

# Neutrophil Function in Selected Surgical Disorders

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MICROBIAL infections continue to cause disability and death in a wide variety of surgical disorders, and analysis of the related etiological factors often fails to provide an adequate explanation for the development of infection. Infections occur infrequently in normal persons postoperatively even though variable degrees of bacterial contamination regularly accompany operations.<sup>6, 8</sup> Conversely, a similar amount of bacterial contamination may pose a serious threat to a patient with an impairment of his host resistance.<sup>4</sup>

Although the diseases which produce these abnormalities of host resistance are both diverse and complex, diminished resistance to infection can be related to abnormalities of inflammatory lesions, opsonic substances, or phagocytic cells.<sup>2</sup> In this trinity of host defense (inflammatory lesion-opsonin-phagocyte), each is important, but the first two provide only ancillary mechanisms to aid the phagocyte.

This investigation was performed to study the importance of the antibacterial function of polymorphonuclear leukocytes as a factor of host defense in patients with selected surgical disorders.

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## Materials and Methods

The technic described by Alexander *et al.*<sup>3</sup> was used for the semiquantitative evaluation of phagocytosis and intracellular killing of bacteria by human neutrophils. Briefly, this technic involved the preparation of duplicate sets of tests in tubes with approximately  $5 \times 10^6$  neutrophils, approximately  $1.5 \times 10^6$  bacteria (*Staphylococcus aureus* 502A), and pooled human serum (10% final concentration) in a culture medium which contained Hank's balanced salt solution, fetal calf serum, and chicken embryo extract. Immediately after mixing, Penicillin and Streptomycin were added to one of the tubes (Falcon plastic Wasserman tubes), and the tubes were capped and incubated on a tilting table aliquot mixer at 37° C. for 4 hours. A similar set of tests from one or more normal individuals was always run as a control. Total viable bacterial counts were measured in the tests in which no antibiotics were added, but only cell associated bacteria could be measured in the tests to which antibiotics had been added.

Comparisons between test subjects and controls were made by dividing the number of viable bacteria which remained in the patient's or experimental animal's test by the number of viable bacteria which remained in the control's test after 4 hours of incubation. For convenience, this value can be expressed as a neutrophil-bacteri-

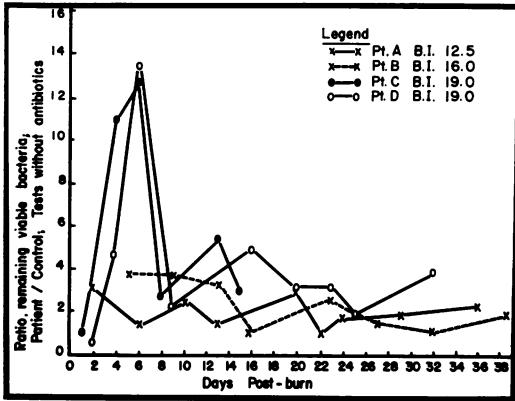


FIG. 1. Serial neutrophil function tests on patients with moderate thermal injuries. The degree of abnormality can be related to the severity of the burn as indicated by the burn index (BI). Note the temporal relation of burn injury to neutrophil function abnormality. Patient A, 6-year-old boy. Patient B, 4-year-old boy. Patient C, 2½-year-old girl. Patient D, 29-year-old man. Ratios (NB indices) of 0.5–2 are normal; 2–3, marginal abnormality; 3–4, mild abnormality; 4–8, moderate abnormality; 8–12, severe abnormality; and above 12, extremely severe abnormality.

cidal index (NB Index) for the samples without antibiotics, and the value can be expressed as a phagocytosis-intracellular killing index (PIK Index) for the tests with antibiotics according to the following formulas:

For the tests without antibiotics,

NB Index

$$= \frac{\text{remaining viable bacteria in patient's test}}{\text{remaining viable bacteria in control's test}}$$

For the tests with antibiotics,

PIK Index

$$= \frac{\text{remaining viable bacteria in patient's test}}{\text{remaining viable bacteria in control's test}}$$

The comparisons in the tests without antibiotics (NB Index) measured total neutrophil bactericidal capacity, and the comparisons in the tests with antibiotics (PIK Index) provided information concerning the type of functional abnormality. Values for the NB index of 0.5 to 2.0 were considered to be normal; values of 2.0 to 3.0 were

considered marginal; values of 3.0 to 4.0, mild abnormalities; values of 4.0 to 8.0, moderate abnormalities; values of 8.0 to 12.0, severe abnormalities; and values above 12.0, extremely severe.

