RESPONSE OF LEUKOCYTES TO PARENTERAL INJECTION OF *PSEUDOMONAS AERUGINOSA* INTO RATS AND MICE

by

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INTRODUCTION

Pseudomonas aeruginosa[1], formerly designated as *Pseudomonas pyocyanea* and *Bacillus pyocyaneus*, generally is considered to be a harmless saphrophyte on the normal skin and a commensal in the intestines of man and animals. The importance of this organism as a pathogen has too often been overlooked. Actually the organism is a dangerous one causing serious and even fatal diseases under certain conditions.

The widespread and somewhat indiscriminate use of antibiotics has disturbed the natural balance between cocci and bacilli in mixed infections and has permitted this relatively non-pathogenic bacterium to multiply and become the secondary invader or sole causative agent in certain serious infectious diseases. *Pseudomonas* infections of human beings are reported to be indolent, and the organisms contribute to the chronicity of mixed infections.

This organism is not invasive or aggressive but rather is an "opportunist" which most frequently attacks an undernourished or debilitated host and affects the very young most severely. The infection reaches the blood stream not by active spreading but through massive seeding from primary foci, most common of which are the gastrointestinal tract, middle ear, nasal cavity, throat, cutaneous lesions or sites of operative or instrumentative procedures.

Occasionally, this organism demonstrates unquestionable pathogenicity in the production of fatal meningitis, endocarditis, pneumonia and septicemia

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[1]The terminology for bacteria mentioned in this thesis follows that employed in Bergey's Manual of Determinative Bacteriology, 6th edition. Where an obsolete epithet is used by a cited author the name is followed in parentheses by the Bergey designation.

as well as local suppuration in otitis, arthritis, osteomyelitis, superficial wounds and burns. It has been implicated in fatal diarrheas of infants.

Pseudomonas aeruginosa has been recovered from air in preparation, treatment and surgery rooms where it may contaminate surface wounds, burns, drugs, surgical instruments and dressings. Through contamination of equipment and solutions, this organism has been introduced into the urinary tract when using instruments, into the meninges during lumbar puncture and into surgical wounds during dressing.

Pseudomonas aeruginosa attacks the skin more frequently than any other tissue of the body and has a predilection for the anal and urogenital regions. It is reported that clinically its disease processes can be confused with a number of skin diseases including pellagra and lupus erythematosus disseminatus and must be differentiated from aleukemic leukemia and the purpuras.

Pseudomonas aeruginosa is unusual in that it has the ability to grow also in plant tissues. The possibility of a public health problem is apparent if Pseudomonas-infected raw vegetables would be eaten by susceptible humans.

Changes in the absolute number of leukocytes and their relative proportions are of great significance as a measure of the responses of the body to invading organisms. In many instances, the alteration of the absolute and relative leukocyte count and of leukocyte morphology are of such a character that the organism may be immediately suspected and treatment may be initiated at once. The early diagnosis of systemic diseases caused by Pseudomonas aeruginosa is essential because the termination is often fatal, and the present-day therapy is specific.

The contradictory reports of clinical hematological observations and the relative paucity of controlled experimental infections in laboratory animals prompted this investigation into the hematological response of rats and mice

to Pseudomonas aeruginosa.

REVIEW OF LITERATURE

The role of Pseudomonas aeruginosa in the pathogenicity of various disease processes in both man and animal is documented in the literature.

Chambers (1900) and Valentine (1924) found the organism associated with otitis media. Fatal cases of infant diarrhea caused by this organism have been investigated by Cooley (1908), Hunter and Ensign (1947), and Geppert et al (1952). Walker (1952) reported one fatal and two prolonged incidents of diarrhea in infants with pre-existing enteritis. Mills and Kagan (1954) observed severe, acute gastrointestinal infections and admitted the possibility of the organism being transferred to newborn infants by carriers. The bacterium was isolated by Lovett (1924) and Dasse (1928) from persons affected with agranulocytic angina. Kline and Mascke (1932) and Geppert et al (1952) reported Bacillus pyocyaneus (Pseudomonas aeruginosa) as the causative agent of fatal cases of ecthyma gangrenosum in infants and children. In diseases of the respiratory tract, the organism has been implicated in bronchopneumonia (Kline and Mascke, 1932), tracheobronchitis (O'Brien, 1950) and bronchiectasis (Geppert et al, 1952). Kearns (1936), DeMuth and Rawson (1948), Coller and Dyer (1951) and Waisbren and Hastings (1953) have encountered the bacterium in fatal cases of endocarditis. Septicemias, which terminated in fatalities, are recorded by Epstein and Grossman (1933), and Kraus and Hunter (1941); and 90 cases verified by antemortem and postmortem blood cultures are reviewed by Kerby (1947). Salvin and Lewis (1946) isolated Pseudomonas species from 45 percent of patients' ears affected with external otitis and identified 21 of the 52 isolates as being Pseudomonas aeruginosa. Jackson et al (1951) described the adverse effect of Pseudomonas

pyocyanea (Pseudomonas aeruginosa) infection in burns. Cases of Pseudomonas meningitis have been described by Jawetz (1952) and Chigier (1953), and two fatal cases of Pseudomonas meningitis following spinal analgesia have been reported by Evans (1945). The bacterium has been encountered in infections of the urinary tract in 39 patients by Carroll et al (1947) and as the causative agent of pyelonephritis by Jawetz (1952) and Erwin et al (1953).

Yow (1952) reported a survey that revealed the incidence of Pseudomonas aeruginosa infections to have increased from 15 percent of the Gram negative bacilli recovered in June, 1944, to 30 percent in January, 1951. The one common factor in the disease investigated by Geppert et al (1952) was prolonged antibiotic therapy.

In the reported hematological response of humans affected with Bacillus pyocyaneus (Pseudomonas aeruginosa), L$_o$vett (1924) and Dasse (1928) observed agranulocytosis in cases where the organism was isolated from agranulocytic angina. In fatal infections, two persons exhibited leukopenia and one agranulocytosis (Kline and Mascke, 1932). Granulocytopenia was recorded by Epstein and Grossman (1933). Leukocyte counts that remained low in endocarditis were observed by Kearns (1936). Wintrobe (1951), p. 207 listed neutrophilia as being characteristic of acute infections with the organism. In a review by Kerby (1947), 90 cases of Pseudomonas aeruginosa bacteremia were studied in which two cases of granulocytosis and three cases of granulocytopenia were reported, and it was stated that granulocytopenia was not a characteristic development. DeMuth and Rawson (1948) and Coller and Dyer (1951) described cases of endocarditis in which leukocytosis was found. In two fatal cases of tracheobronchitis affecting infants, leukocytosis, lymphocytosis and granulocytopenia was recorded by O'Brien (1950). In an acute lower respiratory infection, Geppert et al (1952) found leukocytosis

with eosinophilia. Waisbren and Hastings (1953) reported leukocytosis with 95 percent polymorphonuclear leukocytes in endocarditis.

In animals, Pseudomonas pyocyanea (Pseudomonas aeruginosa) has been isolated as the etiological agent of a highly fatal disease of chickens (Essex et al, 1930), epizootic gastroenteritis in chinchillas (Keagy and Keagy, 1951), otorrhea in dogs (Farrog and Mahmound, 1953) and mastitis in the bovine (Tucker, 1954).

In experiments with laboratory animals, the following reactions to Pseudomonas aeruginosa are recorded. Gheroghiewsky (1899) claimed to have found a leukocyte-destroying ferment in cultures of the organism. An investigation by Waite (1908) revealed the intraperitoneal injection of 0.25 to 1.0 ml of 24-hour broth culture grown at 37°C caused rats, guinea pigs and a rabbit to die within two to three days. In the intraperitoneal inoculation of a broth culture of the organisms, Lovett (1924) caused an exudate in the abdominal cavity of guinea pigs prior to injection of broth. The probable influence of broth was to produce a toxic effect characterized by vacuoles and irregular staining with Wright's stain in the leukocytes from the peritoneal cavity. In addition, the total leukocyte count in the circulating blood decreased with granulocytopenia and apparent degenerative changes in the polymorphonuclear leukocytes. Slight leukocytopenia and severe neutropenia were recorded in further investigation. Meader et al (1925) found strains freshing isolated from human lesions to be highly virulent, while strains recovered from water and old laboratory strains were avirulent. The virulent strains had a pronounced affinity for the genitourinary organs of rabbits and guinea pigs. Cannon et al (1945) commonly found the organism in succinylsulfatheazole-fed guinea pigs and attributed the growth to weakened defenses in the experimental animals. Subcutaneous

injection of Bacillus pyocyaneus (Pseudomonas aeruginosa) into guinea pigs caused slight reduction of the white cell count and always a relative reduction of granulocytes from 86 to 30 percent (Dasse 1928). Elrod and Brann (1942) stated that the amount of inoculum must be either excessive or the host weakened. Moderate doses of virulent strains killed rabbits, guinea pigs, mice and goats while other strains produced mild disease or failed to infect (Smith et al 1952, p. 487). Gorrill (1952) injected three million to 250 million organisms in broth intravenously into mice using 12 strains to obtain septicemic death in 24 to 48 hours, renal abscess and death in three to 14 days or survival. Engley (1954) confirmed the fact that large numbers of organisms in dosages of one million to ten million in saline were necessary to kill mice when injected intraperitoneally.

In a review of the blood studies of normal laboratory animals, Gardner (1947a, 1947b) has compiled the average values of various white cells of both mice and rats. The average total number of leukocytes in the blood is 15,050 per cmm for the adult rat and 14,080 per cmm for mice, both regardless of sex or strain. The average values of various leukocytes for rats and mice are given in Tables 1 and 2.

Table 1. Average values of leukocytes of normal rats, Gardner (1947a).

Leukocyte	Percent	Range
Stab neutrophils	3.85	0-7
Segmented neutrophils	25.7	12-39
Eosinophils	2.08	0-3.4
Basophils	0.09	0-1
Lymphocytes	66.97	53-83
Monocytes	4.92	0.7-7

Table 2. Average values of leukocytes of normal mice, Gardner (1947b).

Leukocyte	Percent	Range
Neutrophils	20.62	8.0-57.9
Eosinophils	2.58	0.0-15.1
Basophils	rare	
Lymphocytes	66.56	36.2-89.8
Monocytes	5.73	0.7-14.0

Klrod and Brann (1942) observed that the organism has the ability to grown in plant tissues and infects such vegetables as onions, cucumbers and potatoes.

EXPERIMENTAL STUDIES

The animals used in this study were bred from stock albino rats and white mice, raised in a thermostatically controlled room at a temperature of 24°C, fed a ration of "Dog Checkers"[2] and identified according to the ear-marking scheme of Bittner (1941), p. 475.

As often as practical, each series of experiments was conducted on siblings which are indicated by the same age groupings in the tables. Otherwise, the animals were grouped in the same weight class or placed in a class of varied weights to determine the effect of animal size to standard dose.

In all experiments, the organism used was Pseudomonas aeruginosa, Northern Regional Research Laboratory strain B23. The bacterium was grown on nutrient agar slants at room temperature for 24 hours and harvested in one ml

[2]A brand of commercial, dog-food biscuit manufactured by Ralston Purina Company, St. Louis 2, Missouri.

of sterile, physiological saline in each tube by carefully scraping the surface of the agar with a flamed loop. The suspension was transferred by pipette to a screw-cap, plastic centrifuge tube which had been previously sterilized by exposure to ultra-violet light for four hours. The bacterial cells were washed three times in ten mls of sterile, physiological saline and sedimented with a Servall angle-head centrifuge at 4000 rpm for ten minutes. The washed cells were transferred to a sterile test tube and then diluted to 800 million cells per ml of sterile, physiological saline by use of a Coleman Model 11 Universal spectrophotometer using a wave length of 500 millimicrons (Fig. 1). Standardized test tubes with physiological saline in the blank were used in the turbidity measurements.

Prior to the injection of Pseudomonas aeruginosa, the stock suspension of 800 million bacterial cells per ml was diluted by the use of sterile, physiological saline and sterile test tubes to the proper concentration to allow the injection of 0.12 ml to four ml of the suspension depending upon the route of injection, dosage and species. The control animals were injected with sterile, physiological saline by the same route and in the largest amount used as a diluent in that group of animals.

