig. 13). The weight of the animals during the experiment revealed an initial drop but gradual recovery to normal levels was observed in the surviving rats. Temperature levels showed little variation throughout the period of the experiment. The mortality results in the 12 rats that were contaminated with the virulent strain of Ps. aeruginosa revealed that seven survived the entire 28 days and were sacrificed. Two of the rats died on the second postburn day, during the shock phase, and the remaining three died on the eighth, tenth and twelfth burn day respectively (Fig. 14). Autopsies revealed lung and splenic abscesses in some animals as well as occasional intestinal ulceration.

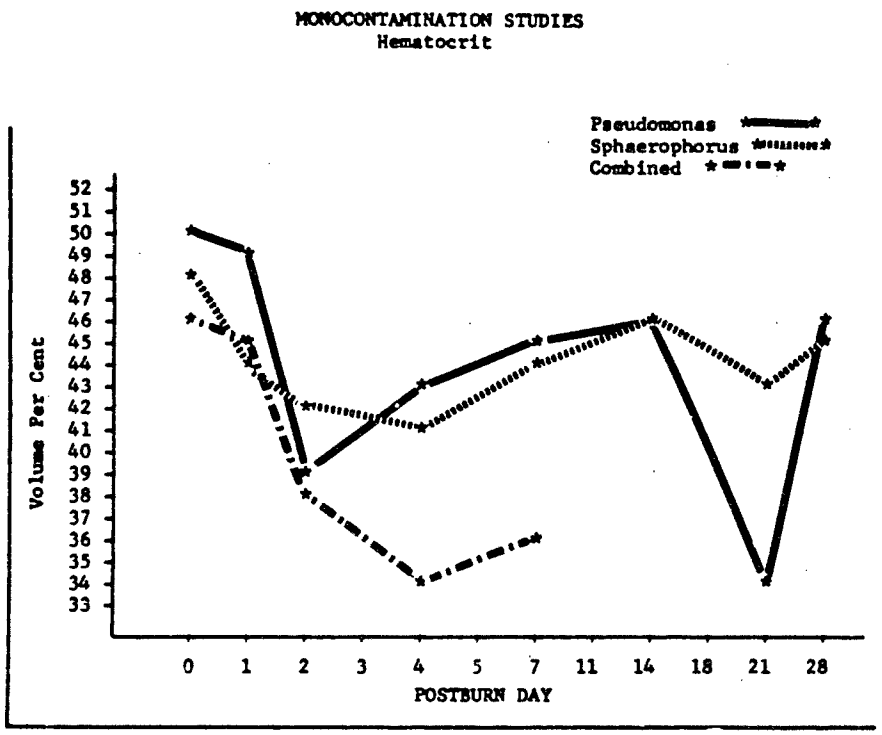

Fig. 12. Graphic comparison of the postburn hemotocrits in rats that had been mono-contaminated with Pseudomonas aeruginosa or Sphaerophorus necrophorus, with a third group of germ-free animals sustaining combined contamination prior to burning. The hematocrit levels in the Sphaerophorus necrophorus contaminated group were very similar to those seen in the germ-free burned rats. The Pseudomonas contaminated group had hematocrit levels, with the exception of an unexplained drop at three weeks postburn similar to those of the germ-free and Saphaerophorus necrophorus contaminated groups. The hematocrit fell sharply in the combined contamination group with little evidence of return toward normal.

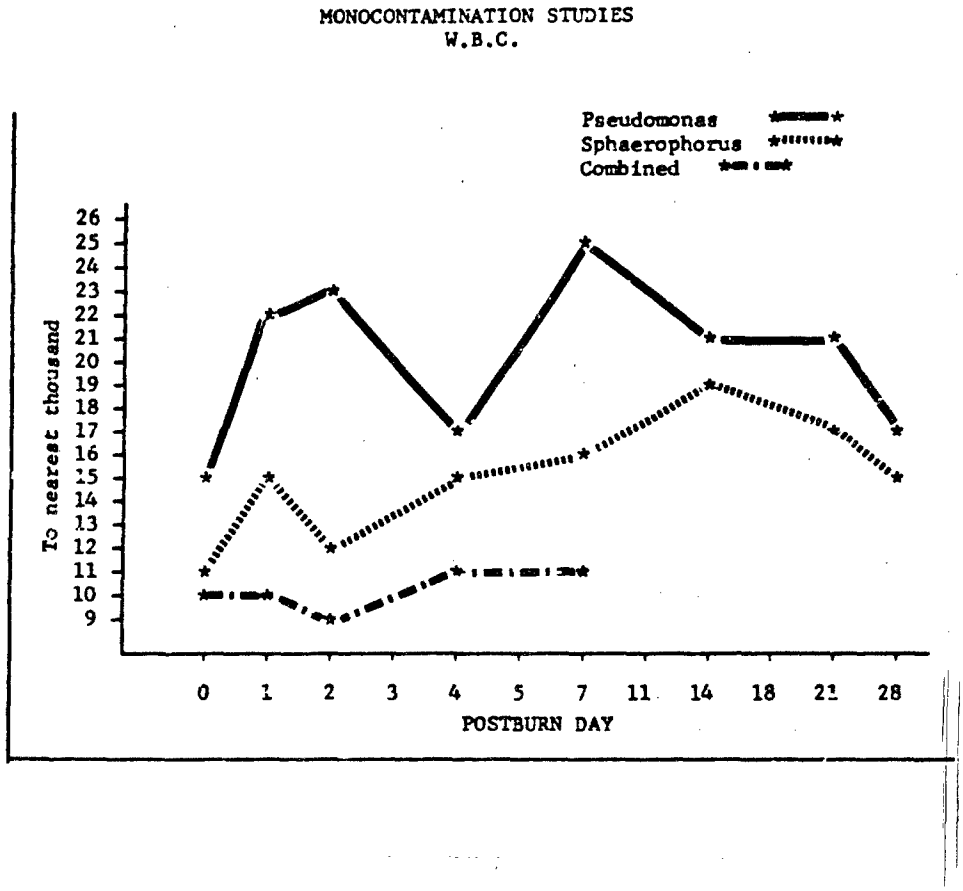

Fig. 13. Compares the postburn WBC in the monocontaminated rats with the group having combined Pseudomonas aeruginosa and Sphaerophorus necrophorus contamination. The WBC in the Sphaerophorus group was similar to the germ-free rats. In the Pseudomonas group the WBC levels were higher but followed the same trend. The WBC in the combined contamination group stayed at low levels.

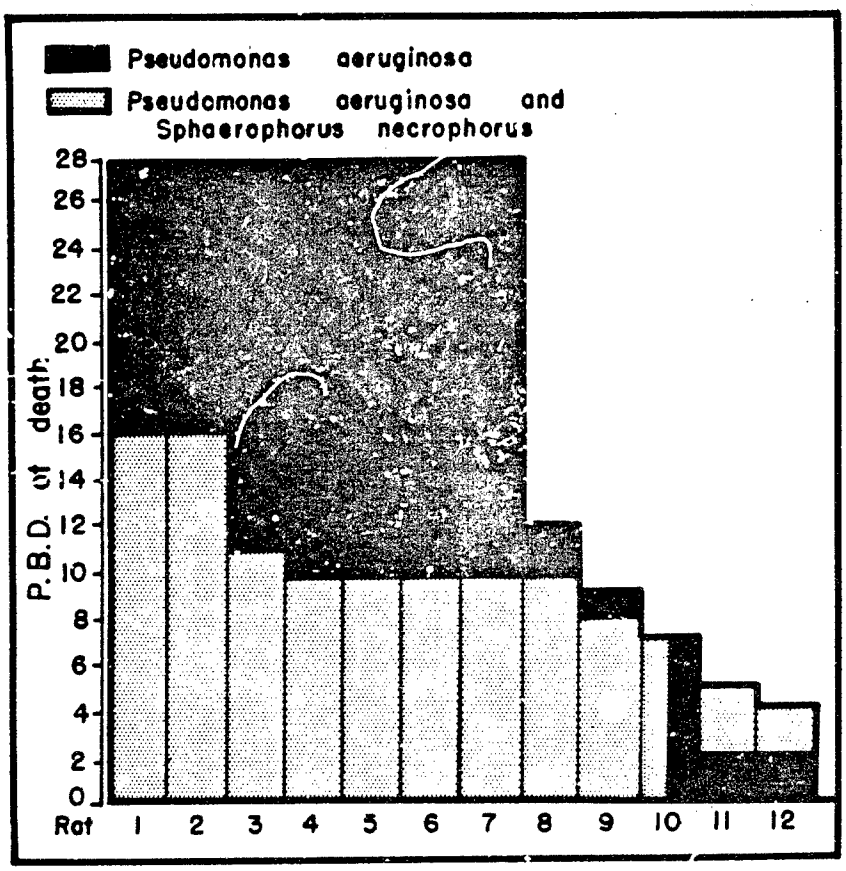

Fig. 14. Demonstration of the survival time of the rats monocontaminated with Pseudomonas aeruginosa and comparison with the shorter survival time of rats having combined Pseudomonas and Sphaerophorus necrophorus contamination.

