The Effect of Differing Ambient Oxygen Tensions on Wound Infection

THOMAS K. HUNT, M.D., MARK LINSEY, B.S., GUNTA GRISLIS, B.A., MINETTA SONNE, B.A., ERNEST JAWETZ, M.D.

From the Departments of Surgery and Microbiology, University of California San Francisco, San Francisco, California 94143

Wound infections were studied in rabbits using two standard inocula ($\doteq 10^4$ and $\doteq 10^6$) of *Pseudomonas aeruginosa* injected into a subcutaneous wound dead space made by implantation of standard wire mesh cylinders. The inoculation was done on the fourth day after implantation of the cylinders in animals kept from the day of implantation in atmospheres of 12%, 21%, or 45% oxygen content. Samples of wound fluid (0.2 ml) were removed for quantitative culture just before inoculation and 3, 7, 14, and 21 days later. No positive cultures resulted from samples taken before inoculation. One uninoculated wound served as a control in each animal. None of these control wounds became infected. Culture counts were significantly highest in the anoxic group and lowest in the hyperoxic group. Established infections were significantly lowest in the hyperoxics and highest in the hypoxics. The percent of wounds showing a significant culture count showed a similar trend. The mechanisms of this effect is not known, but a possible mechanism lies in the relative inability of leucocytes to kill this bacterium under hypoxic conditions.

WOUNDS IN TISSUES with a poor blood supply become infected far more frequently than wounds in tissue that have good perfusion. Severely traumatized and hypoxic patients seem to be unusually susceptible to infection. Experimentally, impairment of tissue perfusion by vasoconstrictors or hemorrhagic shock enhances infections at sites in which bacteria are injected.[2] Tissue trauma at a distance from the wound impairs healing, decreases the already tenuous oxygen supply to the wound, and increases susceptibility to infection,[2,4] but this increased susceptibility to infection can be reduced by restoring the blood-oxygen supply to the wound.[4]

Obviously, there is a good circumstantial argument for associating tissue hypoxia with infectability. However, as far as we are aware, the relationship of infectability of soft-tissue wounds to chronic hypoxia and hyperoxia at atmospheric pressures has never been studied.

Submitted for publication March 26, 1974.
Supported by U.S. Public Health Service Grant GM 12829.

In previous studies from this laboratory the composition of extracellular fluid in wounds of animals exposed to hypoxic, normoxic, and hyperoxic environments was investigated.[8,9] Varying ambient oxygen concentrations profoundly affected wound metabolism and the rate of collagen synthesis. The present study was designed to test susceptibility to wound infection in animals that chronically breathed fixed concentrations of 14%, 20% and 45% oxygen.

Methods

Three or four stainless steel, wire mesh cylinders 5 cm long and 6.5 cm in circumference were implanted under sterile conditions beneath the dorsal skin of 35 anesthetized New Zealand White rabbits by methods previously described.[9] After the skin was closed over the cylinders and the animals awakened from anesthesia, they were placed into lucite boxes approximately 70 L in volume, into which a gaseous mixture was pumped at approximately 3 L per minute per rabbit. By adjusting the inflow rate, the ambient pCO_2 was kept below 0.5%. Temperature remained 24–28 C, and humidity 70–90%.

Three ambient atmospheres were used; hyperoxic, 42% to 46% oxygen, normoxic, 21% (air), and hypoxic, 12% to 14% oxygen. The hyperoxic mixture was obtained by passing pure oxygen through a Venturi type gas mixer and diluting it with air (Puritan,® 40% setting). The 5% increment above the rated value of the instrument was due to a slight resistance in our gas conducting tubes. The normoxic and hypoxic mixtures were obtained by passing air or nitrogen through an identical mixer on the same setting. This method gives reliable gas mixtures over long periods at minimal expense.

On the fourth day after implantation of the cylinders, when the tissues had adhered to the wire mesh, a 0.2 ml sample of wound fluid was aspirated aseptically from each wound cylinder and was cultured. All specimens yielded no growth. Selected wound cylinders were then injected with a known number of Pseudomonas aeruginosa prepared as follows:

An overnight culture of an established strain of *Pseudomonas aeruginosa* was diluted to 10^2 and 10^4 dilutions. Each dilution was sampled for bacterial count by the culture plate technique, and 0.2 ml of the resuspended culture was inoculated into the selected wounds. One wound was inoculated with sterile broth (control), two wounds were inoculated with a small bacterial inoculum (1.6×10^4 to 3.3×10^5) and one wound was inoculated with a large bacterial inoculum (1.3×10^6 to 7.7×10^6). In the few cases in which only 3 wounds were made, one was a control, one received the lower and one the higher inoculum.

