

Intralipid promotes infection, presumably by inhibiting the reticuloendothelial system, thereby suppressing the host's constitutive immunity, whereas Diprivan supposedly promotes infection by supporting bacterial growth and increasing the inoculating dose. This study considers whether bacterial replication alone in Intralipid and Diprivan adequately explains the increased risk of infection associated with these agents or whether other factors might also be involved.

Methods: Staphylococcus aureus was cultured in 10% Intralipid or Diprivan at clinically relevant conditions or in Intralipid containing 0.005% (w/v) sodium EDTA, a current additive, to measure growth. To determine whether Intralipid affected infection, New Zealand white rabbits were injected intravenously with S. aureus with or without Intralipid. Twenty-four hours later, bacteria in lung, liver, spleen, and kidney tissues were enumerated.

Results: S. aureus failed to grow in Diprivan or Intralipid containing 0.005% EDTA. Whereas S. aureus did replicate in plain Intralipid, growth was delayed until the bacteria conditioned the media. Once initiated, growth was slow at clinically relevant temperatures. The administration of Intralipid to rabbits significantly increased the recovery of staphylococci from the kidneys, \( P < 0.001 \), relative to the other tissues 24 h after an intravenous inoculation with S. aureus, compared with rabbits receiving S. aureus with no Intralipid.

Conclusions: These results suggest that Diprivan, and possibly Intralipid, represent poor media for the growth of S. aureus and may promote infection through mechanisms other than increased inoculum size. (Key words: Bacterial growth; culture; temperature; propofol.)

In 1991, six months after gaining the approval of the Food and Drug Administration, Diprivan (Zeneca, Wilmington, DE; a preparation of propofol, in a 10% Intralipid vehicle [Abbott Laboratories, Abbott Park, IL]) was implicated in several staphylococcal and fungal infections reported from four geographically separate areas of the United States.\(^1\) Supplies of Diprivan from the manufacturer were found to be sterile, but lapses in sterile technique during preparation of the drug for administration were documented by the Centers for Disease Control (CDC).\(^1\) In vitro growth studies showed that low inocula of \textit{Staphylococcus aureus} (10-100 colony forming units [CFU]/ml) replicated in Diprivan at 34°C, reaching concentrations of \(10^5\) or \(10^6\) CFU/ml in 24 h.\(^1\) It was therefore assumed that Diprivan had served as a growth medium for the bacteria, resulting in the administration of overwhelming numbers of microorganisms to patients. However, the number of bacteria in Diprivan at the time of its administration has never been determined, and there is some evidence that bacterial replication in Diprivan is limited in clinical settings.

Diprivan uses a 10% emulsion of Intralipid as a vehicle for the anesthetic propofol, and Intralipid itself is associated with a significantly increased risk of staphylococcal and fungal infections in pediatric\(^2,5\) and adult\(^4,7\) populations when used for parenteral nutrition. The
most commonly implicated organisms in infections related to Intralipid are *S. aureus* and fungi, in particular, *Candida albicans,* particularly when there is direct contact between Intralipid and the microbe. This association seems to hold whether Intralipid is given for parenteral nutrition or as a component of Diprivan.

The increased incidence of infection after the administration of Intralipid has been attributed to a depression of reticuloendothelial function. In an effort to describe how Intralipid impedes the constitutive immunity of the host, the effects of Intralipid on phagocytosis, chemotaxis, and oxidative function were evaluated. The influences of Intralipid on bacterial killing and inhibition of lymphocyte blastogenesis to specific antigens also were studied.

Suppressed reticuloendothelial function and a generalized depression of the innate immunity of the host would be expected to result in diverse infections from the many organisms encountered by patients in the hospital. Yet *S. aureus* and *C. albicans* remain the leading infectious agents associated with Intralipid and Diprivan, accounting for a disproportionate number of infections. Hence, divergent hypotheses have been suggested as explanations for what may be a common phenomenon. As a result, infection control measures may not be aimed at the most relevant factors in Diprivan- and Intralipid-associated infections.

These studies were conducted to determine whether *S. aureus* would grow in Intralipid or Diprivan sufficiently during clinically relevant conditions to reach overwhelming numbers. We also evaluated whether Intralipid could promote systemic infection by *S. aureus* in a rabbit model. The formulation of Diprivan was changed in 1994 when 0.005% sodium EDTA was added to the product. Because many studies that investigated these issues were conducted before 1994, we also used Intralipid with and without 0.005% sodium EDTA in our experiments.