The routes of injection were: subcutaneous, intramuscular and intraperitoneal in both the rat and mouse; intracardial in the rat; and intravenous in the mouse. The site of the subcutaneous injection was on the median dorsal line approximately one cm anterior to the tail base. For the intramuscular injection, the inoculum was deposited in the heavy musculature of the posterior region of the right thigh. The intraperitoneal injection was performed to the right of the median line anterior to the ligamentum inguinale. The intracardial injection of the rat was conducted under ether anesthesia and between the fourth and fifth ribs after previously determining

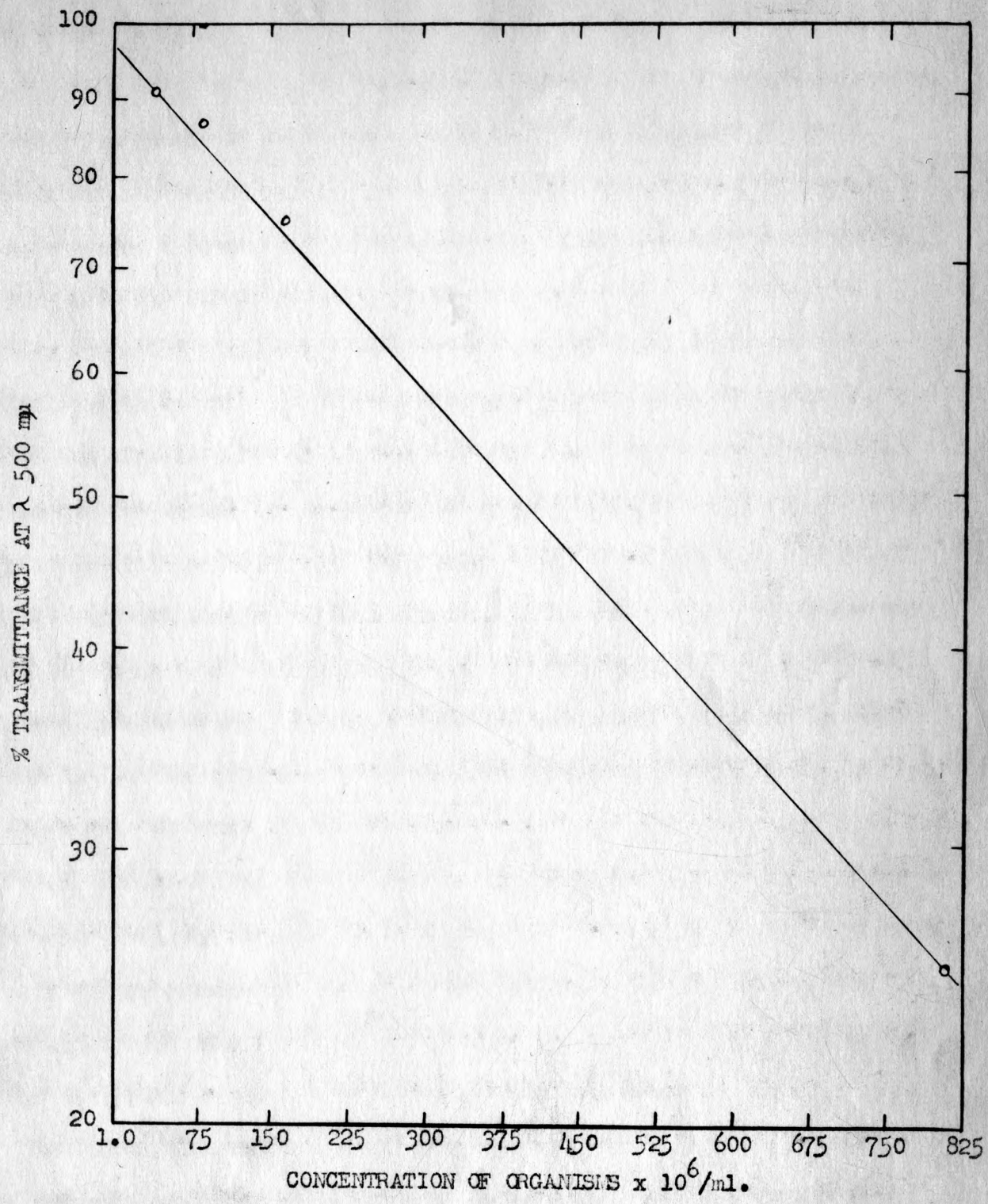


Fig. 1. Calibration curve used for the calculation of the concentration of organisms per ml of saline.

that the needle was in one of the heart chambers. The mouse was injected intravenously into the vein of the tail. For each route of injection, either a sterile tuberculin or sterile one ml or five ml syringe fitted with a 26-gauge sterile needle was used, except during the intravenous injection of the mouse, when a 27-gauge sterile needle was employed. Prior to injection, the skin site was sterilized with 95 percent alcohol.

Blood for the total white cell counts and the blood films for the differential white cell counts was obtained by restraining the rats in a chalk box and the mice in a 100 ml graduate (Plate I). Both devices limited the movement of the animal to reduce physiological "excitement" leukocytosis and yet permitted the protrusion of the tail. Prior to obtaining the blood, the tail was cleansed with 95 percent alcohol, dried with a clean towel and one to two mm of the tail was removed with a Bard-Parker knife blade. The tail was gently stripped until a drop of blood of approximately two mm in breadth was obtained. The blood was transferred to a clean slide previously immersed in alcohol for 24 hours and air-dried.

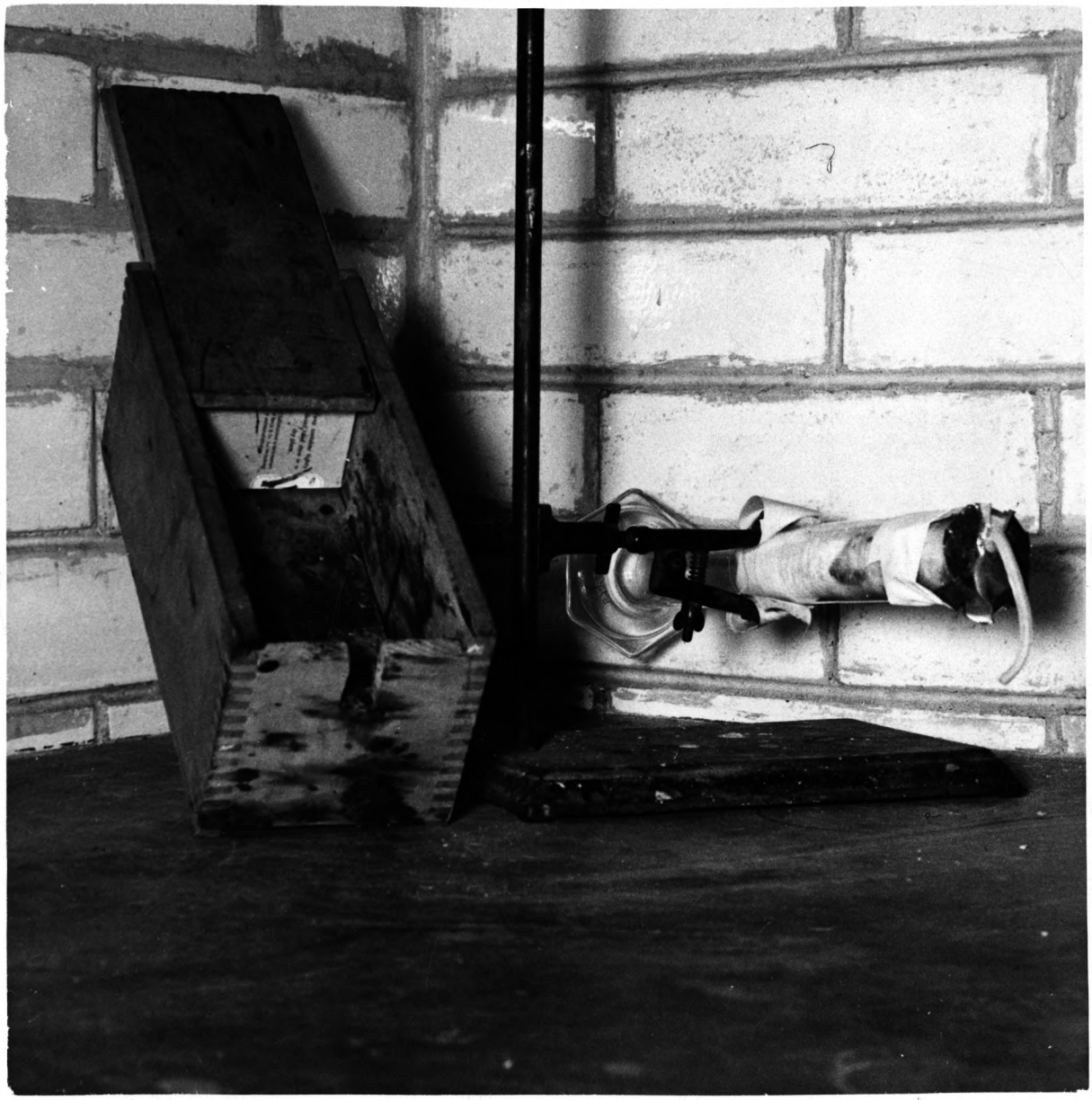
When the blood from the rat coagulated too rapidly to obtain a desirable sample, the tail was immersed in warm water at 45°C for one to two minutes or heart blood was obtained by anesthetizing the rat with ether and inserting a 26-gauge needle into the heart and drawing one ml of blood into a five ml syringe previously rinsed in 20 percent sodium citrate.

The blood films were prepared and inspected and the white cell counts were performed according to the method outlined in Department of the Army Technical Manual, TM 8-227, Methods for Medical Laboratory Technicians, (1951), p. 41, except that the blood for the total white cell count was drawn to the one-tenth mark on the white cell pipette with a resultant dilution of 100. The blood film was stained with Giemsa stain and the leukocytes were identified

EXPLANATION OF PLATE I

Devices used in the restraint of rats (left) and mice (right).

PLATE I



according to the description of Gradwohl (1943), p. 437, Gardner (1947a), p. 81 and Gardner (1947b), p. 173.

Each animal that died during the study was autopsied according to the method described in Department of the Army Technical Manual, TM 8-227, (1951), p. 22 with the exception that the surface of the animal was sterilized with a 1-1000 solution of mercuric chloride. The animals were examined for gross pathological lesions. The lesions, heart and liver were cultured on five percent ovine blood agar, and the blood agar plates were incubated at 37°C for 48 hours. The abscessed kidneys of mice were examined microscopically after fixing in ten percent formaldehyde, dehydrating, sectioning and staining with azure-eosin stain.

RESULTS

Leukocyte Values of Normal Rats and Mice

The average values of leukocytes from the blood of normal rats and mice as listed in Tables 3 and 4 were obtained 24 hours prior to the parenteral injection of Pseudomonas aeruginosa. In compiling these results, the sex of both species was disregarded because no significant differences were observed in total leukocyte counts and differential blood counts of male and female rats and mice. The rats were 55 days to 74 days old and weighed from 120 grams to 221.5 grams. The mice were 41 days to 63 days old and weighed from 17 grams to 23 grams.

Table 3. Average relative and absolute leukocyte counts of 41 normal rats.

Leukocyte	Relative Number* Average	Relative Number* Range	Absolute Number** Average	Absolute Number** Range
Total leukocytes			15448	7500-23750
Lymphocytes	62	47-78	9696	4620-14788
Juvenile neutrophiles	0.18	0-6	28	0-228
Stab neutrophiles	4	0-13	733	0-2938
Segmented neutrophiles	29	15-48	4368	2205-7800
Eosinophiles	1	0-5	156	0-475
Basophiles	not observed			
Monocytes	3	1-7	525	75-1187

*Expressed as number per 100 leukocytes
**Expressed as number per cmm

Table 4. Average relative and absolute leukocyte counts of 50 normal mice.

Leukocyte	Relative Number* Average	Relative Number* Range	Absolute Number** Average	Absolute Number** Range
Total leukocytes			11125	6000-15500
Lymphocytes	58	42-80	6305	3620-8575
Juvenile neutrophiles	0.6	0-4	75	0-580
Stab neutrophiles	6	1-12	696	105-1550
Segmented neutrophiles	31	10-48	3952	1200-7130
Eosinophiles	1	0-5	146	0-470
Basophiles	not observed			
Monocytes	4	0-9	448	0-1305

*Expressed as number per 100 leukocytes
**Expressed as number per cmm

Total Leukocyte Counts in Response to Parenteral Injection

The total leukocyte response of the rats to the parenteral injection of Pseudomonas aeruginosa is summarized in Table 5. The table is a consolidation of results obtained from the injection of the organisms by the intra-

Table 5. Summary of the total white cell count per cmm of blood from rats in response to parenteral injection of Pseudomonas aeruginosa.

Number of Rats	Route of Injection	Dosage (Millions) (of cells)	White cell count on days following injection: Normal	7 hours	1 day	2 days	3 days	4 days	7 days	9 days	11 days	14 days	Remarks
Average of 7 controls	IP, SC, IM, and IC*	.5 ml to 5 ml saline only	13500	16595	16620	18250	15125	17415	18250		16625		Survived
1	IP	1	8500		20250	23250	11500	18750	12500				Survived
1	IP	10	14250		27750	23250	23250	53000					Died within 5 days
1	IP	10	15000		17750	18750		17250					Survived
Average of 2	IP	100	12750		18750	22250	16500	22250	10500				Survived
Average of 2	IP	400	19000	28625	38500	26250	26250	35725	20625		20625		Survived
Average of 5	IP	400	16950	15625	8000	12250	25000						All died within 4 days
Average of 4	IP	800, 800, 1600, 3200	16310										All died first day
Average of 2	SC	200	16750	24500	24000		36125	48125	59875	103625	105000	88500	Both died within 15 days
1	SC	200	18250	34250	19260		60250	53500	42750	33750	10500		Survived
Average of 3	SC	400	15830	7080	13000		38250	70250	129000				All died within 8 days
1	IM	200	13250	26500	20000		29500	21500	14250	23250	24000	12750	Survived
Average of 2	IM	200	15625	13825	8500#		16750#	16750#	75250	93250	91000	118750	Both died within 15 days
Average of 3	IM	400	18750	26330	18250		36000	65000	90250				Both died within 8 days
Average of 3	IC	100	13480	24900	78750		27750#						All died within 4 days
Average of 3	IC	400	16700	45250	66750								All died within 2 days

*IP-Intraperitoneal, SC-Subcutaneous, IM-Intramuscular, IC-Intracardial
#Heart blood

peritoneal, subcutaneous, intramuscular and intracardial routes, respectively. Analysis of the data revealed no significant difference in the total white blood cell counts obtained as the result of the injection of the controls with varied amounts of physiological saline by the four different routes; therefore, these values are combined as one entry for the sake of brevity. Data resulting from all the total leukocyte counts of each rat used in this study may be found in the Appendix (Tables 11, 12, 13 and 14).

Intraperitoneal injection of 800 million, 1600 million, and 3200 million Pseudomonas aeruginosa killed the rats within 24 hours after injection. Smaller numbers of the organisms in the amounts of one million, ten million, 100 million and 400 million resulted in reactions of leukopenia and death or leukocytosis and survival or death. The only rat receiving one million organisms had a definite rise in total white cell count the first day after injection from a normal of 8,500 white blood cells to 20,500 white blood cells. Leukocytosis continued through the fourth day and on the seventh day the total leukocyte count was found to be normal. After the injection of ten million organisms, one rat revealed no hematological response and one rat died within five days. The rat that did not survive showed a twofold increase in leukocyte count over a normal of 14,250 white blood cells the first day. This abnormal leukocyte count was maintained the following two days with a fourfold increase over the normal white cell count developing the fourth day. The rat was dead on the fifth day. Two rats survived the intraperitoneal injection of 100 million organisms. These rats showed a twofold increase of the average normal white cell count of 12,750 by the second day. This range of total leukocyte counts was sustained through the fourth day and returned to a normal value on the seventh day. When 400 million organisms were injected, two rats had an immediate rise in average

total white cell counts within seven hours with a twofold increase over the average normal count of 19,000 white blood cells through the fourth day and a return to a normal total count on the seventh day. This normal white cell count continued through the 11th day with ultimate survival. Five other rats were injected with the same dose and all the rats died within four days. These rats responded with a leukopenia at one day which was one-half of the average normal white cell count of 16,950. There was a rise to a total leukocyte count of 12,250 on the second day and then the final count was 25,000 white blood cells on the third day.

