

Effects of temperature and handling conditions on lipid emulsion stability in veterinary parenteral nutrition admixtures during simulated intravenous administration

Elizabeth J. Thomovsky, DVM; Robert C. Backus, DVM, PhD; F. A. Mann, DVM, MS; Cynthia K. Richmond, BS, RVT, RPh; Colette C. Wagner-Mann, MA, DVM, PhD

Objective—To determine whether lipid particle coalescence develops in veterinary parenteral nutrition (PN) admixture preparations that are kept at room temperature (23°C) for > 48 hours and whether that coalescence is prevented by admixture filtration, refrigeration, or agitation.

Sample Population—15 bags of veterinary PN solutions.

Procedures—Bags of a PN admixture preparation containing a lipid emulsion were suspended and maintained under different experimental conditions (3 bags/group) for 96 hours while admixtures were dispensed to simulate IV fluid administration (rate, 16 mL/h). Bags were kept static at 4°C (refrigeration); kept at 23°C (room temperature) and continuously agitated; kept at room temperature and agitated for 5 minutes every 4 hours; kept static at room temperature and filtered during delivery; or kept static at room temperature (control conditions). Admixture samples were collected at 0, 24, 48, 72, and 96 hours and examined via transmission electron microscopy to determine lipid particle diameters. At 96 hours, 2 samples were collected at a location distal to the filter from each bag in that group for bacterial culture.

Results—Distribution of lipid particle size in the control preparations and experimentally treated preparations did not differ significantly. A visible oil layer developed in continuously agitated preparations by 72 hours. Bacterial cultures of filtered samples yielded no growth.

Conclusions and Clinical Relevance—Data indicated that the veterinary PN admixtures kept static at 23°C are suitable for use for at least 48 hours. Manipulations of PN admixtures appear unnecessary to prolong lipid particle stability, and continuous agitation may hasten lipid breakdown. (*Am J Vet Res* 2008;69:652–658)

Parenteral nutrition is widely used in companion animal medicine, and standardized protocols for preparation of PN admixtures have maximized safety of administration. However, the addition of lipids to PN admixtures still raises concerns about coalescence of lipid particles; such coalescence may result in the formation of particles that are sufficiently large to block small pulmonary blood vessels. To reduce the risk of embolism associated with such coalescence, the FDA presently recommends administration of a single lipid-containing PN admixture preparation at room temperature for no longer than 24 hours.¹ An informal survey of US and Canadian veterinary teaching hospitals

ABBREVIATIONS

PN	Parenteral nutrition
TEM	Transmission electron microscopy
PCS	Photon correlation spectroscopy

conducted by one of the authors (EJT) revealed that the FDA recommendation has been adopted by many institutions but that clinicians at other establishments administer PN solutions for 48 hours or longer.

A lipid-containing solution is considered unsuitable for use when > 0.4% of its lipid particles are larger than 5.0 µm in diameter.^{2–11} The typical diameter of chylomicrons in the bloodstream is 0.4 to 1.0 µm. An abundance of large particles in the admixture puts an animal that is receiving PN at risk for development of lipid-induced pulmonary emboli, because the internal diameter of pulmonary capillaries is 4 to 9 µm.^{2,4,10–14}

Lipid particles have a tendency to coalesce into larger particles. The initial stages of lipid particle degradation include what is termed as creaming, during which lipid particles rise to the surface of an emulsion, and flocculation, during which individual lipid particles become associated with each other in groupings. Both

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From the Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO 65211. Dr. Wagner-Mann's present address is Helias High School, 1305 Swifts Hwy, Jefferson City, MO 65109.

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Address correspondence to Dr. Thomovsky.

of these early stages of degradation can be reversed by agitation of solutions. However, once the particles coalesce into larger particles, degradation is irreversible.

