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

FINAL REPORT

WILLIAM A. ALTEMEIER, M. D.

March, 1970

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U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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University of Cincinnati Medical Center.
Cincinnati, Ohio, 45229

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FINAL REPORT

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March 31, 1970

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U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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During the final period between December 1, 1968 and March 31, 1970, our research program under contract number DA-49-193-MD-2094 has been concerned with the following clinical and laboratory studies in burn patients:

I. Further investigations of the nature and significance of the dynamic bacterial flora of major burns;

II. Investigation of the nature, frequency, and significance of the anaerobic non-sporulating bacteria in sepsis occurring in burn patients and their relationship to synergism in poly-microbic infections;

III. Clinical evaluation of Pseudomonas Vaccine;

IV. Gnotobiotic studies of the response and course of standard burn wounds;

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

VI. Studies in experimental animals of the nature and causes of Curlings' ulcer in the post burn period;

VII. Continuing clinical and laboratory studies of all patients treated on University of Cincinnati Burn Unit;

VIII. Publications.

I. The Nature and Significance of the Changing Bacterial Flora in Servere Burns.

A. Changes in the microbial flora of burn wounds.

Since the annual report of December, 1968, an additional 72 patients have been admitted to the Burn Unit of the Cincinnati General Hospital and have been monitored bacteriologically as an intergral part of our continuing studies on the dynamics of burn wound infections.

As indicated in Table I, Staphylococcus aureus continues to colonize the burn wound with greater frequency than any other organism (see also Annual Report, Dec. 1968), with incidences of 62% for 1968 and 58% during the first half of 1969. There appears to be a trend, however, toward a decrease in the incidence was 83%. This is born out by a relative decrease in the incidence of septicemia in burn patients caused by the hemolytic Staphylococcus aureus as compared to the gram-negative bacilli during the past five years. It is of particular interest to note the continued disapperance of Staphylococcus aureus UC-18 which had been such a signigicant agent of nosocomial infections at this and other hospitals in various parts of the world prior to 1967. This strain has reappeared, however, in burn wounds of patients housed in the Shriners Burns Institute during the past year, with an incidence if 14%. The reasons for the complete disappearance of the UC-18 Staphylococcus from wounds at the Cincinnati General Hospital Burn Unit and the current reappearance at the Shriners Institute are not understood, but there is suggestive

TABLE I

Incidence of recovery of selected organisms from burn wounds.

(Cincinnati General Hospital Burn Unit)

	1966	1967	1968	1969 (Jan.-June)
Total number of patients	75	84	71	26
S. aureus	62 (83%)	56 (67%)	44 (62%)	15 (58%)
S. aureus UC-18	32 (43%)	3 (3%)	0	0
S. aureus 80,81	6 (8%)	7 (8%)	4 (6%)	0
Ps. aeruginosa	31 (41%)	47 (56%)	36 (51%)	13 (50%)
Aerobacter-Klebsiella	41 (55%)	44 (52%)	26 (37%)	9 (35%)
Escherichia sp.	19 (25%)	22 (26%)	15 (21%)	10 (39%)
Proteus sp.	20 (27%)	20 (24%)	13 (18%)	7 (27%)
Beta Streptococci	6 (8%)	7 (8%)	4 (31%)	1 (4%)
"Yeast"	23 (31%)	0	22 (31%)	4 (15%)
Candida albicans	8 (11%)	13 (16%)	16 (23%)	7 (27%)
Candida sp.	0	13 (16%)	0	0

TABLE II

Incidence of recovery of selected organisims from burn wounds.

(Shriners Burns Institute) 3-1-69 - 1-3-70

Organisms	Total Patients: 148	Acute: 50
S. aureus	82 (55%)	42 (84%)
S. aureus UC-18	25 (17%)	24 (48%)
S. aureus 80, 81	0	0
Ps. aeruginosa	35 (24%)	31 (62%)
Aerobacter-Klebsiella	31 (21%)	27 (54%)
Escherichia sp.	22 (15%)	20 (40%)
Proteus Streptococci	13 (9%)	13 (26%)
Beta Streptococci	14 (9%)	3 (6%)
"Yeasts"	34 (23%)	28 (40%)

evidence that this is related to the type of topical antibacterial therapy used.

The incidence of Pseudomonas aeruginosa in infected burn wounds has remained high, being 51% during 1968 and 50% during the first half of 1969 at the Cincinnati General Hospital Burn Unit. (Tables I, II)

The incidences of other organisms listed such as Escherichia, Aerobacter-Klebsiella, Proteus, and B. hemolytic Streptococci have not varied signigicantly.

Problems have become increasingly more apparent concerning the emergence of Candida albicans and other fungi in infected burn wounds with incidences of colonization ranging from 16% to 30% (Tables I, II). Positive blood cultures containing C. albicans were obtained from 4 patients, all of who expired. The importance of candiduria as an early manifestation of systemic invasion by Candida sp. has become evident. The relationship of systemic or topical antibiotic therapy to the development of invasive infection by Candida albicans has been impressive and somewhat disturbing, particularly during gentamicin therapy. When systemic infection or septicemia became evident, the therapeutic agent of choice was intravenous amphotericin B. Protential pathogenicity of yeasts other than C. albicans was suggected also by these data.

I, B. Continuing studies of antibiotic resistance of organisms recovered from burn wounds.

During the past year, there has been a continuing trend toward decreasing resistance of the Staphlicoccus aureus to penicillin,

tetracycline, and chloramphenicol. The cyclic variation in penicillin resistance of the Staphylococcus aureus has been most interesting. This phenomenon was first reported by us (W.A.A.) in 1956 and it has recurred every 2-4 years. As noted earlier, during 1966, 76% of the cultures of Staphylococcus aureus recovered from burn wounds were resistant to penicillin; during the first and second halves of 1967, 1968, and the first half of 1969, the per cent of isolates resistant to pencillin were 84%, 66%, 11%, 14% and 8%, respectively. (Fig, IA, IB, IC). This same trend has been observed to a lesser extent and with less regular cyclic variation with other antibiotics and with other organisms recovered from the surgical infections on the General Surgical Services of the University of Cincinnati Medical Center. Tables III through X indicate the antibiotic resistance of organisms tested during the latter half of 1968 and 1969.

The factors responsible for these cyclic variations are largely unknown, but the importance of defining them and evaluating their effects is obvious. The clinical significance of this knowledge in the treatment of trauma patients with staphylococcal infections is obviously of great importance to us.

Table XI shows the wide variety of phage types of Staphylococcus aureus isolated from a variety of surgical infections, including burns. The significance of the changing phage types is under further investigation.

TABLE III

ANTIBIOTIC RESISTANCE OF ORGANISMS ISILATED FROM BURN WOUNDS

1 July - 31 December, 1968

		Penicillin		Bacitracin		Chloro-mycetin		Erythro-mycin		Novobiocin		Tetra-cycline		Neomycin		Ampicillin	
Disc conc.*		2	10	2	10	5	30	2	15	5	30	5	30	5	30	2	10
Staphlococcus aureus	%	39.1	13.6	49.1	10.9	58.1	0	15.5	0	6.4	0.9	71.8	14.5	63.6	20.0	12.7	0.9
	R	43	15	54	12	64	0	17	0	7	1	79	16	70	22	14	1
	T	110	110	110	110	110	110	110	110	110	110	110	110	110	110	110	110
Streptococcus (enterococcus)	%	38.0	6.0	18.0	4.0	12.0	0	6.0	4.0	34.0	10.0	72.0	22.0	96.0	70.0	8.0	0
	R	19	3	9	2	6	0	3	2	17	5	36	11	48	35	4	0
	T	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Streptococcus (pyogenic)	%	20.0	13.3	13.3	6.7	13.0	0	6.7	6.7	13.3	6.7	13.3	0	93.3	60.0	13.3	13.3
	R	3	2	2	1	2	0	1	1	2	1	2	0	14	9	2	2
	T	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Streptococcus (viridans)	%	16.7	0	66.7	33.3	16.7	0	0	0	33.3	0	33.3	16.7	66.7	50.0	0	0
	R	1	0	4	2	1	0	0	0	2	0	2	1	4	3	0	0
	T	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Diplococcus pneumoniae	%	0	0	0	0	0	0	0	0	0	0	0	0	100	100	0	0
	R	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
	T	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

