

FINAL REPORT

February 1 through June 30, 1965

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"Clinical and Laboratory Studies of Infections in Debilitated States  
Following Ionizing Radiation and Radiomimetic Drugs"

Contract No. DA-49-193-MD-2719

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A B S T R A C T

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Studies of clinical infections among patients with selected malignant disease revealed an overall infection rate of 4.5 infections per 100 patient-months observed. These rates were seen to increase during increasing debility, irradiation, or treatment with chemotherapeutic drugs. Increased rates could not be demonstrated during the period of treatment with adrenal cortical steroids. While x-ray and chemotherapy were associated with similarly high rates of infection in the latter stages of life, in the earlier stages of disease x-ray therapy was associated with much higher rates than chemotherapy. A greater frequency of infection following irradiation from low voltage sources as compared to high voltage sources was noted.

Studies of infection in mice with Neisseria meningitidis revealed increased susceptibility among mice receiving 400 r total body irradiation and challenged three days post-irradiation. Alteration of the meningococcus to produce L-forms resulted in a strain equally as virulent for mice as the parent strain. Penicillin treatment protected both normal and irradiated mice from deaths due to the parent strain. Penicillin treatment only partially protected normal mice and failed to protect irradiated mice from deaths due to the L-forms.

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## Introduction

This is the final report of research contract No. DA-49-193-MD-2719 entitled "Clinical and Laboratory Studies of Infection in Debilitated States Following Ionizing Radiation and Radiomimetic Drugs" covering the abbreviated period February 1, 1963, to June 30, 1965. It is divided into two parts.

Part I is a retrospective study of patients first admitted to the University of Chicago Hospitals in 1960 with selected malignant disease. Infection rates are determined for each of the various conditions studied. These rates are seen to increase two-fold during the period of radiation therapy and chemotherapy when compared with the 30 day period immediately preceding such therapy. An increase in infection rate during treatment with adrenal cortical steroids could not be demonstrated. Debility is associated with increased infection rates independent of the influence of treatment.

There is some suggestion that chemotherapy and radiation therapy increase infection rates by different mechanisms. Infection rates during both forms of therapy are similar in the latter one-half of observation of a deceased patient's life, but rates following radiation therapy exceed by three times the rates following chemotherapy during the initial one-half of the period of observation. In addition, almost all infections following chemotherapy were associated with leukopenia, while only 50 per cent of infections following radiation therapy were associated with leukopenia. Infection following irradiation from low voltage sources occurred more frequently than infections following irradiation with high voltage sources.

Part II is a laboratory study of the influence of irradiation on infection in mice with an altered strain of Neisseria meningitidis. This altered strain was derived from an L colony and demonstrated penicillin resistance and increased potential to L-formation. It showed equal potential to grow as a bacterial form on 5 per cent blood agar and as an L-form on 20 per cent serum agar with added penicillin. The pathogenicity of this strain was established in mice and was shown to be equal to that of the parent strain. Penicillin therapy protected both irradiated and non-irradiated mice from the parent strain, but only partially protected non-irradiated and failed to protect irradiated mice from the altered strain.

### Part I. A Study of Infection Among Patients with Debilitating Diseases at the University of Chicago Hospitals

A retrospective chart survey to determine the incidence of infection and the influence of treatment with ionizing radiation, radiomimetic drugs, or antimetabolic chemotherapeutic agents on infection was done as a preliminary approach to prospective studies of such patients.

## Materials and Methods

All patients first admitted to the University of Chicago Hospitals in 1960 with the diagnosis of chronic lymphatic leukemia, Hodgkin's disease, reticulum cell sarcoma, lymphosarcoma, metastatic carcinoma of the breast, or metastatic carcinoma of the lung were studied. Details of the clinical course, treatment received, and infections encountered were recorded. Exact dates and total dose of x-ray or drug therapy were noted in each instance. For the purposes of this study infection was defined as the occurrence of an adequately documented clinically recognizable infection which caused the physician to change existing therapy or to institute new therapy. Purposefully excluded

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from this survey were upper respiratory infections, acute exacerbations of chronic bronchitis, asymptomatic bacteremia, or the autopsy diagnosis of patchy or focal bronchopneumonia or pyelonephritis.