Because agitation of a lipid-containing admixture reverses lipid particle creaming and flocculation, agitation should slow the progression of coalescence and thereby prolong the period in which administration of such an admixture is considered safe. Similarly, use of an appropriately selected filter during delivery of the PN admixtures should be able to successfully remove coalesced lipid particles of embolic size, which would also potentially extend the duration of use of an admixture preparation. Results of previous research indicate that PN admixtures containing lipid emulsions can be kept for as long as 1 week at refrigeration temperatures (4°C) prior to their safe administration over a 24-hour period to humans, suggesting that keeping a PN admixture at refrigeration temperatures during administration should likewise prolong the preparation's period of safe administration.^{7,15-19}

In published retrospective studies²⁰⁻²² of PN usage in dogs and cats, bags of PN solutions were either administered immediately or stored for as long as 5 days at refrigeration temperatures prior to administration. Although it was not always specifically stated in those reports, it appeared that a single bag of PN was not administered to a patient for more than 48 hours. Mechanical complications (eg, catheter occlusions or dislodgements and development of thrombophlebitis) developed in 21%²¹ to 46%²⁰ of treated animals. Hyperglycemia was another common complication ranging from 20%²² to 75%²⁰ of all patients, with cats seemingly more affected than dogs. Other metabolic derangements detected in animals receiving PN were hypo- and hypernatremia, hypo- and hyperkalemia, hypo- and hypercalcemia, and hypo- and hyperphosphatemia. However, the proportions of the 3 study populations that developed these metabolic derangements were much less than those that developed hyperglycemia: only as many as 10% of dogs and cats in 1 study,²⁰ 34% of cats in another study,²¹ and 11% of cats in a third study.²² There was an overall low rate of septic complications; in 2 studies,^{20,22} septic complications were not detected in cats or dogs, and PN-associated sepsis developed in 6% of cats in another study.²¹ Lipemia was evident in 46% of cats and dogs²⁰ and 13% to 24% of cats.^{21,22} No embolic respiratory complications were noted in any veterinary studies.

The purpose of the study reported here was to determine whether veterinary PN admixtures that are kept at room temperature (23°C) can be used for more than 48 hours after preparation without development of excessive lipid particle coalescence and whether lipid particle coalescence is prevented by filtration, refrigeration, or agitation of the preparations. By use of TEM, the intent was to evaluate the change in lipid particle size distribution in bags of standard veterinary PN admixture over time at room temperature; the goal was to determine whether prolonged duration of bag hanging (> 24 hours) increased the embolic risk of PN administration because of the development of an unacceptable proportion of large lipid particles (ie, those > 5 µm in diameter). We were also interested to investigate whether the duration of safe administration of an admixture could be prolonged by physical manipulations

of the PN admixture. If the duration of safe administration of each lipid-containing admixture can be extended (> 24 hours), the result would be cost savings for clients. At our institution, compounding fees and bag-administration set costs account for as much as 75% of the daily cost of PN administration. Prolonged use of admixture preparations would also be convenient for clinicians and pharmacists, especially over weekends and holidays when pharmacy services may be limited or unavailable. Our hypotheses for the present study were that a standard lipid-containing veterinary PN admixture can be kept in a static position at room temperature for more than 48 hours prior to the development of an unacceptable proportion of large lipid particles (with their inherent increased risk of embolism) and that agitation, refrigeration, and filtration of an admixture preparation will delay increases in size distribution of lipid particles that would be considered unsafe.

Materials and Methods

Admixture preparation—Fifteen 2-L bags^a of PN admixture were prepared according to standard compounding protocols²³ by a pharmacist (CKR) at the University of Missouri-Columbia Veterinary Medical Teaching Hospital. Each bag contained 525 mL of 50% dextrose (1.7 kcal/mL),^b 453 mL of 20% lipid emulsion (2 kcal/mL),^c 840 mL of 8.5% amino acids and electrolyte solution (1 g/11.76 mL),^d and 5 mL of vitamin B complex.^e The admixture volume of each bag would support the resting energy requirement of a 10-kg adult dog for 96 hours, as calculated by use of the following formula: (30 × weight [kg]) + 70 kcal/d. The reported size of the emulsified fat particles in the lipid emulsion was 0.5 µm.²⁴ The admixture was typical of veterinary formulations, in that 50% of the nonprotein metabolizable energy was available as lipid and 50% was available as dextrose (calculated osmolarity, 1,280 mosm/L).