% - Percent of cultures resistant
R - Number of cultures resistant
T - Number of cultutres tested
* - Disc concentrations on Penicillin & Bacitracin in units, others in micrograms

TABLE IV

ANTIBIOTIC RESISTANCE OF ORGANISMS ISOLATED FROM BURN WOUNDS

1 July - 31 December, 1968

		Stc.+	Pro.+	Gentamicin- - -			Kanamycin		Kef-lin	Linco-cin	Naf cillin		Strepto-mycin	Vibramycin	
Disc conc.*		5	1	2	10	30	5	30	30	2	1	2	10	5	30
Staphylococcus aureys	%	0.9	2.7	2.4	0	0	66.4	59.1	1.8	0.9	17.3	69.1	64.6	13.9	0
	R	1	3	1	0	0	73	65	2	1	19	76	71	5	0
	T	110	110	42	97	61	110	110	110	110	110	110	110	36	36
Streptococcus (enterococcus)	%	98.0	98.0	96.2	44.9	25.0	96.0	60.0	10.0	88.0	94.0	92.0	90.0	53.3	46.7
	R	49	49	25	22	4	48	30	5	44	47	46	45	8	7
	T	50	50	26	49	16	50	50	50	50	50	50	50	15	15
Streptococcus pyigenic)	%	33.3	40.0	100	30.8	16.7	93.3	53.3	6.7	20.0	26.7	80.0	66.7	28.6	0
	R	5	6	5	4	1	14	8	1	3	4	12	10	2	0
	T	15	15	5	13	6	15	15	15	15	15	15	15	7	7
Streptococcus (viridans)	%	83.4	83.4		0	0	83.4	33.3	16.7	66.7	83.4	66.7	50.0	50.0	50.0
	R	5	5		0	0	5	2	1	4	5	4	3	1	1
	T	6	6	0	5	2	6	6	6	6	6	6	6	2	2
Diplococcus pneumoniae	%	0	0		0	0	100	0	0	0	0	0	0		
	R	0	0		0	0	1	0	0	0	0	0	0		
	T	1	1	0	1	1	1	1	1	1	1	1	1	0	0

* Disc concentrations of Penicillin & Bacitracin in units, others in micrograms
+ Stc. - Staphcillin
+ Pro. - Prostaphlin
% - Percent of cultures resistant
R - Number of culture resistant
T - Number of culture tested

TABLE V

ANTIBIOTIC RESISTANCE OF ORGANISMS ISOLATED FROM BURN WOUNDS

1 July - 31 December 1968

		Chloro-mycetin		Tetra-cycline		Neomycin		Polymyxin		Colymycin		Ampicillin		Gentamicin			Kanamycin		Kef-lin	Vibramycin	
Disc conc.*		5	30	5	30	5	30	5	30	2	10	2	10	2	10	30	5	30	30	5	30
Pswudemonas aeruginosa	%	94.0	3.0	92.5	6	75.9	3.0	0.8	0	15.8	0.8	98.5	98.5	71.7	0	0	100	72.9	100	100	56.8
	R	125	4	123	30	101	4	1	0	21	1	131	131	38	0	0	133	97	133	45	25
	T	133	133	133	133	133	133	133	133	133	133	133	133	58	118	58	133	133	133	45	44
Pseudomonas fluorescens	%	100	100	0	0	0	0	0	0	0	0	100	100		0		0	0	100	0	0
	R	1	1	0	0	0	0	0	0	0	0	1	1		0		0	0	1	0	0
	T	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1
Pseudomonas species	%	82.1	0	8 .3	67.9	96.4	75.0	10.7	10.7	67.9	10.7	100	42.9	100	81.5	78.9	100	92.9	92.9		
	R	23	0	25	19	27	21	3	3	19	3	28	12	26	22	15	28	26	26		
	T	28	28	28	28	28	28	28	28	28	28	28	28	26	27	19	28	28	28	0	0
Bacterium anitratum	%	96.3	46.3	7.4	0	70.4	31.5	0	0	0	0	96.3	29.6	78.6	76.9	88.9	70.4	9.3	94.4	9.5	4.5
	R	52	25	4	0	38	17	0	0	0	0	52	16	11	40	16	38	5	51	2	1
	T	54	54	54	54	54	54	54	54	54	54	54	54	14	52	18	54	54	54	21	22
Aerobacter-Klobsiella	%	22.9	10.4	33.3	29.2	8.3	2.1	6.3	2.1	6.3	4.2	100	75.0	10.0	2.4	0	4.2	4.2	20.8	65.4	30.8
	R	11	5	16	14	4	1	3	1	3	2	48	36	1	1	0	2	2	10	17	8
	T	48	48	48	48	48	48	48	48	48	48	48	48	10	42	10	48	48	48	26	26
Proteus species	%	33.3	25.0	91.7	77.8	38.9	2.8	97.2	97.2	97.2	97.2	77.8	16.7	9.1	0	0	30.6	2.8	11.1	87.5	87.5
	R	12	9	33	28	14	1	35	35	35	35	28	6	1	0	0	11	1	4	14	14
	T	36	36	36	36	36	36	36	36	36	36	36	36	11	33	9	36	36	36	16	16
Alcaligenes species	%	57.1	4.8	76.2	61.9	95.2	90.5	4.8	0	66.7	4.8	100	47.6	100	81.0	50.0	100	76.2	95.2	18.2	9.1
	R	12	1	16	13	20	19	1	0	14	1	21	10	1	17	2	21	16	20	2	1
	T	21	21	21	21	21	21	21	21	21	21	21	21	1	21	4	21	21	21	11	11

% - Percent of cultures resistant
R - Number of cultures resistant
T - Number of cultures tested
* - Disc concentrations in micrograms

TABLE VI

ANTIBIOTIC RESISTANCE OF ORGANISMS ISOLATED FROM BURN WOUNDS

1 July - 31 December 1968

		Chloro-mycetin		Tetra-cycline		Neomycin		Polymyxin		Colymycin		Ampicillin		Gentamicin			Kanamycin		Kef-lin	Vibramycin	
Disc conc.*		5	30	5	30	5	30	5	30	2	10	2	10	2	10	30	5	30	30	5	30
Escherichia species	%	4.8	4.8	33.3	33.3	0	0	0	0	0	0	76.2	19.0	0	0	0	0	0	0	42.9	42.9
	R	1	1	7	7	0	0	0	0	0	0	16	4	0	0	0	0	0	0	3	3
	T	21	21	21	21	21	21	21	21	21	21	21	21	2	15	5	21	21	21	7	7
Serratia species	%	9.5	4.8	100	9.5	0	0	90.5	85.7	92.2	90.5	100	47.6	6.7	0	0	4.8	14.3	90.5		
	R	2	1	21	2	0	0	19	18	20	19	21	10	1	0	0	1	3	19		
	T	21	21	21	21	21	21	21	21	21	21	21	21	15	19	12	21	21	21	0	0
Hafnia group	%	0	0	0	0	0	0	0	0	0	0	100	0		0		0	0	0		
	R	0	0	0	0	0	0	0	0	0	0	1	0		0		0	0	0		
	T	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	0	0
Other Gram-Negative rods	%	20 0	0	50.0	10.0	80.0	50.0	30.0	20.0	50.0	20.0	90.0	40.0	100	85.7	100	80.0	70.0	80.0	0	0
	R	2	0	5	1	8	5	3	2	5	2	9	4	7	6	2	8	7	8	0	0
	T	10	10	10	10	10	10	10	10	10	10	10	10	7	7	2	10	10	10	1	1

% -
R - Number of cultures resistant
T - Number of cultures tested
* - Disc concentrations in micrograms

