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The Spectrophotometric Absorbance of Intralipid®

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The spectral absorbance of Intralipid®, a phospholipid emulsion, was investigated to discern its effect, when parenterally administered, on the spectrophotometric measurement of hemoglobin (Hb), oxyhemoglobin (HbO_2), and carboxyhemoglobin (HbCO). While accounting for dilutional factors, various concentrations of Intralipid in both water and hemoglobin solutions were analyzed at six wavelengths commonly used to measure Hb, HbO_2 and HbCO. Absorbance increased linearly with Intralipid concentration at all wavelengths, and ranged from 0.034 at 505 nm to 0.019 at 626.6 nm per mg of Intralipid. Therefore, in patients receiving Intralipid, significant errors in Hb, HbO_2 and HbCO measurements can be introduced if these measurements are made by oximetry, and the authors suggest that such measurements should be accomplished by methods other than spectrophotometry. (Key words: Blood: hemoglobin. Measurement techniques: spectrophotometry.)

INCREASED LEVELS of endogenous lipids have been shown to interfere with spectrophotometric measurements of oxyhemoglobin, carboxyhemoglobin and total hemoglobin. In particular, the error in measurement is due to the concentration of chylomicrons and not to the presence of low-density or very-low-density lipoproteins.[1] Intralipid®,§ which is a soybean oil and egg phospholipid emulsion, is carried in plasma in a form physically similar to chylomicrons.[2] The use of Intralipid as a component of parenteral hyperalimentation of the critically ill patient is common, and its use in the preparation of patients for surgical procedures is increasing. Thus, it is becoming increasingly important to question whether hemoglobin determinations using oximetry are reliable in these patients. This study has evaluated the spectrophotometric absorbance of various concentrations of Intralipid.

Methods

The light absorbances of Intralipid, 10 per cent, in distilled water in concentrations of 2.5, 5, 10, 20, and 40 mg/ml were measured at wavelengths of 505, 535, 585.2, 594.5, 600, and 626.6 nm on a Perkin Elmer Model 550®¶ scanning spectrophotometer. These measurements were then repeated at the same wavelengths with equivalent concentrations of Intralipid, 10 per cent, in a solution of hemoglobin (10.1 g/dl). In addition, the light absorbances of pure Intralipid, 10 per cent, and of the pure hemoglobin solution were measured at those wavelengths.

All sets of measurements were made on six samples and the mean values of the spectral absorbances at the respective wavelengths derived. Regression coefficients relating absorbance to concentration of Intralipid were calculated. The amount of change in absorbance per milligram of Intralipid at each wavelength was derived.

Since the increasing concentrations of Intralipid had an increasing dilutional effect on hemoglobin concentration, light absorbance was measured in the hemoglobin solution to which distilled water was added

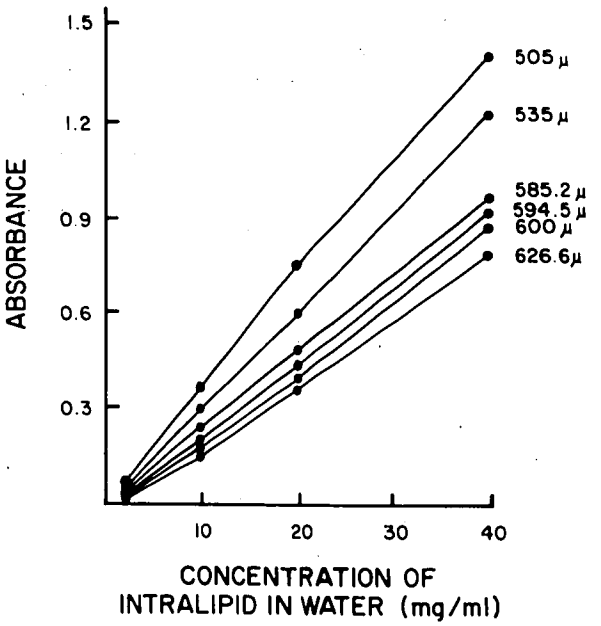

FIG. 1. The relationships of concentrations of Intralipid in water and absorbances at 505, 535, 585.2, 594.5, 600, and 626.6 nm.

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¶ Scientific Products, McGaw Park, Ill.

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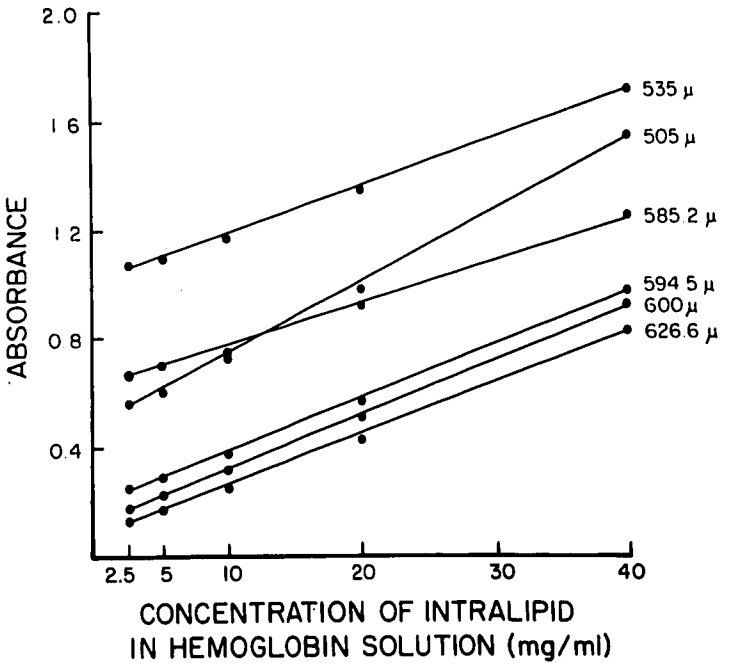

FIG. 2. The relationships of concentrations of Intralipid in hemoglobin and absorbances at 505, 535, 585.2, 594.5, 600, and 626.6 nm.

in volumes equal to the volumes of Intralipid used to prepare the original samples. Readings were again made on six samples of each dilution. The mean values of these spectral absorbances at each wavelength were obtained and regression coefficients relating absorbance to concentration of hemoglobin were derived.