When a high NB index in the tests without antibiotics was associated with a high PIK index in the tests with antibiotics, the defective killing was interpreted as being caused primarily by an abnormality of intracellular killing. When a high NB index was associated with a low PIK index, the results were interpreted as representing primarily a defect in phagocytosis (ingestion). When the NB index was within the normal range, but there was a high PIK index, the results were interpreted as representing a mild defect in intracellular killing which was associated with an increased rate of phagocytosis. If the NB index was abnormally high, but the PIK index was normal, this finding was interpreted as representing a combined defect involving both phagocytosis and intracellular killing. Both tests measure rates of phagocytosis and intracellular killing. Either a low rate of phagocytosis or an impairment of intracellular killing will result in a high NB index in the test without antibiotics whereas in the test with antibiotics, a low rate of phagocytosis will cause a low PIK index, and impaired intracellular killing will cause a relatively high PIK index.

Serial studies were performed on the majority of patients and experimental animals in this study.

## Results

### Thermal Injury

Neutrophil function tests were performed on 11 patients with thermal injuries, the severity of which was estimated by the burn index (the percentage of area of third degree involvement plus 50% of the area of second degree burn). Serial tests on four patients with moderately severe injuries are shown in Figure 1. Each of the four

patients survived, and none developed septicemia or deep, invasive wound infections. Each was treated with topical anti-microbial therapy.

The neutrophil function tests done on patients with severe burn injuries are shown in Figure 2. All of those patients surviving more than 1 week died as a result of septic complications despite vigorous topical antimicrobial treatment of the burn wound and the administration of specific systemic antibiotic therapy. Patients E and F both demonstrated the presence of abnormal neutrophil function which clearly preceded the development of clinical infection. Patients H, I, and J, had septic complications before the first neutrophil function tests were obtained.

An attempt was made to correlate the degree of abnormality of neutrophil function with both the total circulating neutrophil counts and the ratio of segmented to non-segmented neutrophils, but no positive correlation could be demonstrated. An increase in the rate of phagocytosis associated with a mild intracellular killing defect was the type of abnormality which occurred first after burn injury. Between the third and seventh postburn days, the inability of neutrophils to kill ingested bacteria was strikingly apparent in the patients with larger burn injuries. Later during the postburn course, the types of abnormalities encountered were variable. Usually, an intracellular killing defect predominated, often with a very significant increase in the rate of phagocytosis. Combined abnormalities of phagocytosis and intracellular killing were found not infrequently, but in no instance was there an abnormality of antibacterial killing which resulted primarily from a diminution in the rate of phagocytosis of bacteria.

### Physical Trauma

Neutrophil function tests were performed on six patients with various physical in-