S. necrophorus (12 rats). Hematocrit of the S. necrophorus contaminated animals showed an initial drop during the first postburn week and then gradual recovery toward normal levels identical to that seen in burned germ-free rats (Fig. 12). The WBC on these animals after burning were very similar to the germ-free animals with counts rising slightly during the first postburn day and again at the end of the second week and then gradually returning toward normal. The WBC was generally below that of the Pseudomonas contaminated rats and approximately the same as the germ-free rats (Fig. 13). The temperature varied only slightly during the entire course of the burn but the weight dropped during the first week and then gradually returned toward normal. All the animals monocontaminated with S. necrophorus survived the 28 day period (Fig. 15). Autopsies were negative.

Staphylococcus aureus UC-18 (12 rats). In this group of animals, the Hct. dropped sharply during the first two postburn days, some 10 vol. per cent, then gradually stabilized and in the survivors returned towards normal by the end of the four week study period (Fig. 16). The WBC showed the same type of curve as seen in the germ-free rats except that higher levels were reached at the third postburn day and at the end of the first week. The WBC count was returning toward normal, however, at the end of the study period (Fig. 17). Again, there was little variation in temperature during the four week postburn except that some terminal fall in temperature was noted in the immediate antemortem period. Weight dropped precipitously during the first two weeks postburn in this group of animals, then gradually returned toward normal in the survivors. Ten of the twelve animals survived the entire 28 day period. Two animals died,

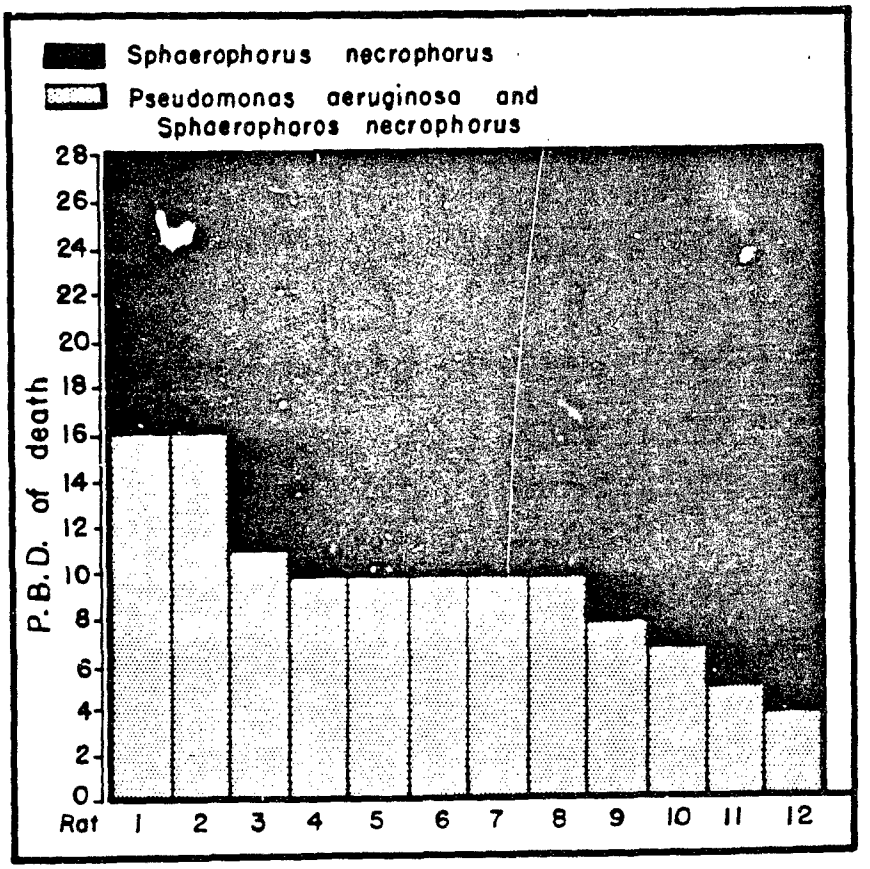

Fig. 15. Demonstration of the survival of all of the rats monocontaminated with Sphaerophorus necrophorus prior to burning and comparison with the shorter survival time of rats having combined Pseudomonas and Sphaerophorus contamination.

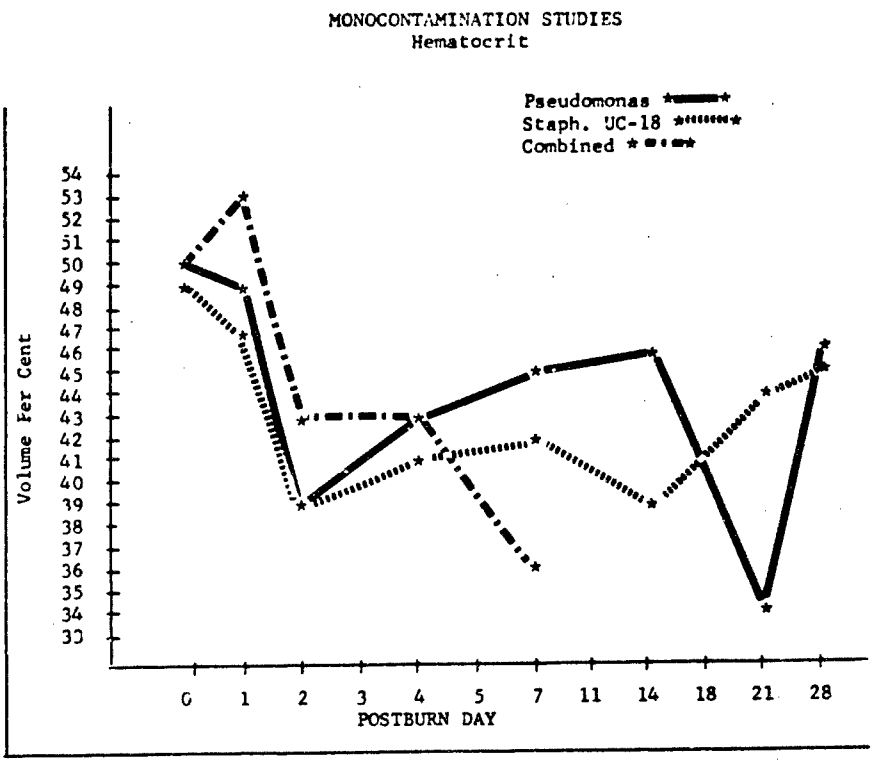

Fig. 16. Comparison of the postburn hematocrits in rats monocontaminated with Pseudomonas aeruginosa and Staphylococcus aureus (UC-18), with a third group having combined contamination. With the exception of a low hematocrit reading on day 21 (Pseudomonas aeruginosa group), both the monocontamination groups had levels similar to the germ-free rats. The combined contamination group had steadily declining hematocrit levels.

