PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 162, 458–463 (1979)

Effect of Intravenous Fat Emulsion on Pentobarbital-Induced Sleep Time in Rats (40704)

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The introduction of Intralipid, a 10% soybean oil emulsion, represented a major clinical advance in parenteral nutrition in that it provides large amounts of energy in relatively small volumes (1). The intravenous administration of fat emulsion appears to be safe and free of serious risks or complications (2–4). However, with intravenous fat emulsion therapy, because of the lipophilic nature of many drugs there is a potential for drug interactions. In order to test this possibility, we examined whether the anesthetic action of the lipid-soluble drug sodium pentobarbital might be altered by the intravenous administration of 10% fat emulsion. We also determined whether the metabolic depressant action of pentobarbital would affect the clearance of the fat emulsion from the plasma.

Materials and methods. Sleeping times. Male Sprague–Dawley rats weighing 300–350 g were used in all experiments. The animals were given food (Purina Rat Chow) and water *ad libitum.* While the animals were under ether anesthesia, a saline-filled PE-50 cannula was placed in the right jugular vein and exteriorized through the skin at the back of the neck. Each animal was allowed 90 min recovery time. Narcosis was then induced by intravenous administration of pentobarbital (30 mg/kg) and the time (minutes) to the return of the righting reflex was determined. When Intralipid emulsion (10%, Cutter Laboratories) was given, it was infused through the venous cannula over 30 sec. A saline volume control was employed for comparison with all doses of Intralipid emulsion tested. Control rats were included with each group of experimental rats in order to control for differences related to time of day or day-to-day variation.

Intralipid emulsion clearance. Our observations on the effect of Intralipid emulsion on pentobarbital-induced sleep times suggested that clearance of the administered fat emulsion from the plasma may be an important determinant of the interaction observed. We, therefore, determined the clearance and half-life of Intralipid emulsion from the plasma according to the procedure described by Carlson and Rössner (5). While the animals were under ether anesthesia, saline-filled cannulas were placed in the jugular vein and carotid artery. Both of these cannulas were exteriorized through the skin at the back of the neck. The rat was placed in a restrainer and allowed 90 min for full recovery. A bolus injection of 10% Intralipid emulsion in a dose of 0.2 g per kilogram of body weight was given via the jugular vein over a period of 15 sec. At time intervals of 0, 2, 5, 10, 20, 25, and 30 min after infusion of the emulsion, 0.2 ml of blood was taken from the carotid artery and placed in 5.0 ml of an isotonic saline solution. The blood samples were centrifuged immediately at room temperature at 700 rpm for 10 min to sediment red blood cells. Two milliliters of the supernatant was placed in culture tubes (10 × 75 mm), and the molecular light scattering index was determined with a PDQ® Laser Nephelometer (Hyland Diagnostics Division of Travenol Laboratories, Inc., Costa Mesa, Calif.). A standard curve with Intralipid emulsion in saline was used to convert the molecular light scattering index values into milligrams of triglyceride per 100 ml of plasma. Zero-time nephelometry reading was subtracted to correct for endogenous light scattering particles. At this dose the disappearance of Intralipid emulsion from the plasma of rats follows first-order kinetics in which removal is an exponential function (6). The log of the plasma Intralipid emulsion concentration (mg/100 ml) plotted against time gives a straight line. The slope of this line by convention defines the rate constant

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of the first-order reaction rate and is expressed as a percentage removed per minute (K_2). The biologic half-life ($t_{1/2}$) in minutes was calculated using the formula $t_{1/2} = 0.693/K_2$. The values for K_2 and $t_{1/2}$ were obtained from standard programs using a Texas Instruments 59 calculator. Data are expressed as mean ± standard error, and the differences between means were analyzed using one-way analysis of variance and least significant difference test of means (7).

Results. Sleeping time. In our initial studies, when 10% Intralipid emulsion (2.5 g/kg, iv) was administered 5 min after pentobarbital-induced narcosis, sleep time (minutes) was prolonged considerably, 87 ± 6 (n = 15) vs 177 ± 15 (n = 6), $P < 0.001$. As the dose of Intralipid emulsion was decreased to 0.16 g/kg, the prolongation of sleeping time was also decreased in a dose-dependent fashion (Fig. 1). When Intralipid emulsion (2.5 g/kg, iv) was given 5 min prior to pentobarbital-induced narcosis, a significant ($P < 0.001$), although slightly smaller prolongation of sleeping time was observed, 87 ± 6 (n = 15) vs 151 ± 14 (n = 6).