Procedures—Each bag of PN admixture was attached to an IV fluid administration set, and its contents were delivered by use of an IV fluid pump^f into a beaker to simulate IV administration to a patient. The rate of administration was 16 mL/h. All PN solution delivered into the beaker was measured daily to verify the evacuation rate.

Bags of PN admixture were allocated to 1 of 5 groups; conditions of temperature and handling for the duration of the experiment differed for each group of bags. Group 1 was composed of 3 bags of PN admixture that were each kept in a static position at refrigeration temperature (4°C). Group 2 was composed of 3 bags of admixture that were each kept at room temperature (23°C) and were placed on a test tube orbital rotating device^g; the device continuously agitated the admixtures in a circular motion (rotation speed, approx 360°/s [60 revolutions/min]). Group 3 was composed of 3 bags of admixture that were each kept at room temperature and were placed on a test tube orbital rotating device^g; the device agitated the admixtures in a circular motion (rotation speed, approx 360°/s [60 revolutions/min]) for 5 minutes every 4 hours. Group 4 was composed of 3 bags of admixture that were each kept in a static position at room temperature; the admixture solution from

each bag was passed through a 5- μm filter^h placed at the approximate midpoint of the IV fluid administration set tubing. At the end of the 96-hour collection period, two 10-mL samples of PN admixture were collected at a location distal to each filter, inoculated into trypticase-soy agar, and submitted for aerobic and anaerobic bacterial culture. Group 5 (control group) was composed of 3 bags of PN admixture that were each kept in a static position at room temperature.

Collection and assessment of PN admixture samples—A 1.0-mL sample was collected by use of aseptic technique from the most distal port on the IV fluid administration set tubing from each bag of PN admixture at 0, 24, 48, 72, and 96 hours. At 0 hours, samples were collected immediately from the distal port of the IV tubing after the tubing was primed with PN solution and before it was placed into the IV pump. All samples were collected during a 3-week period. During each week of that period, 1 of the 3 bags of PN admixture from each group (1 through 5) was compounded and underwent experimentation (including sample collections at the time points specified).

Immediately after collection, each sample of admixture was diluted 1:10 with physiologic saline (0.9% NaCl) solution. The diluted sample was mixed 1:1 with 2% osmium tetroxide solution and allowed to stand for 15 minutes to allow fixation. The osmium oxidized unsaturated bonds of fatty acids in the lipid particles to create a heavy metal, which stained the lipid membranes black. Approximately 5 μL of the resulting mixture was placed on a 200-mesh carbon copper gridⁱ and allowed to adhere to the grid for 5 minutes. Excess solution was wicked off the grid, and the grid was washed 15 to 20 times in sterile water to remove excess fixative and other precipitates that were not affixed to the grid. The grid was then air dried prior to TEM^j examination.

Via TEM, each grid was assessed for density of lipid particles and spatial distribution of particles (ie, clear separation or clumping of particles). Particles that were closely associated with large deposits of background material were not recognized as separate particles by the image analysis software. Thus, fields that were evaluated were those with high particle density and resolution. Electron micrographs of 6 to 8 regions of each sample grid were obtained to capture images of at least 300 discrete lipid particles. The overall goal was to obtain usable images of 1,000 lipid particles from each group over the 3-week study period.

The TEM image negatives were digitized and analyzed by use of software^k with image processing and analysis applications.^l The software enumerated and measured diameters of particles in the electron micrographs. Image processing was conducted by use of standard methods.²⁵ Images were optimized by use of contrast adjustment prior to the application of bi-level thresholding and watershed segmentation to allow discrimination of separate lipid particles from background material. Parameter filters were applied to remove particles < 100 pixels and those that were not round in shape.

Radius and roundness were determined for each particle. Features were counted and included as par-

ticles if they were of a sufficient roundness. Roundness was calculated by use of an equation as follows:

$$\text{Roundness} = 4(\text{area})/\pi(\text{maximum diameter})^2$$

Roundness of 1.0 indicates a perfect circle, and features with a roundness value < 0.66 were not evaluated. The diameter of a particle was determined by doubling the circumscribed radius for a perfect circle drawn around that particle (circle extended to the maximum borders of the particle).