Results

A total of 85 such patients were studied, and 43 of these had a clinical infection during the course of their follow-up (Table 1). Table 2 lists the types of infection encountered in each disease category. There were 47 bacterial, 7 viral, and 5 fungal infections encountered.

Table 1

The Incidence and Type of Clinical Infection Among Selected Patients with Malignant Disease First Treated at the University of Chicago Hospitals in 1960

Disease	Patients	Number with Infection	Total Infections	Type of Infection		
				Bacterial	Viral	Fungal
Chronic lymphatic leukemia	8	7	9	8	1	0
Hodgkin's disease	10	5	6	3	2	1
Reticulum cell sarcoma	4	2	6	5	0	1
Lymphosarcoma	18	10	14	10	3	1
Carcinoma, breast	19	8	10	9	0	1
Carcinoma, lung	26	11	14	12	1	1
	—	—	—	—	—	—
Total	85	43	59	47	7	5

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Table 2

Clinical Infections Among 85 PatientsChronic Lymphatic Leukemia

- \* Staphylococcal abscess, pneumonia, and bacteremia
- \* Staphylococcal pyoderma with septicemia
- Superficial pyoderma and conjunctivitis
- Staphylococcal pyoderma
- Scrotal abscess due to beta streptococcus
- \* Acute pyelonephritis with bacteremia
- Urinary tract infection (2)
- Herpes zoster

Hodgkin's Disease

- \* Bacterial pneumonia (all lobes)
- Acute pneumonia
- \* E. coli bacteremia
- \* Herpes zoster
- Chickenpox
- Monilia infection, mouth

Reticulum Cell Sarcoma

- \* E. coli pneumonia
- Acute bacterial pneumonia
- Acute bronchopneumonia
- Staphylococcal postoperative wound infection
- Acute pyelonephritis
- Monilia vaginitis

Lymphosarcoma

- FUO (responded dramatically to antibiotics)
- \* Bronchopneumonia
- Beta streptococcus pharyngitis (severe)
- \* Acute bronchopneumonia
- + \* Sub-diaphragmatic abscess (postoperative)
- \* Abdominal abscess (postoperative)
- Otitis media due to staphylococcus
- Urinary tract infection (3)
- Herpes simplex (lip, cheek, neck)
- + \* Generalized cytomegalic inclusion disease (salivary gland virus)
- Herpes simplex
- Oral moniliasis

\* Infection associated with death.

+ Infection occurred simultaneously with another.

Carcinoma of Breast

- + \* E. coli pneumonia
- Postoperative pneumonia
- \* Pneumococcal meningitis
- \* Gram negative bacteremia, E. coli
- \* Gram negative bacteremia, Proteus
- Urinary tract infection (3)
- \* Urinary tract infection with pyohydronephrosis
- + \* Candida pharyngitis and esophagitis

Carcinoma of Lung

- \* Staphylococcal pneumonia
- Bronchopneumonia
- Acute pneumococcal pneumonia
- \* Bronchopneumonia
- Tooth abscess and pneumonia
- Pneumonia
- Pneumonia with lung abscess
- \* Acute bronchopneumonia
- Postoperative wound infection
- \* Soft tissue abscess, gamma streptococcus
- Urinary tract infection
- \* E. coli bacteremia
- Herpes zoster
- Oral moniliasis

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Table 3 shows the infection rates per 100 patient-months following each type of therapy. A total of 24 infections occurred during the 936 months patients were observed without therapy for a rate of 2.6 infections per 100 patient-months. This rate was found to increase two-fold during the months of steroid therapy and four-fold during the months of x-ray or chemotherapy.

