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Formulation and Evaluation of Ethiodized Oil Emulsion for Intravenous Hepatography

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Abstract □ A study was conducted to prepare and evaluate an ethiodized oil emulsion for intravenous administration that would selectively opacify the liver. Several formulations with differing globule size were prepared and compared for their *in vivo* activity (degree of liver opacification obtained) and stability. Results of studies conducted in rabbits and monkeys revealed that the oil globules that concentrate most in liver were 2.0–3.0 μm in diameter. The formulation of choice was stable for 6 months at refrigeration temperature (2–6°). In monkeys, this formulation produced an improved diagnostic image of the liver using computerized tomography at a dose of 0.2 ml/kg. The potential use of such emulsions in diagnostic radiology is briefly discussed.

Keyphrases □ Ethiodized oil—various emulsions for intravenous administration prepared, effect of oil globule size on liver opacification in rabbits and monkeys □ Radiopaque media—ethiodized oil, various emulsions for intravenous administration prepared, effect of oil globule size on liver opacification in rabbits and monkeys □ Hepatography, intravenous—various emulsions of ethiodized oil prepared, effect of oil globule size on liver opacification in rabbits and monkeys

There have been numerous previous attempts to opacify the liver and spleen by intravenously administered contrast material. As early as 1930, Keith and Briggs (1) used intravenously injected emulsified oil to opacify the liver and spleen in rats. Degkwitz (2) produced an iodine-containing oily contrast material[1] (I), which was used in clinical studies (3) following experiments in animals.

Experimental work (4) with I in mice, rabbits, and guinea pigs indicated that intravenous injection did opacify the spleen, liver, and placenta. This same contrast material was used in nine patients but was too toxic for routine clinical examinations (5). Emulsified ethyl diiodostearate was injected in 10 patients, most of whom had severe toxic reactions (6). Experimental work (7) in dogs used an emulsified oily contrast medium of ethiodized oil[2]. The average globule size of the emulsion (7) was 0.3 μm, and it opacified the liver and spleen.

Recently, two experimental emulsified forms of iodized oil[3], II*a* and II*b*, were produced. Their basic difference was the average globule size of the emulsion, II*a* (emulsion grossiere) having the larger globule size and II*b* (emulsion fine) the smaller. Emulsion II*b*, mode diameter of 1.3 μm (range 0.16–7 μm), was used intravenously in two clinical studies (8, 9), but up to 75% of the patients had disturbing reactions such as fever, chill, anorexia, nausea, vomiting, and a 10–20% drop in platelet counts. These reactions appeared to be dose related and almost constant above the 1.2-ml/kg dose level. In another study (10) with 32 rhesus monkeys, a 2.0-ml/kg iv dose of the same emulsion produced an opacified diagnostic image of the liver and spleen on X-ray examination.

A recent revolutionary discovery in diagnostic roentgenology, computerized tomography (CT), with its improved contrast resolution, raised hopes that a fraction of the previously utilized dose would opacify the liver sufficiently for diagnostic evaluation without undesirable side effects. With this new equipment, Vermess *et al.* (11) opacified the liver and demonstrated carcinogen-induced hepatic tumor in the rhesus monkey with an intravenous dose as low as 0.1–0.2 ml of II*b*/kg.

The possible use of such small doses in human subjects renewed interest in the ethiodized oil emulsion for clinical hepatography. Unfortunately, II*b* was no longer available. Therefore, a study was initiated to prepare an ethiodized oil emulsion for intravenous hepatography. In addition, since none of the mentioned reports attempted to correlate the size of the oil globules with the opacifying ability and since no quantitative data related the dose to the density of the liver scans, a more systematic study of the subject appeared to be warranted.

The size of the oil globules is of primary importance in the opacification of the various tissues, in accordance with Degkwitz's theory that different tissues absorb oil globules of different sizes (2). This fact compounded the problem

[1] Jodsol.
[2] Ethiodol, iodinated ester of poppyseed oil, iodine content 37% (w/v), is routinely used for lymphography; Savage Laboratories, Houston, TX 77036.
[3] II*a* is AG-52-315 and II*b* is AG 60-99; Lipidol UF, Laboratoires Andre Guerbet, Aulnay sur Bois, France.