Statistical analysis—Particle diameters from each TEM micrograph were determined and tabulated by micrograph number (1, 2, 3, and so forth), bag number (1, 2, or 3), time (0, 24, 48, 72, or 96 hours), and group (1, 2, 3, 4, or 5) on a computerized data worksheet.^m Treatment and sampling time effects were evaluated for mean, SD, median, and maximum values of lipid particle counts and diameters. Post hoc Dunn multiple comparison tests were used to determine significant differences in particle size among sample collection time points. At each sample collection, the significance of effect of treatment (control conditions, refrigeration, intermittent agitation, continuous agitation, or filtration) was determined by use of a Kruskal-Wallis 1-way ANOVA on ranks. The Holm-Sidak method was applied to determine whether there was a significant difference in the number of particles counted at each time point. The Fisher exact test was used to determine whether the data within groups 1 through 4 were distinct from findings for the control group (ie, bags of PN admixture kept at room temperature without agitation or filtration). χ^2 Analysis was used to determine the probability that 0.4% of particles were > 5 μm in diameter in the control samples at 96 hours.

The number of electron micrographs evaluated for each admixture sample (6 to 8) and the number of admixture bags within treatment groups (3) were based on a power analysis in which β was set to 0.8. Frequency mean and variance estimates used in the power analysis were based on previously reported observations.²⁶⁻²⁸ Statistical analyses were conducted by use of commercially available software.ⁿ Significance was set at a value of $P < 0.05$.

Results

Among the initial (0-hour) samples collected from bags of each group, lipid particle diameter mean, median, and maximum values did not differ significantly ($P = 0.65$, $P = 0.82$, and $P = 0.63$, respectively); particles > 5 μm in diameter were not observed. Of the 3,208 particles in all groups evaluated at 0 hours, the largest particle diameter was 1.50 μm . This particle was present in a control group sample, was the largest particle detected at any sample collection time point, and was approximately 6 times as large as the mean diameter of the control particles at 0 hours (0.26 μm ; Table 1).

The total number of particles counted in the 96-hour samples obtained from the control group admixtures was 716. If the proportion of large particles had reached 0.4%, 2 or 3 particles > 5 μm in diameter should have been detected. Because no large particles

Table 1—Mean \pm SD and median (range) values of lipid particle diameter (determined via TEM examination at 0, 24, 48, 72, and 96 hours after preparation) in bags of PN admixture exposed to various conditions of temperature and handling (5 groups of 3 bags each).

Group*	Time point (h)	No. of particles	Particle diameter (μm)	
			Mean \pm SD	Median (range)
1	0	487	0.26 \pm 0.12	0.24 (0.11–1.11)
	24	771	0.24 \pm 0.09	0.22 (0.02–0.80)
	48	1,145	0.24 \pm 0.10	0.21 (0.08–1.01)
	72	381	0.27 \pm 0.13	0.24 (0.01–0.85)
	96	1,275	0.25 \pm 0.10	0.25 (0.11–0.84)
2	0	912	0.25 \pm 0.11	0.23 (0.01–1.03)
	24	542	0.26 \pm 0.41	0.23 (0.11–1.12)
	48	811	0.24 \pm 0.01	0.22 (0.08–0.73)
	72	768	0.23 \pm 0.01	0.21 (0.01–0.96)
	96	1,787	0.16 \pm 0.09	0.21 (0.11–1.07)
3	0	459	0.26 \pm 0.12	0.23 (0.01–1.08)
	24	1,386	0.25 \pm 0.11	0.23 (0.01–1.15)
	48	1,083	0.24 \pm 0.11	0.22 (0.01–1.00)
	72	500	0.24 \pm 0.11	0.21 (0.01–0.92)
	96	549	0.26 \pm 0.11	0.24 (0.10–0.82)
4	0	391	0.25 \pm 0.10	0.24 (0.02–1.05)
	24	1,516	0.24 \pm 0.10	0.23 (0.01–0.98)
	48	784	0.23 \pm 0.09	0.24 (0.08–0.70)
	72	382	0.25 \pm 0.11	0.24 (0.01–0.71)
	96	560	0.27 \pm 0.11	0.25 (0.11–1.43)
5	0	959	0.27 \pm 0.13	0.23 (0.11–1.50)
	24	2,017	0.25 \pm 0.10	0.28 (0.02–0.96)
	48	782	0.25 \pm 0.12	0.22 (0.11–0.84)
	72	782	0.27 \pm 0.12	0.23 (0.11–0.84)
	96	716	0.29 \pm 0.15	0.17 (0.11–0.93)