**Methods**

**Preparation of Bacteria**

*S. aureus* isolated from a wound, and strain 17, which was used in the original growth studies reported by the Centers for Disease Control, were used in these experiments. Bacteria were maintained in L broth (LB) or on L agar (containing 1.5% w/v agar). Stock cultures were stored at -70°C in LB containing 35% (v/v) glycerol. Bacteria were incubated in LB at 37°C overnight without agitation, and the next morning a 1-ml aliquot was inoculated into 24 ml fresh LB and grown at 37°C with agitation for approximately 4 h to ensure that the bacteria were growing exponentially. Bacterial density was then estimated by optical density at 600-nm wavelength. The *S. aureus* culture was centrifuged at 13,800g for 10 min at ambient temperature, and the bacterial pellet was suspended in 25 ml phosphate-buffered saline with 0.01% (w/v) gelatin. Serial dilutions of the bacteria were made to achieve appropriate concentrations of bacteria. A 0.1-ml aliquot was plated to measure the CFU/ml.

**Effects of Temperature on Growth of S. aureus in Intralipid and Diprivan**

To determine the effect of temperature on the growth kinetics of *S. aureus*, 25 ml Intralipid, Diprivan, or LB was inoculated with 0.1 ml phosphate-buffered saline with 0.01% (w/v) gelatin containing 500-1,500 CFU of *S. aureus*. The lipid cultures (Intralipid and Diprivan) were incubated at clinically relevant temperatures 16°C and 17.5°C (operating room temperatures), 22.5°C (ambient temperature), or 30°C (previous methods of others) for 24 h. These cultures were maintained in 50-ml plastic conical centrifuge tubes with the lids loosely closed to simulate the environmental conditions of clinical infusions. To determine the number of viable bacteria, 0.1 or 0.2 ml of the cultures, or appropriate dilutions thereof, was plated in duplicate at frequent intervals for up to 24 h. We compared the growth rate of *S. aureus* in Intralipid and Diprivan with the growth rate in the rich medium LB at each temperature. Repetitions were performed for each experiment to ensure that growth counts were consistent and reproducible.

**Analysis of the Lag Period after Transfer of Exponential Cultures in Intralipid**

To evaluate the extended lag phase in the growth of *S. aureus* in lipids compared to LB, *S. aureus* was grown in LB to achieve an exponential growth phase, as described previously. A 0.1-ml suspension (10⁵ CFU/ml) of *S. aureus* in phosphate-buffered saline was added to 25 ml Intralipid (10⁵ CFU final concentration) and incubated at 22.5°C. Every 2 h for 12 h and then at 4-h intervals up to 48 h, 0.5 ml Intralipid culture was sampled for CFU/ml.

Anesthesiology, V 91, No 5, Nov 1999
ferred to 35 ml Intralipid to yield a bacterial concentration of approximately $10^3$ CFU/ml. Bacterial growth in the second culture was measured by frequent plating, as described previously.

To determine whether limiting carbon dioxide in the Intralipid growth media contributed to the observed lag phases, Intralipid containing 0, 10, and 100 mM sodium bicarbonate was prepared by adding 8.4% (w/v) sodium bicarbonate to the second cultures. Because the bicarbonate solution contributed less than 1% of the final volume of the culture, Intralipid was not diluted appreciably.

**Effects of Intralipid on Rabbit Infection with S. aureus**

After approval was obtained from the University of Florida's Institutional Animal Care and Use Committee, male New Zealand white rabbits (3.0 kg) were injected intravenously via the ear vein with $5 \times 10^7$ CFU S. aureus suspended in 1 ml Intralipid ($n = 6$) or saline ($n = 6$). A third group of animals ($n = 6$) was injected intravenously with 1 ml Intralipid 1 min before intravenous injection of $5 \times 10^7$ CFU S. aureus suspended in saline. Serial blood samples were obtained from the contralateral ear vein immediately before, immediately after, and at 1, 5, 10, 20, 60, and 120 min after inoculation. Intravenous cannulae were removed from the ears, and the animals were returned to their cages and given free access to food and water. Twenty-four hours after inoculation, the rabbits were anesthetized, and biopsies of the liver, lung, spleen, and kidneys were obtained. The tissues were weighed, homogenized in 5 ml phosphate-buffered saline with 0.01% (w/v) gelatin at 4°C, and 0.1 ml was plated for quantitative colony count. The remainder of the sample was stored at 4°C for further dilution or concentration to quantify CFU.