TALBE VII

ANTIBIOTIC REISTANCE OF ORGANISMS ISOLATED FORM BURN WOUNDS

1 July - 31 December 1968

		Penicillin		Bacitracin		Chloro-mycetin		Erythro-mycin		Novobiocin		Tetra-cycline		Neomycin		Ampicillin	
Disc conc.*		2	10	2	10	5	30	2	15	5	30	5	30	5	30	2	10
Staphylococcus auteus	%	37.3	7.8	84.3	5.9	60.8	0	7.8	0	7.8	0	86.3	19.6	72.5	15.7	9.8	0
	R	19	4	43	3	31	0	4	0	4	0	44	10	37	8	5	0
	T	51	51	51	51	51	51	51	51	51	51	51	51	51	51	51	51
Streptococcus (enterococcus)	%	59.5	21.4	26.2	14.3	9.5	2.4	0	0	61.9	38.1	57.1	14.3	90.5	16.9	35.7	7.1
	R	25	9	11	6	4	1	0	0	26	16	24	6	38	26	15	3
	T	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42
Streptococcus (pyogenic)	%	14.3	0	14.3	14.3	0	0	0	0	14.3	14.3	14.3	0	85.7	14.3	0	0
	R	1	0	1	1	0	0	0	0	1	1	1	0	6	1	0	0
	T	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Streptococcus (viridans)	%	33.3	0	33.3	0	33.3	0	0	0	100	66.7	33.3	33.3	66.7	66.7	33.3	33.3
	R	1	0	1	0	1	0	0	0	3	2	1	1	2	2	1	1
	T	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Diplococcus pneumoniae	%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	T	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

% - Percent of cultures resistant
R - Number of cultures resistant
T - Number of cultures tested
* - Disc concentrations of Penicillin & Bacitracin in units, others in micrograms

TABLE VIII

ANTIBIOTIC RESISTANCE OF ORGANISMS ISOLATED FROM BURN WOUNDS

1 January - 1 April 1970

		Stc.+	Pro.+	Genta-mycin	Kanamycin		Kef-lin	Linco cin	Naf cillin	Strepto-mycin		Vibramycin	
Disc conc*		5	1	10	5	30	30	2	1	2	10	5	30
Staphylococcus aureus	%	23.5	2.0	0	84.3	0	0	0	0	86.3	86.3	19.6	0
	R	12	1	0	43	0	0	0	0	44	44	10	0
	T	51	51	31	51	51	51	51	51	51	51	51	51
Streptococcus (enterococcus)	%	97.6	97.6	10.5	97.6	81.0	33.3	66.7	97.6	95.2	78.6	23.8	4.8
	R	41	41	2	41	34	14	28	41	40	33	10	2
	T	42	42	19	42	42	42	42	42	42	42	42	42
Streptococcus (pyogenic)	%	50.0	33.3	50.0	0	0	0	0	16.3	0	0	0	0
	R	3	2	1	0	0	0	0	1	0	0	0	0
	T	6	6	2	7	7	7	7	6	7	7	7	7
Streptococcus (viridans)	%	100	100	0	100	66.7	33.3	33.3	100	100	100	33.3	33.3
	R	3	3	0	3	2	1	1	3	3	3	1	1
	T	3	3	1	3	3	3	3	3	3	3	3	3
Diplococcus pneumoniae	%	100	100		0	0	0	0	100	0	0	0	0
	R	1	1		0	0	0	0	1	0	0	0	0
	T	1	1	0	1	1	1	1	1	1	1	1	1

* Disc concentrations of Penicillin & Bacitracin in units, others in micrograms
+ Stc. - Staphcillin
+ Pro. - Prostaphlin
% - Percent of cultures resistant
R - Number of cultures resistant
T - Number of cultures tested

TABLE IX

ANTIBIOTIC RESISTANCE OF ORGANISMS ISOLATED FROM BURN WOUNDS

1 January - 1 April 1970

		Chloro-mycetin		Tetra-cycline		Neomycin		Polymyxin		Colymycin		Ampicillin		Genta-micin	Kanamycin		Kef-lin	Vibramycin	
Disc conc.*		5	30	5	30	5	30	5	30	2	10	2	10	10	5	30	30	5	30
Pseudomonas	%	96.2	6.6	88.7	22.6	72.6	9.4	0	0	45.3	0.9	100	100	4.3	100	38.9	100	87.7	61.3
aeruginosa	R	102	7	94	24	77	10	0	0	48	1	105	105	2	106	73	106	93	65
	T	106	106	106	106	106	106	106	106	106	106	106	106	47	106	106	106	106	106
Pseudomonas	%	100	16.7	33.3	33.3	33.3	0	0	0	33.3	0	100	100	0	33.3	16.7	100	33.3	33.3
fluorescens	R	6	1	2	2	2	0	0	0	2	0	6	6	0	2	1	6	2	2
	T	6	6	6	6	6	6	6	6	6	6	6	6	1	6	6	6	6	6
Pseudomonas	%	100	0	100	0	100	0	0	0	0	0	0	0		100	0	0	0	0
species	R	1	0	1	0	1	0	0	0	0	0	0	0		1	0	0	0	0
	T	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
Bacterium	%	100	35.1	29.8	0	91.2	17.5	0	0	7.0	0	98.2	35.1	100	98.2	3.5	96.5	3.5	1.8
anitratum	R	57	20	17	0	52	10	0	0	4	0	56	20	20	56	2	55	2	1
	T	57	57	57	57	57	57	57	57	57	57	57	57	20	57	57	57	57	57
Escherichia	%	0	0	9.7	6.5	0	0	0	0	3.2	0	93.5	3.2	0	9.7	0	0	16.1	3.2
species	R	0	0	3	2	0	0	0	0	1	0	29	1	0	3	0	0	5	1
	T	31	31	31	31	31	31	31	31	31	31	31	31	25	31	31	31	31	31
Alcaligenes	%	34.6	0	73.0	19.2	65.4	65.4	0	0	30.8	0	92.3	30.8	57.1	84.6	65.4	76.9	19.2	3.8
species	R	9	0	19	5	20	17	0	0	8	0	24	8	4	22	17	20	5	1
	T	26	26	26	26	26	26	26	26	26	26	26	26	7	26	26	26	26	26

% - Percent of cultures resistant
R - Number of cultures resistant
T - Number of cultures tested
* - Disc concentrations in micrograms

TABLE X

ANTIBIOTIC RESISTANCE OF ORGANISMS ISOLATED FROM BURN WOUNDS

1 January - 1 April 1970

		Chloro-mycetin		Tetra-cycline		Neomycin		Polymyxin		Colymycin		Ampicillin		Genta-micin	Kanamycin		Kef-lin	Vibramycin	
Disc conc.*		5	30	5	30	5	30	5	30	2	10	2	10	10	5	30	30	5	30
Proteus species	%	70.6	17.6	100	82.4	5.9	0	100	100	100	100	100	29.4	0	23.5	0	29.4	100	100
	R	12	3	17	14	1	0	17	17	17	17	17	5	0	4	0	5	17	17
	T	17	17	17	17	17	17	17	17	17	17	17	17	8	17	17	17	17	17
Aerobacter-Klebsiella	%	0	0	7.7	0	0	0	7.7	7.7	23.1	15.4	100	69.2	0	0	0	15.4	7.7	0
	R	0	0	1	0	0	0	1	1	3	2	13	9	0	0	0	2	1	0
	T	13	13	13	13	13	13	13	13	13	13	13	13	8	13	13	13	13	13
Serratia species	%	20.0	0	60.0	0	0	0	100	80.0	100	100	100	80.0	0	0	0	100	0	0
	R	1	0	3	0	0	0	5	4	5	5	5	4	0	0	0	5	0	0
	T	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Hafnia species	%	0	0	25.0	0	0	0	0	0	0	0	100	50.0	0	0	0	50.0	25.0	0
	R	0	0	1	0	0	0	0	0	0	0	4	2	0	0	0	2	1	0
	T	4	4	4	4	4	4	4	4	4	4	4	4	2	4	4	4	4	4
Citrobacter species	%	0	0	0	0	0	0	0	0	0	0	100	0		0	0	0	0	0
	R	0	0	0	0	0	0	0	0	0	0	1	0		0	0	0	0	0
	T	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
Other Gram negative rods	%	28.6	0	14.3	14.3	.4	17.4	.4	57.1	71.4	71.4	85.7	42.9	100	71.4	71.4	57.1	0	0
	R	2	0	1	1	5	5	4	4	5	5	6	3	4	5	5	4	0	0
	T	7	7	7	7	7	7	7	7	7	7	7	7	4	7	7	7	7	7