*Bags of PN admixture were exposed to various experimental conditions; during the exposures, admixture preparations were emptied from each bag via an IV fluid administration set to mimic administration to a patient. For 96 hours, 3 bags of PN admixture were kept in a static position at refrigeration temperature (4°C; group 1); kept at room temperature (23°C) and continuously agitated on a test tube orbital rotating device in a circular motion (rotation speed, approx 360°/s [60 revolutions/min]; group 2); kept at room temperature and agitated intermittently on a test tube orbital rotating device in a circular motion (rotation speed, approx 360°/s [60 revolutions/min]) for 5 minutes every 4 hours (group 3); kept in a static position at room temperature (solution passed through a 5- μm filter placed at the approximate midpoint of the IV fluid administration set tubing; group 4); or kept in a static position at room temperature without further intervention (control group 5). At 0 hours, samples were collected immediately from the distal port of the IV tubing after the tubing was primed with PN solution and before it was placed into the IV pump.

were evident in control group samples collected at any time point, the proportion of large particles would have to have been $< 0.019\%$. The probability of not finding a large particle simply by random chance would be $< 0.1\%$ (χ^2 test; $P < 0.001$) if the true frequency of large particles in the control bags at 96 hours was 0.4%.

Evaluation of particles from each group at each sample collection time point was successfully completed with the exception of 3 of the 75 sample collections. In each of those instances, particles were not evaluated because of poor-quality TEM images in which lipid particles either were not evident or could not be distinguished from the background material despite examination of repeated sample preparations. These poor images were derived from samples collected at 72 hours in the refrigeration group (1) during the third study week, at 72 hours in the intermittent agitation group (3) during the third study week, and at 96 hours in the filtration group (4) during the second study week. From

each of those PN admixture bags, lipid particles from subsequent samples could be evaluated. The cause of the poor image quality could not be identified.

Within a group, the number of particles counted in samples collected at each time point did not differ significantly, except for samples collected from group 2 bags that underwent continuous agitation. Within this group, more particles were detected at 96 hours than at 0 hours (596 ± 108 particles vs 304 ± 272 particles; $P = 0.02$).

Compared with findings at 0 hours, lipid particle mean, median, and maximum diameters for each sample collection at 24, 48, 72, and 96 hours from the control bags (group 5) did not differ significantly ($P = 0.49$, $P = 0.97$, and $P = 0.14$, respectively); those values for subsequent samples were also not significantly different from one another (Figure 1). Of the 5,256 total particles in control group samples evaluated at all time points, the largest particle diameter was 1.50 μm (at 0 hours).