In introducing Pseudomonas aeruginosa via the subcutaneous route, a dose of 200 million organisms in two rats provoked a significant rise in total white cell count of one and one-half times the normal average of 16,750 within seven hours. This total white cell count was maintained through the first day with a twofold and threefold increase over the average normal count on the third day and fourth day. On the seventh day, the average total count increased to four times the average normal, or 59,875 leukocytes, and this count was doubled by the ninth day. This high count ranging from 83,000 white cells to 105,000 white cells was maintained until death of the two rats within 15 days. One other rat that received 200 million organisms subcutaneously and survived had a normal total leukocyte count of 14,250 which doubled by the seventh hour and showed a threefold increase to 62,250 white blood cells on the third day. This range of counts was continued for six days and then a normal leukocyte count was exhibited on the 11th day. The injection of 400 million Pseudomonas aeruginosa resulted in death to three rats within eight days. The average white cell count at seven hours was one-half the average normal count of 15,830. The average total leuko-

cyte count returned to normal one day after injection and continued to rise with a twofold increase of the average normal count on the third day, fivefold increase of the average normal count on the fourth day and an eightfold increase of the average normal count (or a total white cell count of 129,000) on the seventh day.

After the intramuscular injection of 200 million and 400 million Pseudomonas aeruginosa, the average total leukocyte counts of the rats were analogous to that found after the injection of the same number of organisms subcutaneously. Of the three rats injected with 200 million organisms, one survived and two died within 15 days. The surviving rat had a twofold increase in the normal total leukocyte count of 13,250 by the seventh hour. This count was maintained through the 11th day and then returned to normal by the 14th day. The other two rats that received the same dosage as the previous rat revealed a leukopenic response to the injection through the first day with a reduction of the white blood cell count from a normal average of 15,620 cells to 8,500 cells. Because of rapid coagulation of the blood, attempts to obtain satisfactory samples on the third and fourth day resulted in failure. Heart blood was obtained, examined and a normal white cell count resulted. On the seventh day, there was a fivefold increase over the normal leukocyte count of 15,625 which increased through the ninth and 11th days until an eightfold total cell count of 118,700 was found prior to death within 15 days.

The rats responded to the intracardial injection of 100 million and 400 million Pseudomonas aeruginosa with leukocytosis and death. A dose of 100 million injected into three rats resulted in a twofold increase of an average total white cell count of 13,480 on the seventh hour and a threefold increase of the normal leukocyte count after one day. This total white cell

count was maintained until death after the third day. The injection of 400 million organisms was responsible for a threefold increase of the normal count of 16,700 on the seventh hour and a fourfold increase of the average normal count and death within two days.

The total leukocyte response of the mice to the parenteral injection of Pseudomonas aeruginosa is summarized in Table 6. The method of listing the data is the same as for the rats. Results from all the total leukocyte counts of each mouse used in this study may be found in the Appendix (Tables 15, 16, 17 and 18).

As a result of the intraperitoneal injection of doses of one million, ten million, 80 million, 100 million, 200 million, 400 million and 800 million of Pseudomonas aeruginosa, eight mice died within two and one-half hours to 18 hours. Two mice injected intraperitoneally with one million organisms survived the first day with a small average increase in number of leukocytes over the average normal white cell count of 12,000. This leukocyte count increased twofold over the average normal count by the second day and then decreased to within the normal range on the 11th day. Both of these mice died within 12 days. One mouse injected intraperitoneally with four million organisms responded with a two and one-half-fold increase over the normal white cell count of 12,250 within eight hours and maintained a leukocyte count within this range until the 17th day when the total leukocyte count increased to 56,250 white blood cells, and death ensued on the 18th day. After the intraperitoneal injection of a dose of four million organisms, two other mice survived. These mice exhibited a twofold increase over an average total white cell count of 9,375 by the first day, an increase to fourfold of the average normal count on the second day, a decrease to twofold of the average normal leukocyte count on the seventh day

Table 6. Summary of the total white cell count per cmm of blood from mice in response to parenteral injection of Pseudomonas aeruginosa.

Number of Mice	Route of Injection	Dosage (Millions) (of cells)	White cell count on days following injection: Normal	8 hours	1 day	2 days	3 days	7 days	11 days	12 days	13 days	16 days	17 days	23 days	Remarks
Average of 6 controls	IP, IV, IM, and SC*	.12 ml to 1 ml saline only	11175	10875	14450	11000	12125	13900	12425	11875		9500		11750	All survived
Average of 2	IP	1	12000		13500	22250			8750						Both died within 12 days
1	IP	4	12250	30000	20200	18500		32000	35250		37000		56250		Died 18th day
Average of 2	IP	4	9375	13250	21110	32625		21375	13625		13250				Survived
1	IP	8	12250	13750	17500	21250		9500	9250		12750				Survived
Average of 2	IP	8	11375	10225	22500	21250		32500							Both died within 8 days
1	IP	40	13500		22250			29750							Died within 8 days
Average of 8	IP	1, 10, 80 100, 100, 200 400, 800	10340												All died within 1 day
Average of 3	IV	2	10600	16500	17500	22830		15750	12250		11950				All survived
1	IV	2	9000	21250	31000	58500		34750							Died within 8 days
Average of 4	IV	4	11250	22120	15310	22370		44000	37000		21750	37500		61250	All died within 24 days
Average of 2	IM	1	13000	10875	10625		17650	16500		14250		16125			Survived
1	IM	1	10750	14250	21000		35750	63750		30500		40000			Died within 17 days
Average of 2	IM	5	14870	4375	7500		5000								Both died within 4 days
1	IM	5	14500	7950	15250		19250	20500		27750		16750			Survived
Average of 3	IM	10	14660	7580	8000										All died within 2 days
Average of 3	SC	1	10500	13500	16160		19080	15630		10580					All survived
1	SC	5	13250	14000	26500		21000	54500		28500	22750		12750		Survived
Average of 2	SC	5	12500	9125	19750		25870	35370		49750	54500				Both died within 14 days
Average of 4	SC	10	11850	8435	8675		28250								All died within 4 days

*IP-Intraperitoneal, IV-Intravenous, IM-Intramuscular, SC-Subcutaneous

and a return to normal by the 13th day. One mouse survived a dose of eight million organisms. The total count remained within the normal range for eight hours, increased twofold of the normal leukocyte count of 12,250 after one day, remained in this range during the second day, returned to normal by the seventh day, and the mouse subsequently survived. Two other mice did not survive the intraperitoneal injection of eight million Pseudomonas aeruginosa. They showed a small decrease in the average total white cell count of 11,375 at eight hours and then a twofold increase of the normal leukocyte count after the first day. The white cell count of 22,500 white blood cells was maintained through the second day. During the seventh day, the total white cell count had increased threefold over the average total normal leukocyte count and both mice were dead within eight days. One mouse lived until the eighth day after an intraperitoneal injection of a comparatively massive dose of 40 million organisms. This animal exhibited a twofold increase over the normal total white cell count of 13,500 prior to death.

After the intravenous injection of two million Pseudomonas aeruginosa into four mice, three mice survived and one mouse died within eight days. The three mice that survived exhibited a one and one-half fold increase over the average total leukocyte count of 10,600 through the first day with an increase to twofold of the average normal white cell count during the second day and a return to a normal leukocyte count by the 11th day. The mouse that died showed an immediate twofold increase over the normal leukocyte count of 9,000 in eight hours, an increase of three times the normal white cell count at one day, an eightfold increase over the normal leukocyte count in two days and a reduction to a fourfold increase over the normal white cell count of 34,750 the seventh day with death occurring on the next day. The intravenous injection of a dose of four million organisms resulted in death to

each of four mice. These mice exhibited a twofold increase over the average normal leukocyte count of 11,250 within eight hours, and this white cell count continued through the second day. There was an increase to four times the average normal white cell count on the seventh day, and this leukocyte count continued through the 16th day with a total count of a fivefold increase of the average normal count, or 61,250, on the 23rd day. The mouse died the following day.

Varied doses of one million, five million and ten million Pseudomonas aeruginosa were injected intramuscularly into each of three mice in three groups. Of the three mice receiving a dose of one million organisms, two survived and one died. Neither of the two that survived showed any significant change in the average total white cell count. The mouse that died from the injection of one million organisms exhibited a slight rise over the normal white cell count of 10,750 at eight hours, an increase of two times the normal leukocyte count the first day, a threefold increase of the normal white cell count during the third day, a sixfold increase of the normal white cell count on the seventh day, a threefold increase over the normal white cell count on the 12th day, a fourfold increase of the normal white cell count or a total white cell count of 24,000 on the 16th day and death followed on the 17th day. Of the three mice that received five million organisms, two died and one survived. The two mice that died showed a decrease of one-half of the average normal total white cell count of 14,870 at eight hours and the total leukocyte count remained in this range until death within four days. The mouse that survived also exhibited the decrease to one-half of the normal white cell count of 14,500 at eight hours with an increase to normal after the first day. A twofold increase of the normal white cell count was observed on the third day, progressing to a threefold increase over the normal count

on the 12th day and a return to normal by the 17th day. After exhibiting total white cell counts at eight hours and at one day that were one-half of the average normal white cell counts of 14,660 leukocytes, the three mice that received ten million organisms died within two days.

Each of ten mice were injected subcutaneously with varied doses of one million, five million and ten million Pseudomonas aeruginosa. The three mice that received a dose of one million organisms survived and showed a twofold increase in the average normal total white cell count of 10,500 through the third day and then a return to the average normal white cell count by the 12th day. Of the three mice which received five million organisms, one survived and two died. The survivor exhibited no increase in the normal total leukocyte count of 13,250 at eight hours but after one day the total white cell count was doubled and this leukocyte count continued through the third day increasing to four times the normal on the seventh day and then decreasing in the same pattern until a normal white cell count was observed on the 17th day. The average total leukocyte count of 12,500 of the two mice that died decreased at eight hours, increased one and one-half times the normal average white cell count the first day and continued to increase. The number of white cells was double the normal average leukocyte count on the third day, three times the normal average on the seventh day, four times the normal average on the 12th day, the leukocytosis continued through the next day, and death occurred on the 14th day. None of the four mice survived that was injected with a dose of ten million organisms. The average total leukocyte count of 11,850 decreased through the first day and then increased threefold of the normal average white cell count, or to 28,250 white blood cells on the third day. Each of the four mice was dead within four days.

Morphology and Staining Characteristics of Leukocytes of Rats and Mice

Following the staining of the blood films of the rats and mice with Giemsa stain for the differential white cell counts, examination revealed that the morphology and staining characteristics of the leukocytes were closely related to the stained human white cells described by Gradwohl, (1943) and the rat and mice leukocytes described by Gardner, (1947a, 1947b).

The lymphocytes varied in size from large to small. The shape of the nucleus was round to indented and it contained deep-purple staining, blocky chromatin. Occasionally, a nucleolus was observed in the nucleus of the large lymphocyte. The light-blue staining cytoplasm varied from abundant in the large lymphocyte to none at all in the small lymphocyte. The unstained, perinuclear zone described in human lymphocytes was not apparent.

The nucleus of the juvenile neutrophiles varied from slightly indented to bean-shaped, and the chromatin material stained a purple of medium intensity with small clear areas that appeared pink. The cytoplasm stained pink and contained numerous fine granules.

The nucleus and the cytoplasm of the stab neutrophiles showed the same staining characteristics as the juvenile neutrophiles. The shape of the nucleus was characteristic of that described for this cell.

The segmented neutrophiles varied in size from one and one-half times to three times the diameter of the erythrocyte. The nucleus stained a purple color and had small, clear areas that appeared pink. The normal nucleus contained from two to five segments and sometimes appeared twisted. When the animals became severely infected, hypersegmentation of the nucleus appeared with lobulation up to ten lobes. Often, there appeared to be free lobes of chromatin in the cytoplasm with no visible chromatin strands

connecting these to the main body of chromatin. In addition, a severe infection produced toxic granules that appeared as small, dark-blue stained material. The cytoplasm of the segmented neutrophiles stained pink and contained numerous fine granules.

The eosinophiles were identified by the orange-colored, course granules in the cytoplasm.

The nucleus of the monocyte contained fine, lacy chromatin which stained a pinkish-violet. The shape of the nucleus varied from slightly irregular to deeply indented. The cytoplasm was abundant and stained grayish-blue. The cell membrane stained a deep blue. The monocyte was characterized by an irregular contour.

Hemograms of the Rats and Mice

After analysis of the data assembled from the differential leukocyte counts of the rats and mice that responded to the parenteral injection of Pseudomonas aeruginosa, the results were found to be too voluminous to be presented for each individual animal; therefore, this information obtained from the infected animals was averaged to establish the trend of distribution of the different forms of leukocytes in relation to time until normality was reestablished or death occurred. The Schilling hemograms were constructed and interpreted according to Gradwohl (1943), p. 347 and Todd and Sanford (1948), p. 254.

In constructing the hemograms for rats that recovered from an infection caused by the parenteral injection of Pseudomonas aeruginosa (Table 7), the entries were terminated at six days. Differential and total leukocyte counts obtained after this period were normal in the majority of the animals, while only a few animals experienced a protracted leukocytosis. At the time of

seven hours after injection, the hemogram indicates that the rats responded with a "degenerative blood picture" as revealed by the increase in stab neutrophiles. With continued infection, juvenile neutrophiles and a leukocytosis appeared within one day characterizing a "regenerative blood picture". The shift to the left receded and a right shift followed. A normal distribution of the white cells was observed by the sixth day.