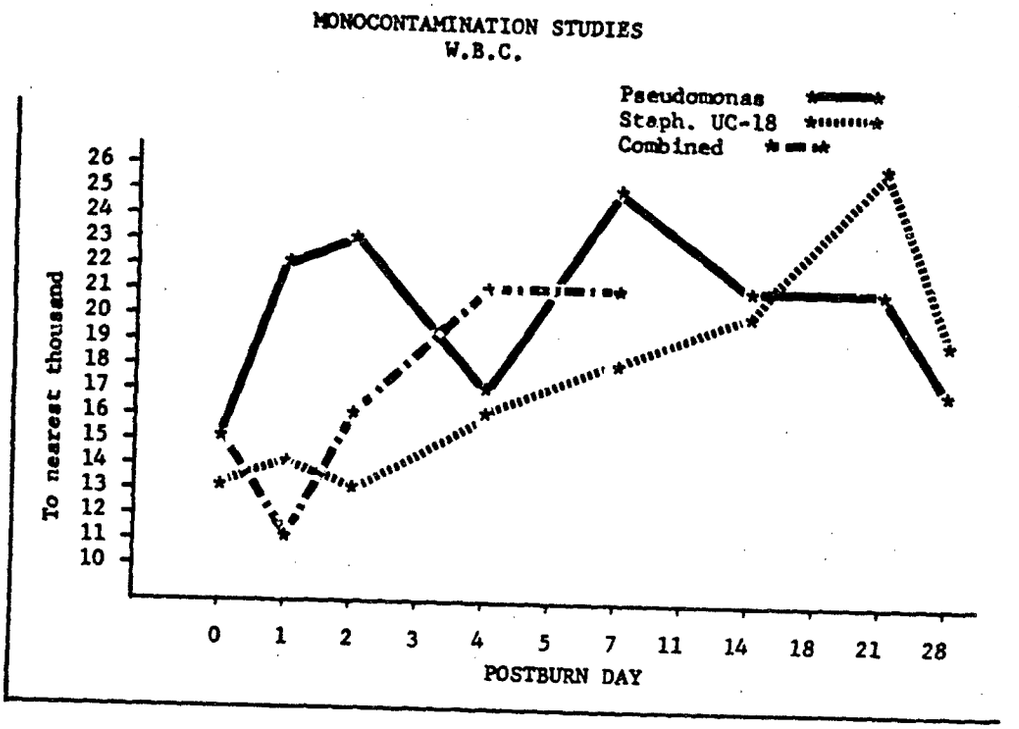

Fig. 17. Comparison of the postburn WBC in rats monocontaminated with Pseudomonas aeruginosa and Staphylococcus aureus (UC-18) with a third group having combined contamination. Both monocontamination groups showed rises until the third week when the WBC levels began to return to normal. The combined contamination group is difficult to evaluate since the surviving rats had high WBC and the others had low WBC prior to death.

one on the sixth and one on the seventh postburn day similar to the experience in the germ-free animals burned (Fig. 18). Autopsies were negative.

In general the three experiments demonstrated that the monocontamination in the group of germ-free rats prior to burning did not cause a precipitous change in the expected mortality rate, not in the Hct, WBC, temperature and weight studies performed during the postburn period. The animals contaminated with Ps. aeruginosa did have a somewhat higher mortality rate, where as those contaminated with Staphylococcus aureus had the same mortality rate as seen in the burned germ-free rats.

B) The next experiments were conducted to determine whether or not synergism might exist between the specific bacterial strains employed in the monocontamination studies of the gnotobiotic rats. Two groups of gnotobiotes were studied employing combined contamination.

Ps. aeruginosa and S. necrophorus (12 rats). The same two strains of these bacteria were introduced into the rats' drinking water as previously described four days prior to burning the animals. All studies as previously described were again performed in this group of animals. Studies of the Hct. revealed a much sharper drop during the first four postburn days to a level some 12 per cent lower than base line levels. There was only minimal improvement at the end of the first week (Fig. 12). The WBC failed to show any elevation throughout the first postburn week averaging a normal level throughout the postburn period (Fig. 13). In the animals expiring, a low WBC was frequently obtained immediately prior to death. There was a steady decline in the weight during the entire

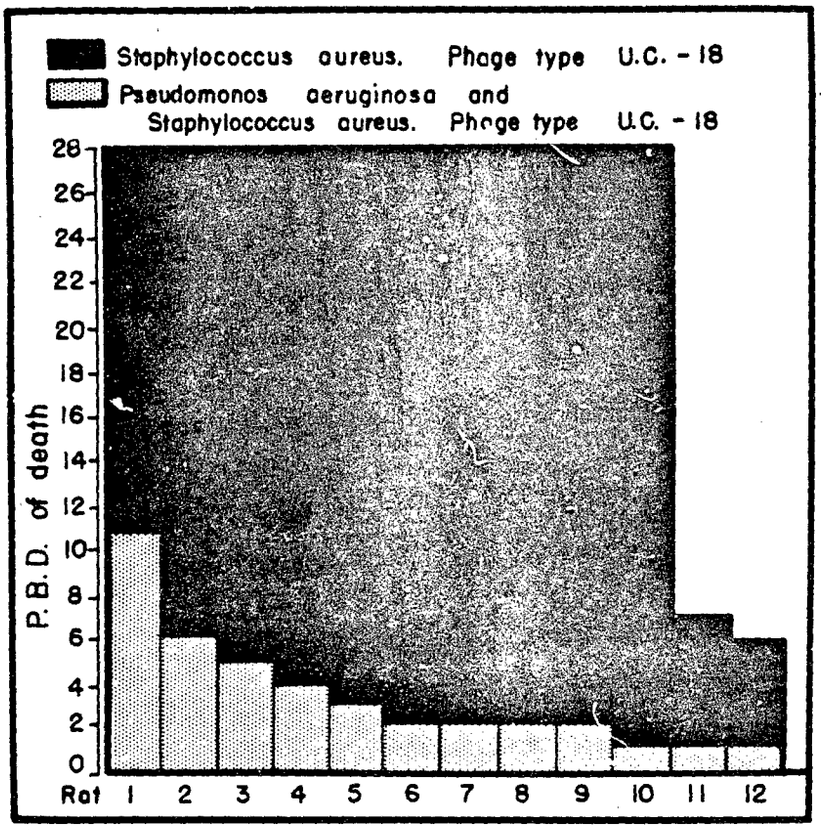

Fig. 18. Comparison of the postburn survival time of the monocontaminated Staphylococcus aureus (UC-18) group with the much shorter survival time in the rats contaminated with both Pseudomonas and Staphylococcus aureus (UC-18).

postburn period averaging up to 50 grams. Temperature variations were minimal, but frequently showed a drop immediately prior to death. There were no surviving rats after 16 days. Two rats expired on the sixteenth postburn day, one on the eleventh postburn day, five on the tenth postburn day and one each on the eighth, seventh, fifth, and fourth postburn days. The animals appeared toxic throughout the postburn period and seemed to die septic deaths. Comparisons of the mortality rates in a combined infection and in infections with S. necrophorus or Ps. aeruginosa alone are seen in Fig. 14 and 15.

Ps. aeruginosa and Staphylococcus aureus UC-18 (12 rats). The next group of animals was contaminated with both Ps. aeruginosa and Staphylococcus aureus UC-18.Burns were again inflicted after four days and all studies repeated . A slight initial rise in Hct. on the first postburn day was followed by a steady decline during the entire postburn period. (Fig. 16). The WBC fell on the first postburn day and then gradually rose to twice normal levels by the end of the first week (Fig. 17). It should be noted that there was great variation in the WBC during this time with the longer surviving animals having markedly elevated counts and the expiring animals usually having low counts immediately prior to death. A sharp fall in weight during the postburn period was noted and temperatures were often subnormal prior to death. Of the 12 rats studied, three expired on the first postburn day and four on the second. One animal expired on the third, fourth, fifth and sixth days respectively, and the last survivor died on the eleventh postburn day. A comparison of the survival rates with the combined infection with those of Ps. aeruginosa and Staphylococcus aureus UC-18 alone are presented in Fig. 18 and 19.

The second group of five germ-free rats that had been contaminated with the combination of Ps. aeruginosa and Staphylococcus aureus UC-18 were allowed to live in this environment for up to four weeks prior to burning in the usual manner. It was thought that these animals might develop some resistance to the inflicting organisms during this period and show improvement in the mortality rate. However, of the five animals burned, two died on the first postburn day and one on the second. The other two animals died on the eleventh and thirteenth postburn days. Laboratory studies in this group were similar to those in the animals contaminated and burned within four days.