The 26-min difference between the mean sleeping times of rats given Intralipid emulsion 5 min before and those given it 5 min after pentobarbital-induced narcosis suggested the importance of clearance of the fat emulsion from the plasma as a factor in the prolongation of pentobarbital-induced sleeping time. When the time of administration of Intralipid emulsion was increased to 60 min prior to pentobarbital anesthesia, the prolongation of sleeping time was reduced in a time-dependent fashion (Fig. 2). If the amount of Intralipid emulsion present in the plasma at any given time is causally related to the prolongation of pentobarbital-induced sleep time then heparin, an agent demonstrated to increase the clearance of triglycerides from the blood (8), would be expected to decrease the prolongation of sleep time at any particular dose of Intralipid emulsion. Results testing this hypothesis are shown in Table I. Heparin (200 units/kg, iv) significantly ($P < 0.01$) decreased the prolongation of sleep time at the two doses of Intralipid emulsion tested.

Since the Intralipid emulsion was administered while the animals were in three different states (conscious, pentobarbital-anesthetized, and heparinized), we examined the clearance and half-life of Intralipid emulsion under these various conditions. The effect of pentobarbital and heparin on Intralipid emulsion clearance is shown in Fig. 3. There was a significant difference ($P < 0.01$) among the pentobarbital-anesthetized, heparinized, and conscious animals in the percentage removal (K_2) of Intralipid emulsion, with heparin augmenting the clearance rate and pentobarbital decreasing it. These values are summarized in Table II, along with the $t_{1/2}$ values and the coefficients of correlation describing the respective straight lines. It can be seen that heparin increases the clearance of Intralipid emulsion from the plasma and decreases the half-life, while pentobarbital decreases the clearance of Intralipid emulsion

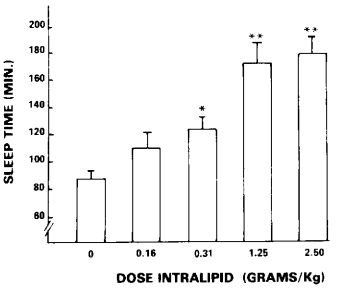

FIG. 1. Effect of Intralipid emulsion (iv) on pentobarbital (30 mg/kg)-induced sleep time. Intralipid emulsion was administered 5 min after pentobarbital anesthesia and the time to the return of the righting reflex was determined. Values are the means ± SEM of six animals at each dose except control (0 Intralipid emulsion) where n = 15. $^*P < 0.05$, $^{**}P < 0.001$ by group comparison with control values.

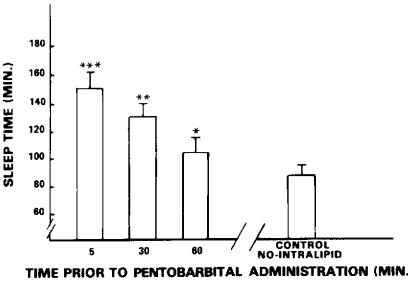

FIG. 2. Effect of Intralipid emulsion (2.5 g/kg, iv) on pentobarbital (30 mg/kg)-induced sleep time. Intralipid emulsion was administered at 5, 30, and 60 min before pentobarbital anesthesia and the time to the return of the righting reflex was determined. Values are the means ± SEM of six animals at each time. $^{***}P < 0.001$, $^{**}P < 0.005$, and $^*P < 0.05$ vs control values.

and increases the half-life.

Discussion. Pentobarbital sleep time in the rat was prolonged in a dose-dependent fashion by the administration of 10% Intralipid emulsion. Although the doses employed in this study were high compared to clinical dosage (4), the high metabolic rate of the rat must be taken into account. The rat utilizes oxygen at about 20 ml/min/kg (9) and man at about 3.5 ml/min/kg (10). In addition, in fasted man, the clearance of Intralipid emulsion following a bolus injection (0.1 g/kg) was reported to be approximately 4%/min (11–14) while we observed a 16.6%/min clearance in the conscious rat.

The mechanism whereby Intralipid emul-

TABLE I. EFFECT OF HEPARIN (200 units/kg) ON THE PROLONGATION OF PENTOBARBITAL-INDUCED SLEEP TIME BY INTRALIPID EMULSION.[a]

Treatment	Sleep time (min)
Control	87 ± 6
Intralipid (2.5 g/kg)[b]	131 ± 10[c]
Intralipid (2.5 g/kg) + heparin[b]	90 ± 5[d]
Intralipid (1.25 g/kg)[e]	169 ± 16[c]
Intralipid (1.25 g/kg) + heparin[e]	112 ± 8[d]

[a] Values are the means ± SEM of six animals with each treatment except control where $n = 15$.
[b] Thirty minutes prior to pentobarbital anesthesia.
[c] $P < 0.01$ vs control value.
[d] $P < 0.01$ vs corresponding Intralipid emulsion (without heparin) value.
[e] Five minutes after pentobarbital anesthesia.