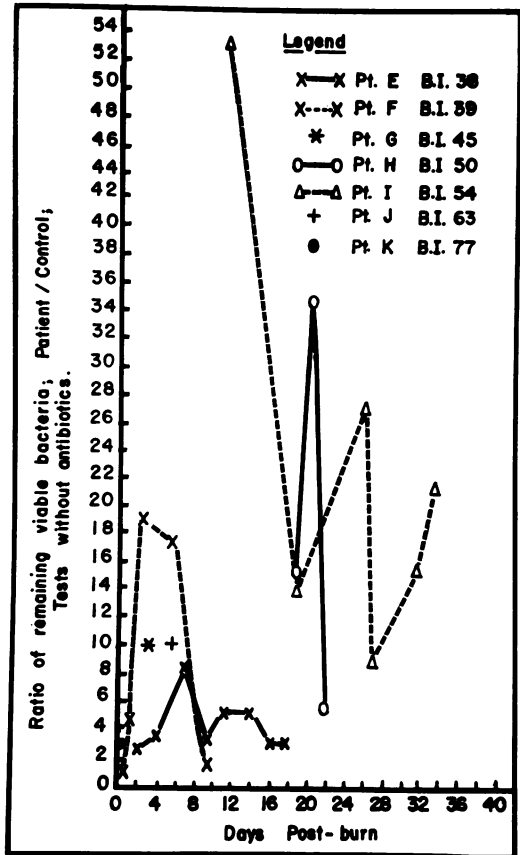


FIG. 2. Neutrophil-bactericidal indices on patients with extreme thermal injuries. Striking abnormalities were found in this group which varied with the extent of burn injury and which were temporally related to the time of injury. Patient E, 47-year-old man. Developed positive blood cultures on 8th postburn day and died on 21st postburn day of pneumonia, *Pseudomonas* septicemia, and sub-arachnoid hemorrhage from rupture of mycotic aneurysm. Patient F, 72-year-old man. On sixth postburn day, developed pneumonia and clinical sepsis. Died on ninth postburn day with gram negative septicemia. Patient G, 28-year-old woman. Died from renal failure on fourth postburn day. Patient H, 7-year-old girl. Marked abnormality of intracellular killing associated with rapid phagocytosis. Positive blood cultures for *Streptococcus viridans* and a *Providencia* group on 22nd postburn day. Died 23rd postburn day with bilateral pulmonary emboli. Patient I, 49-year-old woman. Extreme abnormality of antibacterial killing. Multiple blood cultures positive for *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Died 35th postburn day with bilateral pneumonia, multiple brain abscesses, and pancreatic abscess. Patient J, 6-year-old girl. Died 22nd postburn day from bilateral pneumonia. Patient K, 80-year-old man. Incineration type of injury. Test two hours after injury showed only a marginal abnormality. Died first postburn day with renal failure and diffuse hemorrhage from a coagulation defect.

TABLE 1. *Neutrophil Function Tests on Patients with Surgical Infections*

Number Age/Sex	Diagnosis	Ratio of Viable Bacteria, Patient/Control, 4 Hr. Sample			Interpretation of Tests	Comment
		Postoperative Day or Date	Antibiotics at 0 Min. (PIK Index)	No Anti- biotics (NB Index)		
A 40M	Multiple injuries. Crushed chest. Liver contusion. Retroperitoneal hematoma. Explora- tory Laparotomy.	0.5	0.71	0.44	Normal	
		3	14.29	1.42	Increased phagocytosis. Mild intracellular killing abnormality	
		6	4.86	7.0	Moderate abnormality of intracellular killing.	Developed fever. No localized infection.
		10	—	—	—	Septicemia and pneu- monia diagnosed.
		12	—	—	—	Died—gram nega- tive sepsis. Developed RLL pneumonia.
B 78M	Carcinoma of cecum. No operation.	12/7	—	—	—	Developed RLL pneumonia.
		12/13	0.18	10.0	Phagocytosis decreased	This type of abnor- mality rarely en- countered.
		1/12	—	—	—	Died—extensive pneumonia.
C 67M	Small bowel obstruc- tion treated by nasogastric de- compression.	11/16	—	—	—	Admitted. Urinary catheter inserted.
		11/19	—	—	—	Developed urinary infection (Proteus). Positive blood cul- ture (Klebsiella).
		11/22	8.63	10.77	Severe intracellular killing defect.	
		11/24	0.9	5.50	Abnormality improved.	
		11/29	7.0	2.86	Further improvement.	
D 5M	Recurrent pneumonia of left lung. Resec- tion LLL 8/30/67 for bronchiectasis secondary to aspirated peanut	3/11/68	19.47	3.42	Mildly increased phagocytosis and decreased intracellu- lar killing.	Patient recovered. Three days after onset of relapse. Or- ganism was pneumococcus.
		3/15	0.69	0.87	Normal	Clinical improvement
		3/25	—	3.55	Mild abnormality	No clinical infection
		4/10	4.43	3.08	Mild abnormality	Three days after onset of relapse. Still febrile.
E 46F	Repair ventral hernia 9/13. Developed pneumonia 9/18.	10/6	6.8	5.7	Intracellular killing moderately abnormal.	Clinical recovery by this time.
F 48F	Diabetic. Two years after leg amputa- tion.	9/30	—	—	—	Admitted. Abscess of amputation stump.
		10/4	4.7	8.7	Impaired intracellular killing.	Patient still septic.

juries. The results of the comparisons between patients and normal controls in the tests without antibiotics are shown in Figure 3.

In patient F, a moderately severe abnormality of bacterial killing (NB index) was noted on the second and third postoperative days which returned to normal by the eighth postoperative day. At this time, the patient developed pancreatitis and jaun-

dice. By the tenth postoperative day, the neutrophil abnormality was marked although it returned toward normal as the jaundice and pancreatitis subsided.

### Fasting

Since surgical patients are often subjected to a marked reduction in caloric intake, a normal human volunteer took no caloric intake for a 4-day period during

which time serial neutrophil function tests were performed to see if acute deprivation of nutrients would have an effect on neutrophil function. During this 4-day fast, there was no significant variation in either the test with antibiotics or the test without antibiotics when compared to similar tests obtained before the fast was begun.

### Patients with Surgical Infections

Tests were obtained on six patients with surgical diseases, who developed infections. Their clinical course and test results are outlined in Table 1. Patient A is of particular interest inasmuch as an abnormality of neutrophil function was noted to precede the development of clinical infection.

### Infected Experimental Animals

To determine whether or not abnormalities of neutrophil function were caused by the presence of infection, serial tests were obtained on three adult female dogs in whom infections were induced. Two normal animals served as controls throughout the experiment. Each of the three animals developed clinical infection at the site of inoculation which resulted in extensive cellulitis and abscess formation. The abscesses ruptured spontaneously between the third and fourth day with gradual return of temperature to normal. The results of the tests without antibiotics are shown in Figure 4. In none of the animals did a significant abnormality of neutrophil antibacterial killing develop.