Table 8 lists the data obtained from the differential blood counts of rats that failed to survive the parenteral injection of Pseudomonas aeruginosa. The proportion of cells at seven hours reveals a "regenerative blood picture" but the left shift persisted and the course of infection was ended with death. The terminal blood picture was one of neutrophilia, aneosinophilia, relative lymphopenia and marked leukocytosis.

Tables 9 and 10 listing the average differential count data for mice that survived or that died from the parenteral injection of Pseudomonas aeruginosa reveals hemograms that are essentially the same as for the rats.

Table 7. Hemogram reflecting the average total leukocyte counts and average values of the classes of leukocytes after the parenteral injection of Pseudomonas aeruginosa into rats in which the termination of the course of infection was recovery.

Time After Injection	WBC*	Eosinophiles#	Juvenile Leukocytes#	Stab Leukocytes#	Segmented Leukocytes#	Lymphocytes#	Monocytes#
Normal	15339	1	0.03	5	28	62	3
7 hours	29500		0	25	21	51	2.5
1 day	25143		3	12	46	37	3
2 days	24050		2	8	35	52	2
3 days	29083		2	10	43	42	3
4 days	28536		1	9	27	59	2
5 days	30750		2	7	27	61	2
6 days	16875		1	5	35	58	1

*Expressed as number per cmm
#Expressed as number per 100 leukocytes

Table 8. Hemogram reflecting the average total leukocyte counts and average values of the classes of leukocytes after parenteral injection of Pseudomonas aeruginosa into rats in which the termination of the course of infection was death.

Time After Injection:	WBC*	Eosinophiles#	Juvenile Leukocytes#	Stab Leukocytes#	Segmented Leukocytes#	Lymphocytes#	Monocytes#
Normal	14486	.9		4	27	65	3
7 hours	17650		2	33	33	30	2
1 day	23066		5	16	30	44	3
3 days	29906		5	17	40	34	2
4 days	50125		5	12	62	20	1.5
7 days	82850		5	16	62	16	1
9 days	100166		4	12	71	11	1
11 days	98000		3	13	73	8	1.5
14 days	103625		7	13	71	7	1.5

*Expressed as number per cmm
#Expressed as number per 100 leukocytes

Table 9. Hemogram reflecting the average total leukocyte counts and average values of the classes of leukocytes after parenteral injection of Pseudomonas aeruginosa into mice in which the termination of the infection was recovery.

Time After Injection	WBC*	Eosinophiles#	Juvenile Leukocytes#	Stab Leukocytes#	Segmented Leukocytes#	Lymphocytes#	Monocytes#
Normal	11350	1	1	6	29	57	4
8 hours	13770		3	10	43	39	5
1 day	17562		1	10	37	47	5
2 days	25666		2	12	54	26	5
3 days	19611		2	9	39	43	6
5 days	20438		2	6	52	37	4
7 days	20646		1	5	48	40	5
9 days	20417	0.5	1	4	44	48	2
10 days	25917		1	6	49	39	4
11 days	12208		0.5	4	50	43	4
12 days	12875		1	5	49	39	6
13 days	14250	1	1	8	34	50	7
16 days	15062	1	1	5	35	53	5
17 days	12750		1	7	28	59	5

*Expressed as number per cmm
#Expressed as number per 100 leukocytes

Table 10. Hemogram reflecting the average total leukocyte counts and average values of the classes of leukocytes after parenteral injection of Pseudomonas aeruginosa into mice in which the termination of the infection was death.

Time After Injection	WBC*	Eosinophiles#	Juvenile Leukocytes#	Stab Leukocytes#	Segmented Leukocytes#	Lymphocytes#	Monocytes#
Normal	11130	1	1	7	31	56	4
8 hours	12488		2	13	34	48	3
1 day	16279		1	11	42	41	4
2 days	27541		1	7	56	33	3
3 days	26678		4	16	42	31	7
5 days	35305		1	4	67	24	3
7 days	38434		1	5	74	18	4
9 days	34438		2	5	63	24	5
10 days	48833			6	68	22	4
11 days	27000		1	4	63	29	4
12 days	43000		2	4	75	8	11
13 days	37750		2	8	68	12	9
16 days	37583		1	6	69	16	7
18 days	56250		5	12	63	13	7
19 days	47250		8	12	55	12	12

*Expressed as number per cmm
#Expressed as number per 100 leukocytes

Neutrophilic Granulocyte Response of Rats and Mice

The response of the neutrophilic granulocytes to the parenteral injection of Pseudomonas aeruginosa was the most dramatic of any of the classes of leukocytes. The data for the rats is graphically illustrated in Figs. 2 and 3. The information presented is the average absolute number of the neutrophilic granulocytes per cmm observed at intervals for each route of injection throughout the entire course of the infections that terminated in death.

The injection of varied fatal doses of Pseudomonas aeruginosa by the subcutaneous, intraperitoneal, intramuscular and intracardial routes resulted in neutrophilic granulocytosis which varied only in degree in relation to time. The neutrophilic response to the fatal subcutaneous doses was a re-

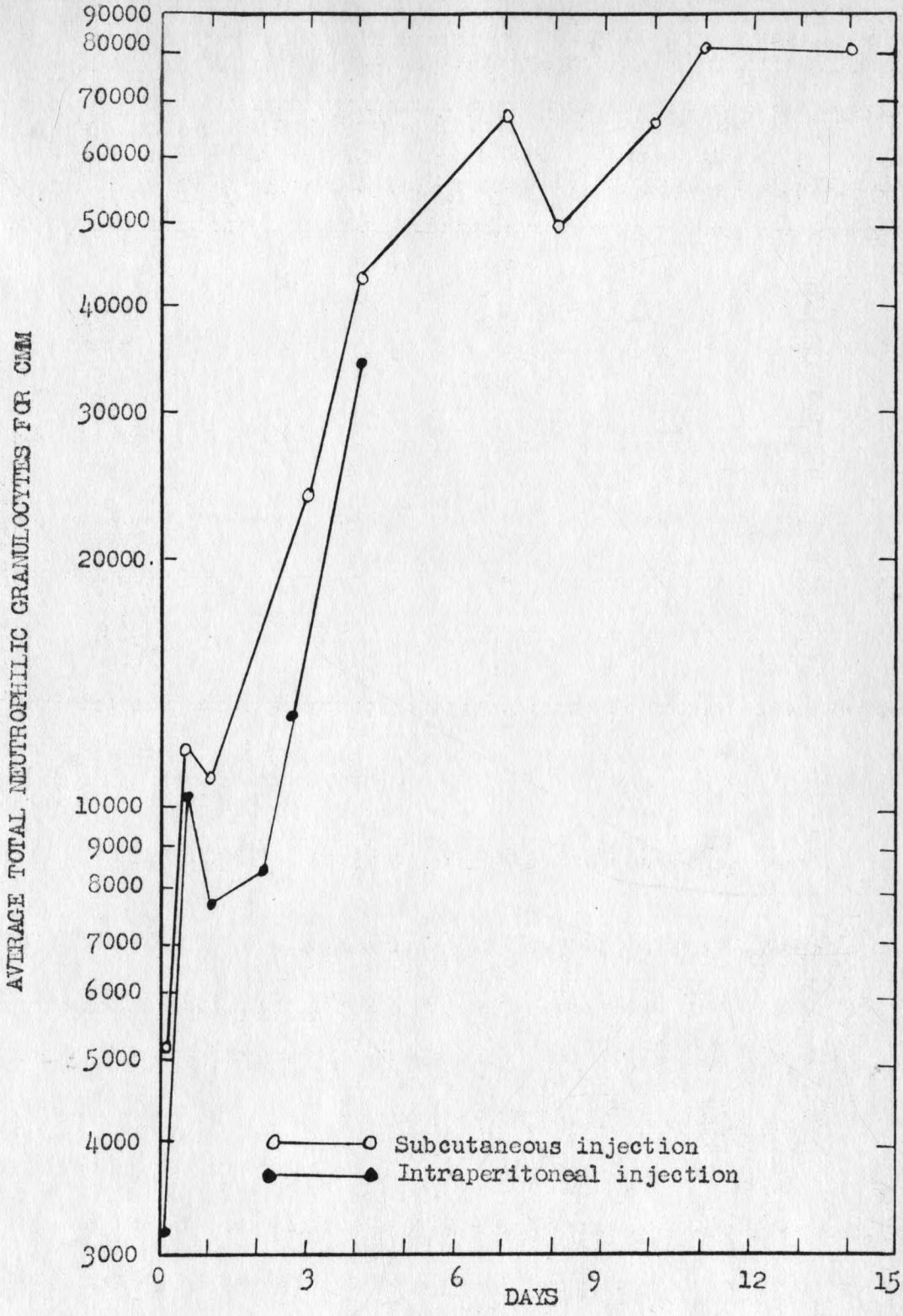


Fig. 2. The average total neutrophilic granulocyte response of rats to subcutaneous and intraperitoneal injection of varied, fatal doses of Pseudomonas aeruginosa.

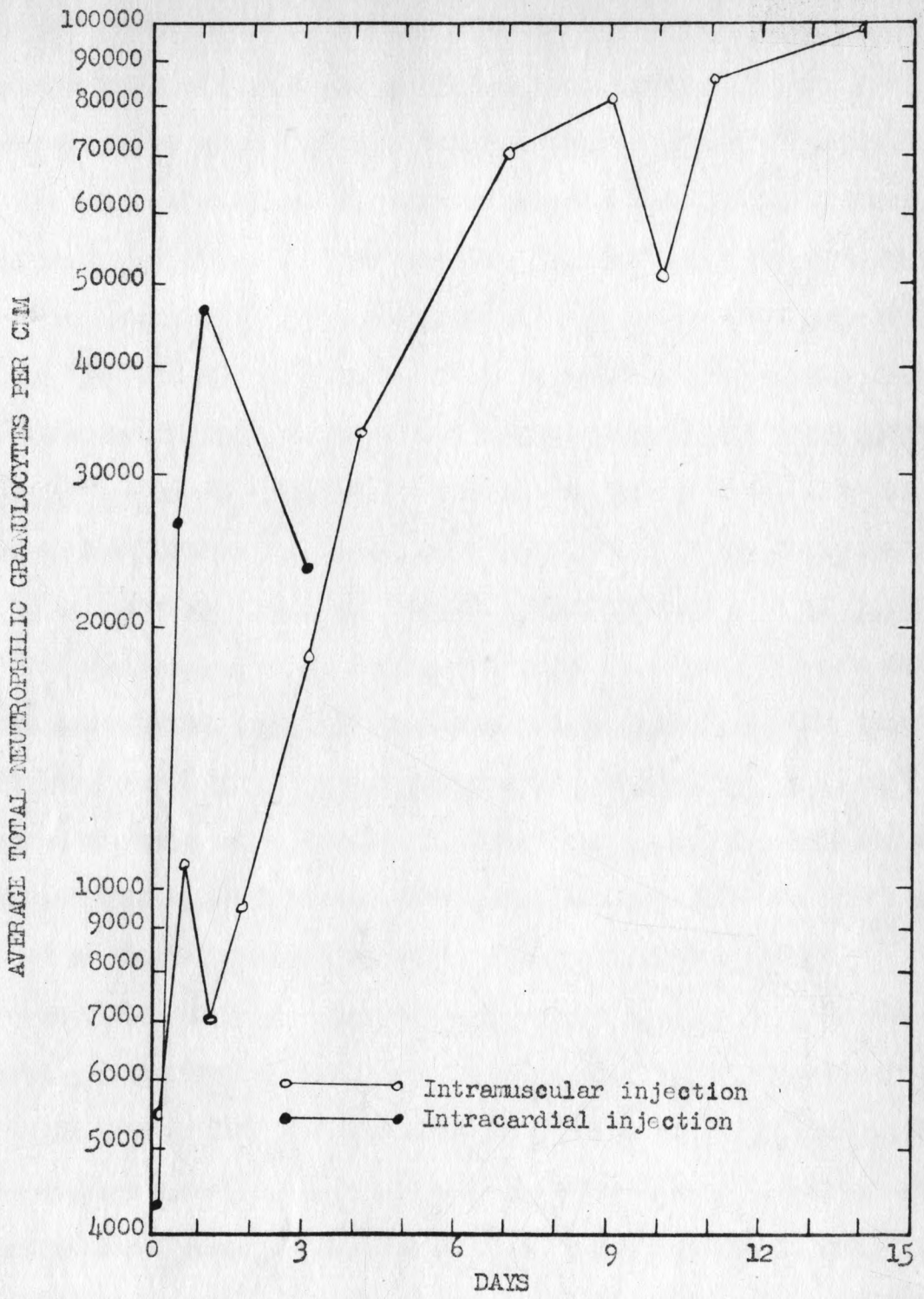


Fig. 3. The average total neutrophilic granulocyte response of rats to intramuscular and intracardial injections of varied, fatal doses of Pseudomonas aeruginosa.

sponse of a twofold increase at eight hours over an average normal absolute neutrophilic count of 5,380 cells. This response remained in the same range of absolute numbers of neutrophiles through the first day after injection. During the third day, a fivefold increase over the average normal absolute count of neutrophiles was observed and this increase continued with absolute counts of neutrophiles being in the range of 81,615 cells to 82,740 cells during the 11th through the 14th day after injection. The response of the neutrophiles to the intraperitoneal and intramuscular injection of Pseudomonas aeruginosa was essentially the same as for the subcutaneous route of injection. The intracardial injection of fatal doses of the organisms resulted in an immediate eightfold increase of neutrophiles over an average normal absolute count of 4,312 cells. The range of average absolute counts of 23,888 neutrophiles to 47,421 neutrophiles was maintained until death.

Figures 4 and 5 graphically illustrate the neutrophilic granulocyte response of the mice to fatal doses of Pseudomonas aeruginosa injected by the intraperitoneal, subcutaneous, intramuscular and intravenous routes. The method of listing the data is the same as used for the rats.