In each group of animals studied, at least two rats were not burned. All of these controls remained healthy and active regardless of the type of contamination employed.

The use of germ-free animals seemed to offer a tool for more accurate evaluation of the effects of burn injury. This method of investigation would seem to offer an opportunity to separate "burn toxemia" as such, from bacterial infection and septicemia which frequently confuses the evaluation of the significance of specific "burn toxins".

At the present time, continued studies are underway to evaluate agglutinin and percipitin levels as well as determining the electrophoretic patterns in germ-free and selectively contaminated burned animals. Studies are also being performed to determine if vaccination of the germ-free rats prior to burning will cause an increased survival rate in the combined contamination groups in which mortality rates have been 100%.

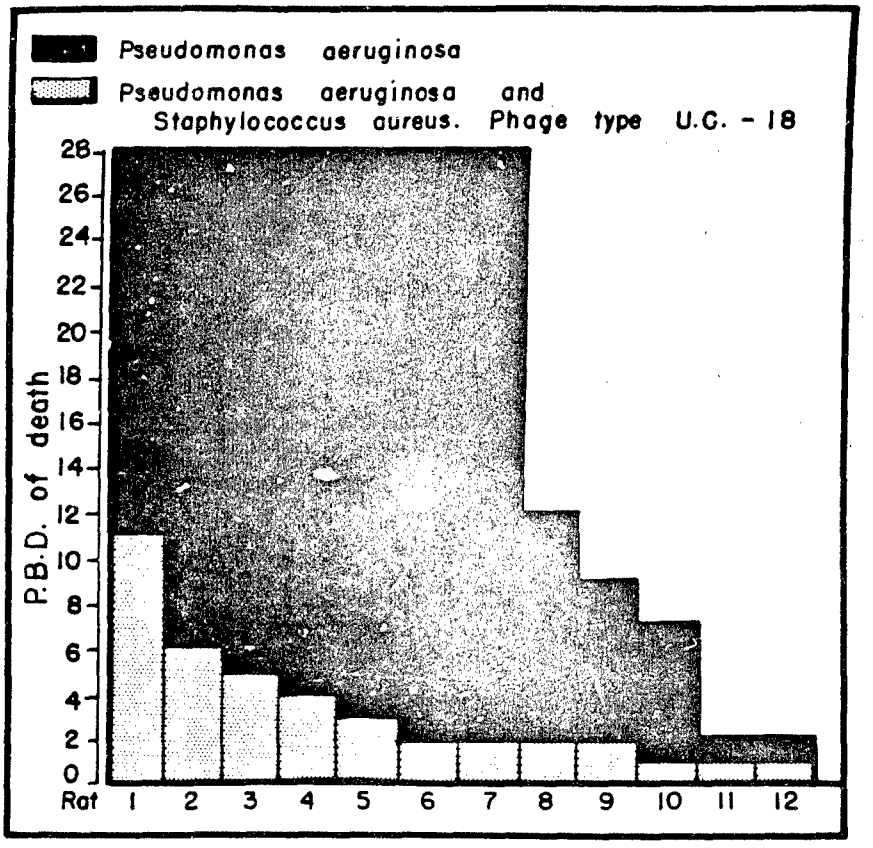

Fig. 19. Demonstration of the survival time in the monocontaminated Pseudomonas aeruginosa group - although less than that Staphylococcus aureus (UC-18) group, it is still much greater than the combined Pseudomonas aeruginosa and Staphylococcos aureus (UC-18) group.

VI. Continuing Clinical and Laboratory Studies of All Burn Patients Treated by the Burn Service of the Cincinnati General Hospital.

In the period between December 16, 1964 and October 15, 1965, there were 166 admissions to the Burn Service. This number represents 133 patients (135 admissions) for the treatment of acute burns and 28 patients (31 admissions) for plastic reconstructive procedures. The range of total cutaneous involvement was from less than 1 per cent to 90 per cent of the total body surface. The mean per cent of total body surface involved was 20 per cent; and the mean full thickness involvement was 10 per cent of the total body surface. Fifty-five (41%) of the acute burns were admitted with only partial thickness involvement; 61 patients (46%) presented with both partial and full thickness involvement; and the remaining 17 patients (13%) were treated for full thickness involvement only. Table XXI. The incidence of age to mortality in the various percentage groups involved can be noted in Table XXII.

Causes of Thermal Trauma. Flames of such varied origin as: outdoor fires, stove burners, lighter fluid, gasoline, cigarettes, playing with matches (one suicide attempt), explosions with flames, and flames of unknown origin, accounted for 50 per cent of all acute burn admissions. Hot liquids (water and coffee) accounted for another 30 per cent of the admissions. The remaining 20 per cent were the result of contact with hot metal (4%), electricity (5%), hot grease and candy (5%), chemical agents (3%), fireworks, gunpowder and flash (3%). See Table XXI for the actual numbers of the patients involved.

TABLE XXI. Patients treated between December 16, 1964 and October 15, 1965.

81	MALE	PATIENTS (133)
52	FEMALE	
38	HOT LIQUIDS (water and coffee)	CAUSE OF BURN
17	OPEN FLAMES (outdoor fires & stove burners)	
14	PLAYING WITH MATCHES	
12	EXPLOSIONS WITH FLAME	
8	ELECTRICITY	
8	HOT GREASE, HOT CANDY	
7	FLAMES OF UNKNOWN ORIGIN	
6	CONTACT WITH HOT METALS	
5	HOUSE FIRES	
4	GASOLINE FLAMES, LIGHTER FLUID	
4	CIGARETTES, SUICIDE	
4	CHEMICAL AGENTS	
4	GUNPOWDER, FIREWORKS, FLASH	
20	TOTAL BODY SURFACE	MEAN PERCENTAGE OF BURN
10	FULL THICKNESS BURN	
39	HEAD AND/OR FACE	AREAS INVOLVED
32	NECK	
68	UPPER EXTREMITIES	
23	HANDS	
65	ANTERIOR TRUNK	
51	POSTERIOR TRUNK	
28	BUTTOCKS	
15	GENITALIA	
65	LOWER EXTREMITIES	
3	SOLES OF THE FEET	
22.5 units	MAXIMUM	WHOLE BLOOD
3 units	MEAN	

TABLE XXII

% area	0-14 years			15-44 years			45-64 years			65+ years		
	NO. of PA-TIENTS	DEATHS	Case MOR-TALITY	NO. of PA-TIENTS	DEATHS	CASE MOR-TALITY	NO. of PA-TIENTS	DEATHS	CASE MOR-TALITY	NO. of PA-TIENTS	DEATHS	CASE MOR-TALITY
0- 4	18	0		3	0		1	0				
5-14	32	0		9	0		6	0		1	0	
15-24	16	0		9	1	11%				1	1	100%
25-34	7	1	14%	2	0					1	1	100%
35-44	8	1	13%	2	0							
45-54	3	0		1	0		1	1	100%			
55-64	2	1	50%	1	1	100%						
65-74	1	1	100%				1	1	100%	1	1	100%
75-84	2	2	100%							1	1	100%
85-94	1	1	100%				1	1	100%	1	1	100%
94+												
AGE GROUP TOTALS	90	7	8%	27	2	7%	10	3	30%	6	5	83%

Areas of Burn Involvement. The areas noted in acute burn involvement were: the head and/or face (29%), neck (24%), upper extremities (51%), hands (17%), anterior trunk (49%), posterior trunk (38%), buttocks (21%), genitalia (11%), the lower extremities (49%), and the soles of the feet (1%). See Table XXI for the number of patients falling into the above groups.

Mortality. The overall mortality rate for this period was 12.8 per cent. In this group of 17 patients with fatal burns, the mean total body surface involvement was 59 per cent, and the mean full thickness burn was 54 per cent. These patients ranged in the age of 2 to 105 years. Death occurred from the day of burn to the 47 postburn day. Causes of death for the individual patient can be ascertained from Table XXIII. Since age and total burn involvement influence mortality rates, some value may be derived from the breakdown in Table XXII.