On days 3, 7, 14, and 21 after inoculation, the skin over the dorsal end of each wound was shaved and treated with tincture of iodine. Two tenths ml of fluid was aspirated from each wound cylinder and was immediately placed into nutrient broth. The bacteria in these specimens were promptly counted by the culture plate technique. The specimens were coded, and the cultures and counts were done by one of us (M.S.) who was unaware of the source of the specimen. Statistical comparisons of all results were done by Fischer's exact test.

Another group of 12 animals was kept in the experimental atmospheres, 4 animals in each atmosphere. The arterial and wound gases were measured by standard techniques on the fourth post-implantation day.

Results

Figures 1 and 2 show the mean bacteria counts found in wounds on selected days. In general, the higher counts occurred in hypoxic animals and the lower counts in the hyperoxic group.

Bacteria counts of 5×10^2 or greater were arbitrarily considered as "infections." Significant differences in the numbers of "infected" wounds between the air and 45% oxygen breathing rabbits ($p < 0.05$) were found on days 3, 7, and 14 in the wounds inoculated with the large inoculum. The difference between the numbers of wounds "infected" in the hypoxic and air groups was smaller and not significant. The differences between the hypoxic and hyperoxic groups were statistically significant ($p < 0.05$) on all test days.

In the low inoculum groups, statistically significant differences in the number of wounds "infected" were obtained between the air and hyperoxic groups only at 7 days. Once again the differences between hypoxia and hyperoxia breathing were greater with p values of less than 0.06 at 7 and 21 days. The small overall "infection" rates account for the small differences found.

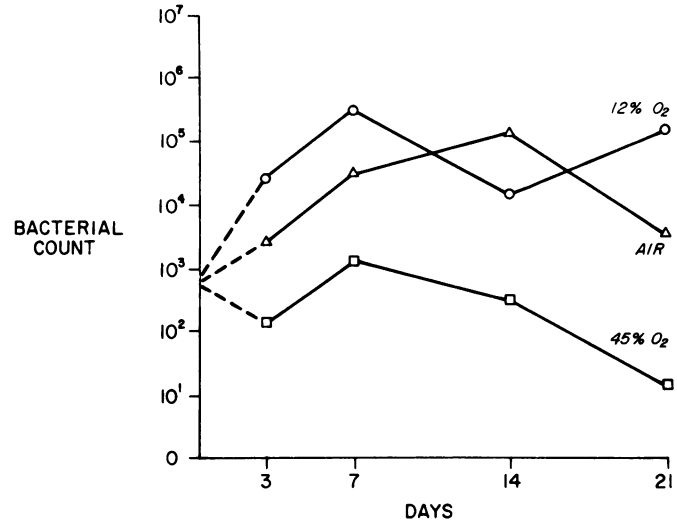

FIG. 1. Mean bacteria count of wounds originally inoculated with 10^4 bacteria.

The distribution of bacteria counts differed also between the groups. Figure 3 shows the range of bacteria counts for the high inoculum groups at day 7. In general, the hyperoxic group had the lowest count, the normoxic group was intermediate, and the hypoxic group had the highest counts. When these and other such data for the other days were plotted on a 2 × 2 matrix using Fischer's exact test, a number of differences were found with p values of less than 0.01 between normoxic and hypoxic groups as well as between hyperoxic and normoxic groups.