The injection of fatal doses of Pseudomonas aeruginosa via the intraperitoneal and intravenous routes resulted in a neutrophilic granulocytosis within eight hours. This was manifested by a threefold to fivefold increase over the average normal absolute counts. The general rise in average absolute neutrophilic count persisted throughout the course of infection until a range of an average absolute neutrophilic count of 44,000 cells to 53,000 cells was reached prior to death in 14 days to 23 days. The subcutaneous and intramuscular injection of fatal doses of the organism differed in that a neutrophilic granulocytopenia resulted at eight hours followed by a neutrophilia after one day and then the same general increase of neutrophilic

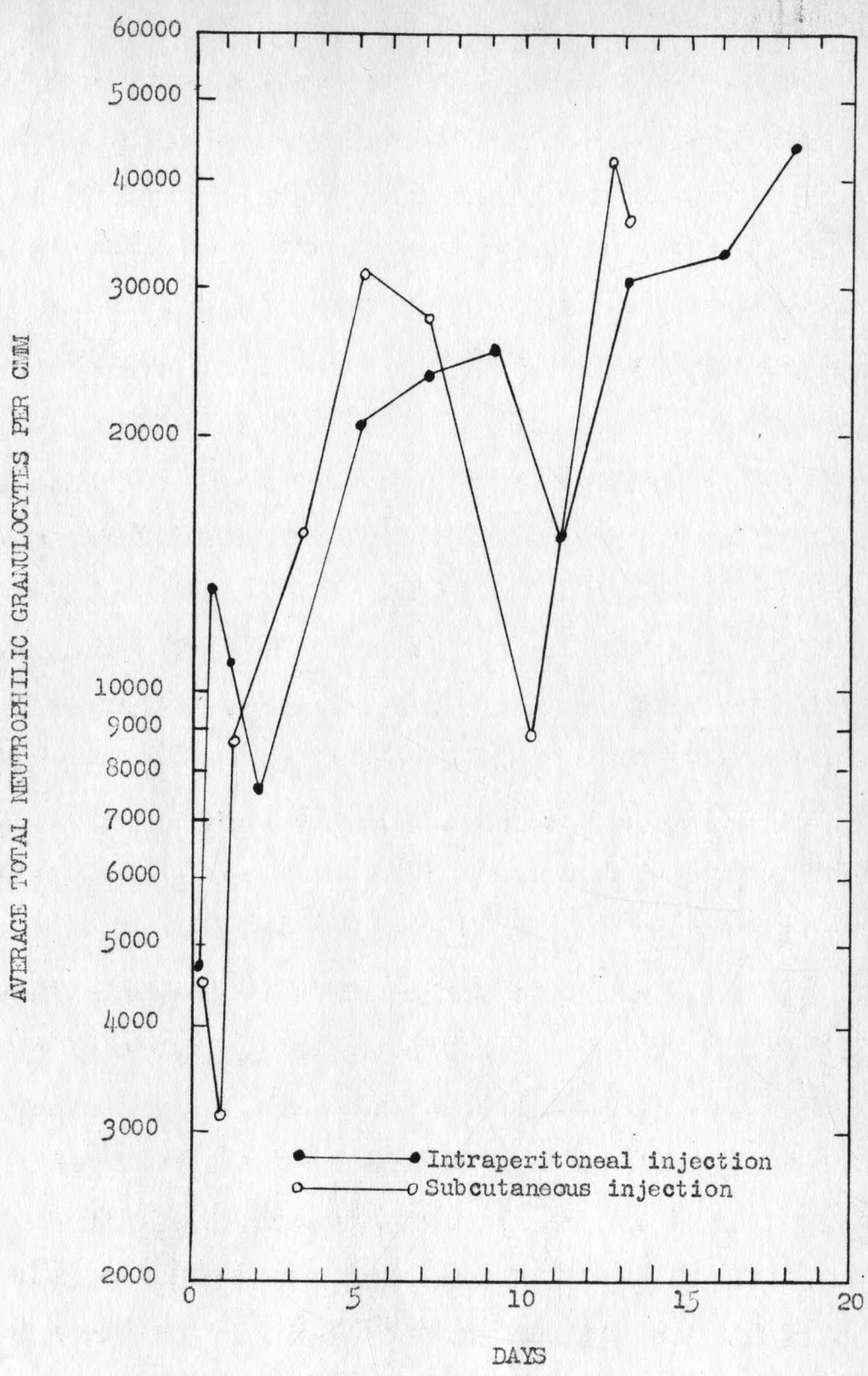


Fig. 4. The average total neutrophilic granulocyte response of mice to intraperitoneal and subcutaneous injections of varied, fatal doses of Pseudomonas aeruginosa.

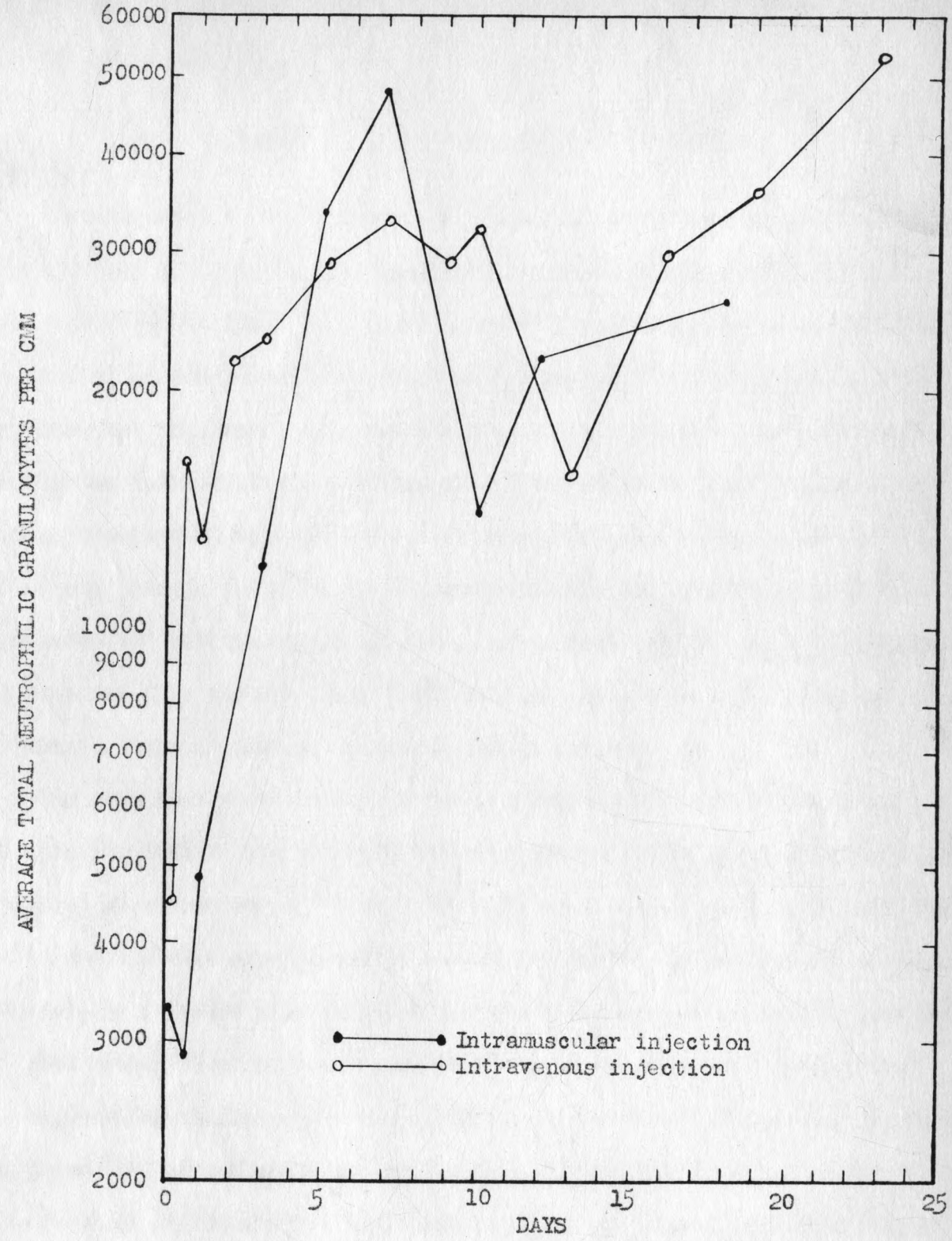


Fig. 5. The average total neutrophilic granulocyte response of mice to intramuscular and intravenous injections of varied, fatal doses of Pseudomonas aeruginosa.

granulocytes as observed after the intraperitoneal and intravenous injections of the organisms.

Pathogenicity, Symptoms and Morbid Anatomy

Massive doses of Pseudomonas aeruginosa in the amounts of 800 million, 1600 million and 3200 million organisms introduced intraperitoneally into rats caused death within the first day after injection. The rats exhibited symptoms of marked prostration and a mucoid secretion from the eyes. After the injection of lesser doses ranging from one million to 400 million organisms by the intraperitoneal, subcutaneous, intramuscular, and intracardial routes, the rats exhibited slight to moderate indications of infection and subsequent recovery or moderate to severe indications of infection which terminated in death in two to 15 days. After death, Pseudomonas aeruginosa was cultured from the heart and liver of each rat and from the sites of subcutaneous, intramuscular and intraperitoneal injections.

The rats that survived the intraperitoneal dose of one million, ten million, 100 million and 400 million organisms exhibited apathy, anorexia, dyspnea, and mucoid secretion from the eyes after the first day, through the fourth day and then experienced an uneventful recovery. The one rat that became fatally infected with a relatively small dose of ten million organisms and died within five days developed peritonitis characterized by serosanguinous, peritoneal fluid, fibrous adhesions and extreme inflammation of the peritoneum and serous membranes of the intestines. The five rats that received a dose of 400 million organisms by the same route died within four days. These rats were found lying stupefied on the floor of the cage. Upon necropsy, acute inflammation of the peritoneum and serous membranes of the intestines was observed. All other membranes and internal organs appear-

ed normal.

Two rats injected subcutaneously with a dose of 400 million Pseudomonas aeruginosa died within the first day after nonspecific symptoms of marked prostration and mucoid secretion from the eyes. Another rat receiving the same dose responded in a milder manner and after the first day a slight swelling was seen at the site of injection which became extremely swollen the third day. This condition continued through the seventh day. On the eighth day, the infection terminated in death. Autopsy revealed serosanguinous fluid between the skin and the fascia at the site of injection, pale friable liver and congestion of the spleen. The one rat that received a dose of 200 million organisms and recovered manifested the same symptoms except that the swelling persisted through the seventh day followed by suppuration (Plate II, Fig. 1). On the tenth day, an area the size of an equilateral triangular of approximately two cmm sloughed and the animal experienced a complete recovery. Two rats that received a dose of 200 million organisms and died showed the same symptoms as described for the previous rat until the seventh day when extreme tumefaction was observed over three-quarters of the animals' bodies. Inflammatory edema and cyanosis of the hind legs were observed in these animals. Suppuration appeared at the site of injection and these symptoms remained until death of one rat on the tenth day and the other on the 15th day. Post mortem examination revealed serosanguinous edema between the skin and fascia, a pale, friable liver and petechial hemorrhages of the epicardium. No other pathological lesions were observed.

Each of six rats were injected intramuscularly with varied doses of 200 million and 400 million Pseudomonas aeruginosa. One rat which received 200 million organisms exhibited a contracted, moderately swollen leg and death

EXPLANATION OF PLATE II

Fig. 1. Suppurating wound of a rat seven days after subcutaneous injection of 200 million Pseudomonas aeruginosa.

Fig. 2. Swollen right leg of a rat two days after intramuscular injection of 200 million Pseudomonas aeruginosa.

PLATE II

FIG. 1

FIG. 2

resulted within the first day. Another rat showed the same symptoms (Plate II, Fig. 2) for three days and then recovered. The third rat receiving 200 million organisms experienced the same symptoms through the first day and the leg became immobile due to pain and swelling by the third day. The leg became extremely edematous and cyanotic by the seventh day. This condition was so painful that the rat practiced autophagia (Plate III, Fig. 1). Necrosis appeared on the ninth day and dry gangrene developed by the 12th day. The condition of gangrenous myositis (Plate III, Fig. 2) was terminated by death within 15 days. Autopsy revealed moderate rigor mortis, thin, watery blood, emaciation and a pale, friable liver. The tail blood from this rat coagulated too rapidly on the first, third and fourth days to obtain a satisfactory sample; therefore, heart blood was withdrawn. Pseudomonas aeruginosa was isolated from the heart blood. The intramuscular injection of 400 million organisms into three rats resulted in death of two rats within 18 hours. The third rat died within seven days. Plate IV shows the circumscribed area of necrotic muscular tissue resulting from the intramuscular injection of 400 million organisms.

The intracardial injection of six rats with Pseudomonas aeruginosa resulted in rapid, toxic deaths. Of the three rats receiving a dose of 400 million organisms, two died the first day and the other died during the second day. A dose of 100 million organisms resulted in the death of one rat within the first day, another rat the second day and the third rat lived until the fourth day. The blood of the third rat was characterized by rapid coagulation.

Upon post mortem examination, the mice that succumbed to Pseudomonas aeruginosa infection revealed the same lesions as did the rats but in a lesser degree. Abscess formation of the kidneys was found in one mouse in-

EXPLANATION OF PLATE III

Fig. 1. (Left) A rat manifesting swelling of the site of subcutaneous injection of 400 million Pseudomonas aeruginosa.

(Right) View of a rat practicing autophagia twelve days after the intramuscular injection of 200 million Pseudomonas aeruginosa.

Fig. 2. Stages in the course of Pseudomonas aeruginosa infection after the intramuscular injection of 200 million organisms. (Right) Edematous and cyanotic right hind leg of a rat seven days after injection. (Left) Gangrenous myositis of the right hind leg twelve days after injection.

PLATE III

FIG. 1

FIG. 2

EXPLANATION OF PLATE IV

Circumscribed area of necrotic muscular tissues of the right hind leg of a rat seven days after the intramuscular injection of 400 million *Pseudomonas aeruginosa*.

PLATE IV

jected intraperitoneally. The organism was recovered from the heart and liver of all the mice that died and also from the abscessed kidneys.