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of making the emulsion; aside from the usual safety problems associated with intravenous emulsions (12, 13), the globule size needed to be within a narrow range so that maximum selective opacification of the liver could be obtained.

Various intravenous emulsions of different globule sizes were prepared and tested in rabbits and monkeys. Since such emulsions lose their effectiveness with age (10, 14), probably due to the instability of the emulsion, studies also were conducted to determine the stability and the reproducibility of various emulsion formulations with respect to globule size, distribution, and retention of *in vivo* activity. The feasibility of preparing a sterile emulsion also was investigated.

EXPERIMENTAL

Materials and Equipment—Water for injection[4], ethiodized oil[2], polysorbate 80[5], sorbitan monooleate[5], phosphatidyl choline[6], and particle-free electrolyte solution[7] were used as received. All other chemicals were reagent grade.

An alcoholic extract of lecithin was prepared as follows. About 200 ml of absolute ethanol was added to 50 g of soy lecithin-refined[8], and the mixture was stirred for 20 min and filtered through a fine muslin and then through a filter paper[9]. The remaining slurry and filters were rinsed with enough alcohol to give a final volume of 200 ml of filtrate. When analyzed for solid content (by drying in a steam bath to constant weight), each milliliter of the extract was found to contain 45 mg of solids.

Emulsions were prepared using a homogenizer[10], and their globule size was evaluated using an electronic counter[11]. Computerized tomographic scans for liver were performed with a computerized body scanner[12].

Preparation of Emulsions—In each emulsion, the contents of four 10-ml ampuls of ethiodized oil (total volume of 40 ml, 53 g) were emulsified and brought to a total volume of 100 ml. Thus, the emulsions contained 53% (w/v) of ethiodized oil, equivalent to approximately 0.2 g of iodine/ml of emulsion. Emulsions were prepared by dissolving water-soluble ingredients and emulsifiers in water and oil-soluble emulsifiers in oil. The alcoholic extract of lecithin, when required in the formulation, was also added to the oil phase.

Each phase was heated separately, with mechanical stirring, to 60°. The oil phase was then poured slowly into the stirring aqueous phase. Stirring was continued for 3 min to form the primary oil-in-water emulsion. The volume of the mixture was adjusted to 100 ml with water, and the mixture was then poured into a 500-ml homogenizer flask and milled at specific speed settings (medium, medium high, or high) for a specified time. The rotor shaft of the homogenizer contained two sharp propeller blades, each 3 cm long and 1.5 cm apart at an angle of 30° to each other. The lower blade was fixed at the extreme end of the shaft and adjusted to 6 mm above the bottom surface of the homogenizing flask.

Sterile emulsions were prepared by passing each phase through sterile 0.22-μm filters[13] into sterile flasks and carrying out the remaining procedures (as already described) using the aseptic technique and sterile equipment and supplies where needed. The emulsions were then aseptically transferred to sterile 50-ml multiple-dose vials, which were stoppered with sterile rubber closures and crimped with aluminum caps. The vials were stored at room and refrigeration (2–6°) temperatures.

Experimental Evaluation *In Vitro*—*Gross Observation*—Each emulsion was periodically checked visually for sedimentation, viscosity changes, and redispersibility of layers.

Microscopic Observation—Approximately 0.5 ml of a well-shaken emulsion was diluted with 20 ml of distilled water and again mixed well. One drop of this dilution was placed on a microscope slide and observed at ×1125 magnification. A grid attachment (1.8 μm/grid square) in the ocular lens was used to measure globule size.

In general, a good emulsification resulted in a maximum number of globules in the 1–3-μm size range. Particular attention was given to the number of globules above 5 μm (diameter) or to any unusual or undissolved particles.