### Renal Transplantation

Severe infections not infrequently complicate immunosuppressive therapy for transplantation procedures. The clinical course, leukocyte counts, neutrophil function tests, and immunosuppressive treatment are outlined for three patients in Figures 5, 6, and 7. Notable on each of the charts are the variations in neutrophil function which tended to occur at somewhat regular inter-

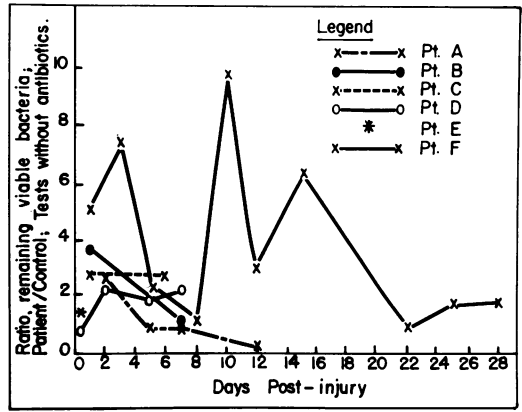


FIG. 3. Neutrophil-bactericidal indices on patients with physical trauma. Severe abnormalities were found in only one patient. The degree of abnormalities tended to correspond to the extent of injury. Patient A, 54-year-old man. Laparotomy for multiple stab wounds. No infection. Patient B, 18-year-old man. Gunshot wound of upper abdomen with minor intraabdominal injuries. Mild abnormality first postoperative day. Minimal pneumonia on second postoperative day. Responded to antibiotic therapy with recovery. Patient C, 59-year-old man. Stab wound of abdomen with evisceration. Febrile postoperative days 1-3, no cause found. Patient D, 36-year-old man. Multiple fractures and lacerations. No infection. Patient E, 47-year-old man. Gunshot wound of heart with prolonged period of shock. Cardiac arrest and death on second postoperative day. Patient F, 30-year-old woman. Extensive lacerations of liver from blunt trauma. See text.

vals despite the administration of immunosuppressive drugs. The second patient (Fig. 6) had an acute failure of the transplanted kidney, similar to those patients recently described by Starzl *et al.*<sup>11</sup> A small perinephric abscess which had been clinically inapparent was discovered at the time of removal of the transplant. Similar to the patients with thermal and physical injuries, no correlation could be made between the antibacterial capability and the maturity of the peripheral neutrophils.

### Malignant Neoplasms

Seven patients with malignant neoplasms of various types were studied. The neutrophil function tests and clinical course of these patients are outlined in Table 2. It was apparent that malignant neoplasms, in

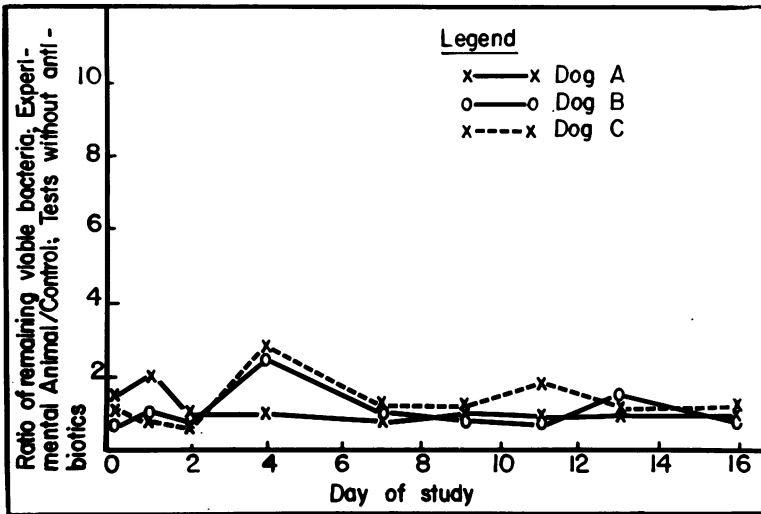


FIG. 4. Serial neutrophil-bactericidal indices in infected dogs. There was no evidence that active infection caused an abnormality of neutrophil function. Dog A was injected subcutaneously with 7.0 ml. of an overnight broth culture of *Pseudomonas aeruginosa*. Dogs B and C were inoculated subcutaneously with 10.0 ml. of a heavy saline suspension of stool.

themselves, did not cause any consistent abnormality of neutrophil function.