% - Percent of cultures resistant
R - Number of cultures resistant
T - Number of cultures tested
* - Disc concentrations in micrograms

Table XI

Phage Types of Staphylococcus aureus Isolated
from Surgical Infections
1969

Purulent Infections. Total Cultures: 246 Burns and other Wounds

Phage Type	Number of Isolates
29	10
29,52	2
29,52,52A	1
29,52,52A,80	2
29,52,52A,80,81	1
29,52,80	2
52,52A,80	4
52,52A,80,81	10
52,80	2
79	1
80	1
80,53,77,84	1
80,81	17
3A	1
3A,81	1
55,71	4
71	3
71,18	1
6,47,53,54,75,83A,UC-14,UC-16,UC-18	1
6,47,54,75,UC-14,UC-18	1
6,53,UC-13,UC-14,UC-18	1
6,53,UC-14	1
47,53,54,75,77,83A,UC-13,UC-14,UC-18	1
47,53,54,75,77,84,85,UC-13,UC-14,UC-18	1
53,77,84	1
53,77,84,85,UC-13,UC-14	1
53,83A,UC-13,UC-14,UC-18	3
53,84	1
53,84,UC-13,UC-14	1
53,85,UC-20	1
53,UC-13,UC-14,UC-18	1
53,UC-14	1
83A,85	1
83A85,UC-16,UC-20	

Table XI (Continued)

Putulent Infections (continued)

Phage Type	Number of Isolates
83A,UC-13,UC-14,UC-18	1
187	1
84	9
84,85	33
84 85,UC-18	5
84,UC-13,UC-14	1
84,UC-13,UC-14,UC-18	2
84,UC-14,UC-18	1
85	4
84,UC-18	1
UC-13,UC-14	1
UC-13,UC-14,UC-18	3
UC-16	2
UC-20	5
Nontypable	95

Stool. Total Cultures: 9

Phage Type	Number of Isolates
52,52A,80,81	1
52,80,81	1
79,42E,53,81,16	1
47,53,54,75,UC-13,UC-14,UC-18	1
UC-16	2
Nontypable	3

Blood. Total Cultures: 2

Phage Type	Number of Isolates
80,81	1
Nontapable	1

Table XI (Continued)

Sputum. Total Cultures: 101

Phage Type	Number of Isolates
29	4
29,52	2
29,52,52A,80	3
29,52,52A,55,71,UC-13,UC-14	1
52	1
52,52A,80	1
52,52A80,81	12
52,80	1
52,81	2
80	1
80,53,77,84,85,UC-13,UC-14,UC-20	1
80,81	4
3A	1
3A,3C,55,71	1
3C	2
3C,55,71	1
71	1
6,47,53,54,75,UC-13,UC-14,UC-18	1
47,53,54,75,84,85,UC-13,UC-14,UC-18	1
47,53,54,75,77,83A,84,85,UC-13,UC-15,UC-18	1
53,77,84,85,UC-13,UC-14	1
53,83A,84,UC-14,UC-16,UC-20	1
77,84,UC-16	1
83A,85	1
83A,85,UC-16,UC-20	1
83A,UC-13,UC-14,UC-18	1
81	1
84	2
84,85	3
84,85,UC-18	2
84,UC-13,UC-14,UC-16	1
84,UC-13,UC-14,UC-18	2
84,UC-14,UC-18	1
UC-13,UC-14,UC-18	1
UC-14	1
UC-18	2
Nontypable	37

Table XI (Continued)

Urine. Total Cultures: %

Phage Type	Number of Isolates
52,62A,80,81	1
55,71	1
47,54,75,83A,UC-13,UC-14,UC-18	1
Nontypable	2

*Others. Total Cultures: 17

Phage Type	Number of Isolates
29,52	1
52,52A,80,81	1
52,80	1
80,81	1
84,85	1
85	1
UC-13,UC-14,UC-18	1
Nontypable	10

*Includes peritineal, pleural, synovial fluids, etc.

II. A. Nature, Frequency, and Significance of Anaerobic Non-sporulating Bacteria in Polymicrobic Infections.

With continued use of culture methods developed in this laboratory and preciously decribed, a total of 663 specimens from surgical patients on the Surgical Services of the Cincinnati General Hospital have been cultured. Of these, 328 have been specimens of infected tissue exudate, of which 307 have been positive by culture. Of the 307 positive cultures, 176 (57%) have contained at least one anaerobe. The anaerobic streptococci (Peptostreptococcus sp.). Bacteroides melaninogenicus, Bacteroides sp. and Sphaerophorus sp. continue to occur with the greatest incidence of 26%, 23%, 21%, and 20% respectively. Peptococcus sp. and Corynebacterium sp. have occurred with incidence of 11% and 7% respecitively. Tables XII and XIII list the incidence of all aerobic and anaerobic organisms recovered from this group of specimens. The high incidence of coexistant anaerobic bacteria in mixed culture with the aerobic types is of interest in relation to their symbiosis and possible synergism.

Of further interest has been the incidence of the non-sporulating anaerobes in body fluids other than blood, such as pleural, peritoneal, subderal, and joint fluids of patients having a variety of surgical infections. Of 28 specimens positive by culture, 12 (43%) contained at least one anaerobe. Sphaerophorus sp. and Corynebacterium sp. each were recovered with an incidence of 18%, followed by the anaerobic streptococci and Bacteroides sp., each with an incidence of 11%, and B. melaninogenicus and C. perfringens, each with an incidence of 7% (Table XIV).

The incidences of all aerobic and anaerobic organisms recovered from urine, feces, and sputum of surgical patients are listed in Tables XV through XVII.

The anaerobic component of infections in burns and other wounds is frequently missed because of incomplete or inadequate bacteriologic methods of cultivation. Our data, however, indicate the prevalence and importance of these anaerobes as infecting bacteria in trauma, and we are seeing an increasing number of patients with Bacteroides and anaerobic streptococcal septicemia and metastatic abscesses during the past six months.

Table XII

Incidence of Recovery of Aerobic and Anaerobic Organisms from Surgical Infections

Aerobes	Isolates	Incidence	Percent Incidence
Aerobacter aerogenes		11	3.6
Aerobacter cloacae		10	3.3
Aerobacter sp.		2	0.7
Aerobacter-Klebsiella		10	3.3
Alcaligenes sp.		2	0.7
Bacillus sp.		30	9.8
Bacterium anitratum		3	1.0
Corynebacterium sp.	32	30	9.8
Escherichia coli	87	86	28.0
Escherichia freundii		2	0.7
Escherichia intermedia		5	1.6
Escherichia sp.		1	0.3
Gaffkya sp.		13	4.2
Hafnia sp.		2	0.7
Klebsiella sp.		20	6.5
Micrococcus sp.	25	24	7.8
Neisseria sp.		2	0.7

Table XII
(continued)

Aerobes	Isolates	Incidence	Percent Incidence
Paracolobactrum:			
aerogenoides		4	1.3
coliforme		6	2.0
intermedium		2	0.7
Proteus inconstans		2	0.7
Proteus mirabilis		39	12.7
Proteus morganii		3	1.0
Proteus vulgaris		6	2.0
Proteus sp.		3	1.0
Pseudomonas aeruginosa	48	45	14.7
Pseudomonas fluorescens		2	0.7
Pseudomonas sp.	7	6	2.0
Salmonella sp.		2	0.7
Sarcina sp.		2	0.7
Serratia marcescens		1	0.3
Serratia sp.		14	4.6
Staphylococci:			
aureus	78	75	24.4
epidermidis	59	58	18.9
species		17	5.5
Streptococci:			
Alpha hemolytic	19	18	5.9
Beta hemolytic		9	2.9
Non-hemolytic		11	3.6
Enterococci	51	50	16.3
Lactic		4	1.3
Pyogenic		8	2.6
Viridans		11	3.6
Mold		3	1.0
Yeast		11	3.6
Candida albicans		8	2.6
Candida sp.		3	1.0
Unidentified			
Gram-negative rods		7	2.3
Gram-positive rods		1	0.3