As we and others have previously shown, occasional cultures, even in wounds which appeared grossly infected, were negative. For purposes of analysis, then, a wound was

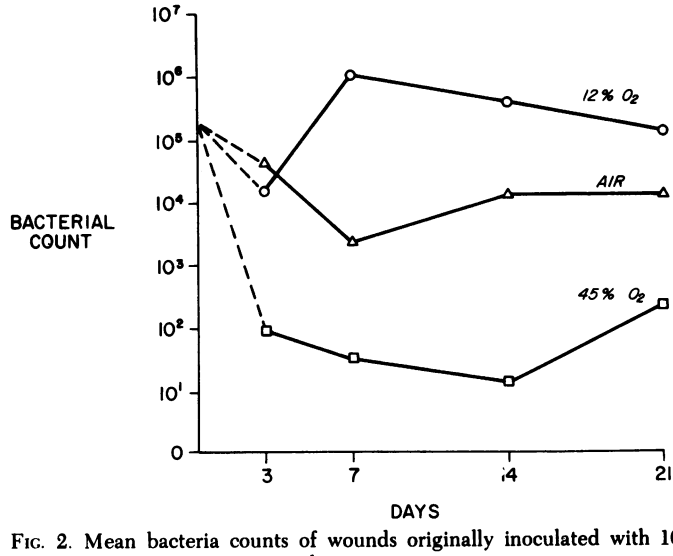

FIG. 2. Mean bacteria counts of wounds originally inoculated with 10^6 bacteria.

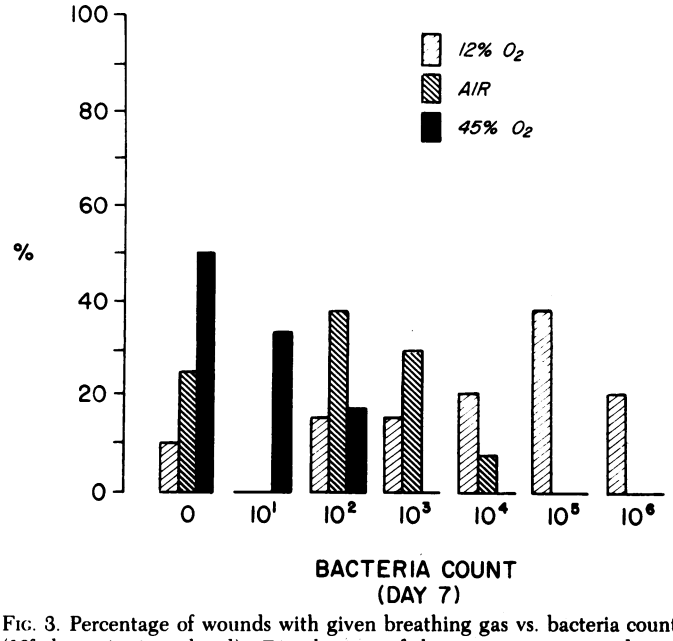

FIG. 3. Percentage of wounds with given breathing gas vs. bacteria count (10^6 bacteria inoculated). Distribution of bacteria counts on day 7 between the three groups. The medians of the hyper and hypoxic groups are more than 1 log apart from the air group.

considered to harbor an established infection when any two of the four samplings had a bacteria count greater than 5×10^2. When this criterion was adopted, as shown in Table 1, a ratio of infected to non-infected wounds could be constructed.

In no case was a control wound infected by any criterion, therefore, cross contamination was not a significant experimental problem.

The respiratory gas tensions in arteries and wounds at the time of inoculation are shown in Table 2. These values were determined in a separate group of animals studied concurrently and kept in identical environments as described above.

Discussion

Previous research on infection and its relation to tissue oxygenation has been performed almost entirely with intermittent hyperbaric oxygen. Oxygen at high partial pressure inhibits growth of some aerobic and anaerobic organisms in culture,[5] but the tensions to which bacteria were exposed in such experiments far exceeded those likely ever to be reached in tissue. Survival time of mice with pneumococcal septicemia was prolonged when they were treated with hyperbaric oxygen shortly after intraperitoneal contamination.[17] Hyperbaric oxygen applied continuously to the surface of experimental wounds infected with *Staphylococcus aureus* or *Pseudomonas aeruginosa* inhibited bacterial growth, but the effect seemed to cease as soon as the treatment was stopped.[10]

Several reports have now documented resolution of chronic osteomyelitis in both animals and man after intermittent hyperbaric oxygen therapy.[16,20]

A mixed result was recently obtained when hyperbaric oxygen was used to treat experimental staphylococcal osteomyelitis. In the animal model tested, the incidence of acute osteomyelitis was unchanged, but accelerated healing of established infections was noted.[7] On the other hand, hyperbaric oxygen failed to alter survival rates in dogs with gram negative peritonitis.[15] In another study, hyperbaric treatment actually seemed to increase the mortality from staphylococcal peritonitis in rates.[6]

Hyperbaric oxygen can be given only intermittently because exposure to hyperbaric oxygen pressures for more than a few hours has toxic effects on the host. We have shown in unpublished studies, there is also a risk of local acidosis from extreme hyperoxia. On the other hand, continuous, relatively mild hyperoxygenation increases wound pO_2 to a lesser degree, but the effect can be maintained indefinitely without progressive wound acidosis or host toxicity.