**Statistical Analysis**

**Bacterial Growth.** We measured and analyzed the doubling time during the exponential phase of growth and the minimal lag period necessary before exponential growth occurred. The minimum lag period was defined as the time preceding the first statistically significant doubling of CFU/ml after inoculation. The doubling time during exponential phase growth was determined by plotting the log$_{10}$ CFU/ml versus time and identifying that portion of the growth curve after the lag phase when at least three points formed a line. Linear regression was used to determine the slope of that line, and the slope was transformed to a doubling time. When more than three points could be chosen to determine the line, and therefore the doubling time (slope), the three points along the line with the highest correlation coefficient were used.

**Distribution of S. aureus in Tissues of Rabbits**

The data for the number of bacteria recovered from tissues 24 h after inoculation are presented as the "Infection Index." Animals received slightly different numbers of S. aureus ($1.5 \times 10^7 - 1.5 \times 10^8$ CFU), and the weight of the rabbits varied by approximately $\pm 400$ g. To normalize for these factors, we present the data as the Infection Index (recovered CFU/g of tissue)/(inoculated CFU/g body weight). Repeated-measures analysis of variance was performed comparing the vehicle (saline vs. Intralipid vs. Intralipid pretreatment) and number of bacteria recovered from tissues (liver, spleen, lung, and kidney) after performing a log transformation with the raw data to stabilize variance. The Tukey multiple pairwise comparison procedure was used to maintain an experiment-wise significance level of 0.05 for pair-wise comparisons among means.

**Results**

**Growth of S. aureus in Intralipid from L Broth**

The doubling times of both S. aureus strains in LB at 37°C were similar: approximately 27 min. When exponential phase LB cultures at 37°C were diluted into fresh LB, there was no lag phase. We then evaluated the growth kinetics of S. aureus in Intralipid at various temperatures (fig. 1, table 1). Growth kinetics of S. aureus PL001 and strain 17 were similar; we present only our most complete data from PL001. Growth in Intralipid was highly erratic, with many experiments failing to achieve replication at any temperatures; we present only those data from the experiments in which the most reproducible and rapid growth occurred. Lag phases were generally longer and growth rates were slower at lower temperatures. At 16°C, the temperature of our operating rooms, there was no net growth even 25.5 h after inoculation of Intralipid.

At 30°C, lag phases ranged from 3 h to 6 h ($\leq 4$ h for 8 of 12 runs), during which there was a decrease in CFU/ml. After this lag period, there was a rapid increase in CFU/ml over the next 2 h, which was not sustained (fig. 1). Cultures then settled into a net doubling time of 3.0 $\pm$ 0.4 h (fig. 1). Although it appears that the staphylococci replicated more rapidly at 22.5°C than at 30°C.
GROWTH OF S. AUREUS IN DIPRIVAN AND INTRALIPID

Fig. 1. Growth of S. aureus PL001 in Intralipid at various temperatures. Bacteria were grown to exponential phase in L broth, suspended in phosphate-buffered saline with 0.01% (w/v) gelatin, and then inoculated into Intralipid at the indicated temperatures. Initial bacterial concentrations for the representative experiments shown were approximately 10^5 CFU/ml. Intralipid cultures were sampled at various times, diluted, and plated to enumerate CFU/ml.

Table 1. Summary of Growth Kinetics in Intralipid

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Doubling time (h)</th>
<th>Minimum lag time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.5°C (n = 8)</td>
<td>5.22 ± 0.48</td>
<td>4-8</td>
</tr>
<tr>
<td>22.5°C (n = 9)</td>
<td>1.54 ± 0.34</td>
<td>3-8</td>
</tr>
<tr>
<td>30°C (n = 12)</td>
<td>3.00 ± 0.36</td>
<td>3-6</td>
</tr>
</tbody>
</table>

Fig. 2. Lack of growth of S. aureus PL001 in Diprivan or Intralipid containing 0.005% EDTA. Bacteria were grown to exponential phase in L broth, suspended in phosphate-buffered saline with 0.01% (w/v) gelatin, and then inoculated into Diprivan or Intralipid containing 0.005% EDTA at 22.5°C. Initial bacterial concentrations for the representative experiments shown were between 10^5 and 10^6 CFU/ml. Cultures were sampled at various times, diluted, and plated to enumerate CFU/ml.