The Extensively Burned Patient. For purposes of this report, the extensively burned patient is defined as the patient with a Burn Index (1% 3° = 1; 1% 2° = ½) of 20 or more. Thirty patients (23%) fell into this classification. This group of patients was treated by conventional methods. The mean per cent of total body surface and full thickness involvement was 50 and 39 per cent, respectively. The mean day of initial grafting, for those patients surviving to grafting, was the 68th postburn day. Excluding three patients who were admitted more than three months after the burn was incurred, the mean day of initial grafting was the 27th postburn day. The mean day of autoganous skin coverage for those patients surviving the burn was the 75th postburn day.

TABLE XXIII. CAUSES OF DEATH

PATIENT	AGE	SEX	% BURNED 3°	% BURNED TOTAL	DEATH (PBD)	CAUSE OF DEATH
1. E.W.	105	F	12	19	30	Pulmonary emboli, bilateral Infected pulmonary infarcts, LUL, RLL
2. L.Y.	4	F	80	84	8	Septicemia, Ps. aeruginosa Curling's ulcer Lower nephron nephrosis
3. I.S.	74	F	60	76	4	Pneumonia, severe
4. R.A.	2	M	60	75	22	Septicemia, Prot. mirabilis Pulmonary infarction, L.L.L.
5. G.H.	3	M	25	30	21	Septicemia, Ps. aeruginosa Pneumonia, lobular
6. F.B.	45	M	40	46	46	Septicemia, Staphylococcus aureus Bleeding diathesis
7. R.A.	44	F	7	18	33	Cardiac arrest
8. I.B.	63	F	84	90	1	Incineration
9. W.O.	28	M	60	60	14	Septicemia, Aerobacter-Klebsiella Pneumonia
10. H.M.	45	M	70	70	11	Septicemia, Ps. aeruginosa Pneumonia, lobular
11. T.H.	5	F	30	36	24	Septicemia, Ps. aeruginosa
12. B.A.	70	F	65	65	3	Incineration
13. T.R.	6	F	90	90	3	Incineration
14. S.P.	78	M	90	90	Init.	Incineration
15. R.M.	12	M	50	60	44	Pneumonia, RUL, RLL, LLL Multiple septic infarcts, both kidneys Splenic infarct, massive; Septicemia
16. B.S.	6	M	70	70	47	Septicemia, Pc. aerogenoides
17. A.S.	65	F	25	30	8	C.V.A.
Mean	39		54	59	19	

Again excluding the patients admitted more than three months postburn, the mean day of autogenous skin coverage was the 38th postburn day.

The mortality rate for patients within this classification was 47 per cent. The mean day of death was the 17th postburn day. Details relevant to each individual patient are recorded in Table XXIV.

Tracheostomy. Tracheostomies were performed in 12 of the 133 acutely burned patients. Six were performed immediately after admission (in the Operating Room) for inhalation burns combined with extensive or deep face and neck burn involvement; one was performed 16 hours postburn, one on the first postburn day, and two on the second postburn day due to obviously increasing respiratory distress; and the remaining two were performed on the 29th and 44th postburn days to facilitate tracheobronchial toilet. Three of the above were admitted to the Cincinnati General Hospital with tracheostomy tubes in place.

Fluid Therapy. Some form of intravenous fluid replacement was administered to 45 per cent of these 133 acutely burned patients. Intravenous fluids are given initially to almost all patients who have sustained a burn injury of more than 10 per cent of the total body surface. The replacement fluids are calculated by using the Evan's and Brooke formulas; with modifications as necessary after frequent clinical and laboratory determinations (i.e. central venous pressure, hematocrit, urine specific gravity and hourly urine output). Colloids in the form of whole blood, plasma and dextran were given to 30 per cent of the patients admitted with acute burns. Thirty-two patients received approximately 96 units of whole blood (mean requirement = 1500 cc's per patient. The

TABLE XXIV. EXTENSIVELY BURNED PATIENT TREATED BY CONVENTIONAL METHODS

PATIENT	AGE	SEX	% 3° BURN	% TOTAL BURN	AUTO-GRAFTS	HOMO-GRAFTS	P.B.D. COMPLETE COVERAGE	P.B.D. DISCHARGE	DEATH P.B.D.	DEATH CAUSE
1. M.G.	3	F	40	48	2	1	50	56	-	-
2. W.K.	7	M	31	50	2	0	141	186	-	-
3. T.S.	2	F	20	36	1	0	58	59	-	-
4. F.B.	53	M	38	46	3	2	-	-	46	Septicemia, lower nephron nephrosis
5. L.Y.	4	F	76	84	-	-	-	-	8	Septicemia, Curling's ulcer
6. I.S.	74	F	60	75	-	-	-	-	4	Pneumonia
7. C.S.	7	F	35	60	3	0	104	104	-	-
8. C.W.	6	F	30	30	1	0	49	79	-	-
9. G.H.	3	M	25	30	-	-	-	--	21	Septicemia, Pneumonia
10. R.A.	2	M	60	75	-	-	-	-	22	Septicemia, Pulmonary infarts
11. P.P.	5	F	30	30	1	0	57	67	-	-
12. B.J.	4	F	40	40	2	0	370	447	-	-
13. L.C.	21	M	0	35	0	0	44	44	-	-
14. W.F.	23	M	0	45	0	0	49	49	-	-
15. R.W.	28	M	0	40	0	0	46	46	-	-
16. I.B.	63	F	84	90	-	-	-	-	1	Incineration
17. F.M.	3	F	15	30	1	0	52	67	-	-
18. T.H.	5	F	30	36	-	-	-	-	24	Septicemia
19. H.M.	45	M	50	69	-	-	-	-	11	Septicemia, Pneumonia
20. R.J.	2	M	15	35	1	0	35	53	-	-
21. B.A.	70	F	70	70	-	-	-	-	3	Incineration
22. T.R.	6	F	90	90	-	-	-	-	3	Incineration
23. S.P.	78	M	90	90	-	-	-	-	Init.	Incineration
24. D.S.	7	M	30	35	1	0	34	51	-	-
25. L.O.	7	M	21	37	1	0	46	64	-	-
26. B.S.	6	M	60	70	3	2	-	-	47	Septicemia
27. R.M.	12	M	50	60	-	-	-	-	44	Infarcts, kidneys, Pneumonia
28. B.F.	7	F	25	30	1	0	-	-	-	-
29. D.W.	7	F	30	35	1	-	-	-	-	-
30. A.S.	65	F	25	30	-	-	-	-	8	C.V.A.

maximum amount of whole blood given to any one patient was 11,250 cc's. Plasma (4,466 cc's) was given to seven patients; and dextran was given to four patients.

Tetanus prophylaxis. All patients admitted with acute burns are carefully questioned with regard to a history of active immunization against tetanus. Those with active immunizations were given tetanus toxoid; and those without active immunization were initially protected with hypertet toxoid until active immunity could be established.

Wound Care. All patients admitted during this period were treated conservatively by exposure (37%) or with occlusive dressings (63%). Initial treatment of the burn wounds (all patients) consists of gently cleansing with a soapy solution to remove any loose debris, and rinsing well with normal saline solution. When dressings are employed, fine mesh gauze (plain or impregnated with vaseline or an antibiotic ointment) is applied to the clean, blotted surface, followed by an occlusive dressing. The dressings were subsequently changed in two to five days, usually every second to third day. Wound cultures are taken initially and at each subsequent dressing change. N.B. It should be noted that three of the above patients were also treated with Silver Nitrate 0.5% dressing for a short time before their deaths. This information is presented elsewhere in more complete detail.

Antibiotic Therapy. Local antibiotic therapy during this period consisted primarily of polysporin ointment used to sparingly impregnate fine mesh gauze which was applied directly to the burn wound. In a few special cases, chloromycetin and gentamycin ointments were used in

preference to the polysporin.

Systemic antibiotics were administered to 79 per cent of these 133 patients. Patients with more than 10 per cent total body burn involvement usually receive penicillin initially. In the larger burn, specific systemic antibiotics were administered as indicated (i.e. by clinical condition and laboratory confirmation). See Table XXV for the list of antibiotics used to treat these patients.