Electronic Counter Analysis—The electronic counter was used for quantitating the globule size of the emulsions. To prepare samples for analysis, the emulsions were diluted to a 1:40,000 concentration by first taking 50 μl of an emulsion and then diluting it with 2 ml of electrolyte solution[7]. A 20-μl aliquot of this dilution was further diluted to 20 ml with electrolyte solution[7] in a disposable, particle-free, plastic beaker[14]. Counts were then performed using a 30-μm apperture tube and a 50-μl sampling volume, which generally contained from 80,000 to 100,000 globules above 0.75 μm in diameter.

The counter was calibrated with 2.02-μm monosized, polystyrene beads[15]. Counts representing the number of globules above a specified diameter were obtained[16]. The count between any size range was calculated by the difference between the cumulative counts above the extremes. The average volume of the globules in a given size range was calculated by the geometric mean of the extremes of the range. Globules below 0.75 μm in diameter were not considered in calculations or evaluation because they were thought to be unimportant in opacifying the liver and because the total volume of this range would not significantly contribute to error in the volume calculations of the larger globules (the contribution of d^3 factor decreases rapidly as diameter, d, decreases). Both the number and volume distribution of the oil globules in the emulsions were calculated.

A potential source of error with this counting procedure should be discussed. During the counting, it appeared that counts for globules larger than 2.0 μm decreased with time (in the diluted emulsion) whereas counts for particles around 1.0 and 1.5 μm remained relatively constant. This result is contrary to the general behavior of emulsions, which, after dilution with saline solutions, usually give higher counts of larger globules, apparently due to coalescence of smaller globules (15, 16). No explanation is apparent for the unusual behavior of ethiodized oil emulsion diluted in electrolyte solution. To minimize this error, the counts were taken as quickly as possible after mixing, starting from the largest size (7 μm) and working down to the 1-μm level. All data presented are the average of at least three runs.

Experimental Evaluation *In Vivo*—The *in vivo* evaluation of the emulsions was performed in three stages. Not all emulsions were examined at all three stages; many were eliminated at the first- or second-stage level.

First-Stage Experiments—First-stage experiments were done with groups of four New Zealand White rabbits, 1.0–3.3 kg. Following the cannulation of an ear vein, the animals were lightly anesthetized with a 2% solution of thiamylal sodium. The initial dose was 1 ml/kg; additional small doses were administered as required to keep the animal under light anesthesia. A 2-ml/kg dose of the experimental emulsion was then injected through the same venipuncture, with the injection rate approximately 1 ml/min.

Prior to the intravenous injection of the emulsion, a preliminary abdominal radiograph was obtained on each animal. Following the completion of the injection, additional X-rays were obtained at 0.5, 1, 1.5, 2, and 3 hr. The increase of the opacity of the livers and spleens was then visually compared, and the effectiveness of the emulsion was evaluated on the basis of the comparison.

Second-Stage Experiments—New Zealand White rabbits were again utilized in groups of four. The preliminary anesthesia was performed as in Stage I. Three of the four rabbits received 0.5 ml/kg of the test emulsions *via* the intravenous cannula, with the injection rate the same as in Stage I. One rabbit in the group was used as a control and injected with 0.5 ml of normal saline/kg. Following the injection, the animals were kept under light anesthesia for 1 hr and then sacrificed by rapid intravenous injection of thiamylal sodium until the cessation of respiration and heartbeat.

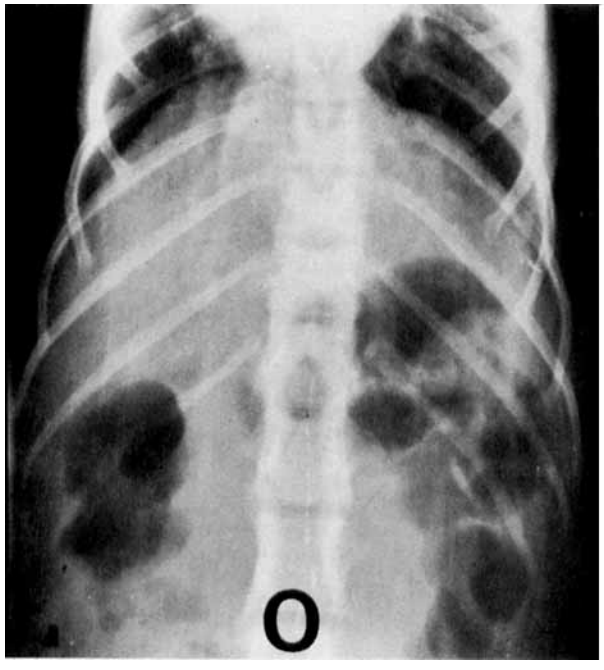
The dead animals were refrigerated for 2 hr, after which computerized tomographic scans of the livers and, occasionally, the spleens were obtained. The computerized tomographic scans were performed with a computerized body scanner[12] utilizing the 25.4-cm scanning circle and