### Biliary Disease

Serial studies were done on two patients with benign biliary tract disease. One of these patients (patient A) had severe diabetes mellitus. The presence of biliary tract obstruction in patient B appeared to have had an adverse effect on neutrophil function since relief of the jaundice resulted in return of the neutrophil function test to within normal limits.

### Normal Controls

During the period between October 3, 1967, and March 22, 1968, six normal adults were used for controls on 71 separate occasions. Greater than 98% of the inoculum was killed by the leukocytes during a 4-hour period of incubation in 77.5% of the tests without antibiotics. Greater than 97% of the inoculum was killed in 90% of the tests. Conversely, in 10% of the tests, fewer than 96% of the bacteria were killed. In a previously reported study,<sup>2</sup> neutrophil function in normal controls appeared to be definitely within the abnormal range approximately 10% of the time. These abnormalities of neutrophil function were found at

periodic intervals of approximately three weeks in men, and were consistently greater in normal males than normal females.

### Discussion

Surgical therapy affords many opportunities for bacterial contamination and colonization of wounds, and microbial contamination has been the rule after traumatic or surgical disruption of the cutaneous or mucosal barriers. The removal and destruction of these bacteria depend on a complex sequence of physiological events which includes the deposition of adequate numbers of normally functioning phagocytic cells to areas of contamination. In humans and higher animals, neutrophils are the most important of these phagocytic cells.<sup>7</sup> Despite their obvious importance in the pathophysiology of surgical infections, little attention has been given to documenting abnormalities of the antibacterial function in surgical disorders. The regular occurrence of opportunistic infections in certain groups of patients, such as those receiving large doses of immunosuppressive drugs or suffering burn injury, adds emphasis to the need for additional studies of the mechanisms of host resistance in surgical patients. Although the technic used for studying

TABLE 2. *Neutrophil Function Tests on Patients with Malignancies*

Number Age/Sex	Diagnosis	Ratio of Viable Bacteria, Patient/Control, 4 hr. Sample			Interpretation of Tests	Comment
		Post-operative Day or Date	Anti- biotics at 0 Min. (PIK Index)	No Anti- biotics (NB Index)		
A 48F	Carcinoma breast. Extensive metas- tases. Bilateral adrenalectomy	5	4.70	7.50	Moderate intracellular killing defect.	No clinical infection
B 52F	Carcinoma breast. Extensive metas- tases. Bilateral adrenalectomy	-6 -3 1 4	2.20 0.84 1.92 5.26	1.56 1.78 1.68 1.88	Normal Normal Normal Slight increase in phagocytosis.	No infection dur- ing hospital stay. Died 3 weeks after operation from metastatic disease.
C 65M	Carcinoma cecum, multiple myeloma	12/13	0.96	2.45	Probably normal.	
D 56M	Carcinoma of lung. Refused operation.	11/20 11/22	1.81 1.96	0.86 0.77	Normal Normal	
E 79M	Extensive carcinoma of colon. Not re- sectable. Diverting colostomy done on 10/12	11/22 11/24 11/29 12/1 12/4 12/6 1/23 1/30	2.75 2.30 6.00 2.67 0.84 2.96 2.93 3.38	2.00 4.00 3.14 1.38 1.84 1.18 0.26 2.44	Normal Abnormal intracellular killing. Abnormal intracellular killing. Normal Normal Normal Normal Abnormal intracellular killing.	This patient prob- ably demon- strates normal cyclic variation.
F 12M	Neuroblastoma with extensive metas- tases	5/15 5/23	2.34 113.00	1.60 1.30	Normal Marked increase in rate of phagocytosis. Intracellular killing slightly decreased.	
G 12F	Choriocarcinoma with extensive metastases	5/9 5/15	0.39 0.42	0.5 0.51	Normal Normal	

antibacterial function was relatively crude inasmuch as pure populations of neutrophils could not be used, it represents an improvement over previously available techniques and it has yielded consistent results. It must be emphasized, however, that neutrophil function as measured by this test represents only one important aspect of host resistance.

The serial studies of neutrophil function done on patients with thermal injuries dem-

onstrated that little if any abnormality occurred within the first few hours. During the first week, however, moderate to extremely severe abnormalities were demonstrated which tended to improve between the seventh and tenth postburn day. These findings are consistent with the derangements of leukocyte lysosomal enzymes that have been found to occur following burn injury.<sup>1</sup> Abnormalities during the subsequent course of the patients were variable,