Results

Absorbance increased linearly at all wavelengths with increasing concentrations of Intralipid in water (fig. 1, regression coefficient = 0.99) and in a solution of hemoglobin (fig. 2, regression coefficient = 0.99).

The increases in absorbance per mg of Intralipid were 0.034 at 505 nm; 0.029 at 535 nm; 0.022 at 585.2 nm, 594.5 nm and 600 nm; and 626.6 nm for the solution of Intralipid in water. A similar pattern was found for the Intralipid–hemoglobin solution, where absorbance per mg of Intralipid increased by 0.027 at 505 nm and by 0.019 at 535, 585.2, 594.5, 600, and 626.6 nm.

The method of preparing the various concentrations

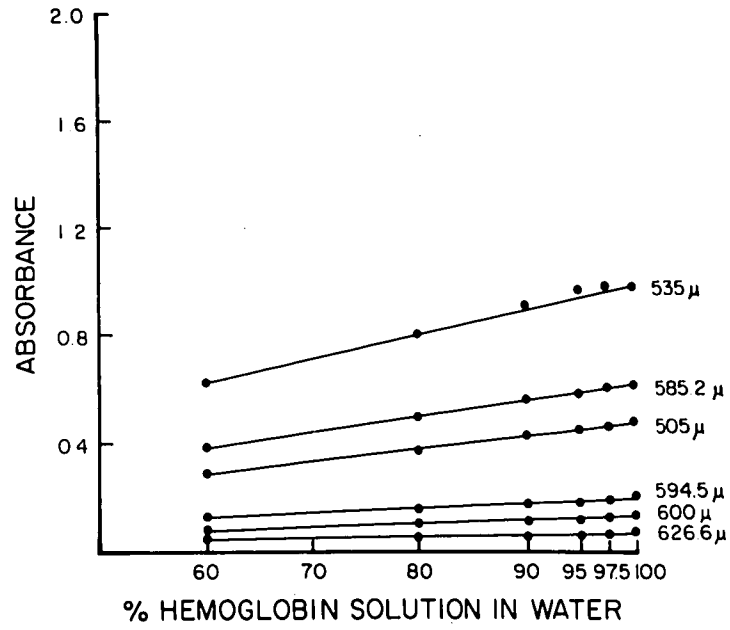

FIG. 3. The relationships of absorbances and hemoglobin concentrations at 505, 535, 585.2, 594.5, 600, and 626.6 nm.

of Intralipid in hemoglobin solution resulted in decreasing the hemoglobin concentration as the Intralipid concentration increased. This dilutional effect, which decreased the contribution of hemoglobin to the measured absorbance, explains the difference between the changes in absorbance per mg of Intralipid prepared in water and in hemoglobin solution (fig. 3, regression coefficient = 0.99–0.95).

Discussion

The concentrations of Intralipid studied are similar to those measured in clinical situations[3] and are believed to represent probable concentrations in patients receiving lipid hyperalimentation.

The absorbance wavelengths analyzed in this study are those employed in two commonly used oximeters, the Radiometer OSM 2 CO-Oximeter®,** which analyzes hemoglobin at 505 and 600 nm, and the IL 282 CO-Oximeter®,†† which analyzes hemoglobin at 535, 585.2, 594.5, and 626.6 nm. Absorbancy values obtained at these wavelengths are used to measure hemoglobin, oxyhemoglobin, carboxyhemoglobin, and methemoglobin. The respective concentrations are determined by the solution of simultaneous equations relating absorbance and concentration at two or more wavelengths, as described by the Lambert-Beer law. Examples of these equations are represented thus:

$$A(n_1)/L = (\epsilon HbO_2[n_1] \times cHbO_2) + (\epsilon Hb[n_1] \times cHb)$$

$$A(n_2)/L = (\epsilon HbO_2[n_2] \times cHbO_2) + (\epsilon Hb[n_2] \times cHb)$$

A = absorbance at given wavelength

n = specific wavelength

ϵ = molar absorption coefficient

c = substance concentration

L = length of cell path

The specific wavelengths (n_1 and n_2) chosen are those at which the various forms of hemoglobin have widely differing absorbances. It is, therefore, apparent that if the serum concentration of Intralipid were known, the change in absorbance per mg of Intralipid, as measured in this study, could be applied as correction factors for absorbance in the equations.

At present, serum Intralipid concentrations are not readily available to the clinician. However, it is clear that Intralipid has sufficient spectral absorbance to make oximetry measurements of hemoglobin pigments inaccurate. The evaluation of arterial and tissue oxygenation in critically ill patients requires accurate measurements of hemoglobin, oxyhemoglobin, and carboxyhemoglobin. It is suggested that in patients receiving Intralipid these measurements ought to be accomplished by methods other than spectrophotometry.

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