Table 3  
Infection Rates During Various Types of Therapy

Type of Therapy	Observed Patient-Months	Infections	Rate per 100 Patient-Months
None	936	24	2.6
X-ray	135	16	11.9
Steroid	160	8	5.0
Chemotherapy	55	6	10.9
Combined	<u>44</u>	<u>5</u>	<u>11.4</u>
Total	1,322	59	4.5

Patients are often only treated, however, at a time when their disease is active. Debility may be greater, therefore, during the periods of therapy than during the periods of no therapy. A more precise effect of therapy on infection rates is seen when these rates are compared for the 30 day period immediately prior to therapy, the period during therapy, and the 30 day period immediately following therapy. In Table 4 infection rates are seen to double during x-ray or chemotherapy when compared in this manner. No increase during steroid therapy was seen.

Table 4  
Infections Occuring Immediately Prior to, During, and Immediately Following Therapy

Type of Therapy	Prior			During			Following		
	Months	Inf.	Rate	Months	Inf.	Rate	Months	Inf.	Rate
X-ray	121	7	5.8	102	12	11.8	142	8	5.6
Chemotherapy	51	4	7.9	91	12	13.2	41	4	9.8
Steroid	39	6	15.3	243	12	4.5	14	3	21.2

Table 5 shows the number of infections occurring among patients who received steroid therapy. Of 113 months when these patients were not taking the drug there were 9 infections, while only 12 infections occurred in the 204 months in which they did take the drug.

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Table 5

## Clinical Infections Among Patients Treated with Steroids

Disease	Months not Receiving Steroids	Infections	Months Receiving Steroids	Infections
Chronic lymphatic leukemia	24	1	95	5
Hodgkin's disease	24	2	18	1
Reticulum cell sarcoma	1	0	3	0
Lymphosarcoma	64	6	88	6
	—	—	—	—
Total	113	9	204	12

While therapy was shown to have a definite effect on infection rates in these patients, a comparison of deceased patients and patients still alive (Table 6) reveal that debility also leads to increased infection rates. Of 865 patient-months observed for patients still alive at the time of this study, there were 13 infections for a rate of 1.5 infections per 100 patient-months. This rate was increased five-fold in deceased patients in whom 47 infections were observed in 607 patient-months for a rate of 7.8. This effect was also seen when infections were tabulated for each patient according to the first half of the period of observation and the last half of the period of observation (Table 7).

Table 6

Incidence of Clinical Infection Among Patients Still Living  
Compared with Those Deceased

Disease	Deceased Patients			Live Patients		
	Pt.-Months Observed	Inf.	Inf. per 100 Pt.-Months	Pt.-Months Observed	Inf.	Inf. per 100 Pt.-Months
Chronic lymphatic leukemia	94	6	6.4	148	4	2.7
Hodgkin's disease	72	4	5.6	236	2	0.9
Reticulum cell sarcoma	6	1	-	100	1	1.0
Lymphosarcoma	103	12	11.7	289	6	2.2
Carcinoma, breast	164	10	6.1	92	0	-
Carcinoma, lung	<u>169</u>	<u>14</u>	<u>8.3</u>	<u>-</u>	<u>-</u>	<u>-</u>
Total	608	47	7.8	865	13	1.5