[4] Abbott Laboratories, North Chicago, Ill.
[5] Atlas Chemical Industries, Wilmington, Del.
[6] Phospholipon-80, containing 80% phosphatidyl choline, American Lecithin Co., Atlanta, Ga.
[7] Isoton II, conducting fluid for Coulter Counter measurements, Coulter Diagnostics, Hialeah, Fla.
[8] ICN Pharmaceuticals, Cleveland, Ohio.
[9] No. 2, Whatman, Clifton, N.J.
[10] Homogenizer 45, Virtis Research Equipment, Gardiner, N.Y.
[11] Model B, Coulter Electronics, Hialeah, Fla.
[12] Model EMI 5005, Electric and Musical Industries, Northbrook, Ill.
[13] Millipore Corp., Bedford, Mass.
[14] Accuvette II, Coulter Diagnostics, Hialeah, Fla.
[15] Coulter Electronics, Hialeah, Fla.
[16] Corrected for coincidence as directed in the instrument manual.

Table I—Formulation of Emulsions

Emulsion	Emulsifier/100 ml	Mix Speed[a]	Comments
III	Polysorbate 80, 4.5 g, and sorbitan monooleate, 1.5 g	Medium	Opacified spleen but did not significantly opacify liver; average globule size smallest of all emulsions and least effective
IV	Polysorbate 80, 2.25 g, and sorbitan monooleate, 0.75 g	Medium high	Opacified liver well but not as well as V and VI
V	Alcoholic extract of lecithin[b], 10 ml	Medium high	Most effective of all emulsions in liver opacification, reproducibility, and clarity
VI	Lecithin in ethanol, 3.0 g/10 ml	Medium high	Opacified as well as V but not as reproducible
II*b*	Lecithin, 3 g, and polyoxyl 40 stearate, 1.2 g		Unavailable commercial product, used for comparison purposes

[a] The mix time was 3 min for Emulsions III–VI. [b] Extract from 2.5 g of lecithin; representing 0.45 g of extracted solids.