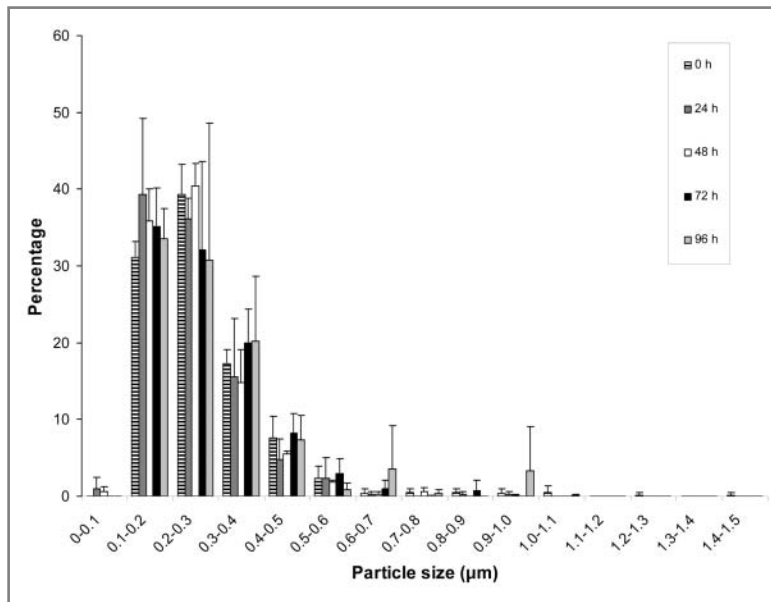


Figure 1—Mean \pm SD percentage distribution of lipid particle diameters (determined via TEM examination at 0, 24, 48, 72, and 96 hours after preparation) in bags of PN admixture that were kept static at room temperature (approx 23°C; control group) for 96 hours while admixtures were dispensed to simulate IV fluid administration (rate, 16 mL/h). At 0 hours, samples were collected immediately from the distal port of the IV tubing after the tubing was primed with PN solution and before it was placed into the IV pump.

Within each group (1 through 4), lipid particle mean, median, and maximum diameters at 24, 48, 72, and 96 hours were not significantly different from findings at 0 hours and values did not differ significantly among sample collection time points. Mean, median, and maximum lipid particle diameters in samples from bags exposed to the experimental treatments were also not significantly different from paired observations in samples from bags of the control group at any sample collection time point (Table 1). Furthermore, at each sample collection time point, the mean, median, and maximum particle diameters among the treatment groups did not differ significantly.

Visual inspection of each lipid admixture was conducted daily when samples were collected for analysis. Regardless of other experimental conditions, all bags kept in a static position had a visible cream layer at the admixture surface by 72 hours. The PN solutions in the group 2 (continuously agitated) bags consistently had separated into distinct layers, developing a visible oil layer in the bag, the IV fluid administration set, and the cartridge loaded in the IV pump at 72 hours. Culture of samples of the group 4 PN solutions collected at the end of the 96-hour collection period yielded no aerobic or anaerobic bacterial growth.

Discussion

For quantitative evaluation of lipid particles in aqueous emulsions, 3 techniques are generally accepted: light obscuration particle measurement, PCS, and TEM.^{26,27,29} Transmission electron microscopy and PCS are equivalently accurate techniques and are superior to light obscuration particle measurement for particle-size evaluation.²⁷ However, particles < 100 nm in diameter are more easily identified via TEM, whereas particles

< 120 to 150 nm in diameter are difficult to detect via PCS.³⁰ A potential limitation of all particle-sizing techniques is that sample sizes are exceedingly small and, therefore, could be construed as not representative of the entire admixture.⁵ Nevertheless, TEM imaging is considered an accurate and reliable means for identifying particle size in lipid emulsions.

For our purposes, TEM particle sizing was considered sufficient for identifying a distribution of lipid particle diameters in PN admixtures that would be associated with increased risk of embolism. Any emulsion is considered unsuitable for IV administration when the proportion of large particles (> 5 µm in diameter) in an emulsion exceeds 0.4%. In the present study, initial statistical plans included categorizing particles as < 5 µm in diameter versus \geq 5 µm in diameter. If the proportion of large particles had reached 0.4% in the control bags at the 96-hour time point, 21 particles > 5 µm in diameter would have been observed. Despite counting and sizing 5,256 particles in 15 control bag samples during the entire study period, no particles were > 5 µm in diameter in any control bag sample

at any time point. This result was unexpected, particularly in samples collected after the bags of admixture had been suspended and in use for 24 hours (the current recommended period of safe use of a PN preparation) and especially after 96 hours when particle coalescence was anticipated to be the greatest. Even after 96 hours, lipid particles in admixture samples collected from the IV fluid administration sets attached to control bags appeared to present no embolic risk.

In addition to finding an absence of particles > 5 µm in diameter in the control bags at any sample collection time point, there was no difference in mean lipid particle diameters in control group samples collected at 0 and 96 hours. This result was also unexpected because the lipids were anticipated to coalesce over time, causing a shift in the distribution of lipid particle diameters toward notably greater sizes. These findings support the conclusion that, over time, the standard veterinary PN solutions used in our study that underwent no special temperature or handling manipulations did not develop a lipid particle diameter distribution associated with increased risk of embolism.