Fig. 3. Analysis of lag phase during transfer of S. aureus from exponential growth in Intralipid to fresh Intralipid. The primary culture of S. aureus in Intralipid was prepared as for figure 1. At 24 h postinoculation (arrow), when bacterial density was approximately 10^5 CFU/ml, a portion of the primary culture was diluted into secondary Intralipid emulsions containing 0, 10, or 100 mM sodium bicarbonate. Bacterial growth in all cultures was measured by dilution and plating. This figure is a representative curve for one run.

(continued...)
slightly different inocula and body weights of rabbits. "Infection
index = [recovered CFU/g tissue]/[inoculated CFU/g body weight]" to compensate for
Fig.

presence of Intralipid, the distribution of the bacteria
different
significantly different from each other, but were $significantly
Index for kidneys from both Intralipid-treated groups were not
nously during the following conditions:
intravenously inoculated rabbits. Rabbits were injected intrave-
did not observe replication of S. aureus in Diprivan at any of these
temperatures. Because previous reports from other laboratories described replication of S. aureus in
and because the formulation of Diprivan was changed in 1994 to include 0.005% w/v sodium
EDTA, we used Intralipid without EDTA and with
0.005% EDTA added as an approximation of the original
preparation and its reformulation.
In Intralipid, we observed a temperature-dependent
lag phase before bacterial replication, followed by slow
and erratic growth (table 1 and fig. 1). The extended lag
phase after inoculation of S. aureus into Intralipid is
consistent with data from Crocker et al.,22 who reported
a colony count equal to the inoculating dose after 6 h of
incubation in Intralipid at 25°C. Sosis and Braverman19
grew S. aureus in Diprivan (before 1994) at 27°C and
found that the bacteria had not doubled after 6 h,
whereas Tessler et al.18 showed that S. aureus had
not doubled 8 h after inoculation in Diprivan at 25°C. Ar-
duino et al.17 similarly reported growth curves showing
lag periods exceeding 6 h at 30°C. One study showed
almost immediate growth by S. aureus at 20°C,20 but the
bacteria were not removed from their initial growth
medium before inoculation in Diprivan, which could
have precluded a lag phase because the bacteria essen-
tially were in dilute broth. Hence, our data are consistent
with virtually all previous studies with respect to the
presence and duration of the lag period.

Once initiated, S. aureus grows poorly in Intralipid.
Our studies reveal a doubling time of 1.5 h at 22.5°C.
Crocker et al.22 and Mally et al.21 reported doubling
times of nearly 3 h for S. aureus in Intralipid at 25°C,
whereas Arduino et al.17 showed that the doubling time
of S. aureus in Diprivan (before 1994) was approxi-
mately 2 h at 30°C. Our shorter doubling times undoubt-
edly arise from the manner in which they were calcu-
ated. Unlike previous investigations, we did not simply
average the doubling time over 24 h. Rather, we chose
three time points after the lag period from which to
generate a regression line with the highest correlation
coefficient and used the slope of that line to compute
the doubling time for the most rapid and sustainable
growth period.

Discussion
Before Intralipid- and Diprivan-associated infections
can be effectively controlled, the basis for the infections
must be understood. Recent studies have focused on the
ability of Diprivan to serve as a growth medium for
bacterial pathogens17-20 but these studies have not al-
ways evaluated bacterial growth at clinically relevant
temperatures. We therefore measured the replication of
S. aureus in Diprivan and Intralipid at clinically relevant
Anesthesiology, V 91, No 5, Nov 1999
phase recurred (fig. 3). Because the staphylococci were
adapted to Intralipid and were growing exponentially
before transfer, we suspected this second lag phase
occurred while the bacteria conditioned the medium
before exponential growth ensued. Bacteria need a finite
concentration of carbon dioxide to grow because car-on dioxide is a nonconsumed reactant of lipid metab-
olism.25 We postulated that the growth of S. aureus
might be limited until sufficient carbon dioxide accumu-
lated in the medium from bacterial metabolism to allow
the bacteria to enter the exponential growth phase. We
tested this hypothesis by adding sodium bicarbonate to
provide an immediate source of carbon dioxide to In-
tralipid. Addition of bicarbonate nearly abolished the lag
period during transfer from a primary Intralipid culture
in exponential phase of growth to fresh Intralipid (fig. 3).