The present study is unique in that it involved continuous exposure to only 45% oxygen at one atmosphere and specifically investigates both the susceptibility of soft tissue wounds to infection as well as their ability to clear established infection. Furthermore, the techniques used allow us to measure the actual wound environment and relate it to infectability.

These experiments seem to indicate that resistance to in-

TABLE 1. *Ratios of "Infected" to "Uninfected" Wounds Using the Arbitrary Criteria for Infection as 5×10^2 Bacteria or More in 2 of the 4 Samplings*

Large Inoculum	Hyperoxia	0/12 = 0%	Sign.	$p < .015$	$p < .012$
	Normoxic	7/11 = 64%	NSD.	$p < .27$	
	Hypoxic	7/10 = 70%			
Small Inoculum	Hyperoxic	0/18 = 0%	Sign.	$p < .05$	$p < .04$
	Normoxic	5/19 = 26%	NSD.	$p < .28$	
	Hypoxic	4/12 = 33%			

TABLE 2. *Wound pO_2, pCO_2 and pH on the Day of Inoculation (See Methods)*

Blood Gasses At Time of Inoculation			
	PaO_2	$PaCO^2$	PaH
Hyperoxia	191 ± 20	62 ± 8*	7.32 ± 0.02
Normoxia	69 ± 6	31 ± 3	7.46 ± 0.02
Hypoxia	39 ± 2	26 ±2	7.39 ± 0.02
Wound Gases At time of Inoculation			
	pO_2	pCO_2	pH
Hyperoxia	13.6 ± 0.9	85.6 ± 4	7.19 ± 0.01
Normoxia	10.6 ± 0.7	67 ± 2	7.20 ± 0.01
Hypoxia	6.2 ± 0.4	55 ± 2	7.11 ± 0.02

fection in the wound is oxygen dependent. However, the mechanism is not readily apparent. The variations in wound environment between hypoxic and hyperoxic animals in this study are relatively small. The difference between the normoxic and hyperoxic environments is no greater than 100 mm Hg, and pseudomonas are not inhibited by partial pressures of oxygen up to 760 mm Hg.[5,16] The decreased susceptibility to infection of the hyperoxic group, therefore, must be related to a host factor.

We cannot exclude the possibility that the relative "maturity" of the hyperoxic wounds enhanced resistance. Reciprocally, the "immaturity" of the wounds in the hypoxic group may have contributed to susceptibility. Investigation of this possibility is being carried out. However, the role of "maturity" of the wound in resistance to infection is unclear. All that can be said is that in some way, "mature" granulation tissue seems quite resistant to infection.

The most likely explanation for our findings seems to be found in the reaction of the phagocytic defense system to changes in oxygen environment. White cells ingest bacteria in a wide variety of environments, even anaerobic ones. However, phagocytosis of bacteria by polymorphs is followed by a burst of oxygen consumption which seems to be important to intracellular killing.

Neutrophils derive energy for particle ingestion from glycolytic metabolism.[3,11,13] Inhibitors of oxidative pathways, such as cyanide and hypoxia do not inhibit particle ingestion *in vitro*.[3,18] Killing of ingested organisms, on the other hand, is greatly impaired under anaerobic conditions. For instance, Cline has shown that intracellular killing of Listeria by human macrophages is diminished by one-third when the pO_2 of the medium is changed from greater than 100 mm Hg to less than 15 mm Hg.[3] The pO_2 of wound dead spaces after the first few days is below 10 mm Hg.