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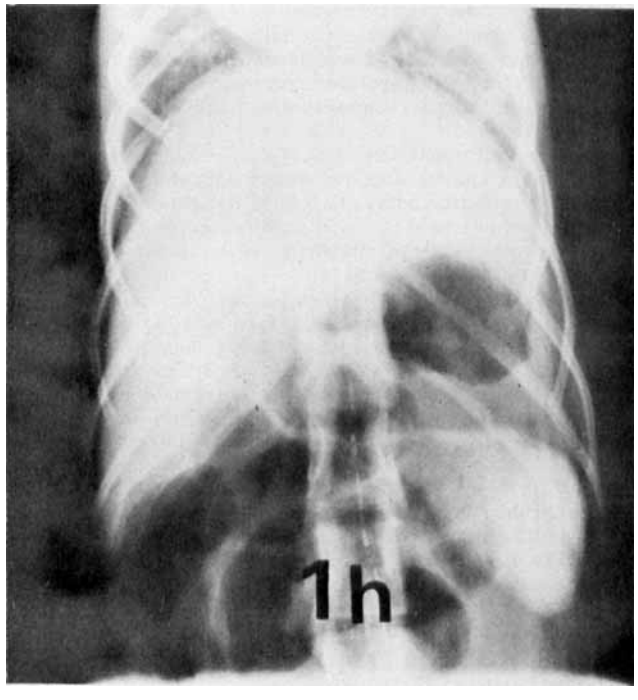
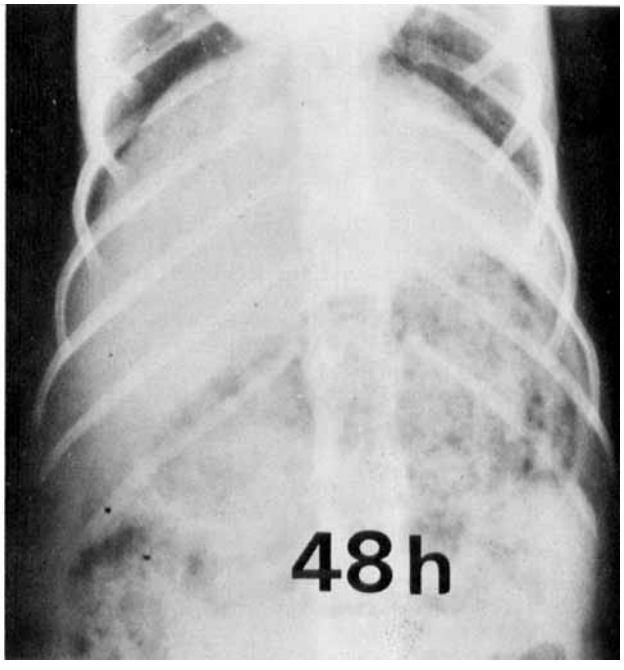


b



c

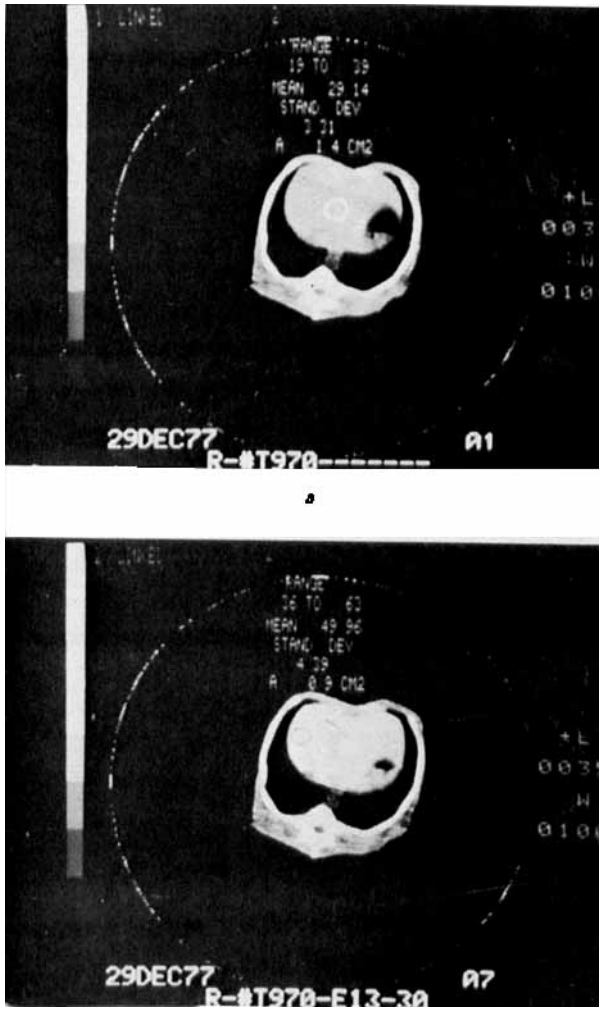
Figure 1—*Radiographs of rhesus monkey livers before* (a), *1 hr* (b), *and 48 hr* (c) *after the intravenous injection of 2 ml of Emulsion V/kg. Note marked increase in the density of liver and spleen after 1 hr and almost complete clearance of the contrast material 48 hr after the injection.*

80-sec scanning time. The slice thickness of the scans was 13 mm, and the scans were performed at 1-cm distances. The number of slices necessary to obtain proper visualization of the liver parenchyma varied according to the size of the livers. The densities of the livers and, occasionally, the spleens were measured both visually on the measure mode and by the computer-generated mean attenuation value of a selected homogeneous area of the liver on the independent viewing console.