Total number of specimens: 328

Total number of specimens positive by culture: 307 (93.6%)

Total number of specimens with one or more anaerobe: 176 (53.7%)

Table XIII

Incidence of Recovery of Aerobic and
Anaerobic Organisms from Surgical Infections

Anaerobes	Isolates	Incidence	Percent Incidence
Actinomyces bovis		1	0.3
Actinomyces israelii		1	0.3
Actinomyces sp.		2	0.7
Bacteroides			
melaninogenicus		70	22.8
species	80	64	20.8
Catenabacterium sp.		1	0.3
Clostridium			
bifermentans		3	1.0
butyricum		1	0.3
chauvoei		1	0.3
fallax		1	0.3
histolyticum		4	1.3
novyi		1	0.3
paraputrificum	2	1	0.3
perfringens		10	3.3
sphenoides		1	0.3
tertium		1	0.3
tetani		1	0.3
species		6	2.0
Corynebacterium sp.	23	22	7.2
Dialister sp.		1	0.3
Fusobacterium sp.		12	3.9
Peptococcus sp.	38	33	10.7
Peptostreptococcus sp.	98	79	25.7
Ramibacterium sp.		5	1.6
Sphaerophorus sp.	74	61	19.9
Veillonella sp.		5	1.6
Unidentified			
Gram-positive rods		4	1.3

Table XIV

Percent incidence of recovery of organisms from body fluids (other than blood) of surgical patients

Aerobes	Isolates	Incidence	Percent Incidence
Aerobacter cloacae		1	3.6
Aerobacter-Klebsiella		1	3.6
Bacillus sp.		2	7.1
Corynebacterium sp.		1	3.6
Escherichia coli		7	25.0
Escherichia freundii		1	3.6
Gaffkya sp.		1	3.6
Haemophilus influenzae		1	3.6
Klebsiella sp.		1	3.6
Proteus mirabilis		1	3.6
Pseudomonas aeruginosa		4	14.3
Sarcina sp.		1	3.6
Serratia marcescens		1	3.6
Staphylococci:			
aureus		1	3.6
epidermidis		3	10.7
species		2	7.1
Streptococci:			
Beta hemolytic		1	3.6
Non-hemolytic		1	3.6
Enterococci	5	4	14.3
Pyogenic		1	3.6
Candida sp.		1	3.6
Yeast		2	7.1
Unidentified			
Gram-positive coccus		1	3.6

Table XIV
(continued)

Anaerobes	Isolates	Incidence	Percent Incidence
Bacteroides:			
melaninogenicus		2	7.1
species		3	10.7
Clostridium:			
difficile		1	3.6
perfringens		2	7.1
Corynebacterium sp.		5	17.9
Peptococcus sp.		1	3.6
Peptostreptococcus sp.		3	10.7
Sphaerophorus sp.	6	5	17.9
Veillonella sp.		1	3.6
Total number of specimens:	37		
Total number of specimens positive by culture:	28	(75.7%)	
Total number of specimens with one or more anaerobe:	12	(32.4%)	

Table XV

Percent Incidence of Recovery of Aerobic & Anaerobic Organisms from Urine Specimens of Surgical Patients

Aerobes	Isolates	Incidence	Percent Incidence	Anaerobes	Isolates	Incidence	Percent Incidence
Alcaligenes sp.		1	3.1	Bacteroides:			
Bacillus sp.		1	3.1	melaninogenicus		6	18.7
Corynebacterium sp.		4	12.5	species		4	12.5
Escherichia coli		11	34.4	Clostridium sp.		1	3.1
Escherichia intermedia		1	3.1	Corynebacterium sp.		4	12.5
Gaffkya sp.		3	9.4	Peptococcus sp.		4	12.5
Klebsiella sp.	3	2	6.3	Peptostreptococcus sp.	5	4	12.5
Micrococcus sp.		3	9.4	Sphaerophorus sp.		1	3.1
Paracolobactrum coliforme		3	9.4	Veillonella sp.		1	3.1
Proteus mirabilis		4	12.5	Unidentified Gram-positive coccus		1	3.1
Proteus vulgaris		1	3.1	Unidentified Gram-positive rod		1	3.1
Proteus sp.		1	3.1				
Pseudomonas aeruginosa		5	15.6				
Pseudomonas sp.		1	3.1				
Sarcina sp.		1	3.1				
Staphylococci:							
aureus		1	3.1				
epidermidis		13	40.6				
species		1	3.1				
Streptococci:							
Alpha hemolytic		5	15.6	Total number of specimens:		37	
Beta hemolytic		1	3.1				
Non-hemolytic		1	3.1				
Enterococci		7	21.9	Total number of specimens positive by culture:		32	(86.5)
Lactic		1	3.1				
Pyogenic		1	3.1				
species		1	3.1				
Candida sp.		1	3.1	Total number of specimens with one or more anaerobe:		14	(37.8)
Yeast		1	3.1				

Table XVI

Percent Incidence of Recovery of Aerobic & Anaerobic Organisms from Stool Specimens of Surgical Patients

Aerobes	Isolates	Incidence	Percent Incidence	Anaerobes	Isolates	Incidence	Percent Incidence
Aerobacter aerogenes		6	17.6	Bacteroides:			
Aerobacter-Klebsiella		2	5.9	melaninogenicus		12	35.3
Bacillus sp.		5	14.7	species	27	21	61.8
Bacterium anitratum		1	2.9	Catenabacterium sp.		4	11.8
Corynebacterium sp.		3	8.8	Clostridium:			
Escherichia coli		23	67.6	difficile		2	5.9
Escherichia sp.		1	2.9	histolyticum		1	2.9
Gaffkya sp.		4	11.8	perfringens		5	14.7
Klebsiella sp.		6	17.6	sporogenes		1	2.9
Paracolobactrum:				species		5	14.7
coliforme		1	2.9	Corynebacterium sp.		2	5.9
intermedium		1	2.9	Fusobacterium sp.		1	2.9
Proteus mirabilis		10	29.4	Lactobacillus sp.		1	2.9
Proteus morganii		3	8.8	Peptococcus sp.		4	11.8
Pseudomonas aeruginosa		4	11.8	Peptostreptococcus sp.	13	11	32.4
Staphylococci:				Ramibacterium sp.		4	11.8
aureus		6	17.6	Sphaerophorus sp.	26	22	64.7
epidermidis		6	17.6	Veillonella sp.		1	2.9
species		2	5.9	Unidentified			
Streptococci:				Gram-positive rod		1	2.9
Alpha hemolytic	8	6	17.6				
Non-hemolytic		4	11.8	Total number of specimens:		34	
Enterococci		14	41.2				
Lactic		2	5.9	Total number of specimens positive by culture:		34	(100.0)
Pyogenic		2	5.9				
Viridans		1	2.9				
Candida albicans		2	5.9	Total number of specimens with one or more anarobe:		31	(91.2)
Yeast		6	17.6				

Table XVII

Percent Incidence of Recovery of Aerobic Organisms from Sputum Specimens of Surgical Patients

Aerobes	Isolates	Incidence	Percent Incidence
Aerobacter aerogenes		1	2.0
Aerobacter cloacae		3	5.9
Aerobacter-Klebsiella		1	2.0
Alcaligenes sp.		1	2.0
Bacillus sp.		4	7.8
Bacterium anitratum	2	1	2.0
Corynebacterium sp.		11	21.6
Diplococcus pneumoniae		4	7.8
Escherichia coli		2	3.9
Flavobacterium sp.		1	2.0
Gaffkya sp.		9	17.6
Hafnia sp.		1	2.0
Klebsiella sp.		3	5.9
Micrococcus sp.		10	19.6
Neisseria sp.	14	13	25.5
Proteus mirabilis		7	13.7
Proteus rettgeri		1	2.0
Pseudomonas aeruginosa		10	19.6
Pseudomonas fluorescens		1	2.0
Pseudomonas sp.		1	2.0
Serratia sp.		2	3.9
Staphylococci:			
aureus		9	17.6
epidermidis		13	25.5
species		1	2.0
Streptococci:			
Alpha hemolytic	10	8	15.7
Beta hemolytic		3	5.9
Non-hemolytic		6	11.8
Enterococci		4	7.8
Lactic		1	2.0
Pyogenic	16	12	23.5
Viridans	17	15	29.4
Candida albicans		13	25.5
Yeast		2	3.9