TABLE 3. *Neutrophil Function Tests on Patients with Biliary Disease*

Number Age/Sex	Diagnosis	Ratio of Viable Bacteria, Patient/Control, 4 Hr. Sample			Interpretation of Tests	Comment
		Post- operative Day	Antibiotics at 0 Min. (PIK Index)	No Anti- biotics (NB Index)		
A 42F	Juvenile diabetic. Cholelithiasis, No jaundice.	-7	0.94	2.67	Mild intracellular killing defect.	Blood sugar 388
		2	2.72	2.69	Mild intracellular killing defect.	Blood sugar 368
		5	1.16	3.33	Mild intracellular killing defect.	Blood sugar 356
		9	0.42	0.81	Normal	Blood sugar 284
B 87M	Common duct stone.	-1	3.62	7.56	Moderate intracellular killing defect.	Bilirubin 12.4
		3	4.72	7.56	Moderate intracellular killing defect.	Bilirubin 7.0
		5	7.06	4.87	Moderate intracellular killing defect.	
		7	5.60	4.00	Moderate intracellular killing defect.	
		10	5.24	6.88	Moderate intracellular killing defect.	Bilirubin 4.9
		12	62.00	11.71	Severe intracellular killing defect. In- creased phagocytosis.	
		14	7.78	2.06	—	—
		17	1.89	1.80	Normal	Bilirubin 1.05
19	1.48	0.78	Normal			
24	0.76	1.30	Normal			

depending somewhat upon the extent of the burn injury. The extent of burn injury correlated well with the degree of neutrophil abnormality, and there was also a pronounced temporal relationship. Interestingly, clinical infection, when present, was usually diagnosed 1 or 2 days following the greatest degree of abnormality of neutrophil function. These findings contrast sharply with those of Balch,<sup>5</sup> probably because of differences in technic.

In patients with physical injury, the abnormalities of neutrophil function were mild in degree but tended to be related to the extent of the injury. In the patient with a massive liver injury, a moderate abnormality of neutrophil function occurred on

the third postoperative day but returned to normal values by the eighth postoperative day only to recur again subsequent to the development of pancreatitis and jaundice. The only other patient studied with obstructive jaundice also had a moderate abnormality of neutrophil function which returned to normal after relief of the biliary obstruction and disappearance of the jaundice.

The group of patients with malignant tumors showed only occasional abnormalities indicating that advanced malignancy, *per se*, was not associated with an abnormality of neutrophil function. From these patients and those with injury, it was apparent that an operative procedure in itself



did not regularly cause any abnormality of neutrophil function. Sbarra *et al.*<sup>9,10</sup> showed that abnormalities of neutrophil function can occur in certain types of malignancies.

The most important and interesting of the patients studied were those with established surgical infections. In each of six patients, there was a definite abnormality of neutrophil function which could be demonstrated readily by the tests. In five, the abnormal tests were obtained after the infection had become established. In the sixth, however, the abnormality of neutrophil function was shown to have preceded the development of clinical infection, suggesting that it was etiologically important. The inability to induce any significant abnormality of neutrophil function by the creation of moderately severe infections in experimental animals provides further evidence that abnormalities of neutrophil function are a cause rather than a consequence of infection. Additional experience in dogs undergoing experimental renal allografting with immunosuppression has also shown that abnormalities of neutrophil function precede the development of infection. Uremia had no effect on neutrophil function in either humans or animals.

Neutrophil function tests were considered to be abnormal approximately 10% of the time in the subjects used for controls. At such times, any abnormalities found in patients were minimized by comparison with the controls, and errors in the reported results would be toward normal values. The tests on the controls in this study tend to confirm the conclusions from a previous study that abnormalities of neutrophil function occur in normal men at periodic intervals approximating 3 weeks.<sup>2</sup> These were usually mild abnormalities but occasionally reached what was considered to be a moderate severity. In a few instances, abnormalities in normal controls have been associated with minor cutaneous

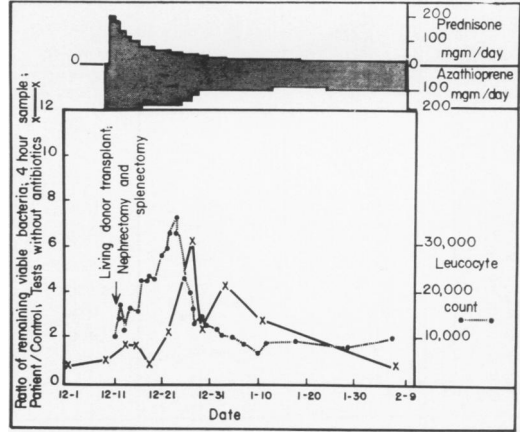


FIG. 5. Chart of tests on a 37-year-old man with a renal allograft from a living related donor. Note the moderate abnormality of neutrophil function 16 days after the transplant.

infections. It is apparent from our studies in both humans and experimental animals that abnormalities in males tend to be of a greater proportion than in females. No adequate systematic study has been done to determine the interval of periodic vari-