Results of the present study were in contrast to those of other studies^{6,31,32} of lipid particle size. In 1 study,³¹ the percentage of large particles (1.6 to 25.4 µm in diameter) significantly increased between 0 and 72 hours both in samples kept at refrigeration temperatures and those kept at room temperature. That study did not include direct assessment of particle size, but relied on Coulter counter analysis of numbers of particles at various time points. In another study,⁶ admixtures containing lipid emulsion that were kept at room temperature developed particles with diameters > 5 µm within 30 hours, as determined via a dynamic light scatter technique. Breakdown of the admixture solutions was vis-

ible in both of those studies. Another study³² in which PN admixtures were examined by use of a light extinction method revealed variation in particle sizes over time; with longer time intervals, greater particle sizes as well as visual precipitation of components were detected. The reason for variance of results of those studies from the findings of the present investigation is not completely clear; however, it is important to note that in each of the previous studies, lipid particles were not directly viewed and measured to determine their diameters.

The findings of our study are not completely without precedent. By use of scanning electron microscopy, investigators examined PN admixtures that had been stored at refrigeration temperatures for 28 days followed by administration at room temperature during a 2-day period and found that the mean diameter of lipid particles was not significantly increased from day 0 to day 30.¹⁵ In that study, mean initial particle diameter was $0.27 \pm 0.08 \mu\text{m}$ and particle diameter on day 30 was $0.36 \pm 0.11 \mu\text{m}$; the largest particle detected was $2.74 \mu\text{m}$. Additionally, other studies^{7,16-19} in which PN solutions were evaluated after storage at refrigeration temperatures for as long as 28 days and then administered during a 1- to 2-day period at room temperature revealed that there were no significant changes in mean particle diameter over time, and the proportion of lipid particles $> 2.0 \mu\text{m}$ in diameter did not exceed 0.4%. The techniques used in those studies included scanning electron microscopy, light microscopy, PCS, and computerized particle counting methods. Results of other studies³³⁻³⁵ of PN admixture stability involving dynamic light scattering and light extinction methods of lipid particle examination indicated that although there was a gradual increase in particle size over time, the proportion of large particles that could be associated with risk of embolism did not significantly increase after 30 hours at room temperature.

Variation in admixture mineral composition may account for the reported differences in lipid particle stability and may provide a reason for the long-term stability of the PN admixtures of the present study that were kept at room temperature. Typically, the electrostatic charge (zeta potential) on the surface of lipid particles inhibits coalescence.⁴ This repulsive charge is bestowed on the lipid particles by the phospholipid surfactant in the lipid emulsion. However, the presence and amount of ions in the PN admixture, especially the presence of divalent cations such as calcium²⁺, can alter these protective electrostatic charges.^{3,31,34} Additionally, phosphorus, a standard component of PN admixtures, can precipitate with calcium and result in lipid emulsion breakdown.^{3,32,35} The PN admixtures used in our study did not have calcium as a component, whereas most admixtures administered to humans (including those analyzed in other studies^{4,7,15,17,18,32-34}) routinely contain calcium. It is possible that calcium hastened the breakdown of PN admixtures in previous studies. An interesting future investigation would involve the addition of calcium to the admixtures used in our study to determine whether calcium induces significant changes in lipid particle size over time.

Another intriguing finding of the present study was that after 72 hours, the PN admixture in the continu-

ously agitated bags (group 2) had separated into distinct layers within the bag and the chambers of the infusion set and IV tubing. The uppermost layer appeared to be an oil layer, indicating coalescence of lipid particles. This is similar to the finding of an investigation by Bettner and Stennett,³¹ in which rings of oil began forming by 48 hours and progressed to free-floating oil layers by 1 week in admixtures kept refrigerated and at room temperature. In our study, no large lipid particles were detected via TEM examination in the PN admixtures in group 2 bags, despite the presence of an oil layer. Comparison of mean and maximum particle sizes over time in the continuously agitated bags and control bags did not indicate any difference in particle-size distribution.