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Table 7

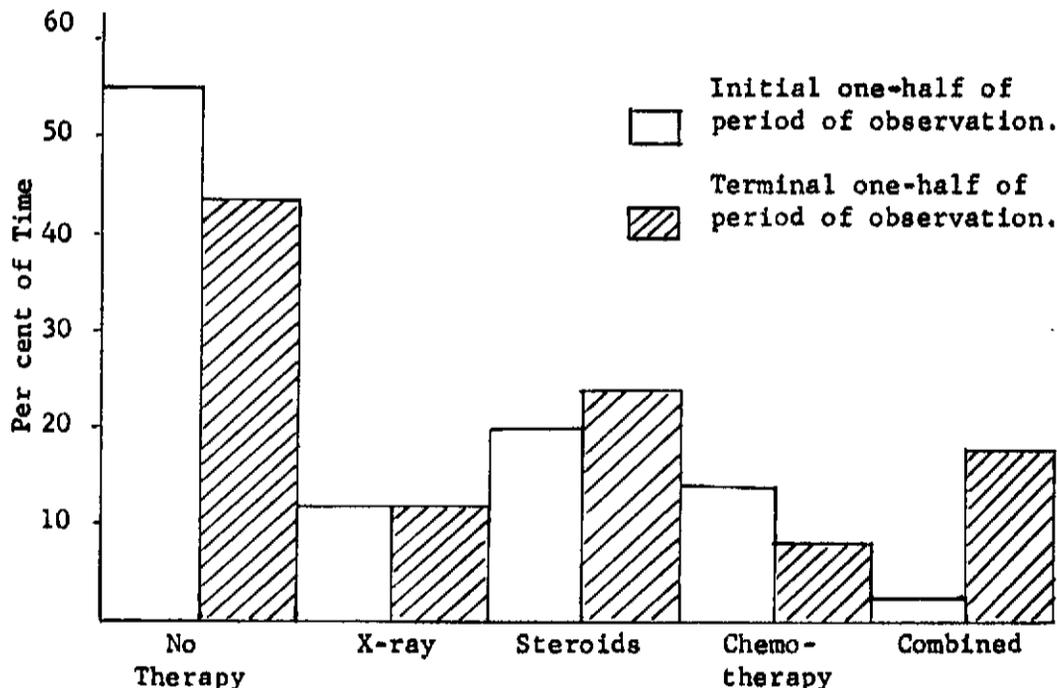
Clinical Infections During Total Period of Observation of Deceased Patients Distributed by Time of Occurrence

Disease	Patients	Months Observed	Average Longevity	Observation Period		Inf. per 100 Pt.-Months
				1st Half	Last Half	
Chronic lymphatic leukemia	4	94	27.0	1	5	7.0
Hodgkin's disease	4	72	18.0	0	4	5.6
Reticulum cell sarcoma	2	6	3.0	0	1	-
Lymphosarcoma	11	100	9.1	5	7	12.0
Carcinoma, breast	11	93	8.5	4	6	10.8
Carcinoma, lung	9	70	7.8	$\frac{3}{13}$	$\frac{4}{27}$	10.0

That the increased rates observed in the latter period of observation and in deceased patients was not due to increased amount of time exposed to radiation or chemotherapy by these patients is shown in Figure 1 which graphically represents the amount of time spent by this group of patients undergoing either x-ray, steroid, chemotherapy, combined, or no therapy during the initial one-half or terminal one-half of the period of observation. As can be seen there is no marked difference in the amount of time spent undergoing treatment in these two periods.

Figure 1

Percentage of Time Spent Under Various Therapeutic Regimens by Deceased Patients Distributed in the Initial One-Half and Terminal One-Half of the Total Period of Observation



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Infection rates following radiation therapy and chemotherapy given during the initial and terminal periods of observation are given in Table 8. Infection rates during the terminal one-half of the period of observation were quite high and similar for both types of therapy (26.9 and 27.2 per 100 patient-months). During the initial period of observation, however, the infection rate following chemotherapy was found to be 4.2 as compared to 12.9 per 100 patient-months following radiation. Almost all infections following chemotherapy were associated with depression of the total leukocyte count below 5,000 per mm<sup>3</sup> while infections following x-ray occurred equally as frequently among individuals whose leukocyte count was not depressed (Table 9).

Table 8

Incidence of Clinical Infection Associated with Chemotherapy and X-ray in the Initial One-Half Compared to the Terminal One-Half of the Period of Observation

Treatment	Initial One-Half			Latter One-Half		
	Pt.-Months	Inf.	Rate/100	Pt.-Months	Inf.	Rate/100
Chemotherapy	48	2	4.2	44	12	27.2
X-ray	62	8	12.9	41	11	26.9

Table 9

Clinical Infection Following Treatment in Relation to Effect on Leukocyte Count

Treatment	Below 5000		Not below 5000	
	Number	Infections	Number	Infections
Chemotherapy	22	12	11	1
X-ray	44	11	40	8

Table 10 shows the frequency of infection following high voltage (linear accelerator or Co<sup>60</sup>) sources as compared to low voltage (250 KV) sources. Of 60 treatments with high voltage sources there were 10 infections for a rate of 16.7 per cent. Of 40 treatments with a low voltage source there were 11 infections for a rate of 27.5 per cent.