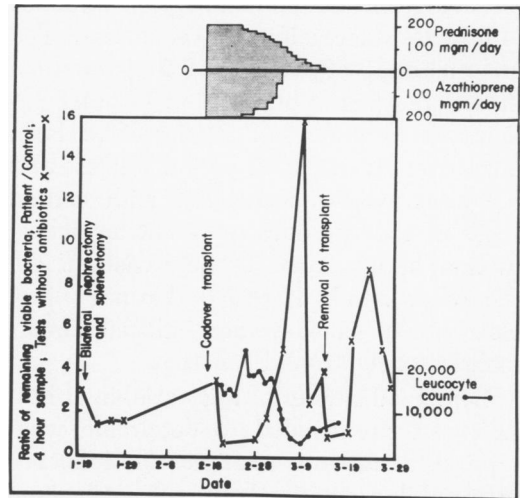


FIG. 6. Chart of tests on a 43-year-old man who had a renal allograft from a cadaveric donor. An extremely severe abnormality occurred on his 20th postoperative day which seemed to be related to a minor toxicity to azathioprene. However, a severe abnormality was found again 17 days later, long after all immunosuppressive drugs had been stopped. Note the tendency of the abnormalities to occur at periodic intervals.

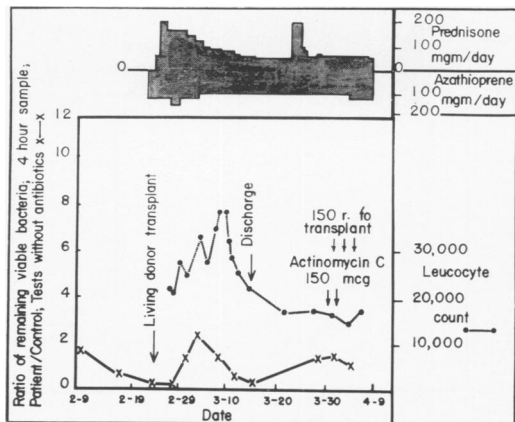


FIG. 7. Chart of tests on a 17-year-old girl with a renal transplant from a living related donor. None of the neutrophil function tests were considered to be abnormal, but the periodic variation in function is quite apparent.

ations in the female or its relationship to the menstrual cycle.

The finding that there was a periodic variation in neutrophil function in normal individuals may possibly offer an explanation for the development of septic complications in patients in whom analysis of the physiological and immunological variables of host resistance offers no other basis. The abnormalities of neutrophil function seen in the transplant patients and in other patients in whom serial studies have been done over an extended period suggest that the abnormalities associated with certain types of therapy may represent an accentuation of a normal cyclic variation. On the other hand, extensive thermal injury clearly may cause severe induced abnormalities of neutrophil function.

Continued studies of this variable of host resistance are needed to determine what types of therapeutic manipulation influence neutrophil function both adversely and positively.

### Summary

A test which provides separate semi-quantitative measurements of the processes of phagocytosis and intracellular killing of

bacteria by human neutrophils has been developed and used to evaluate neutrophil function in a variety of surgical disorders. Significant abnormalities have been demonstrated in patients with thermal trauma, physical injury, biliary obstruction, bacterial infection, and immunosuppressive therapy. In patients with thermal burns, the degree of abnormality could be related directly to the extent of injury and had a temporal relationship to the time of injury.

Abnormalities detected by the tests were usually characterized by an increase in the rate of phagocytosis and an impairment of intracellular killing. Operations of the magnitude of a laparotomy were associated with only slight or no abnormalities.

A normal cyclic variation in neutrophil function has been demonstrated in healthy persons, and it appears that this type of variation can be adversely affected by certain types of therapy.

Abnormalities of neutrophil function may be of etiological importance in the development of microbial infections in surgical patients where no other explanation can be found by analysis of other immunological and physiological variables.

### Bibliography

1. Alexander, J. W.: Serum and Leukocyte Lysosomal Enzymes. Derangements Following Severe Thermal Injury. *Arch. Surg.*, **95**:482, 1967.
2. Alexander, J. W.: Surgical Infections—Pathogen vs Host. *J. Surg. Res.*, **8**:225, 1968.
3. Alexander, J. W., Windhorst, D. B. and Good, R. A.: Improved Tests for the Evaluation of Neutrophil Function in Human Disease. *J. Lab. Clin. Med.*, **72**:136, 1968.
4. Altemeier, W. A., Todd, J. C. and Inge, W. W.: Gram-Negative Septicemia: A Growing Threat. *Ann. Surg.*, **166**:228, 1967.
5. Balch, H. H.: Resistance to Infection in Burned Patients. *Ann. Surg.*, **157**:1, 1963.
6. Culbertson, W. R., Altemeier, W. A., Gonzalez, L. L. and Hill, E. O.: Studies on the Epidemiology of Postoperative Infection of Clean Operative Wounds. *Ann. Surg.*, **154**:599, 1961.
7. Hirsch, J. G.: Neutrophil and Eosinophil Leukocytes. In Zweifach BW, Grant L and McCluskey, R. T. (Eds). *The Inflammatory*