Table XVII

Percent Incidence of Recovery of Anaerobic Organisms from Sputum Specimens of Surgical Patients

Anaerobes	Isolates	Incidence	Percent Incidence
Bacteroides melaninogenicus		19	37.3
species	11	9	17.6
Corynebacterium sp.	16	14	27.4
Fusobacterium sp.		2	3.9
Peptococcus sp.		3	5.9
Peptostroptococcus sp.	23	20	39.2
Ramibacterium sp.		2	3.9
Sphaerophorus sp.	7	6	11.8
Veillonella sp.		10	19.6
Unidentified Gram-positive rods		2	3.9
Total number of specimens		52	
Total number of specimens positive by culture:		51	(98.1)
Total number of specimens with one or more anaerobe:		42	(80.8)

II. B. Studies on pathogenecity or virulence mechanisms of Pseudomonas aeruginosa.

Pseudomonas aeruginosa is one of the predominant organisms isolated from cases of bronchopneumonia reportedly responsible for up to 90% of the deaths of patients suffering from cystis fibrosis (P. A. di Sant'Agnese, 1956, Am. J. Med. 21:406). Ps. aeruginosa isolated from cystic fibrosis patients have been shown to produce a "slime" or "capsular" material chemically similar to the abnormal, ethanol insoluble mucoid material secreted by the exocrine glands of the cystic fibrosis patient. (Doggett, et al, 1965, J. Bact. 89: 476). Doggett speculated that the metabolism of the pseudomonas is altered by the infected host so that it produces a mucous similar or identical to that mucous produced by the host. In light of our difficulties over the years to demonstrate differences in virulence for animal isolates of Pseudomonas aeruginosa from human infections, Doggett's suggestion becomes intriguing; what effects do host factors play in pathogenecity of this enigmatic organism. It seemed worthwhile for us to investigate the possibility that the unique environment provided by the cystic fibrosis patient might provide a tool for such investigations. Since the mucous secretions of the cystic fibrosis patients was shown to contain significant concentrations of DNA, our preliminary approach was to determine whether the differences in production of mucoid slime layer by cultures of Ps. aeruginosa from cystic fibrosis patients might be genetically controlled; ie: is the DNA of the organism altered by the host environment to "endow" the capacity to produce this abnormal muco-polysaccharide? To this end, the quanine-cytosine base ratios

of DNA extracted from cultures of Ps. aeruginosa isolated from cystic fibrosis patients and burn patients were compared by determining the density gradients in cesium chloride, using the Spinco Model E Ultracentrifuge. The technique of Meselson, et al was employed (Proc. Nat. Acad. Sci., Wash, 43:631), and the buoyant density was calculated as described by Schildkraut et al (J. Mol. Biol. 4:430, 1962), and mouse liver DNA was used as a standard for all determinations.

As indicated in Table XVIII there were no apparent differences in the DNA of the organisms isolated from the cystic fibrosis patient when compared to those isolated from the burn patient.

It was observed during these studies that strains of Ps. aeruginosa from burn patients produced very mucoid "slime" when cultured under appropriate conditions. The production of such slime by strains isolated from cystic fibrosis patients has caused some workers to characterize these as a different species, Ps. nebulosa. Our studies will continue, firstly, to compare chemically and serologically the slime layers produced by the organisms isolated from different types of infection, and secondly, to employ other methods which will enable us to determine whether or not the pathogenicity of Ps. aeruginosa can indeed be altered by its human-host environment, whether by induction of enzymes or "toxins", or in fact by genetic alterations which the G/C ratio determinations do not detect. Continous culture studies also have been initiated to study the effects of environment of pathogenicity of this species.

Table XVIII

Densities of Pseudomonas aeruginosa DNA as determined by density gradient in Cesium Chloride

Source of Culture: Cystic Fibrosis		Burn wound	
DNA 1a	1.731 g cm^{-3}	DNA 7	1.733 g cm^{-3}
DNA 1b	1.730	DNA 8	1.732
DNA 2	1.731	DNA 10	1.732
DNA 3	1.731	DNA 12	1.732
DNA 5	1.731	DNA 20	1.732
		*DNA 19	1.733
DNA 11	1.732		
DNA 13	1.732	Heat denatured	
DNA 16	1.732	DNA 9	1.733
DNA 15	1.732	DNA 10	1.732
*DNA 22	1.733		
Heat denatured			
DNA 2	1.732		
DNA 5	1.732		
Mean:	1.7325 g cm^{-3}	Mean:	1.7323 g cm^{-3}
Average deviation from the mean:	0.0005 g cm^{-3}	Average deviation from the mean:	0.0004 g cm^{-3}

*Note: DNA 22 & 19 were done in the same run

**Significant to four places only

III. Clinical Evaluation of Pseudomonas Vaccine.

1. One of the greatest problems facing the injured patient in either military or civilian life is the danger of infection. Trauma is the fourth leading cause of death in civilian life, and post-trauma infection continues to be a serious and essentially unsolved problem. Its development leads to prolonged morbidity, increased mortality, excessive cost of hospitalization, unnecessary loss of limbs, delay in wound healing, cosmetic desfigurement, less of the injured person's capabilities to the military or industry, and increased legal liability to physician, hospital, and murse.

2. While antibiotics have given us dramatic and effective methods of treating many established infections after injury, antibiotic therapy has not eliminated the incidence of the development of serious and life threatening infections even after a quarter of century of its use.

3. In fact, there has been an increase in the number of infections and a shift in the types of infections from the preciously more important gram-positive bacterial ones to the now more important gram-negative varieties, In our studies and our travels, it has become apparent that there has developed a fourteen-fold increase in thenumber of cases of gram-negative sepsis during the past 12 years. This was reported last year and the trend has continued.

4. One of the most important causes of such gram-negative infections has been the Pseudomonas aeruginosa. In injuries such as burns, compound fractures, and other large wounds, infections by the gram-negative bacillus

Pseudomonas aeruginosa have reached major proportions. Antibiotic theraoy has proven to be an insufficient answer to the problem of prevention or adequate treatment for Pseudomonas infections.

5.

. For this reason we have turned to the possibility of preventing Pseudomonas infection by increasing the host-resistance of the injured patient through vaccination using a newly developed vaccine. This vaccine which was described last year is being prepared by the Parke-Davis Company. It has been used for immunization of burn patients to evaluate its effectivementss in precenting and controlling various types of Pseudomonas infection.

A study of host resistance factors in the development of Pseudomonas infections has been made in one-hundred consecutive surgical patients with burn injuries of 20% of the body surface or greater in the Department of Surgery of the University of Concinnati Medical Center. Lack of specific antibody to the corresponding immunotype of Pseudomonas, abnormalities of intracellular killing by neutrophills, and heavy colonization of the burn wound each contribute greatly to the development of Pseudomonas sepsis following a burn injury. (Eighty of these patients were vaccinated with a new polyvalent Pseudomonas vaccine developed by Parke-Davis and Company.) Correction of the first determinant by specific immunization has resilted in a marked decrease in the incidence of Pseudomonas sepsis in the group of patients.

Evaluation of the vaccine was done in three phases. In phase I the patients were randomized for vaccination and control. The vaccinated patient were given low does of antigen. In phase II, all patients were

vaccinated, but this was during a period of experimentation with dosage and route of administration. In phase III, all patients were given multiple injections of antigen (25.5 u/kg/injection with a total of 10 injections). The overall mortality from Pseudomonas sepsis has been decreased to approximately 1/3 of the frequency which occurred in non-vaccinated similar patients including 103 patients during the 18 months immediately preceeding the onset of the study. Of the four mortalities from Pseudomonas infection that occurred in the vaccinated group, all were given low doses of antigen, and all but one had a poor response to immunization associated with subcutaneous administration of the drug, a route no longer used. In the last 32 patients, all of whom received a high dose of vaccine, there has been no Pseudomonas associated mortality. (Ten of the patients were excluded from consideration either because they had established Pseudomonas sepsis at the time of admission or because they died within the first five days after admission before satisfactory response to immunization could be expected).