Table 10

Clinical Infection Following Therapy with Low Voltage and High Voltage Radiation

Type	Treatments	Infections	Per cent
Low voltage	40	11	27.5
High voltage	60	10	16.7

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Conclusions

The calculation of rates of infection in various disease states is a beginning approach to epidemiological studies of infections occurring among individuals receiving various potent therapeutic programs which alter host defenses and immune mechanisms. The experience with infections among this selected sample of patients is not unlike the experience of others working in large cancer centers. That infection rates are increased at the time of x-ray or chemotherapy is not unexpected. Somewhat unexpected, however, is the failure to demonstrate increased infection rates with steroid therapy. It would appear that the role of steroids in infection warrants reconsideration. Sparberg and Kirsner (Sparberg, M. and Kirsner, J.B. Steroid therapy and infections. J.A.M.A. 188:680, May 18, 1964) have recently rendered this opinion in an editorial based on their huge experience in clinical gastroenterology. Perhaps there is beneficial effect of adrenal cortical steroids in debilitated patients.

Debility among this group of patients was considered to correspond to the latter one-half of the period of observation prior to their death. Twice as many infections were encountered in this period as were encountered in the initial one-half of the period of observation. Infection rates were considerably higher among patients deceased than among those still living. It is believed that these differences are not due to a greater intensity of treatment prior to death, since surprisingly little difference in total time spent under therapy could be detected during the two periods. Highest infection rates were encountered among debilitated patients (patients in the terminal one-half of the period of observation) undergoing x-ray or chemotherapy.

The demonstration of increased infection rates following x-ray or chemotherapy fails to give any lead as to the mechanism by which such infections arise. Nevertheless there is some suggestion that whatever the mechanism, it is probably not the same. While both x-ray and chemotherapy give similar infection rates in debilitated patients, the rate of 12.9 infections per 100 patient-months following x-ray therapy in the initial one-half of the period of observation exceeds by three-fold the rate of 4.2 following chemotherapy in that same period. Furthermore, almost all of the infections encountered following chemotherapy were associated with leukopenia while this association could not be made for infections following x-ray.

Among this group of patients x-ray therapy with low voltage sources was associated more frequently with infection than therapy with high voltage sources. The greater skin erythema effect of low voltage x-ray may account for this increase. At least three of the eleven infections following low voltage irradiation were infections directly invading through the area of skin or mucous membrane being irradiated.

Part II. Laboratory Studies of the Influence of Radiation on Infection in Mice with Neisseria meningitidis

Recent studies have indicated that L-phase variants of bacteria may arise in vivo in response to antibiotic therapy and be the cause of persistent infection or relapse following therapy. If such is true, these L-phase variants may be especially important in debilitating diseases at the time of radiation or chemotherapy which depress host resistance.

In our laboratory we have altered a strain of meningococcus to increased potential for L-formation and to resistance to antibiotics whose primary mode of action is cell wall inhibition. This altered strain has equal potentiality

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to grow on ordinary bacteriologic media indistinguishable from its parent strain except for antibiotic resistance and to grow in the typical characteristic *i*-form on media suitable for such growth. The following experiments were performed to compare the infections with the parent and altered strain of meningococcus in irradiated mice.

#### Materials and Methods

Mice: CF-1 females, 4 to 6 weeks old, were used. All the mice challenged at one time came from a single shipment.

Irradiation: Mice were irradiated with a 250 KV, 30 ma maxitron 250 (General Electric) machine using 0.25 mm copper and 1 mm aluminum filters at a target distance of 79 cm and a dose rate of approximately 60 r per minute.