The burst of oxygen consumption following phagocytosis is associated with greatly increased glucose oxidation through the hexose monophosphate shunt. Thus, the leucocyte is able to switch from anaerobic to aerobic conditions after phagocytosis, provided oxygen is available.[1,13]

Hypoxia reduces resting oxygen consumption of guinea pig monocytes and polymorphonuclears as shown by Stähelin, Sutter and Karnovsky.[21] They showed oxygen consumption fell by more than 80% when pO_2 of the medium was reduced from 150 to 8 mm Hg. They also noted that oxygen consumption, lactate production and viability of polymorphonuclears fell considerably as pH fell below 7.5, although monocytes were not as severely affected. In the present experiments, wound pH fell from a control level of 7.2 to less than 7.1 in hypoxia and rose slightly with the hyperoxic environment. Kempner also has reported the depressed resting respiration by leukocytes subsequent to lowered oxygen tension.[12]

The enzyme system which relates to intracellular killing is extremely complex. In general, however, investigators agree that the production of hydrogen peroxide and the reduction of NADPH by the NADPH oxidase system with the aid of a halide ion is a major component of the killing mechanism.[11,14] Allen, Stjernholm, and Steele have demonstrated that the active principle in the peroxide reaction is derived from atmospheric oxygen.[1] Therefore, the system would seem to be pO_2 dependent. Furthermore, sepsis of several types has been related to white cells which are myeloperoxidase deficient. Furthermore, the often fatal chronic granulomatous disease is apparently due to a failure of the peroxide producing mechanism.[11] Thus, there is considerable circumstantial evidence indicating that the hypoxia which characterizes injured tissue may well be responsible for the concomitant increased susceptibility to infection.

One can hypothesize that bacteria, fibroblasts, and phagocytes compete for what little oxygen is available in the wound space. Infection results when sufficient bacteria are present to overwhelm the phagocytic response or to diminish its already poor oxygen supply to the point that intracellular killing becomes insufficient to prevent infection. Provision of extra oxygen in the normally hypoxic wound apparently enhances phagocytic killing. Hypoxemia in addition to the normal wound hypoxia probably puts the phagocytic response at a further disadvantage.

This project was undertaken to investigate a possible mechanism for the increased infectability of wounds in trauma patients. It was intended to extend Conolly's experiment in which he showed that remote trauma (which diminishes wound oxygen supply) doubled wound infectability.[4] The wound pO_2 produced in the present experiment by hypoxemia was designed to be the same as that produced by remote trauma. Inocula and organisms were identical with the previous study. However, where the infection rate was doubled by trauma, it rose only about 40% in the current study. The reason for this difference may be apparent in the wound gas data. The effect of remote trauma is to diminish would perfusion, to decrease pO_2, but also to *increase* pCO_2 in the wound by diminishing the means of its removal.[22,23] Pure hypoxia without hypoperfusion *decreases* pO_2 but also *decreases* pCO_2. Thus, hypoxia is not the equal

of hypoperfusion. Of the two, hypoperfusion seems the most powerful potentiator of infection. This observation correlates with those of Siegel, Goldwyn, and Friedman who noted that the highest mortality rates in sepsis occurred in those patients whose mixed venous pCO_2 was markedly elevated.[19].

Because of the recent discovery that enhanced oxygen delivery can accelerate healing in both soft tissue and bone[8] we have been tempted as has Hamblen[7] to conclude that the cure of chronic osteomyelitis with hyperbaric oxygen is merely a result of enhanced healing. The present study suggests, however, that enhancement of phagocytic killing of bacteria may be part of the mechanism.

It would seem a simple matter to advocate that enriched oxygen mixtures be given to all patients at risk for wound infection since we have shown in unpublished studies that breathing oxygen elevates wound pO_2 in humans. In practice oxygen breathing can enhance tissue oxygenation; but it will do so only if blood volume is maintained, if vasoconstriction is minimized, if blood supply is adequate, and if fluid overloads and tissue edema are avoided. If tissue oxygen transport is kept normal, however, hyperoxia could possibly become a practical method of enhancing resistance to infection in otherwise susceptible wounds. Certainly, the avoidance of hypoxia and hypovolemia would seem important in prevention of infection.