Third-Stage Experiments—These experiments were conducted on three female and two male normal rhesus monkeys, 3–6 kg. The animals were sedated and anesthetized as described previously (11). To prevent respiratory motion during the 80-sec exposure of the computerized tomographic scanning, the animals were intubated and connected to a respirator. Spontaneous respiration was eliminated by intravenous administration of 3 mg of tubocurarine chloride. Preliminary scans of the liver and, occasionally, the spleen were obtained utilizing the equipment and technique described in Stage II.

Following the preliminary scans, 0.2 ml of the experimental emulsion/kg was slowly injected (over approximately 1 min) into a peripheral vein. Thirty minutes after completion of the injection, the scans of the liver were repeated. In most experiments, the animals were also rescanned 60 min after the injection.

The density of the livers was measured on preliminary, 30-, and 60-min scans, as described in the Stage II experiments, both visually and by the computer-generated mean attenuation expressed in EMI Units (EU) on the 500 scale. The increases in the density of the livers after 30 and 60 min were compared to the preliminary scan and evaluated.



a b

Figure 2—*Computerized tomographic images of rhesus monkey livers before* (a) *and 1 hr after* (b) *the intravenous injection of 0.2 ml of Emulsion V/kg. Computer-generated density measurements indicated an increase in liver attenuation by 21 EMI Units.*

RESULTS AND DISCUSSION

The success of Emulsion II*b* in opacifying the liver (10) prompted efforts to formulate a similar emulsion. With the same formulation (3% purified soy lecithin and 1.2% polyoxyl 40 stearate as emulsifier), initial attempts were made to prepare an emulsion with a maximum number of globules around 1.3 μm in diameter. However, considerable difficulty was encountered during preparation of the emulsion. The lecithin was not completely soluble, even in hot (60°) ethiodized oil, and, more important, the resulting emulsion contained large spongy masses (8–10 μm) that could not be filtered through a 5-μm stainless steel filter. Therefore, several different procedures were attempted.

Table I lists the formulations and procedures used in preparation of five emulsions along with their relative *in vivo* results. The data on Emulsion II*b* are included for comparison. However, at the time of *in vivo* testing, II*b* was at least 1 year old and was not representative of the true potency of a freshly prepared compound.

Emulsion III (Table I) was prepared using the formulation of Hom *et al.* (17). This emulsion, when examined under a microscope, revealed that most of the globules were around 1 μm in diameter; a few were between 1.5–2.0 μm, and very few were above 2 μm. Emulsion III, at a dose of 2.0 ml/kg, was tested in rabbits by the conventional radiographic technique and showed only minimal opacification of the liver. An emulsion with a globule size smaller than that of Emulsion III also opacified the liver poorly. Therefore, an emulsion with a larger globule size was investigated.

Emulsion IV was made with the same polysorbate 80–sorbitan monooleate emulsifier combination as Emulsion III, but a smaller amount of emulsifier was used (Table I). On microscopic observation, Emulsion IV showed a good number of globules in the 1.5–2.0-μm range and very few above the 3–5-μm range. Although Emulsion IV opacified the liver in rabbits, it was somewhat less effective than II*b*. Thus, this emulsion showed promise and probably could have been improved further by changing the emulsifier concentration to increase the globule size. However, work on these emulsifier combinations was discontinued because of the lack of toxicity data on their intravenous use in humans.

The use of lecithin was investigated once again. To eliminate the major problem of spongy masses observed with refined soy lecithin, the lecithin was purified further by extraction in ethanol. When the alcoholic extract was used along with the addition of enough alcohol to make the final alcohol concentration 10% (v/v) in the emulsion, it no longer contained spongy masses and appeared clean under the microscope. Therefore, variations of the formulation were examined by changing the emulsifier concentration or stirring speed, which resulted in the preparation of Emulsion V (Table I).