Challenge organism: A group C strain of Neisseria meningitidis isolated from the spinal fluid of a patient with meningococcal meningitis was used. This strain was maintained by weekly subpassage on 5 per cent rabbit blood agar incubated in 5 per cent CO<sub>2</sub> (candle jar). Mouse virulence was maintained by repeated passage in mice. This strain is herein termed the parent strain.

The parent strain was altered by producing typical L-colonies from a large inoculum on trypticase soy agar + 20 per cent horse serum + 1000 mcg penicillin per ml. After L-colonies were well established, 1000 units penicillinase was added to the media. After a period of three to five days the edge of the L-colony could be seen to swarm. At this stage the growth was picked up by a wire loop and plated on 5 per cent blood agar. The growth of the altered strain could be distinguished from the parent strain by the ease by which it produced L-colonies in the presence of penicillin and by the ease with which such L-growth could produce bacterial type growth on blood agar.

Inoculums: An 18-hour culture from a blood agar plate was suspended by means of a wire loop in sterile isotonic saline to a standard density (approximately 10<sup>9</sup> organisms per ml). Ten-fold dilutions were made in gastric mucin (pH adjusted to 7.2) and the bacterial count checked by plating 0.1 ml of the 10<sup>-6</sup> and 10<sup>-7</sup> dilutions.

All injections were given in 1.0 ml amounts by intraperitoneal inoculation.

Autopsies: Every mouse which died was autopsied for culture of the heart's blood unless it had been eaten by its cage mates. A few mice which died with negative blood culture were excluded from the mortality data.

#### Results

The meningococcus is nonpathogenic for mice. Even large numbers of organisms (up to 10<sup>7</sup> per ml) suspended in saline failed to cause death in normal or irradiated animals. If meningococci are suspended in gastric mucin, however, a single organism may suffice to cause death. Table 11 shows a titration of the parent strain suspended in saline and various concentrations of gastric mucin in both normal and irradiated mice. Within the limits of these experiments mice receiving 400 r total body irradiation and challenged on the third day post irradiation were more susceptible than normal non-irradiated controls. In this series 10<sup>3</sup> organisms suspended in 2 per cent gastric mucin killed nine of ten irradiated animals, while only two of five normal animals succumbed to a similar dose of organisms.

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Table 11

Inoculation of Parent Strain Neisseria meningitidis into Mice

Number of Organisms Inoculated	Deaths/Number Inoculated	
	Unirradiated	400 r
10 <sup>7</sup> in saline	0/4	0/4
10 <sup>4</sup> in 1% mucin	3/4	3/4
10 <sup>3</sup> in 1% mucin	0/4	0/4
10 <sup>3</sup> in 2% mucin	2/5	9/10
10 <sup>2</sup> in 4% mucin	4/4	
10 in 4% mucin	3/4	
1 in 4% mucin	2/4	
4% mucin control	0/10	0/4

Table 12 shows similar data for the altered strain of meningococcus and confirms the pathogenicity of this organism. Irradiated mice were also more susceptible to this strain. Of 10 mice receiving 10<sup>3</sup> organisms in 2% mucin, seven died while only four of fifteen normal animals succumbed to a similar dose.

Table 12

Inoculation of Altered Strain Neisseria meningitidis into Mice

Number of Organisms Inoculated	Deaths/Number Inoculated		
	Unirradiated Controls	400 r	500 r
10 <sup>3</sup> in 2% mucin	4/15	7/10	
10 <sup>3</sup> in 4% mucin	10/10	5/5	5/5
10 <sup>2</sup> in 4% mucin	5/5		
10 in 4% mucin	8/9		
1 in 4% mucin	0/4		
Mucin control	0/10	0/4	1/2*

\* Died 16 days post radiation with coliform infection.

Penicillin therapy uniformly protects mice against death due to meningococcal infection. If the altered strain is an L-form organism then penicillin therapy should fail to protect mice infected with such organisms. A summary of experiments in penicillin treated animals is shown in Table 13. In one series of experiments all animals received 10<sup>5</sup> organisms in 4 per cent gastric mucin and were treated with 50 mcg penicillin subcutaneously 2 and 6 hours after challenge. All mice receiving the parent strain survived including those receiving 400 r total body irradiation. Of 20 normal mice receiving the altered strain 5 succumbed to infection despite treatment, while 14 of 20 mice receiving 400 r and 11 of 12 mice receiving 500 r total body irradiation died of infection.