References

1. Allen, R. C., Stjernholm, R. L. and Steele, R. H.: Evidence for the Generation of an Electronic Excitation State(s) in Human Polymorphonuclear Leukocytes and its Participation in Bactericidal Activity. Biochem. Biophys. Res. Commun., 47:679, 1972.
2. Burke, J. F.: Wound Infection and Early Inflammation. Monogr. Surg. Sci., 1:301, 1964.
3. Cline, M. J.: Bactericidal Activity of Human Macrophages: Analysis of Factors Influencing the Killing of Listeria Monocytogenes. Infect. Immun., 2:156, 1970.
4. Conolly, W. B., Hunt, T. K., Sonne, M. and Dunphy, J. E: Influence of Distant Trauma on Local Wound Infection. Surg. Gynecol. Obstet., 128:713, 1969.
5. Gottlieb, S. F.: Effect of Hyperbaric Oxygen on Microorganisms. Ann. Rev. Microbiol., 25:111, 1971.
6. Grogan, J. B.: Effect of Hyperbaric Oxygen on Experimental Infections. Arch. Surg., 92:740, 1966.
7. Hamblen, D. L.: Hyperbaric Oxygenation: Its Effect on Experimental Staphylococcal Osteomyelitis in Rats. J. Bone Joint Surg., 50-A:1129, 1968.
8. Hunt, T. K. and Pai, M. P.: Effect of Varying Ambient Oxygen Tensions on Wound Metabolism and Collagen Synthesis. Surg. Gynecol. Obstet., 135:561, 1972.
9. Hunt, T. K., Twomey, P., Zederfeldt, B. and Dunphy, J. E.: Respiratory Gas Tensions and pH in Healing Wounds. Am. J. Surg., 114:302, 1967.
10. Irvin, T. T., Norman, J. N., Suwangul, A. and Smith, G.: Hyperbaric Oxygen in the Treatment of Infections by Aerobic Microorganisms. Lancet, 1:392, 1966.
11. Karnovsky, M. L., Simmons, S., Karnovsky, M. J., *et al.*: Comparative Studies on the Metabolic Basis of Bactericidal Activity in Leukocytes. *In* Phagocytic Mechanisms in Health and Disease. R. C. Williams, Jr. and H. Hugh Fudenberg, editors. New York, Intercontinental Medical Book Corporation, 67, 1972.
12. Kempner, W.: Effect of Oxygen Tension on Cellular Metabolism. J. Cell. Physiol., 9-10:339, 1936–37.
13. Klebanoff, S. J.: The Myeloperoxidase—Mediated Antimicrobial Systems. *In* Phagocytic Mechanisms in Health and Disease. R. C. Williams, Jr., and H. Hugh Fudenberg, editors. New York, Intercontinental Medical Book Corporation, 3, 1972.
14. Lehrer, R. I.: The Role of Phagocyte Function in Resistance to Infection. Calif. Med., 114:17, 1971.
15. Ollodart, R. M., Seitz, C. R., Blair, E., *et al.*: Effect of Hyperbaric Oxygen on Gram Negative Bacilli. Clin. Res., 12:37, 1964.
16. Perrins, D. J. D., Maudsley, R. H., Colwill, M. R., *et al.*: OHP in the Management of Chronic Osteomyelitis. *In* Proceedings of the Third International Congress on Hyperbaric Medicine. I. W. Brown and B. G. Cox, editors. Publication 1404 of the National Academy of Sciences, National Research Council, Washington, D.C., 578, 1966.
17. Ross, R. M. and McAllister, T. A.: Protective Action of Hyperbaric Oxygen in Mice with Pneumococcal Septicaemia. Lancet, 1:579, 1965.
18. Sbarra, A. J. and Karnovsky, M. L.: The Biochemical Basis of Phagocytosis. J. Biol. Chem., 234:1355, 1959.
19. Siegel, J. H., Goldwyn, R. M. and Friedman, H. P.: Pattern and Process in the Evolution of Human Septic Shock. Surgery, 70:232, 1971.
20. Slack, W. K., Thomas, D. A. and Perrins, D.: Hyperbaric Oxygenation in Chronic Osteomyelitis. Lancet, 1:1093, 1965.
21. Stahelin, H., Suter, E. and Karnovsky, M. L.: Studies on the Interaction Between Phagocytes and Tubercle Bacilli. J. Exp. Med., 104:121, 1956.
22. Zederfeldt, B.: Studies on Wound Healing and Trauma with Special Reference to Intravascular Aggregation of Erythrocytes. Acta Chir. Scand. Suppl., 224, 1957.
23. Zederfeldt, B. H. and Hunt, T. K.: The Effects of Trauma on Respiratory Gases in Healing Wounds. *In* Current Topics in Surgical Research, Vol. 1. George D. Zuidema and David B. Skinner, editors. New York and London, Academic Press, 297, 1969.