Our analysis of antibody level with the vaccinated and non-vaccinated patients indicated that circulating IgG was the predominant type of antibody which provides protection against infection. Burn injury has been associated with a generalized depression of neutrophil function and an accentuation of a physiological cyclic dysfunction. The period cyclic dysfunction of the ability of neutrophils to kill ingested bacteria seemed to explain the sporadic development of invasive infection in burn patients. Indeed, in those burn patients developing systemic infections who have been carefully studied by serial neutrophil function tests, the development

of infection has been associated with an abnormality of neutrophil function in each instance. The presence of a high level of circulating antibody will partially compensate for the abnormality associated with cyclic dysfunction of neutrophils, making it easier for the neutrophils to ingest and kill bacteria. It is partially because of this that vaccination against Pseudomonas aeruginosa has been effective in preventing Pseudomonas sepsis.

Although several new antibiotics have been introduced recently as effective drugs against Pseudomonas aeruginosa, there are increasingly frequent reports of the emergence of strains highly resistant to these antibiotics. The emergence of these resistant strains makes it increasingly desirable to develop alternate methods for the prevention of infections caused by Pseudomonas aeruginosa, and the immunological approach to this problem seems currently to be the most promising.

IV. Studies of Response and Course of Standard Burns in Germ-Free Animals

This period was one of extensive research into various technical aspects of our laboratory procedures. Work during this time resulted in development of new and more efficient techniques in preparing the animals for planned experiments, more efficient and accurate methods of harvesting and processing blood and body fluids, new methods of empolying improved anesthetic agents, etc. During this quarter a group of germ-free Sprague-Dawley rats were contaminated with Staphylococcus aureus (UC-18) and subjected a 20% body surface emergent burn. The experiment was performed not only to collect the data on the individual animals but to check out the new techniques developed

for handling the germ-free colony. The usual studies of hematocrit, white blood cell count, total protein, weight, temperature, activity and electrophoretic studies were performed. A mortality rate of 20% compared favorably to similar studies performed in the past. The new anesthetic techniques were much better than the previous intraperitoneal sodium pentothal. It was determined that shaving the animals with gas sterilized rechargeable clippers was a great improvement over previous methods of shaving with sterile razor blades. This new method of shaving prior to burning was approximately 7 times as fast as previous method and much more efficient. There was much less trauma to the animals. No pHisoHex was used thus eliminating any possibility of pHisoHex toxicity. There were no lacerations which did occur with the blade method. No deaths have been attributable to the anesthetic and shaving procedure, whereas they had occurred by previous methods employed.

Problems developed with the germ-free colony of rats which seem to be related to a vitamin deficiency. The natural vitamins in the food content is greatly destroyed by the autoclaving procedures prior to passing the feed into the isolators. Methods of introducing sterile vitamin solutions into the isolator to supplement the diet of the germ-free animals was necessary. The vitamin solution was prefiltered in a disposable Nalgene Filter unit containing a 0.45 micron grid membrane to remove the large contaminants. The vitamin solution was then introduced into a Seitz Pressure Filtration apparatus which contained a 0.2 micron membrane. The entire apparatus was gas

sterilized prior to introduction of the vitamin solution. An air compressor was used to obtain a filtration pressure of ten pounds per square inch and the vitamins were thus passed into individual vials within the isolator. To date the methods seem to be suitable and has greatly improved the nutritional status of the germ-free colony.

Tests were also conducted on the various types of laboratory media employed in the laboratory to support the germ-free experiments. The problem seemed to be that the benzylammonium chloride compound presently used for surface disinfecting of the isolator will prevent microorganisms from growing in the usual media even if only small amounts of the germicidal solutions are present. After extensive experiments that was determined that the addition of small concentration of inactivating agents such as, lecithin will inactivate the disinfectant and allow organisms to grow. The addition of inactivating compound, such as, lecithin and Tween 80 will help eliminate the possibility of false negative cultures.

An experiment with 15 germ-free animals was carried out using a new technique developed early during the year to evaluate the pathogenicity of Sera-Type 3 Ps. aeruginosa. The animals were anesthetized, then received a third degree burn over 20% of their body surface. The burn surface was then painted with 2 cc. of various dilutions of the Type 3 Ps. aeruginosa. Results of the experiment were as predicted.

The control animals that were burned but not contaminated survived the entire one month period, the control animals that were not burned but were contaminated with full strength Ps. aeruginosa survived throughout the entire period. The animals contaminated with the 10^{-1} and 10^{-2} dilutions of Sera-Type 3 Ps. aeruginosa died within 4 days. The animals contaminated with 10^{-3} dilution survived an average of two weeks in the 10^{-4} an average of three weeks postburn. Although all laboratory tests are not completed on this group of animals, the specific agglutination titers to Type 3 Ps. aeruginosa were low in the entire group with no significant differences between survivors and those animals that expired early in the experiment.

Preparations are being made with new groups of germ-free animals to continue the evaluation of the various Sera types of Ps. aeruginosa and compare their pathogenicity one to another. Further studies in electrophoresis and agglutination titers will also be carried out, although the studies to date have not been as productive as we had hoped. Concurrently the gnotobiotic techniques that have proved effective research methods in the laboratory are being extended to the clinical wards. An entire reverse isolation therapy program in the treatment of burns has been established to eliminate exogenous sources of infection and prevent the emergence of resistant endogenous bacteria therby reducing the mortality secondary to infection. We have determined that with the proper equipment and techniques reverse isolation with complete elimination of exogenous bacteria is possible in the treatment of burns. The chief source of contamination of the

burns treated in reverse isolation seems to be from the gastro-intestinal tract of patients and this source of contamination can only be partly controlled by oral antibiotic therapy and sterile techniques within the unit. The suppression of the usual endogenous gastrointestinal flora has been followed by the overgrowth of yeast in many cases. Although complete reverse isolation is not recommended for the routine burn care of burn patients, it would seem to have excellent potential in certain phases of bacteriologic and immunologic research.

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

Theoretically, the early removal of burn eschar by excision has always been an appealing concept. Septicemia is rarely observed before the fifth postburn day. The peak of the infection does not occur until the 11th postburn day. If the 3 to 5 week period during which eschar separates by autolysis could be shortened by excision, the morbidity as measured by hospital stay would be markedly decreased. In line with this thinking, a program of massive excision of burns was instituted in the University of Cincinnati Medical Center in January of 1958. Since that time, 10 patients with burns between 25 and 50% total body surface had been treated in this fashion. Two patients survived, but of the remaining 8, 4 expired from septicemia and 4 expired from cardio-pulmonary complications. At this point, excision was limited to burns of less that 15% of the total body surface area. Seventeen patients were treated up to December 1, 1968. An additional

patient with a 3% burn of the buttock has been treated in this fashion during the period covered by this report. Excisional therapy is preferentially used for small electrical burns and for localized third degree burns. Improved topical therapy is felt to contraindicate the use of excision in burns in questionable thickness.

A new program for evaluation of massive excision for very large burns has been proposed. The death rate in large burns (in excess of 70% third degree) is essentially 100%. Dr. J. Wesley Alexander proposed that due to the unavailability of donor sites in such patients, typed tissue donors from the patient's family might be used in combination with immune suppressive therapy with antilymphocyte globulin to secure prolonged coverage with skin other than the patient's own, until such time as autografting could be completed. In six patients in whom this procedure has tentatively been contemplated, none survived the autolysis phase. None of these patients reached a point at which grafting could be considered with any hope of success.

It has, therefore, been suggested that in the large third degree burn (in excess of 70%), massive early excision might be carried out within the first 72 hours. Coverage with Tannered skin from matched donors, probably relatives of the patient, could then be applied. Work in this area has indicated that immune response in the gravely burned patient falls off markedly after the first week. It is, therefore, suggested that coverage by skin from the matched donor not be applied until after

the first week. Excision should, however, be done as soon postburn as possible. As a temporary measure, therefore, wound coverage would be necessary between the period of operative excision and the period of coverage with typed homografts. It is felt that it would be desirable to cover the patient with skin which would not antigenically sensitize the patient to the transplant from the matched donor. This would contraindicate the use of cadaver homografts.