Wound Closure. In an effort to get the burn wound closed in the earliest possible time, great effort is expended in trying to prevent conversion of partial thickness wounds to full thickness; and to prevent massive colonization of the burn wound. Autograft skin has been used exclusively in all patients except three, in accomplishing complete autogenous skin coverage. The mean day of initial grafting (for all patients grafted here) was the 49th postburn day. If all patients who were admitted more than 21 days after incurring the burn are omitted, the mean day of initial grafting is the 33rd postburn day.

Homografts. Homografts have been used in the temporary closure of the burn wound in five patients. The indication for using homografts was generalized debility in all five cases. Further information can be obtained by referring to Table XXVI.

Complications. Approximately 24 per cent of the patients treated in this group of 133 patients developed one or more complications in their postburn courses (Table XXVII). Eleven of the 32 complications in this group are represented by proven cases of septicemia. In this group there were two survivors and ten deaths. The organism involved, their

TABLE XXV

ANTIBIOTIC	PATIENTS	
	NUMBER	PER CENT
Penicillin	105	79
Chloroamphenicol	15	11
Colimycin	12	9
Erythromycin	5	4
Prostaphlin	3	2
Polymyxin B	3	2
Gentamycin	3	2
Polycillin	2	1.5
Amphotericin B	1	1

Antibiotics used to treat 79 per cent of the 133 patients admitted between December 16, 1964 and October 15, 1965.

TABLE XXVI. HOMOGRAFTS

PATIENT	NO. TIMES USED	TYPE	LENGTH OF STORAGE	LENGTH OF SURVIVAL	INDICATIONS FOR USE
18. S.R.	2	Stored	4 days	12 days(k)	General debility
		Stored	9 days	7 days(k)	General debility
19. P.S.	1	Stored	8 days	11 days(k)	General debility
20. M.G.	1	Fresh		16 days(k)	General debility
21. F.B.	2	Fresh		2 days(k)	General debility
		Stored	2 days	12 days(k)	General debility
22. B.S.	3	Fresh		3 days(k)	General debility
		Stored	3 days	2 days(k)	General debility
		Stored	5 days	2 days(k)	General debility

TABLE XXVII

SYSTEM	NUMBER OF PATIENTS
RESPIRATORY	
Pneumonia	4
Pulmonary infarct	1
Pulmonary emboli	1
GASTRO-INTESTINAL	
Curling's ulcer	1
CARDIO-VASCULAR	
Cerebral vascular accident	1
Cardiac arrest	1
Lymphedema	1
GENITO-URINARY	
Lower nephron nephrosis	2
Urethral fistula	1
MUSCULO-SKELETAL	
Loss of right hand	1
Loss of both hands	1
BLOOD	
Septicemia	11
Bacteremia	2
Bleeding diathesis	1
SENSITIVITY REACTIONS	
Penicillin	2
Roseola	1

Complications occurring in 133 acutely burned patients between December 16, 1964 and October 15, 1965.

TABLE XXVIII Proven Cases of Septicemia

PATIENT & AGE	% BURNED 3o	% BURNED TOTAL	POSTBURN DAY ONSET	POSTBURN DAY DEATH	AUTOGENOUS SKIN COVER	CAUSATIVE ORGANISM	SENSITIVITY	ANTIBIOTIC THERAPY ANTIBIOTIC	DAILY DOSAGE	TOTAL DAYS
SURVIVORS:										
S.R. 5 yrs	64	64	31	-	86	Ps. aeruginosa	Gent.	Pen.	1.2 M.u.	2
								Col.	32 mgm.	23
								Gent.	60 mgm.	17
								Pen.	1.2 M.u.	3
P.S. 6 yrs.	53	59	21	-	87	Ps. aeruginosa NePx(BTCTeCol)		Pen.	1.6 M.u.	37
								PxB	30 mgm.	20
								PenVee	500 mgm.	17
M.G. 3 yrs.	40	48	4	-	48	Ps. aeruginosa	Px(NeCol)	Pen.	1.6 M.u.	20
								Col.	10 mgm.	1
								PxB	30 mgm.	22
								PenVee	500 mgm.	26
M.F. 5 yrs.	25	45	27	-	39	Ps. aeruginosa	Gent.	Pen.	2.4 M.u.	25
								Gent.	30 mgm.	25
								PenVee	1.0 Gm.	4
								Cmc.	500 mgm.	4
									750 mgm.	15
								Pros.	750 mgm.	20
								Poly.	750 mgm.	9
C.T. 25 yrs.	20	40	21	-	55	Aerobacter-Klebsiella SATCTeNe(ENo)		Pen.	2-15 M.u.	11
								Cmc.	1-3 Gms.	11
								PenVee	2.0 Gms.	49
								Cms.	2.0 Gms.	41
								Poly.	1.0 Gm.	8
Mean	40	51	21	-	63			Pen.	100%	35
								Col.	40%	12
								PxB	40%	21
								Gent.	40%	21
								Cmc.	40%	35
								Prost.	20%	20
								Poly	40%	9

TABLE XXVIII (Continued)

PATIENT & AGE	% BURNED 3o	% BURNED TOTAL	POSTBURN DAY ONSET	POSTBURN DAY DEATH	AUTOGENOUS SKIN COVER	CAUSATIVE ORGANISM SENSITIVITY (Mod.)	ANTIBIOTIC THERAPY ANTIBIOTIC	DAILY DOSAGE	TOTAL DAYS
DEATHS:									
F.B. 53 yrs.	38	46	(9) 37	45	-	(Ps. aerug. CNePxCol) Staph. aureus ANo (BTCETeNe)	Pen.	2-5 M.u.	35
							PenVee	1.0 Gm.	2
							Col.	150 mgm.	36
							Cmc.	2.0 Gm.	9
L.Y. 4 yrs.	76	84	7	7		Ps. aeruginosa NePxCol	Pen.	1.8 M.u.	8
							Col.	30 mgm.	2
G. H. 3 yrs.	25	30	21	21	-	Ps. aeruginosa PxCol (CNe)	Pen.	1.2 M.u.	8
							Cmc.	750 mgm.	9
							Col.	24 mgm.	9
R.A. 2 yrs.	60	75	22	22	-	Proteus mirabilis	Pen.	1.0 M.u.	2
W. O. 28 yrs.	60	60	3	14	-	Aerobacter-Klebsiella ATCTeNePxCol	Pen.	3.0 M.u.	14
							CMC	210 M.u.	12
T.H. 5 yrs.	30	36	9	24	-	Ps. aeruginosa PxCol (Ne)	Pen.	1.4 M.u.	12
							CMC	400 mgm.	18
							Col.	100 mgm.	15
							Staph.		12
							Amph.B	11 mgm.	5
I. S. 74 yrs.	60	75	4	4		Proteus mirabilis	Pen.	8.0 M.u.	5
							Col.	30 mgm.	2
H. M. 45 yrs.	70	70	7	11	-	Ps. aeruginosa NePx (CCol)	Pen.	3-10 M.u.	12
							CMC	1.0 Gm.	4
							Col.	300 mgm.	3
B. S. 6 yrs.	60	70	18	47	-	Pc. aerogenoides CNePx (G)	Poly.	2.0 Gms.	39
							Gent.	24 mgm.	31
R. M. 12 yrs.	50	60	43	44	-	(Gram positive coccus)	Pen.	1.0 Gm.	1
							CMC	1.0 Gm.	1
Mean	53	61	17	24					

sensitivity patterns, the antibiotic therapy administered and the clinical course of these patients are detailed in Table XXVIII. N.B.: There are four survivors listed in this table - two are patients included in the last report who developed Septicemia during this period.

The mean extent of total burn in the five survivors was 51 per cent, with 40 per cent representing third degree. The mean day of autogenous skin coverage was the 63rd postburn day. The mean day of onset was the 21st postburn day.

The mean extent of total burn in those patients who expired was 61 per cent, and the mean area of third degree burn was 53 per cent. The mean day of onset of septicemia was the 17th postburn day, and the mean day of death was the 24th postburn day.