Our data show a lag period in excess of 6 h, a doubling
time of 1.5 h at 22.5°C (5 h at 17.5°C), and a maximal
concentration of approximately $1 \times 10^8$ CFU/ml, which
is consistent with four previous studies that evaluated
the growth of S. aureus in Intralipid and Diprivan before
its reformulation in 1994.1,17-20-22 These data suggest
that contamination of Diprivan with small numbers of
staphylococci should not create an overwhelming inoc-
ulum for patients, either in the operating room or in
other hospital units. Interestingly, the manufacturers of
these products recommend that the drugs be adminis-
tered within 6 h of preparation, presumably to prevent
potential microbial replication from reaching clinically
significant levels.24

The original report of Diprivan-related infections
stated that the same syringe of Diprivan was used
together in the operating room.1 In none of
the cases was the syringe held overnight and used
the following day. If the lag phase for growth is at least 6 h
(certainly longer at typical operating room temperatures
of 16°C to 17.5°C), and the doubling time is more than
4 h, it is difficult to understand how the bacteria could
have multiplied over the original contamination to more
readily infect patients. Furthermore, the Centers for Dis-
case Control documented that all of the cases were
related to the use of 60-ml syringes in syringe pumps.
Even if the patients were small (50 kg) and the infusion
rates necessary were low (100 $\mu g \cdot kg^{-1} \cdot min^{-1}$), the
syringe would have to be refilled every 2 h. Our data
indicate that S. aureus grows poorly during transfer
to fresh Intralipid. Therefore, adding fresh Diprivan to a
syringe, analogous to diluting the remaining bacteria,
should result in another extended lag period.

The possibility of the Intralipid in Diprivan affecting
the host was evaluated using a rabbit model for staphy-
lococcal infection. Equal doses of S. aureus suspended
in either Intralipid or saline were administered to rabbits
intravenously and were cleared from the blood equally
well (in less than 10 min). However, rabbits given S.
aureus in Intralipid appeared to become more ill, more
rapidly than those who received the bacteria in saline.
Although this observation was qualitative only, the quanti-
tative distribution of bacteria differed significantly
when rabbits were exposed to Intralipid (fig. 4). In the
absence of Intralipid, S. aureus delivered in saline was
recovered about equally from reticuloendothelial tissues
(liver and spleen), lung, and kidney, whereas S. aureus
delivered in either Intralipid or in saline 1 min after
pretreatment of rabbits with Intralipid was primarily
recovered from the kidney (fig. 4). It is conceivable that
S. aureus delivered with Intralipid may be preferentially
distributed to the kidney rather than to tissues in which
they would normally be destroyed. If this is true, then
bacteria may escape destruction in vivo and compro-
mise the animal from a relatively sequestered nidus. It is
interesting to note that Curry and Quie25 also reported
that bacteria were recovered most often from the kid-
neys of patients who had infection after the administra-
tion of Intralipid, although these cultures were obtained
postmortem.

Based on our data, we question whether simple bac-
terial multiplication to increase the inoculating dose can
adequately explain the increased incidence of infection
associated with the clinical use of Diprivan or Intralipid.
Other investigators also have questioned how well bac-
teria actually grow in Diprivan during clinical condi-
tions,26 yet many practitioners simply use Diprivan im-
mEDIATELY after it is prepared before growth can occur,
believing this will alleviate the potential for infection.
Our findings strongly suggest that Intralipid affects the
distribution of the bacteria in vivo and by doing so
potentially increases their virulence. How the altered
distribution of bacteria contributes to the risk of infec-
tion associated with Intralipid and Diprivan is not
known. Nevertheless, the results of our studies suggest
that changing the means by which lipid-based infusions
are handled before administration may not entirely solve
the problem of the infections associated with the use of
these agents. Further studies in specific pathogen-free
rabbits are needed to evaluate this completely. In the
mean time, greater care may be needed for general
infection control in patients receiving these infusions,
because the host defenses or some other aspect of the

Anesthesiology, V 91, No 5, Nov 1999
host–pathogen relationship may be disturbed by Intralipid and Diprivan.

References