Dosages of one million, ten million, 80 million, 100 million, 200 million, 400 million and 800 million injected intraperitoneally resulted in death of the mice within two and one-half hours to 18 hours. One mouse of the same group survived a dose of 40 million organisms for seven days. This mouse exhibited apathy, anorexia and toxemia one day prior to death. One mouse which was injected with a dose of eight million organisms and survived, exhibited a slight infection through the total and differential blood counts but remained active and showed no symptoms throughout the infection. Another mouse of this group remained active until the seventh day and then manifested anorexia, cachexia and death within eight days. Multiple abscesses of the kidneys were found during the post mortem examination.

Of the three mice that received an intraperitoneal injection of four million organisms, two mice survived and exhibited an infection according to the blood picture but remained active throughout the course of the disease. By blood examination, the other mouse showed marked leukocytosis within eight hours. This mouse remained active until the 13th day when it exhibited the usual symptoms of septicemia, and death occurred following the 18th day. On autopsy, the kidneys were found to be abscessed.

In response to the intramuscular injection of Pseudomonas aeruginosa, a dose of ten million organisms produced a marked toxemia and death of four mice within two days. When a dose of five million organisms was injected, two of the three mice lived through the second day. One mouse exhibited a moderately swollen leg and died after three days, and the other developed an edematous leg which turned cyanotic by the tenth day followed by suppuration the 12th day and returned to activity by the 17th day. Each of three

mice was injected with a dose of one million organisms. One mouse revealed no response either clincally or through hematologic examination. Two other mice became infected as shown by the blood picture but remained active. Both of the mice had ruffled coats and patchy, necrotic areas over the entire body by the 12th day. One of the two mice died on the 16th day and the other survived.

The subcutaneous injection resulted in severe to slight infections depending upon the size of the dosage. A dose of ten million Pseudomonas aeruginosa caused death in each of four mice within four days. Each mouse exhibited a contracted leg and mucoid secretion from the eyes by the eighth hour. Two mice died within 15 hours. One mouse showed an extreme, toxic reaction and died within 28 hours and the remaining mouse with the same reaction lived until the fourth day. Post mortem examination revealed an accumulation of serosanguinous fluid at the site of subcutaneous injection. A dose of five million organisms resulted in survival of one mouse and death to the other two within 14 days. Each of the three mice exhibited an infection by the end of the second day as revealed by the total and differential blood counts but remained active. On the sixth day, a moderate swelling at the site of injection was in evidence and the coats of the mice became ruffled. The swelling of the site of injection became extreme on the eighth day. A suppurating wound appeared on one mouse the ninth day, and recovery from the infection was evident by the 16th day. The area of swelling did not drain in the other two mice, and death resulted after 12 days and 14 days. Post mortem examination revealed an accumulation of blue-green pus between the skin and fascia and septicemic lesions of the internal organs. Of the three mice that received a dose of one million organisms, two revealed moderate hematological response and the other none. No clinic-

al symptoms were observed, and the three survived.

Eight mice were injected intravenously with two groups of four each receiving doses of two million and four million Pseudomonas aeruginosa. The four mice in the group that received a dose of four million organisms died. One day after injection, three of the mice became lethargic and mucoid secretion from the eyes developed. All were dead within two days from toxemia. After showing no clinical symptoms through the first day after injection, the remaining mouse of this group exhibited a severe encephalitis on the third day. While at rest, this condition was characterized by involuntary, jerky movements of the head and protruding, glassy eyes. Also, this mouse indicated itching of the head and eyes by scratching these parts with the hind foot. When the mouse was disturbed, it would circle alternately to the right then the left until exhausted and then assume the opisthotonos position before again exhibiting the symptoms shown at rest. When the animal was suspended by its tail, the body of the mouse would gyrate in a horizontal plane with the tail being the axis. This hypersensitive condition continued for three days after which the mouse became lethargic with only moderate, spasmodic movements of the head. This symptom with the additional one of a ruffled coat persisted from the sixth day until death within 19 days. Because of extremely rapid decomposition, only multiple abscessation of both kidneys was observed. Four mice received a dose of two million organisms, two of these exhibited clinical symptoms of apathy and mucoid secretion from the eyes during the second day after injection and then they made an uneventful recovery. One mouse receiving the same dosage became clinically ill on the second day revealing symptoms of lethargy, mucoid secretion from the eyes and ruffled coat and died within eight days. This mouse showed post mortem lesions of septicemia. The

fourth mouse that received a dose of four million organisms exhibited encephalitis in the same manner as previously described in that mouse that received a dose of two million organisms with the exception that it survived and continued to show hyperkinesis throughout an observation period of 47 days.

The macroscopic appearance of the abscessed kidneys from the mice that received intraperitoneal and intravenous injections of Pseudomonas aeruginosa was one of abscesses protruding above the capsule of the kidneys. The size of the abscesses ranged from pinpoint to one mm in diameter. The number of abscesses ranged from a few to so numerous that they appeared to be coalescent.

The microscopic examination revealed the following pathological conditions. The general state of the infection was one of early abscessation. There were numerous foci of bacterial infection on the surface and in the cortical and medullary portions of the kidneys. An overall infiltration of leukocytes was observed with the most numerous cells being polymorphonuclear leukocytes. Severe hemorrhage was found in the intertubular and intratubular spaces. Many tubules had dilated. In a moderate number of tubules, epithelial cells had undergone pyknosis and apparent fatty degeneration.

DISCUSSION

The average values of the leukocytes of normal rats and normal mice (Tables 3 and 4) obtained in this study are essentially the same as those reported by Gardner, (1947a, 1947b) as shown in Tables 1 and 2. The only divergence of consequence was in the average number of total leukocytes per cmm of blood of mice which was found to be approximately 3,000 cells

less. This is probably due to the relatively narrow age group of young mice used in this study in comparison to an overall average of leukocyte counts in which age was disregarded in Gardner's work.

As illustrated in Tables 5 and 6, the results of the average total white cell counts were not influenced by physiological "excitement" leukocytosis. The periodic, average, total leukocyte counts of the control rats and control mice remained within the normal range of 7,500 to 23,750 white blood cells per cmm for the normal rats and 6,000 to 15,000 white blood cells per cmm for normal mice. In addition, the injected rats and mice exhibited total leukocyte counts within the normal range for each species after a return to normalcy as confirmed by lack of the clinical symptoms of infection and normal hematological observations.

The summaries of the total leukocyte counts per cmm of blood of both rats and mice in response to the parenteral injection of Pseudomonas aeruginosa as shown in Tables 5 and 6 indicated that leukocytosis or leukopenia and survival or death are dependent upon the route of injection and the number of organisms in the inoculum. The intracardial and intramuscular injections of 400 million organisms into rats provoked a leukocytosis and death within two to four days. The subcutaneous and intraperitoneal injections of a like number of organisms caused a leukopenic reaction within seven hours to two days with subsequent leukocytosis and death. Two rats survived 400 million organisms injected intraperitoneally after they exhibited a leukocytosis within seven hours. Smaller doses in the amounts of one million, ten million, and 100 million intraperitoneally, 200 million subcutaneously and intramuscularly, and 100 million intracardially were followed by a leukocytosis and subsequent survival or death.

After intramuscular and subcutaneous inoculations of Pseudomonas

aeruginosa into mice, this pattern of an initial leukopenia and subsequent leukocytosis was comparable in the majority of animals to the response in rats. Ten million organisms injected subcutaneously and intramuscularly into mice caused leukopenia with a subsequent leukocytosis and death. When five million organisms were injected by the above two routes, four of six mice experienced leukopenia (with or without subsequent leukocytosis) and death. One of the six mice was an exception to this pattern. Leukopenia was observed but the mouse survived. The other mouse that survived revealed a definite leukocytosis. When one million, four million, eight million organisms were injected intraperitoneally, or two million and four million organisms were injected intravenously, or one million organisms were injected subcutaneously and intramuscularly, the response of the mice was one of leukocytosis (without an initial leukopenia) and survival or death.

From the above observations, an unfavorable prognosis is in order whenever a large enough number of Pseudomonas aeruginosa to reveal initial leukopenia are introduced into rats by the intraperitoneal, subcutaneous and intramuscular routes. This is likewise true for mice injected by the subcutaneous and intramuscular routes.

The total white cell count indicating leukopenia in the above animals was due to the injection of comparatively overwhelming doses of Pseudomonas aeruginosa and the high degree of positive chemotaxis of the phagocytes from the blood stream to the site of injection resulting in a temporary decrease in total number of circulating leukocytes in the majority of mice and a continued leukopenia until death in others. This reaction of the rats and mice probably explains the leukopenic response to Bacillus pyocyaneus (Pseudomonas aeruginosa) reported by Lovett (1924) and Dasse (1928). They

reported death of guinea pigs within 24 hours following injection of an undetermined number of organisms and leukocyte counts only through the period of 24 hours after injection. Also, this early leukopenic response to a comparatively large number of Pseudomonas aeruginosa may be a factor in the reported human hematological reactions of leukopenia when this organism was isolated as the causative agent of disease. The multiplication of the organism in primary foci may have been so rapid that it resulted in an overwhelming number of the organisms and death prior to the time that the human body had an opportunity to institute defensive leukopoiesis.

In Tables 5 and 6, it can be noted that the degree of leukocytosis in relation to time after injection is dependent upon the portal of entry and the number of Pseudomonas aeruginosa introduced into the normal rats and mice. The intracardial injection of 100 million and 400 million organisms resulted in a substantial rise in numbers of leukocytes in the circulating blood within seven hours. After the injection of the organisms in the same range of numbers by the intraperitoneal, subcutaneous and intramuscular routes, there is a comparatively slower rise in leukocytosis that does not reach a maximum number until three days when the rats survive or until approximately seven days or at the terminal stage of the infection when the rats succumb to the infections. In general, the same scheme of response of the leukocytes was observed in the mice. The intravenous injection of two million and four million organisms caused an initial leukocytosis within eight hours with a subsequent rise in number in those mice that died. In those that survived the intraperitoneal injection of two million organisms, the maximum degree of response of the leukocytes was not until the second day. The injection of one million, four million and eight million organisms by the intraperitoneal route and one million and five million organisms by

the intramuscular and subcutaneous routes was followed by a leukocytosis which was of a maximum at two days to seven days in those mice that survived and a maximum number of approximately seven days or at the time of death in those mice that died from the infection. These observations of leukocytosis initially or after a maximum period of three days subsequent to injection of Pseudomonas aeruginosa into rats and mice agrees with the reports of leukocytosis in humans by DeMuth and Rawson (1948), Coller and Dyer (1951), O'Brien (1950), Geppert et al (1952) and Waisbren and Hastings (1953).

The average results of the differential blood counts as presented in Tables 7, 8, 9 and 10 indicate that both the rats and mice respond to Pseudomonas aeruginosa infection in the same manner as do human beings and large animals to most acute infectious diseases and severe intoxications due to bacterial toxins. The rats and mice that recovered from the infections exhibited hemograms that are interpreted as being favorable at seven hours and eight hours, respectively. Leukocytosis is coupled with a "degenerative blood picture" in the rats and a "regenerative blood picture" is associated with leukocytosis in the mice. After one day, the redistribution of the leukocytes of the rats caused a change in the blood picture to one of a "regenerative blood picture". The retrogression of the existing shift to the left resulted in recovery of both the species. At the second day, the rats and mice that did not recover from the injection of Pseudomonas aeruginosa revealed hemograms that were essentially the same blood picture for both the species and the hemograms of the rats and mice that recovered from the infection. This continued blood picture of a shift to the left characterized by a subsequent aneosinophilia, lymphopenia, neutrophilia and leukocytosis can be considered as unfavorable from this study. This

terminal blood picture is comparable to the one termed a septic "acute leukemia" by Gradwohl (1943), p. 353.

In both the rats and mice, the different classes of leukocytes varied in their response to fatal doses of Pseudomonas aeruginosa. The lymphocytes were characterized by a relative lymphopenia, but the absolute count was within the normal range. The eosinophiles totally disappeared from the circulating blood. The relative and absolute numbers of the monocytes remained constant or decreased. The neutrophilic granulocytes increased both in relative and absolute number. The most numerous cell of the neutrophilic granulocytes was the segmented leukocytes which increased from an average normal of 33 percent to an average maximum of 73 percent in the rats and from an average normal of 31 percent to an average maximum of 74 percent in the mice.

This study indicates that after the injection of Pseudomonas aeruginosa into rats and mice in fatal doses not large enough to cause rapid, toxic deaths, the terminal blood picture is one characterized by neutrophilic leukocytosis. This observation is in disagreement with reports of human hematological reactions by Lovett (1924), Dasse (1928), Kline and Masche (1932), Epstein and Grossman (1933), Kearns (1936), and O'Brien (1950), and with reports in guinea pigs by Lovett (1924) and Dasse (1938); but it is in accordance with findings in humans by Wintrobe (1951), DeMuth and Rawson (1948), Coller and Dyer (1951), and Waisbren and Hastings (1953).

The morphological changes in the leukocytes observed during moderate and severe infections by Pseudomonas aeruginosa were not different from those described in other acute, infectious diseases; so therefore, no pathological alteration in the leukocytes was noted that could be considered as pathognomonic for a Pseudomonas infection.

Biological variations to the size of the dose of Pseudomonas aeruginosa appeared in each route of injection. Massive doses of 800 million, 1600 million and 3200 million organisms caused marked prostration and mucoid secretion from the eyes in rats and subsequent toxic death within one day. Smaller doses in the range of one million to 400 million organisms injected by the intraperitoneal, subcutaneous, intramuscular and intracardial routes provoked varied reactions from survival to septicemic death within two days to 15 days. After the intraperitoneal injection of one million organisms, the rat survived. A dose of ten million organisms caused one rat to die within five days and another to survive. After a dose of 100 million organisms, two rats survived. A dose of 400 million organisms resulted in the survival of two rats and the death of five within four days. The subcutaneous injection of 200 million Pseudomonas aeruginosa caused two rats to die within 15 days and one rat to survive. After a dose of 400 million organisms, the uniform results of death occurred in three rats within eight days. After the intramuscular injection of 200 million organisms, one rat survived and two died within 15 days. The dose of 400 million organisms caused death to three rats within eight days. Doses of 100 million and 400 million organisms via the intracardial route resulted in death to six rats within four days. The rats proved to be most susceptible to the organisms entering their bodies by the intracardial route. The second most susceptible route was intraperitoneal. Via the subcutaneous and intramuscular routes, the susceptibility to the organisms was essentially the same and the virulence of the organisms was less than by the intracardial and intraperitoneal routes.