Invasive Infection. The widespread prophylactic use of antibiotic agents has apparently not only failed to decrease the incidence of infection, but has created additional problems through the development of infections produced by emergent antibiotic-resistant bacteria and sensitization of an appreciable segment of patients to the antibacterial drugs. The accumulative concentration of these antibiotic-resistant and virulent bacteria in the hospital environment has increased the threat of overwhelming infection refractory to specific chemotherapy.

In an attempt to compare the effect of the introduction and use of chemotherapeutic and antibiotic agents on the course of infections in burns, three groups of patients have been studied. Group I consisted of 132 patients who were treated between 1942 and 1944 with systemically administered sulfonamides. Group II consisted of 80 patients who were

treated between 1951 and 1953, when penicillin and broad-spectrum antibiotics were used extensively. Group III consisted of 428 patients studied between 1958 and 1964, when the newer antistaphylococcal antibiotics were in use (Table XXIX).

The predominant and most important infecting bacteria during these study periods were the Staphylococcus (coagulase-positive), the beta hemolytic Streptococcus, the Proteus, and Pseudomonas aeruginosa. These organisms accounted for 50 per cent of all bacteria recovered from the infected burn wound or the blood stream and represented 90 per cent of the bacteria causing death by infectious complications.

There was a significant increase in the incidence of strains of the Staphylococcus (coagulase positive) from 20 to 50 per cent in the burn wounds of Group I treated between 1942 and 1944. This high incidence persisted through the second and third weeks while many of these patients were under active systemic sulfadiazine therapy.

During the period of 1951 to 1953, when the patients in Group II were under antibiotic therapy with penicillin and chloramphenicol, the incidence of the Staphylococcus (coagulase positive) in the infected wound was less, being 32 per cent for the first week, 16 per cent for the second week, and 12 per cent for the third week. Similarly, in the third period of 1958 to 1964, when the patients of Group III were treated with penicillin and specific antistaphylococcal antibiotics, the incidence was additionally reduced to 18 per cent during the first week, 7 per cent during the second week, and 9 per cent during the third week.

TABLE XXIX - Comparison of the percentage of bacteria cultured from the burn wounds of Groups I, II, and III during the first, second and third postburn weeks.

	Group I 1942 to 1944 (132 cases)			Group II 1951 to 1953 (80 cases)			Group III 1958 to 1964 (428 cases)		
	1st week	2nd week	3rd week	1st week	2nd week	3rd week	1st week	2nd week	3rd week
Staphylococcus (coagulase positive)	46	44	50	32	16	12	18	7	9
Beta hemolytic Streptococcus	9	33	0	31	1	12	13	4	4
Proteus	18	16	0	21	16	50	6	14	14
Pseudomonas aeruginosa	11	16	50	36	38	50	25	27	52

This relative decrease in incidence found for the Staphylococcus aureus was not noted for the gram-negative bacteria during the latter two periods, particularly in the case of the Proteus and Pseudomonas groups. Instead, there has been a continuing high incidence of these two groups of gram-negative bacteria which was progressive over the three-week postburn period. Both types of organisms reached an incidence of approximately 50 per cent of all cultures during the third week in all three groups. This is particularly striking when it is recalled that these organisms were infrequently found as contaminants in the initial wound cultures (Table XXX). The probable source of this high incidence of gram-negative bacterial infection is most likely one or all of the following sources of contamination: the patient's gastrointestinal tract, the personnel caring for the patient, airborne contamination, dressing carts and dressing routines, and the residual hospital reservoir.

An analysis of 39 cases of proven septicemia occuring between 1958 and 1964 reveals that during this six-year period, there were ten cases of proven gram-positive septicemia and 29 cases of proven gram-negative septicemia on the burn service of the Cincinnati General Hospital. Pseudomonas aeruginosa was responsible for 23 of the 29 proven cases of gram-negative septicemia (Table XXXI). There were four survivors in the ten patients who had proven gram-positive septicemia and only six survivors in the 29 patients who had proven gram-negative septicemia. The antibiotic therapy used to treat this group of ten survivors is shown in Table XXXII.

The overwhelming threat of gram-negative organisms to the burn patient is clearly shown, and the relative ineffectiveness of the antibiotic

TABLE XXI

The percentage distribution of the dominant bacterial flora of fresh burn surfaces in patients studied between 1942 to 1944, 1951 to 1953, and 1958 to 1964 at the Cincinnati General Hospital.

	1942-44 (132 cases) Per cent	1951-53 (80 cases) Per cent	1958-64 (428 cases) Per cent
Staphylococcus (Coag. +)	20	37	45
Staphylococcus (Coag. -)	56	15	25
Beta hem. strep.	14	0	5
Ps. aeruginosa	3	4	24
Proteus	3	4	5
E. coli	11	11	15

TABLE XXXI

PROVEN CASES OF SEPTICEMIA TREATED AT THE CINCINNATI GENERAL HOSPITAL
1960 - 1965

	Cases / Survivors							
	'60	'61	'62	'63	'64	9/12 '65	Totals	
Staphylococcos (Coag. +)	2/1	1/0	0/0	1/1	1/1	1/0	6/3	(6/3)
Ps. aeruginosa	3/0	3/1	4/0	3/1	11/4	5/1	29/7	
Proteus	1/0		1/0		1/0	1/0	4/0	
Pc. aerogenoides			1/1			1/0	2/1	(40/10)
Pc. intermedium	1/0						1/0	
A. aerogenes					1/0		1/0	
Aero. Klebsiella					1/1	2/1	3/2	
								(46/13)

ANTIBIOTIC THERAPY
46 Patients

Cincinnati General Hospital
1960 - 1965

Antibiotic	Per cent
Penicillin	96
Chloramphenicol	65
Colistin	46
Polymyxin B	20
Tetracycline	15
Erythromycin	11
Staphcillin	11
Garamycin	7
Streptomycin	4
Oxytetracycline	4
Ampicillin	4
Vancomycin	2
Prostaphlin	2
Amphotericin B	2

agents used prophylactically or therapeutically in these patients is clearly demonstrated. Recent experiences with topical applications of sulfamylon and the systemic use of gentamycin against the Pseudomonas aeruginosa organisms suggest that these agents are more effective antibacterial agents than those currently in use.

After analyzing the influence of chemotherapy and antibiotic therapy in patients treated since 1942 at the Cincinnati General Hospital, a rational program of antibiotic therapy has evolved and is shown in Table XXXIII.

Systemic antibiotic therapy consists of the routine use of penicillin administered intravenously during the first week to all patients sustaining major burns. Penicillin therapy is continued through the autolytic period and the granulating period by the intramuscular route.

Additional adjunctive therapy in the form of specific anti-staphylococcal antibiotics is instituted when specific indications for these antibiotics present themselves as a result of recovery of resistant organisms from either the wound or blood cultures. When possible, not more than two antibiotics are administered at a time, these being selected on the basis of controlled disc sensitivity studies.

Antibiotic therapy for the gram-negative infections has consisted of Polymyxin B, colimycin, amphocillin and chloramphenicol. However, this antibiotic program has failed to have a favorable effect upon the established gram-negative burn infections.

This failure of antibiotic therapy against the gram-negative organisms causing fatal burn infections should only re-emphasize the importance of

Table XXXIII

Preferred antibiotic therapy used in the treatment of the major burn at the Cincinnati General Hospital.

	Local		Systemic	
	Indications	Antibiotic	Indications	Antibiotic
Initial 0 to 7 postburn days	None	None	Beta hemolytic Streptococcus	Penicillin
Autolytic 8 to 21 postburn days	Beta hemolytic Streptococcus	Bacitracin	Staphylococcus (coag. +)	plus Chloramphenicol Oxytetracycline Erythromycin Tetracycline Novobiocin Vancomycin Staphcillin
Granulating 22 to 30 postburn days	Pseudomonas	Polymyxin B	Pseudomonas Pseudomonas	Polymyxin B Colimycin
			Proteus	Chloramphenicol Amphocillin

an aggressive attack upon the burn wound so that early closure of the wound can be accomplished.

Statistical review. Table XXXIV details the burn distribution by year, the mean per cent of burn varying between 16 and 24 per cent of the total body surface during the peried of 1942 and 1964. The causes of death during the same period are noted in Table XXXV. The mortality rate has ranged from a low of 8 per cent to a high of 22 per cent, or a mean mortality rate during this entire period of 19 per cent.