Although it is unclear why continuous agitation of group 2 bags in our study led to breakdown of the emulsion without evidence of large lipid particles in samples collected from the distal IV port of the administration sets, we support recommendations not to administer such separated emulsions to patients to avoid pulmonary embolism.¹⁻³ Agitation may have disrupted the surfactant layer in the lipid emulsion and nullified the repulsive electrostatic charges on the lipid particles, thereby promoting coalescence. Alternatively, agitation may have overwhelmed the repulsive forces of the particles, leading to collision between and eventual coalescence of lipid particles into a visible oil layer. Because of lower density, coalesced particles may have essentially floated away from the outflow of smaller, more-dense intact particles in solution to create a poorly mixed oil layer. If this were the case, the less-dense large particles or oil layer may have been more likely to separate from the smaller, more-dense particles in the IV fluid administration set components where the admixture was held relatively static for short periods of time. Our assumption is that the smaller, more-dense particles would have continued to move through the IV fluid administration set and were collected in samples from the distal port, whereas the oil layer remained behind. This explanation could be tested by mixing the contents of the bag daily before sample collection and also by collecting samples directly from the bag rather than from the IV administration set tubing and pump; those samples would then be examined via TEM to ascertain whether large particles were present.

In the present study, the creaming that developed in the PN bags in groups 1, 4, and 5 after 72 hours was expected and consistent with previous reports^{1-4,8,31} of changes in lipid admixtures that remained static. Because creaming does not indicate coalescence of lipid particles, it was expected that the lipid particles examined microscopically would not exceed the $5\text{-}\mu\text{m}$ -diameter cut-off point in any of the samples from these groups. It is widely held that gentle agitation can reblend mixtures that have a cream layer without safety concerns regarding their administration to patients.

The finding of no aerobic or anaerobic bacterial growth in cultures of samples collected from admixtures that were held static at room temperature in our study is consistent with previous reports^{2,3,13,36} that bacterial growth in properly prepared and handled PN admixtures is rare and usually results from patient or hospital-related contamination. Therefore, taking into account

our findings regarding bacterial contamination, microscopic lipid particle size, and the visual appearance of admixtures, we conclude that properly compounded PN admixtures of the type used in the present study that are kept static at room temperature are unlikely to develop large lipid particles with an inherent risk of embolism when administered for longer than 48 hours, without the need for other interventions.

- a. 3-in-1 mixing container, Abbott Laboratories, North Chicago, Ill.
- b. 50% dextrose injection, Baxter Healthcare Corp, Deerfield, Ill.
- c. Intralipid 20%, Baxter Healthcare Corp, Deerfield, Ill.
- d. Aminosyn 8.5% sulfite-free crystalline amino acid solution (Na⁺, 78 mEq/L; Cl⁻, 86 mEq/L; K⁺, 66 mEq/L; Mg²⁺, 10 mEq/L; acetate, 61 mEq/L; and PO₄⁻, 30 mEq/L), Abbott Laboratories, North Chicago, Ill.
- e. Vitamin B injection solution, Phoenix Pharmaceutica Inc, St Joseph, Mo.
- f. Abbott Plum A+ infusion IV pump system, Abbott Laboratories, Abbott Park, Ill (used in groups 2 through 5) and Heska Vet/IV 2.2, Heska Corp, Fort Collins, Colo (used in group 1).
- g. Lab-Line lab rotator (31 × 31 cm), Barnstead International Products, Dubuque, Iowa.
- h. Supor sterile syringe filter, Baxa Corp, Englewood, Colo.
- i. Carbon support film on specimen grid on 200 mesh copper, Electron Microscopy Sciences, Hatfield, Pa.
- j. TEM-JEOL 1200 EX, Japan Electron Optics Limited, Tokyo, Japan.
- k. Adobe Photoshop, version CS2, Adobe Systems Inc, San Jose, Calif.
- l. Fovea Pro, version 4.0, Reindeer Graphics, Asheville, NC.
- m. Microsoft Excel, Microsoft Corp, Redmond, Wash.
- n. SigmaStat, version 3.0, SPSS Inc, Chicago, Ill.

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