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Table 13

The Effect of Penicillin Treatment on Infection in Mice with the Parent and an Altered Strain of Neisseria meningitidis

Number of Organisms Inoculated in 4% Mucin	Deaths/Number Inoculated				
	Parent Strain		Altered Strain		
	Unirradiated Controls	400 r	Unirradiated Controls	400 r	500 r
10 <sup>5</sup> Rx A	0/14	0/4	5/20	14/20	11/12
10 <sup>5</sup> Rx B			0/6	4/6	6/6
Mucin controls	0/10	0/4		0/4	1/2

Rx A - Mice received total of 100 mcg penicillin G subcutaneously 2 and 6 hours after inoculation.

Rx B - Mice received total of 300 mcg penicillin G subcutaneously given in divided doses 2, 6, 24, and 48 hours after inoculation.

Continuing penicillin therapy in another series of experiments for 48 hours post challenge protected six normal animals, but ten of twelve irradiated animals succumbed to infection with the altered strain despite this lengthened duration of penicillin therapy.

Conclusions

A strain of Neisseria meningitidis has been altered to increased potential for L-formation. To our knowledge this is the first successful production of meningococcal L-forms. Of greater significance is the demonstration that this altered strain of meningococcus will kill normal mice. As such it should serve as an experimental model for the study of the role of L-forms in disease particularly under conditions of depressed host resistance. The development and characteristics of this altered strain will be the subject of a published report. The observation is not limited to a single strain, as we have successfully produced similar variants in three strains from two different groups of Neisseria meningitidis. It is our belief that this phenomenon is universal to all bacterial species.

In this series of experiments it appears that penicillin therapy at least partially protects normal mice from deaths due to the altered strain. In this regard, L-forms have been shown to grow more slowly than their parent strains. We believe that the altered strain with its equal potentiality to grow as a classical bacterial type or as a classical L-phase variant multiplies initially as a classical bacterial cell. As such it is at least partially susceptible to the action of penicillin. In normal mice the enhancing factor of gastric mucin is operative only for a period of six to eight hours. After that period then mice are naturally resistant to infection with even massive numbers of organisms. If the host defenses are sufficiently altered for a longer period of time as with total body irradiation, then the slower growing L-form is able to initiate

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infection leading to death. Whether or not the altered strain is isolated from deaths occurring early or late, penicillin treated or not treated, the recovered organism retains fully the properties of the altered strain. Studies will be continued to more completely document this sequence of events.

#### Summary

Studies of clinical infections among patients with selected malignant disease first seen at the University of Chicago Hospitals in 1950 revealed an overall infection rate of 4.5 infections per 100 patient-months observed. These rates are seen to have increased during increasing debility, irradiation, or treatment with chemotherapeutic agents. Highest rates were observed during the period of x-ray or chemotherapy in the latter stages of life. Increased infection rates could not be demonstrated during the period of treatment with adrenal cortical steroids.

While x-ray and chemotherapy were associated with similarly high rates of infection in the latter stages of life, in the earlier stages x-ray therapy was associated with infection rates exceeding by three times the rates associated with chemotherapy. A greater frequency of infection following irradiation from low voltage sources as compared to irradiation from high voltage sources was noted.

Studies of infection in mice with Neisseria meningitidis revealed increased susceptibility among mice receiving 400 r total body irradiation and challenged three days post irradiation. A strain of Neisseria meningitidis was altered to increased L-formation. This strain was equally as virulent for mice as the parent strain. Penicillin treatment protected both normal and irradiated animals from deaths due to the parent strain. Penicillin treatment only partially protected normal mice and failed to protect irradiated mice from deaths due to the altered strain. Organisms recovered from all dead mice infected with the altered strain demonstrated fully the properties of the altered strain and grew with equal facility as a bacterial cell or as an L-form.

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