TABLE XXXIV.

YEAR	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964
TOTAL ADMISSIONS	77	91	79	73	99	83	79	72	81	106	109	103	82	104	108	114	112	83	83	90	71	64	142
BURN DISTRIBUTION 0- 5	10	12	10	13	15	17	9	14	13	11	12	9	6	7	16	15	21	13	23	21	19	19	22
6-20	35	49	34	37	47	41	43	28	31	51	54	53	49	63	70	67	63	47	37	51	28	28	54
21-30	18	16	16	16	17	13	14	14	20	20	21	14	16	15	10	13	14	10	7	10	6	7	11
31-40	5	3	6	0	8	4	6	7	9	12	5	7	6	6	3	7	5	4	4	2	9	3	15
41-50	1	2	4	1	6	3	4	5	2	3	3	3	2	4	0	3	3	3	3	0	3	3	13
51 & over	8	9	9	6	6	5	3	4	6	9	14	17	3	9	9	9	6	6	9	6	6	4	27
Mean Per Cent Body Surface	23	20	18	18	19	18	16	21	20	21	22	24	19	20	16	18	16	18	23	19	23	21	22

Distribution and extent of surface area involved in 2095 burn patients between Jan. 1, 1942 & Dec. 31, 1964.

TABLE XXXV.

Causes of death occuring in 2,095 thermal burns treated between 1/1/42 & 12/1/64

CAUSE OF DEATH	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964
TOTAL NO. OF DEATHS	10	12	13	13	14	16	9	12	12	13	17	20	11	16	13	11	9	11	20	11	11	9	25
Septicemia	1	4	2	2	1	2	2	0	0	1	2	0	1	3	1	6	4	5	6	3	5	3	8
Pneumonia	0	3	0	1	2	2	2	0	2	1	1	3	2	1	1	0	0	1	1	3	3	2	1
Pyelonephritis	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	1
Fluid imbalance	1	0	4	0	3	3	1	0	0	1	1	2	1	5	6	2	0	1	0	0	0	0	0
Incineration	6	5	5	4	2	5	1	6	4	3	3	9	4	4	1	1	2	1	2	2	3	2	8
Respiratory burn	1	0	2	1	3	2	1	3	3	5	1	3	1	1	1	2	1	2	5	1	2	2	4
Curling's ulcer	0	0	0	1	0	1	0	0	0	1	1	2	0	1	0	0	0	0	0	0	0	0	1
OTHERS:																							
Cardiac failure	1			1				1	1	1			1				1	1	3				3
Cardiac arrest											1	1			1		1		1	1			1
Myocardial infarct														1	1				1				0
Pulmonary infarct				1	1			2		1	5				1				2		1		1
C.V.A.							1							1						1	1		0
Aspiration Asphyxia					1				2		1								2	1			0
Fat embolism						1					2												0
Cerebral thrombosis							1																0
Per Cent MORTALITY	13	13	16	18	14	22	11	17	15	12	16	19	13	15	12	10	8	13	22	16	18	14	19

Recent Publications

1. The Dynamics of Infection In Burns, Altemeier, W. A. and MacMillan, B. G. Am. Inst. Biol. Sci., No. 9, pp. 203-211, 1962.

2. The Rationale of Specific Antibiotic Therapy in Management of Major Burns, Altemeier , W. A., MacMillan, B. G., and Hill, E. O. Surg. 52:240-249, 1962.

3. Staphylococcal Enterocolitis Following Antibiotic Therapy. Altemeier, W. A., Hummel, R. P., and Hill, E. O. Ann. Surg. 157:847-858, 1963.

4. Methods for the Isolation and Identification of Non-sporulating Anaerobic Bacteria from Clinical Specimens, Dowell, V. R., Hill, E. O., and Altemeier, W. A. Bact. Proc., 1962, p. 90.

5. Effects of Environment on the Morphology of Sphaerophorus necrophorus Grown in Continuous Culture. Dowell, V. R., and Hill, E. O. Bact. Proc., 1964, p. 29.

6. Changing Phage Patterns in Staphylococcal Enterocolitis, Hummel, R. P., Hill, E. O., and Altemeier, W. A. Surg. Forum Vol. XIII, 1962.

7. Development of New Bacteriophages for Staphylococcal Typing, Thomas, D. C., Hill, E. O., Culbertson, W. R., and Altemeier, W. A. Surg. Forum Vol. X, 1960.

8. Constancy of Deoxyribonucleic Acid Base Composition in the Transition of Sphaerophorus necrophorus from Bacilli to Large Bodies, Dowell, V. R., Loper, J. C., and Hill, E. O. J. Bact. Vol. 88, No. 6, 1964.

9. "L Forms" of Bacteria Isolated from Surgical Infections, Hill, E. O. and Lewis, S. Bact. Proc., 1964, p. 48.

10. Frequency of Occurrence and Antibiotic Resistance of Staphylococcus aureus, Phage Type UC-18, in Hospital Infections, Hill, E. O., Lewis, S., and Altemeier, W. A. To be published in Antimicrobial Agents and Chemotherapy, 1964.

11. Staphylococcal Enterocolitis: Studies in Bacteriophage Typing Patterns, Hummel, R. P., Hill, E. O., and Altemeier, W. A. J. Surg. Res. 3:289-292, 1963.

12. Iatrogenic Staphylococcal Enterocolitis, Hummel, R. P., Altemeier, W. A., and Hill, E. O. Ann. Surg. 1960:551-560, 1964.

13. Constancy of Deoxyribonucleic Acid Base Composition in the Transition of Sphaerophorus necrophorus from Bacilli to Large Bodies. Dowell, V. R., Loper, J. C., and Hill, E. O. J. Bact. 88:1805-1807, 1964

14. Use of Phenethyl Alcohol in Media for Isolation of Anaerobic Bacteria, Dowell, V. R., Hill, E. O., and Altemeier, W. A. J. Bact. 88:1811-1813, 1964.

15. Septicemia. Altemeier, W. A. To be published in Current Therapy, 1966.

16. Newer Aspects of Septicemia in Surgical Patients, Altemeier, W. A., Todd, J. C., and Inge, W. W. To be published in Archives of Surg. 1966.

17. The Growing Threat of Gram-negative Septicemia - A Study of 400 Cases, Altemeier, W. A., Todd, J. C., and Inge, W. W. To be published.

References

1. Altemeier, W. A.: Ann. Surg. 104:212-219, 1936.

2. Altemeier, W. A.: Ann. Surg. 107:517-528, 1938.

3. Altemeier, W. A.: Surg. 11:374-384, 1942.

4. Altemeier, W. A. and Carter, B. N.: Ann. Surg. 115:1118-1124, 1942.

5. Altemeier, W. A. and Culbertson, W. R.: Surg., Gynec. & Obstet. 87:206-212, 1948.

6. Altemeier, W. A. and Gaebler, P.: Unpublished Data, 1939-40.

7. Barile, M. F., Graykouski, E. A., Driscoll, E. J. and Riggs, D. B.: Oral Surg., Oral Med. & Oral Pathol. 16:1395, 1963.

8. Burton, K.; Biochem, J.: 62:315, 1956.

9. Herbert, D., Elsworth, R., and Telling, T. D.: J. Gen. Microbiol. 14:601, 1956.

10. Jevons, M. P. and Parker, M. T.: J. Clin, Path. 17:243-250, 1964.

11. Kabat and Mayer's Experimental Immunochemistry, Second Edition. Charles C. Thomas, Springfield, Ill., 1961.

12. Klieneberger-Nobel, Pleuropneumonia-like Organisms (PPLO) Mycoplasmataceae. Academic Press, New York, 1962.

13. Kundsin, R. B., Walter, C. W., and Morin, P.: Science 45:1322-1323, 1964.

14. Marmur, J., Falkow, S., and Mandel, M.: Ann. Rev. Microbiol. 17:329, 1963.

15. Mejbaum, W.: Z. Physiol. Chem., Hoppe-Seyler's 258:117, 1939.