TABLE II. EFFECT OF HEPARIN AND PENTOBARBITAL ON THE FRACTIONAL REMOVAL RATE (K_2) AND HALF-LIFE ($t_{1/2}$) OF INFUSED INTRALIPID EMULSION (0.2 g/kg).[a]

	Control	Heparin (200 units/kg)	Pentobarbital (30 mg/kg)
K_2[b]	16.6 ± 1.6	37.2 ± 3.0[c]	8.7 ± 1.2[c]
$t_{1/2}$[d]	4.3 ± 0.4	1.84 ± 0.2[c]	8.2 ± 0.9[c]
r[e]	0.980	0.980	0.970
n	14	6	7

[a] Values are the means ± SEM.
[b] K_2 expressed as percentage removed/minute.
[c] $P < 0.01$ vs control values.
[d] $t_{1/2}$ was calculated from $0.693/K_2$.
[e] r = correlation coefficient describing fit to a first-order reaction rate.

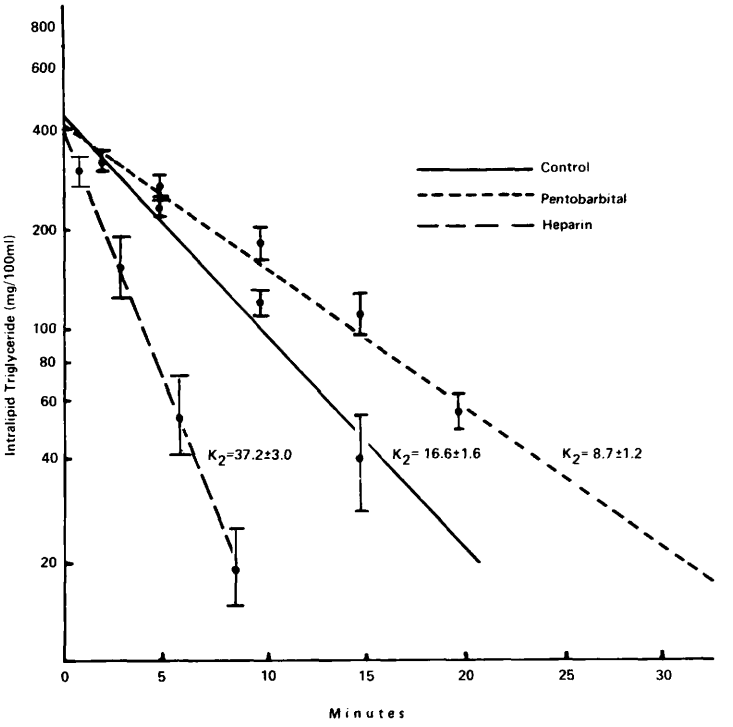

FIG. 3. Effect of heparin (200 units/kg, iv) and pentobarbital (30 mg/kg, iv) on fractional removal rate (K_2) of infused Intralipid emulsion (0.2 g/kg). Each point is the mean ± SEM. Animals in each group were as follows: control ($n = 14$), heparin ($n = 6$), and pentobarbital ($n = 7$).

sion prolongs sleep time is not known at present, but several possibilities exist. Plasma triglycerides or chylomicrons increase after an iv injection of fat emulsion. This plasma pool of lipid may serve as a depot for pentobarbital just as tissue lipids do. Fat emulsion in the blood may take up pentobarbital because of its lipophilic nature. As long as pentobarbital is contained or trapped within the lipid emulsion, it may not be available for metabolism by the liver. Likewise, pentobarbital in the lipid emulsion would not be available to exert an anesthetic effect. Consequently, although the duration of anesthesia may be prolonged, the depth of anesthesia may be reduced. Heparin, by increasing the clearance of Intralipid emulsion from the plasma, may free the "protected" pentobarbital and accelerate its metabolic degradation, thus decreasing its pharmacologic effect.