In the mice, the same variation in susceptibility to varied doses of Pseudomonas aeruginosa occurred in the different routes of injection and

also from size of the doses within each route of injection. After intraperitoneal injection, one million, ten million, 80 million, 100 million, 200 million, 400 million and 800 million organisms caused the same symptoms of marked prostration and mucoid secretion from the eyes and subsequent death within one day. By the same route, one million organisms caused two mice to die within 12 days, four million organisms resulted in two mice surviving and one mouse dying within 18 days. The dose of eight million was followed by survival of one mouse and death to two mice within eight days. A relatively massive dose of 40 million organisms by the above route did not cause death in one mouse until eight days. The injection of two million organisms intravenously resulted in survival of three mice and death to one mouse within eight days. Four mice failed to survive a dose of four million organisms by the same route. After the intramuscular injection of one million, five million and ten million Pseudomonas aeruginosa varied responses resulted. Two mice survived and one mouse died within 17 days from a dose of one million organisms. After a dose of five million organisms, one mouse survived and two mice died within four days. A dose of ten million organisms resulted in no survivals. The findings after the subcutaneous injection were comparable to the above. A dose of one million organisms resulted in survival of three mice, five million organisms produced survival of three mice and death of one mouse within 14 days, and ten million organisms caused the death of four mice within four days. With minor exceptions, an increase in numbers of organisms caused earlier deaths in both the rats and mice.

Even though a majority of the test animals died from the organisms, relatively large doses were necessary to accomplish this action. Another evidence of the low pathogenicity of this organism for normal, healthy rats

and mice is that not one of the controls exhibited symptoms or hematological evidence of an infection. These control animals shared common drinking tubes and food racks and huddled together in close contact with the suppurating wounds.

In general, the rats and mice revealed different clinical symptoms to infection by Pseudomonas aeruginosa. After infection as substantiated by the blood picture, the rats would exhibit marked prostration and anorexis. During an infection comparable to that affecting the rats, most mice would remain active until approximately one day prior to death. In both the rats and the mice, massive doses of Pseudomonas aeruginosa caused death by an extreme toxemia. In the case of smaller doses of organisms, the cause of death was acute septicemia either introduced directly into the blood stream during the experiment or from massive seeding from primary foci. Absorption of toxic products formed in the necrotic lesions was a contributing factor to death in these animals that developed a gangrenous myositis.

After death of the animals as the result of this study, the organism was cultured from the heart and liver, from the site of intraperitoneal, subcutaneous and intramuscular sites of injection and from the abscessed kidneys of the mice. This lends evidence to the fact that the host is seeded with Pseudomonas aeruginosa from primary foci of infection.

The resultant encephalitis in two mice injected via the intravenous route confirmed the findings of a similar condition termed "rolling disease" by Gorrill (1952). The symptoms and course of the disease in this study were similar with the exception that no movements were observed by the author that could be interpreted as rolling, and one mouse recovered from the infection as observed by a normal blood picture. Although this latter

mouse recovered from the infection, it continued to show symptoms of encephalitis throughout an observation period of 47 days.

Admittedly, the recovery of one mouse from the acute infection with continued derangement is not conclusive, but this result may be a clue to permanent damage caused by Pseudomonas aeruginosa in man and animals and is worthy of further consideration.

After intraperitoneal and intravenous injections, the observation of abscessed kidneys and confirmation by microscopic examination adds credulence to the fact that all cases of nephritis caused by Pseudomonas aeruginosa are not of the ascending type but may orininate hematogenically.

SUMMARY

The parenteral injection of Pseudomonas aeruginosa Northern Regional Reserve Laboratory strain B23 into rats and mice resulted in responses of leukopenia or leukopenia with subsequent leukocytosis or leukocytosis depending upon the route of injection and the number of organisms in the inoculum. In the rats, the introduction of 400 million organisms via the intraperitoneal and subcutaneous routes resulted in an initial leukopenic response with subsequent leukocytosis and death. Doses of one million, ten million, 100 million and 400 million organisms intraperitoneally, 100 million and 400 million organisms intracardially and 200 million organisms intramuscularly and subcutaneously provoked a leukocytosis and survival or death. Similar responses were observed in the mice. Ten million organisms injected subcutaneously and intramuscularly caused leukopenia with subsequent leukocytosis and death. When five million organisms were injected via the above two routes, the result was leukopenia with or without leukocytosis and death in four of six mice. The injection of one million, four million, eight

million organisms intraperitoneally, two million and four million organisms intravenously, and one million organisms subcutaneously and intramuscularly resulted in an initial leukocytosis and survival or death.

The degree of leukocytosis in relation to time is dependent upon the portal of entry and the number of organisms. In the rats, the intracardial injection of 100 million and 400 million organisms resulted in a substantial rise in numbers of leukocytes within seven hours. After injection of the organism in the same range of numbers by the intraperitoneal, subcutaneous and intramuscular routes, there was a comparatively slower rise in leukocytosis that did not reach a maximum number until three days when the rats survived and approximately seven days or at terminal stage of the infection when the rats succumbed. A comparable response of the leukocytes was observed in the mice. The intravenous injection of two million and four million organisms provoked an initial leukocytosis within eight hours with a subsequent rise in numbers in those mice that died. In those that survived the intraperitoneal injection of two million organisms, the maximum degree of response was not until the second day. The injection of one million, four million and eight million organisms via the intraperitoneal route and one million and five million organisms by the intramuscular and subcutaneous routes resulted in leukocytosis which was of a maximum at two days to seven days in the mice that survived and a maximum number at approximately seven days or at the time of death in those mice that died from the infection.

After the parenteral injection of fatal doses of Pseudomonas aeruginosa, the differential blood counts revealed a blood picture of aneosinophilia, lymphopenia and neutrophilia. The increase in leukocytes can be termed a neutrophilic leukocytosis. Relative numbers of the neutrophiles of the rats

and mice were in the maximum range of 73 percent to 74 percent.

During moderate and severe infections caused by Pseudomonas aeruginosa, no pathological alterations of the leukocytes were observed that could be considered pathognomonic for Pseudomonas infection.

Massive doses of 800 million, 1600 million and 3200 million organisms injected into rats and 80 million, 100 million, 200 million, 400 million and 800 million organisms injected into mice resulted in death within the first day. Lesser doses by the various routes of injection caused septicemic deaths of the rats and mice in from two days to 23 days or manifestations of infections and survival.

As the result of parenteral injection of Pseudomonas aeruginosa, gangrenous lesions of the skin were observed in ten percent of the rats and five percent of the mice. Abscess formation of the kidneys was found in two mice injected by the intraperitoneal route and in one mouse injected by the intravenous route indicating nephritis of hematogenic origin. Via the intravenous route, two mice revealed symptoms of encephalitis. One of these mice died in 23 days. Although the blood picture had returned to normal, the other mouse continued to manifest hyperkinesis during an observation period of 47 days.

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APPENDIX

Table 11. Total leukocyte count per cmm of blood from rats injected intraperitoneally with varied doses of Pseudomonas aeruginosa.

Rat No.	Age (Days)	Sex	Weight (Grams)	Dosage (Millions of cells)	White cell count on days following injection: Normal	7 hours	1 day	2 days	3 days	4 days	6 days	7 days	11 days	Remarks
16C	65	F	178	1 ml saline	15250	14500	16250	20250		13250	16750	13250	17250	Survived
1B	62	M	170	1 ml saline	15250		20500	18750	17250	21500				Survived
9A	58	M	170.5	4 ml saline	16250		18000	15750	17500	18000	17250			Survived
4AA	63	F	189	4 ml saline	16250	17250								Survived
6A	48	M	177.5	1	8500		20250	23250	11500	18750	18250	12500		Survived
5A	48	M	178	10	14250		27750	23250	23250	53000				Died within 5 days
11B	62	M	179	10	15000		17750	18750		17250				Survived
12A	58	M	178	100	8250		16750	17250	20750	22500	10500			Survived
8B	62	F	168	100	21250		20750	27250	12250	22250				Survived
6C	56	M	159	400	15250	31000	26750	24000	30000	22750		12750	18250	Survived
4C	56	M	176	400	22750	26250	52250	28500	22500	38750		28500	23000	Survived
3A	58	M	179.5	400	17500		12000	12250	25000					Died within 4 days
5C	56	F	120	400	18250	14500	4000							Died within 2 days
7D	56	F	174	400	13500	16750								Died within 1 day
4B	62	F	165	400	11750									Died within 1 day
1AA	63	F	175	400	23750									Died within 1 day
2B	62	M	185	800	18750									Died within 1 day
5AA	63	F	183.5	800	19500									Died within 1 day
3AA	63	F	189	1600	16250									Died within 1 day
2AA	63	M	221.5	3200	10750									Died within 1 day

Table 12. Total leukocyte count per cmm of blood from rats injected subcutaneously with varied doses of Pseudomonas aeruginosa.

Rat No.	Age (Days)	Sex	Weight (Grams)	Dosage (Millions of cells)	White cell count on days following injection: Normal	7 hours	1 day	3 days	4 days	7 days	9 days	10 days	11 days	14 days	Remarks
7B	56	M	222	.5 ml saline	10750	13500	17500			16000			16000		Survived
4D	62	F	147	200	15250	12500	22750	44500	49750	74250	71250	83000	105000	88500	Died within 15 days
34D	62	F	147	200	18250	34250	19260	60250	53500	42750	33750				Died within 10 days
14D	62	F	147	200	18250	36500	25250	27750	46500	45500	136000	24500	10500		Survived
10C	56	F	123	400	13750	5250	17750	38250	70250	129000					Died within 8 days
5C	56	F	147	400	18250	7500	10750								Died second day
3C	56	M	172	400	15500	8500	20500								Died second day

Table 13. Total leukocyte count per cmm of blood from rats injected intramuscularly with varied doses of Pseudomonas aeruginosa.

Rat No.	Age (Days)	Sex	Weight (Grams)	Dosage (Millions of cells)	White cell count on days following injection: Normal	7 hours	1 day	3 days	4 days	7 days	9 days	10 days	11 days	14 days	Remarks
7C	56	F	165	.5 ml saline	16250	17500	14500		16750	15500					Survived
19D	71	F	153.5	200	13250	26500	20000	29500	21500	14250	23250	15700	24000	12750	Survived
9D	74	F	163.5	200	16250	11250	15750*	16750*	16250*	75250	93250	52250	91000	118750	Died within 14 days
6D	68	F	165.5	200	15000	14500	8500								Died within 1 day
14C	56	M	178.5	400	18250	19000	23000	36000	65000	90250					Died within 8 days
3C	56	F	143	400	15250	32250	13500								Died within 2 days
1C	56	F	164	400	22750	28500									Died within 1 day

* Heart blood

Table 14. Total leukocyte count per cmm of blood from rats injected intracardially with varied doses of Pseudomonas aeruginosa.

Rat No.	Age (Days)	Sex	Weight (Grams)	Dosage (Millions of cells)	White cell count on days following injection: Normal	7 hours	1 day	3 days	4 days	7 days	9 days	Remarks
16D	70	M	211	.5 ml saline	11250	19500	16750	12750	15750			Survived
24D	55	F	131.5	100	15000	50750	69000	27750*				Died within 4 days
2D	55	F	137	100	12750	16750	87500					Died within 2 days
1D	55	F	121	100	12750	7250						Died first day
37C	65	M	206.5	400	12250	45250	66760					Died within 2 days
9C	56	M	167	400	20250							Died within 5 hours
7C	56	F	122	400	17500							Died within 5 hours

* Heart blood

Table 17. Total leukocyte count per cmm of blood from mice injected intramuscularly with varied doses of Pseudomonas aeruginosa.

Mouse No.	Age (Days)	Sex	Weight (Grams)	Dosage (Millions of cells)	White cell count on days following injection: Normal	8 hours	1 day	3 days	5 days	7 days	10 days	12 days	16 days	Remarks
5C	63	M	23	.25 ml saline	12250	11250	18750	11500	17500	15750	11500	13500	9500	Survived
3C	63	M	22	1	13500	5250	8250	23500	32250	26250	25250	18750	16250	Survived
1C	63	M	23	1	10750	14250	21000	35750	46000	63750	35250	30250	30500	Died within 17 days
11C	63	M	23	1	12500	16500	13000	12250	11500	6750	12000	9750	16000	Survived
14C	41	F	21	5	14500	7750	15250	19250	23250	20500	27750	17250	16750	Survived
2C	41	F	20.5	5	8500	3500	7500	5000						Died within 4 days
7C	41	F	21.5	5	11250	5250								Died within 1 day
19C	63	F	22	10	11500	4100	2500							Died within 2 days
13C	63	F	21	10	14500	7500	8000							Died within 2 days
16C	63	M	21	10	9500	6000								Died within 1 day
12C	63	F	21.5	10	10000	9250								Died within 1 day

Table 18. Total leukocyte count per cmm of blood from mice injected subcutaneously with varied doses of Pseudomonas aeruginosa.

Mouse No.	Age (Days)	Sex	Weight (Grams)	Dosage (Millions of cells)	White cell count on days following injection: Normal	8 hours	1 day	3 days	5 days	7 days	10 days	12 days	13 days	17 days	Remarks
10C	45	M	20.5	.25 ml saline	8250	11000	13000	12750	11250	15750	13500	10250			Survived
6C	45	F	17	1	8000	11000	12750	14500	20000	17500	15500	12000			Survived
8C	45	F	21	1	13000	18000	22750	23000	14250	20000	13750	11500			Survived
1C	45	F	20.5	1	10500	11500	13000	19750	10750	9500	12000	9250			Survived
3C	45	M	23	5	10250	13500	26750	25250	45000	37500	70000	43500	54500		Died within 14 days
9C	45	M	23	5	13250	14000	26500	21000	34500	54500	67750	28500	22750	12750	Survived
7C	45	F	20.5	5	12750	4750	12750	26500	31000	33250	41250	55000			Died within 13 days
2C	42	M	20.5	10	9750	9250	14000	28250							Died within 4 days
5C	42	M	20.5	10	11000	7000	4750								Died within 1 day
14C	42	F	21	10	14500	8750									Died within 2 days
4C	42	F	20	10	11750	8750									Died within 1 day

Table 17. Total leukocyte count per cmm of blood from mice injected intramuscularly with varied doses of Pseudomonas aeruginosa.