- Process. Academic Press, New York, 1965, p. 245.
8. Mulholland, J. H., Ed.: Postoperative Wound Infection: The Influence of Ultraviolet Irradiation of the Operating Room and of Various other Factors. Report of an Ad Hoc Committee of the Committee on Trauma, Division of Medical Sciences, National Acad. of Sciences, National Research Council. Ann. Surg., 160:1 (Supp), 1964.
  9. Sbarra, A. J., Shirley, W., Selvaraj, R. J., Ouchi, E. and Rosenbaum, E.: The Role of the Phagocyte in Host-Parasite Interactions. I. The Phagocytic Capabilities of Leukocytes from Lymphoproliferative Disorders. Cancer Res., 24:1958, 1964.
  10. Sbarra, A. J., Shirley, W., Selvaraj, R. J., McRipley, R. J. and Rosenbaum, E.: The Role of the Phagocyte in Host-Parasite Interactions. 3. The Phagocytic Capabilities of Leukocytes from Myeloproliferative and other Neoplastic Disorders. Cancer Res., 25:1199, 1965.
  11. Starzl, T. E., Lerner, R. A., Dixon, F. J., Groth, C. G., Brettschneider, L. and Terasaki, P. I.: Shwartzman Reaction After Human Renal Transplantation. New Eng. J. Med., 287:642, 1968.

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### DISCUSSION

DR. HENRY H. BALCH (Washington): I have not seen the manuscript and am not able to comment on the technic used but I have been involved in this type of work in the past and would like to make a few observations.

It is tempting to feel that there are basic defects in host defense against infection in a variety of surgical states, traumatic or otherwise.

I have studied neutrophil function and antibody synthesis in a wide selection of surgical disease and have had difficulty in finding significant defects in host defense. My observations have shown that severely traumatized and very ill patients can synthesize large quantities of antibody globulin on stimulus. Furthermore, neutrophils from such patients can phagocytose and destroy pathogenic micro-organisms as well as neutrophils from normal subjects and in acutely burned patients better than those from normal subjects.

Technic of studying blood bactericidal capacity have to be carefully controlled to be able to compare one study with another. Ratios of white cells to bacteria and the bacterial species used significantly influence the end result. The coagulase positive staphylococcus is particularly hard to work with because of the difficulty in obtaining a satisfactory control baseline for comparison. With this micro-organism there is a wide variety in bactericidal effect in studying leucocytes from different normal subjects and in consecutive studies of leucocytes from individual normal subjects. The coagulase negative staphylococcus is easier to work with but even here the ratio of leucocytes to bacteria in the test system must be about 10 to 1 to ensure a 95% kill. If this ratio is adjusted in favor of the bacteria then the percentage destruction of bacteria is much less.

My studies showing a marked increased bactericidal effect of leucocytes-plasma suspensions from severely burned patients during the early

days following burn injury were all done in triplicate and I am sure that the observations were accurate. They reflect therefore in an *in vitro* system the functional capacity of leucocytes from burned patients suspended in their own plasma. In the paper presented this afternoon leucocytes were removed from the blood of burned patients and resuspended in heterologous immune serum—also an *in vitro* study but an artificial system. Findings from such a study may or may not reflect the functional capacity of leucocytes suspended in their natural environment.

Another significant finding of mine in acutely burned patients is that the mobilization of white blood cells to peripheral tissues is markedly depressed following burn injury for a number of days. Such cells as do appear in response to repeated minor injury during this 7- to 10-day period are mostly juvenile neutrophils but they are actively phagocytic and as stated, have an increased capacity to kill test bacteria. However, there is a marked delay in the appearance of lymphocytes during this period. This impaired cellular response following burn injury is probably very important in permitting invasive bacterial infection as the bacterial growth in the burn wound increases, changing the ratio of leucocytes to bacteria in favor of the latter. If pathogenic bacteria such as coagulase positive staphylococci are present in the burn wound, then invasive infection is probably easier because these micro-organisms have a well known capacity for producing serious infection in apparently normal subjects in the right circumstances. This is even more likely in the burned patient as the early increased post-burn blood bactericidal capacity is returning to normal levels after the first week.

DR. HARRIS B. SHUMACKER, JR. (Indianapolis): I never thought I would relate the story which I am about to tell, but since it is fairly close to the cocktail hour and since we have just