Since lipid solubility is an important determinant of whether a drug is metabolized by the microsomal enzyme system, fat emulsion may competitively inhibit the microsomal drug-metabolizing enzyme system when administered in very high doses. A drug interaction may occur between lipid-soluble compounds because one may competitively inhibit the metabolism of the other. It has been demonstrated that ethyl alcohol, which is not normally metabolized by the microsomal system, can inhibit drug hydroxylations when administered acutely (15). In the same study it was postulated that high concentrations of ethyl alcohol in liver cells competitively inhibit the microsomal hydroxylase system. While the acute administration of alcohol decreases pentobarbital metabolism, chronic administration increased it (16). There are many compounds that inhibit the metabolism of a drug when given acutely and stimulate the metabolism of the same drug after chronic administration (17, 18). Although it is not known whether Intralipid emulsion under different modes of administration would have these varying effects on microsomal function, it has been demonstrated that chronic administration of fat in the diet can augment microsomal drug metabolism. Caster *et al.* (19) reported that, as the intake of corn oil was increased to 3% of the diet, there was an increase in the ability of the liver, in an *in vitro* system, to metabolize drugs. Increases in drug microsomal metabolism and in the amount of cytochrome *P*-450 in the hepatic microsomes of rats fed high levels of corn oil were also reported by Norred and Wade (20). Other workers (21, 22) have shown that the rate of microsomal metabolism of various drugs is increased when rats are fed diets containing polyunsaturated as opposed to more saturated fats. Whether chronic administration of Intralipid emulsion would augment microsomal metabolism of drugs, in a manner similar to the way in which chronic dietary administration of fat does, remains to be determined.

Another possible explanation for the prolonged sleep times may be that free fatty acids generated by the breakdown of fat emulsion inhibit hepatic microsomal metabolism of pentobarbital. Di Augustine and Fouts (23) demonstrated *in vitro* that unsaturated fatty acids competitively inhibit microsomal O-demethylation and N-demethylation.

Since the prolongation of sleep time was found to be related to both the dosage of Intralipid emulsion (Fig. 1) and the time of administration (Fig. 2), the concentration of Intralipid emulsion in the plasma at any given time appears to be an important determinant of its interaction with pentobarbital. Thus the time-dependent nature of Intralipid emulsion's ability to prolong sleep time may be related to the amount of fat removed from the plasma which increases as the time between administration of the emulsion and the time of pentobarbital anesthesia increases from 5 to 60 min. This is further supported by the fact that the prolongation of sleep time at various dosages of Intralipid emulsion decreases following the administration of heparin (Table I). We (Fig. 3) and others (7) have shown that heparin increases the clearance and decreases the half-life of Intralipid emulsion in the blood.

The mechanism by which pentobarbital delays the removal of Intralipid emulsion from the blood is not clear. Similar results were obtained using chylomicrons labeled with [^{14}C]palmitic acid (24). Heparin augments the clearance of Intralipid emulsion from the plasma by releasing the lipoprotein lipase that is present at the luminal surface of the capillary endothelium in various extrahepatic tissues (25). Lipoprotein lipase pro-

motes the breakdown of triglycerides present in fat emulsion, so that its fatty acid constituents can be oxidized or stored. This enzyme is the major pathway for assimilation of intravenously administered lipid and is extremely sensitive to various physiological and pharmacological perturbations (26). Whether pentobarbital can depress lipoprotein lipase activity is not known at present. Mallov and Cerra (27) could demonstrate no effect on cardiac lipoprotein lipase by prolonged sodium pentobarbital anesthesia.

A significant amount of the total lipoprotein lipase in the rat is found in skeletal muscle (28), and a blood flow reduction to this area may decrease the rate of interaction between enzyme and substrate and result in the decreased removal of Intralipid emulsion from the blood. However, this mechanism does not explain the action of pentobarbital on fat emulsion clearance from the plasma since pentobarbital anesthesia does not alter blood flow to skeletal muscle (29).

It has also been demonstrated that pentobarbital anesthesia depresses total body oxygen consumption and this decrease is largely the result of decreased skeletal muscle metabolism (30). It is possible that lipoprotein lipase activity in skeletal muscle is reduced secondary to this effect, resulting in a decreased removal rate of Intralipid emulsion from the blood.

Whatever the mechanism, since pentobarbital decreases the clearance of Intralipid emulsion, it may, in turn, enhance Intralipid emulsion's effect on pentobarbital-induced narcosis. Thus, these studies have demonstrated a reciprocal interaction between Intralipid emulsion and pentobarbital, with Intralipid emulsion prolonging pentobarbital anesthesia, and pentobarbital anesthesia decreasing the clearance of Intralipid emulsion.

Summary. A 10% soybean oil emulsion (Intralipid) when given intravenously was found to prolong pentobarbital-induced sleep time in rats. The prolongation of sleep time was dose and time dependent. Heparin increased the clearance of Intralipid emulsion from the plasma and decreased the amount of time by which sleep was prolonged. It was further demonstrated that pentobarbital anesthesia decreased the clearance of intravenously administered Intralipid emulsion from the plasma. Thus, a reciprocal interaction was observed whereby pentobarbital prolonged the half-life of Intralipid emulsion and Intralipid emulsion prolonged the narcosis induced by pentobarbital.

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