Mouse No.	Age (Days)	Sex	Weight (Grams)	Dosage (Millions of cells)	White cell count on days following injection: Normal	8 hours	1 day	3 days	5 days	7 days	10 days	12 days	16 days	Remarks
5C	63	M	23	.25 ml saline	12250	11250	18750	11500	17500	15750	11500	13500	9500	Survived
3C	63	M	22	1	13500	5250	8250	23500	32250	26250	25250	18750	16250	Survived
1C	63	M	23	1	10750	14250	21000	35750	46000	63750	35250	30250	30500	Died within 17 days
11C	63	M	23	1	12500	16500	13000	12250	11500	6750	12000	9750	16000	Survived
14C	41	F	21	5	14500	7750	15250	19250	23250	20500	27750	17250	16750	Survived
2C	41	F	20.5	5	8500	3500	7500	5000						Died within 4 days
7C	41	F	21.5	5	11250	5250								Died within 1 day
19C	63	F	22	10	11500	4100	2500							Died within 2 days
13C	63	F	21	10	14500	7500	8000							Died within 2 days
16C	63	M	21	10	9500	6000								Died within 1 day
12C	63	F	21.5	10	10000	9250								Died within 1 day

Table 18. Total leukocyte count per cmm of blood from mice injected subcutaneously with varied doses of Pseudomonas aeruginosa.

Mouse No.	Age (Days)	Sex	Weight (Grams)	Dosage (Millions of cells)	White cell count on days following injection: Normal	8 hours	1 day	3 days	5 days	7 days	10 days	12 days	13 days	17 days	Remarks
10C	45	M	20.5	.25 ml saline	8250	11000	13000	12750	11250	15750	13500	10250			Survived
6C	45	F	17	1	8000	11000	12750	14500	20000	17500	15500	12000			Survived
8C	45	F	21	1	13000	18000	22750	23000	14250	20000	13750	11500			Survived
1C	45	F	20.5	1	10500	11500	13000	19750	10750	9500	12000	9250			Survived
3C	45	M	23	5	10250	13500	26750	25250	45000	37500	70000	43500	54500		Died within 14 days
9C	45	M	23	5	13250	14000	26500	21000	34500	54500	67750	28500	22750	12750	Survived
7C	45	F	20.5	5	12750	4750	12750	26500	31000	33250	41250	55000			Died within 13 days
2C	42	M	20.5	10	9750	9250	14000	28250							Died within 4 days
5C	42	M	20.5	10	11000	7000	4750								Died within 1 day
14C	42	F	21	10	14500	8750									Died within 2 days
4C	42	F	20	10	11750	8750									Died within 1 day

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Pseudomonas aeruginosa, which generally is considered to be harmless, is actually a dangerous organism causing serious and even fatal diseases under certain conditions.

The organism is not invasive or aggressive but rather an "opportunist" which most frequently attacks an undernourished or debilitated host and affects the very young most severely.

Pseudomonas aeruginosa has demonstrated unquestionable pathogenicity in the production of fatal meningitis, endocarditis, pneumonia and septicemia as well as local suppuration in otitis, arthritis, osteomyelitis, superficial wounds and burns. The organism has been introduced into the urinary tract when using instruments, into the meninges during lumbar puncture and into surgical wounds during dressing.

Changes in the absolute number of leukocytes and their relative proportions are of great significance as a measure of the responses of the body to invading organisms. In many instances, the alteration of the absolute and relative leukocyte count and of leukocyte morphology are of such a character that the organism may be immediately suspected and treatment may be initiated at once. The early diagnosis of systemic diseases caused by Pseudomonas aeruginosa is essential because the termination is often fatal, and the present-day therapy is specific.

The contradictory reports of clinical hematological observations and the relative paucity of controlled experimental infections in laboratory animals prompted this investigation into the hematological response of rats and mice to Pseudomonas aeruginosa.

The organism used in this study was Pseudomonas aeruginosa, Northern Regional Research Laboratory strain B23. Washed, enumerated suspensions of the organisms in sterile, physiological saline were parenterally intro-

duced into normal rats and mice. The routes of injection were: subcutaneous, intramuscular and intraperitoneal in both the rat and the mouse; intracardial in the rat; and intravenous in the mouse. Throughout the course of the response to Pseudomonas aeruginosa by the different routes and in varied doses, the rats and mice were observed for clinical symptoms of infection and periodic, hematological examinations were completed. The total leukocyte and the differential blood counts were performed in accordance with standard methods. After death of the experimental animals, an autopsy was conducted. The gross pathological lesions of the tissues, internal organs and membranes were observed. The abscessed kidneys of mice were microscopically examined. The lesions, heart and liver were cultured to recover the causative agent of death.

The parenteral injection of Pseudomonas aeruginosa into rats and mice resulted in responses of leukopenia or leukopenia with subsequent leukocytosis or leukocytosis depending upon the route of injection and the number of organisms in the inoculum. In the rats, the introduction of 400 million organisms via the intraperitoneal and subcutaneous routes resulted in an initial leukopenic response with subsequent leukocytosis and death. Doses of one million, ten million, 100 million and 400 million organisms intracardially and 200 million organisms intramuscularly and subcutaneously provoked a leukocytosis and survival or death. Similar responses were observed in the mice. Ten million organisms injected subcutaneously and intramuscularly caused leukopenia with subsequent leukocytosis and death. When five million organisms were injected via the above two routes, the result was leukopenia with or without leukocytosis and death in four of six mice. The injection of one million, four million, eight million organisms intraperitoneally, two million and four million organisms intravenously, and one

million organisms subcutaneously and intramuscularly resulted in an initial leukocytosis and survival or death.

The degree of leukocytosis in relation to time is dependent upon the portal of entry and the number of organisms. In the rats, the intracardial injection of 100 million and 400 million organisms resulted in a substantial rise in numbers of leukocytes within seven hours. After the injection of the organisms in the same range of numbers by the intraperitoneal, subcutaneous and intramuscular routes, there was a comparatively slower rise in leukocytosis that did not reach a maximum number until three days when the rats survived and approximately seven days or at terminal stage of the infection when the rats succumbed. A comparable response of the leukocytes was observed in the mice. The intravenous injection of two million and four million organisms provoked an initial leukocytosis within eight hours with a subsequent rise in numbers in those mice that died. In those that survived the intraperitoneal injection of two million organisms, the maximum degree of response was not until the second day. The injection of one million, four million and eight million organisms via the intraperitoneal route and one million and five million organisms by the intramuscular and subcutaneous routes resulted in leukocytosis which was of a maximum at two to seven days in the mice that survived and a maximum number at approximately seven days or at the time of death in those mice that died from the infection.

After the parenteral injection of fatal doses of Pseudomonas aeruginosa, the differential blood counts revealed a blood picture of aneosinophilia, lymphopenia and neutrophilia. Relative numbers of the neutrophiles of the rats and mice were in the maximum range of 73 percent to 74 percent. The increase in leukocytes may be termed a neutrophilic leukocytosis.

During moderate and severe infections caused by Pseudomonas aeruginosa,

no pathological alterations of the leukocytes were observed that could be considered pathognomonic for Pseudomonas infection.

Massive doses of 800 million, 1600 million and 3200 million organisms injected into rats and 80 million, 100 million, 200 million, 400 million and 800 million organisms injected into mice resulted in death within the first day. Lesser doses by the various routes of injection caused septicemic deaths of the rats and mice in from two to 23 days or manifestations of infections and survival.

As a result of parenteral injection of Pseudomonas aeruginosa, gangrenous lesions of the skin were observed in ten percent of the rats and five percent of the mice. Abscess formation of the kidneys was found in two mice injected by the intraperitoneal route and in one mouse injected by the intravenous route indicating nephritis of hematogenic origin. Via the intravenous route, two mice revealed symptoms of encephalitis. One of these mice died in 29 days. Although the blood picture had returned to normal, the other mouse continued to manifest hyperkinesis during an observation period of 47 days.

Table 15. Total leukocyte count per cmm of blood from mice injected intraperitoneally with varied doses of *Pseudomonas aeruginosa*.

Mouse No.	Age (Days)	Sex	Weight (Grams)	Dosage (Millions of cells)	White cell count on days following injection: Normal	8 hours	1 day	2 days	5 days	7 days	9 days	11 days	13 days	16 days	18 days	Remarks
1D	43	F	22	.5 ml saline	13250	12000	11500	11250	10250			11500				Survived
7A	41	M	18	.25 ml saline	8250		11750		15000	10250						Survived
5B	57	M	23	1 ml saline	13000		11500	13250	11000		11500	13250				Survived
3B	57	F	22	1	8500		13500	22250	7750		12000	8750				Died within 12 days
1A	41	F	16.5	1	15500											Died within 1 day
2D	43	M	23	4	9500	12000	29500	25750	24500	28000	28750	12750	13000			Survived
5D	43	M	21	4	9250	14500	12750	39500	15000	14750	28750	14500	15500			Survived
4D	43	M	21	4	12250	30000	20250	18500	13000	32000	32250	35250	37000	41250	56250	Died within 19 days
8D	43	M	23	8	11500	9250										Died within 1 day
10D	43	M	22.5	8	11250	11500	22500	21250	53750	32500	58500					Died within 10 days
11D	43	M	22	8	12250	13750	17500	21250	17250	9500	17000	9250	12750			Survived
2A	41	M	17.5	10	8250											Died within 1 day
3A	41	M	18	40	13500		22250		42750	29750						Died within 8 days
4A	41	M	18.5	80	11000											Died within 1 day
2B	57	M	23	100	11750											Died within 1 day
5A	41	F	17	100	13250											Died within 1 day
6A	41	F	16.5	200	6000											Died within 1 day
1B	57	F	20.5	400	8750											Died within 1 day
6B	57	F	22	800	8250											Died within 1 day

Table 16. Total leukocyte count per cmm of blood from mice injected intravenously with varied doses of *Pseudomonas aeruginosa*.

Mouse No.	Age (Days)	Sex	Weight (Grams)	Dosage (Millions of cells)	White cell count on days following injection: Normal	8 hours	1 day	2 days	3 days	5 days	7 days	9 days	11 days	13 days	16 days	19 days	23 days	Remarks
7D	57	M	22.5	.25 ml saline	12750	9250		9750	11250	10500			12500				11750	Survived
19D	55	M	22.5	2	10750	20750	18750	26000	29500	16750	14500	13500	9000	11500	17500	19000	17500	Survived
6D	55	M	22	2	11500	22250	19750	23250	14500	11750	9750	13250	11000	12750				Survived
5D	55	F	20	2	10250	16500	14000	18250	11500	25000	23000	21250	16750	11500				Survived
4D	55	F	21.5	2	9000	21250	31000	58500	28250	55750	34750							Died within 9 days
13D	55	F	21.5	4	11000	24500	10250	24500	27750	22750	44000	35000	37000	21750	37500	42250	61250	Died within 24 days
1D	55	F	22	4	9750	13250	17500	20250										Died within 3 days
2D	55	F	22	4	13000	29750	22250											Died within 2 days
3D	55	M	23	4	11250	21000	19250											Died within 2 days

Table 17. Total leukocyte count per cmm of blood from mice injected intramuscularly with varied doses of Pseudomonas aeruginosa.

Mouse No.	Age (Days)	Sex	Weight (Grams)	Dosage (Millions of cells)	White cell count on days following injection: Normal	8 hours	1 day	3 days	5 days	7 days	10 days	12 days	16 days	Remarks
5C	63	M	23	.25 ml saline	12250	11250	18750	11500	17500	15750	11500	13500	9500	Survived
3C	63	M	22	1	13500	5250	8250	23500	32250	26250	25250	18750	16250	Survived
1C	63	M	23	1	10750	14250	21000	35750	46000	63750	35250	30250	30500	Died within 17 days
11C	63	M	23	1	12500	16500	13000	12250	11500	6750	12000	9750	16000	Survived
14C	41	F	21	5	14500	7750	15250	19250	23250	20500	27750	17250	16750	Survived
2C	41	F	20.5	5	8500	3500	7500	5000						Died within 4 days
7C	41	F	21.5	5	11250	5250								Died within 1 day
19C	63	F	22	10	11500	4100	2500							Died within 2 days
13C	63	F	21	10	14500	7500	8000							Died within 2 days
16C	63	M	21	10	9500	6000								Died within 1 day
12C	63	F	21.5	10	10000	9250								Died within 1 day

Table 18. Total leukocyte count per cmm of blood from mice injected subcutaneously with varied doses of Pseudomonas aeruginosa.

Mouse No.	Age (Days)	Sex	Weight (Grams)	Dosage (Millions of cells)	White cell count on days following injection: Normal	8 hours	1 day	3 days	5 days	7 days	10 days	12 days	13 days	17 days	Remarks
10C	45	M	20.5	.25 ml saline	8250	11000	13000	12750	11250	15750	13500	10250			Survived
6C	45	F	17	1	8000	11000	12750	14500	20000	17500	15500	12000			Survived
8C	45	F	21	1	13000	18000	22750	23000	14250	20000	13750	11500			Survived
1C	45	F	20.5	1	10500	11500	13000	19750	10750	9500	12000	9250			Survived
3C	45	M	23	5	10250	13500	26750	25250	45000	37500	70000	43500	54500		Died within 14 days
9C	45	M	23	5	13250	14000	26500	21000	34500	54500	67750	28500	22750	12750	Survived
7C	45	F	20.5	5	12750	4750	12750	26500	31000	33250	41250	55000			Died within 13 days
2C	42	M	20.5	10	9750	9250	14000	28250							Died within 4 days
5C	42	M	20.5	10	11000	7000	4750								Died within 1 day
14C	42	F	21	10	14500	8750									Died within 2 days
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