Accordingly, it is felt that porcine heterograft skin could be used for this purpose. A program of procurement of porcine heterografts using pig skin purchased from a local packing house was accordingly developed fully prior to attempting massive excision. Porcine heterografts have been used as a substitute for cadaver homografts. In 35 patients in whom this material was used, it was felt to be a satisfactory substitute for cadaver homografts. Bacteriologic, clinical and antigenic evaluation of this material proceeds. On completion of the clinical evaluation of heterografts, the proposed massive excisions can be attempted.

VI. Curling's Ulcer

The rational treatment of patients with ulceration of the upper gastrointestinal tract has evolved from the study over a ten-year period of a group of 1,202 patients admitted to the Burn Unit of the Cincinnati General Hospital and the Shriners Burns Institute, a retrospective pathological review of 81 autopsy patients carried out over a ten-year period, and a prospective study of 90 upper gastrointestinal studies performed on a group of 85 patients. In this group of patients there were 182 deaths.

1. When all erosions and ulcerations demonstrated in the pathological material are added to the patients who had guaiac positive stools, melena or frank episodes of bleeding, the incidence of potential ulceration reaches a figure of some 80 percent in burn patients.

2. Less than 1 percent of this population required surgical intervention for the treatment of complications from ulcerative disease of the upper gastrointestinal tract. The most frequent complication was bleeding; however, isolated cases of obstruction and perforation were encountered.

3. The low incidence of operative intervention can be explained by a rational medical management program used to treat these patients, which consisted of a combination of milk and antacid therapy, the use of antispasmodics in selected cases, and the control of the complications of burns by aggressive antibacterial therapy for burn wound sepsis and nutritional support for metabolic depletion.

4. Obstructive complications were rare; however, when they occurred, they resulted from spasm of the pylorus and were effectively

treated by decompression of the stomach with a nasogastric tube. The most insidious and difficult complication resulting from ulcerative disease of the upper gastrointestinal tract was perforation. A high index of suspicion, a change in the clinical picture of the patient, and the use of x-rays were the most helpful criteria for establishing the diagnosis of this complication.

5. Two ulcers of the upper gastrointestinal tract were found to be associated with four cases of superior mesenteric artery compression. These ulcers responded to conservative medical management following rather prompt improvement from the duodenal obstruction and thus did not require surgical intervention. When obstruction secondary to superior mesenteric artery compression cannot be relieved medically, a bypass procedure consisting of a jejunoduodenostomy may be of help in decreasing the incidence of perforation from such ulcers in either the duodenum or the stomach secondary to stasis.

6. Massive bleeding from the upper gastrointestinal tract was treated successfully in four patients by vagotomy and pyloroplasty after the bleeding point in the duodenum was oversewn. Recurrent bleeding occurred in one (or 25%) of these cases and required a secondary hemigastrectomy for control of hemorrhage.

7. Nutritional support by hyperalimentation and aggressive control of infection are important in reducing the complications following surgical procedures, since none of this group of patients died as a result of complications from their ulcer or as a result of their operative procedure.

8. In a prospective study of 85 patients, 9 patients (or 11%) were shown to have ulcerations of the upper gastrointestinal tract. In 70

patients who had normal upper GI series, 32 (or 46%), had a 4+ guaiac stool at some time during their hospital course and 3 (or 4%), had clinically significant upper gastrointestinal bleeding.

9. In a retrospective study of autopsy findings, over a ten-year period, of the gastrointestinal tracts in 81 burn patients, erosive lesions were found in 46.9 percent of all cases and frank ulcers in 23.5 percent. Esophageal lesions were present in 18.5 percent, gastric lesions in 27.7 percent, and duodenal lesions in 11.1 percent. The incidence of frank ulceration was noted to be 7.4 percent in the esophagus, 11.1 percent in the stomach, and 8.6 percent in the duodenum.

10. Rational programs of medical and surgical management have been reported for the treatment of the classical Curling's ulcer of the upper gastrointestinal tract secondary to burns. It is hoped that this conservative approach will continue to minimize the complications from the ulcerative disease of the upper gastrointestinal tract of burn patients.

11. If that incidence of patients who have postburn gastrointestinal ulceration, as diagnosed by barium upper gastrointestinal radiologic examination, is accumulated with cases of acute postburn ulceration found at necropsy examination and by other diagnostic methods, our studies indicate an incidence of postburn gastrointestinal ulceration in the neighborhood of 25 percent incidence for severe acute burn injury in which the thermal trauma is of a burn index of 30 or over.

VII. Continuing Clinical Evaluation of Patients Treated on the Burn Service of the Cincinnati General Hospital.

During this report period, 83 patients were treated on the Burn Service of the Cincinnati General Hospital. The extent of burn in these patients ranged from 1% through 87% of the total body surface area. Ages of the patients ranged from 1 to 80 years of age.

Thirty-nine patients were burned by flame, either from fire or burning liquids. Twenty-three patients were scalded. Four injuries resulted from electrical burns and 10 from contact with hot metals. Five were of chemical origin. The head was involved in 31 cases, the neck in 33, the upper extremities in 48, lower extremities in 37, the trunk in 78, and the hands in 22 cases. Local treatment was confined to three basic agents, gentamicin in 59 patients, Sulfamylon in 5 patients and silver sulfadiazine in 9. Ten other patients were treated with other means including open exposure and Polysporin dressings. These patients usually had small second degree burns. Full thickness excision was used in one patient.

In the period in question, resuscitation was carried out with Ringer's Lactate solution during the first 24 hours. After this time, the patient was switched to maintenance fluids with balanced electrolyte solution. Colloid therapy is rarely employed during the first 48 hours. Blood is rarely used in the acute period. When required, albumin is now used instead of plasma. Central venous pressure lines are used on large burns and in those patients with pre-existing heart or lung disease.

Wounds are prepped with Dreft and hydrogen peroxide solution and gentle debridement before dressings. Both open and occlusive dressing

techniques are used. Homografts are used as an attempt to control local infection, reduce fluid loss from the burn surface and to prepare the donor site to accept an autograft. An active program of cadaver recruitment for homograft donors is maintained. During the period in question, 25 par[illegible]ere treated with cadaver homografts. Such grafts are changed at 2 to 5 day intervals. As soon as the donor sites are prepared, autografting is performed. The expanded mesh autografts using the Tanner unit are employed by choice in the wide majority of the cases. Sheet grafts are still used preferentially on the face and hands. Twenty-nine patients received autografts during the period in question.

Systemic antibiotic therapy consists of penicillin for the first five days of hospitalization in acute burns. Seventy-three patients received penicillin as part of their antibiotic coverage and in the majority, this is the only antibiotic which was given. Erythyromycin was used early in those patients who were allergic to penicillin. Specific antibiotic therapy was received by those patients with infectious complications including septicemia, pneumonia and urinary tract infections. Those patients with burns over 20% of their body surface received the Pseudomonas vaccine.

In the entire group, 14 deaths occurred during the period of this report, for a total of 16%. Of the 59 patients in the group treated with topical gentamicin, there were 11 deaths. Five of these were due to Pseudomonas septicemia, 1 patient having a combined sepsis with Pseudomonas aeruginosa and Bacillus anitratum. Three patients expired

due to pulmonary complications, either pneumonia or pulmonary edema, two died of renal failure, 1 case being complicated by acute hyperglycemia of unknown etiology. One patient died of a myocardial infarction. In the 9 patients treated with CF 100, there were three deaths, two from incineration and one from a respiratory burn. In the Sulfamylon group of 5 patients, there were no deaths. Complications observed during this period, in those patients who survived their burns, included upper GI bleeding in 1, urinary tract infections in 4, pneumonia in 2, pulmonary edema in 1, respiratory burns in 1, purulent scleritis in 1, chicken pox in 1 and otitis media in 1.

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