Emulsion V, at doses of 2.0 ml/kg for conventional X-ray and 0.2 ml/kg for computerized tomography, produced denser liver scans than Emulsions III and IV. Microscopic observation of Emulsion V revealed that the globule sizes were generally larger than those in Emulsions III and IV, and a large number of globules were in the 2-μm range. A further increase in the size of the globules in Emulsion V (obtained by changing the stirring speed or homogenizing time) resulted in an emulsion that gave unsatisfactory liver scans.

Since Emulsion V was made using an alcoholic extract of soy lecithin, it was thought that a purer commercial extract of lecithin, used without further processing, would give a more reproducible emulsion. Therefore, various alcohol-soluble lecithins containing various amounts of phosphatidyl choline were evaluated. Phosphatidyl choline 80%[6] was studied most because of its high alcohol solubility. Emulsion VI (Table I), prepared with 3% (w/v) phosphatidyl choline, resulted in globule sizes that were larger than those of Emulsion V, but a good number of globules were in the 2–2.5-μm range. However, relatively fewer globules were below 1.5 μm and more globules were larger than 3 μm compared to Emulsions III–V.

The potency of Emulsion VI in opacifying the liver was comparable to that of Emulsion V. However, during the preparation of Emulsion VI, the temperature of the oil and water phases had to be very strictly adhered to (otherwise the primary emulsion gelled), and the procedure did not prove to be reproducible. Furthermore, the concentration of the emulsifier in Emulsion VI was much higher than that in Emulsion V (3% compared to 0.45% in Emulsion V), and attempts to reduce it resulted in an even poorer emulsion. Therefore, Emulsion V was chosen for toxicity and clinical studies.

Animal Studies—Figure 1 shows the successive stages of opacification of liver using Emulsion V in conventional X-ray radiography. Maximum opacification was reached in about 1 hr. Other emulsions (Emulsions IV–VI and II*b*) behaved qualitatively the same way, although their scan density was different. The ability of Emulsion V to opacify the liver at a dose of 0.2 ml/kg in monkeys when analyzed by computerized tomography is shown in Fig. 2 and concurs with previous reports (11). This dose is only one-tenth of the previously used doses (2.0 ml/kg) and should reduce the potential toxicity problems associated with ethiodized oil emulsion. Detailed toxicological studies of Emulsion V are being conducted on rats and primates[17].

Stability of Emulsions—Emulsions IV–VI after storage at 4–8° for 6 months showed slight flocculent sedimentation, which rapidly redispersed on mild shaking. The emulsions did not show any significant changes in globule size distribution or hepatographic potency when tested in animals during this storage period. The stability of these emulsions at room temperature also appeared to be similar; but based on experiences with storage of other intravenous lipid emulsions, storage at refrigeration temperatures is recommended. Freezing broke the emulsion and should be avoided.

Sterilization of Emulsions V and VI by autoclaving in the final container was attempted. Autoclaving for 15 min at 121° or for 3.5 min at 132° was tried. In both cases, emulsions were completely broken. Emulsion VI could not be redispersed on shaking. Emulsion V was redispersed on shaking, but redispersed Emulsion V showed large globules (up to 30 μm in diameter) along with smaller globules (1–4 μm). Therefore, sterilization by autoclaving was judged to be unsuccessful.

[17] Toxicity studies at Hazelton Laboratories, Vienna, VA 22180, carried out on rats showed the LD_{50} at 15.6 ml/kg.

Table II—Globule Size Distribution of Emulsions

Size Range, μm	Emulsion III Number, %	Emulsion III Volume, %	Emulsion IV Number, %	Emulsion IV Volume, %	Emulsion V Number, %	Emulsion V Volume, %	Emulsion VI Number, %	Emulsion VI Volume, %	Emulsion IIb Number, %	Emulsion IIb Volume, %
0.75–1.0	59.77	28.1	41.67	10.73	46.94	6.03	31.09	2.62	31.43	2.31
1.0–1.5	33.92	45.1	34.61	25.21	24.9	9.05	28.75	6.86	26.96	5.61
1.5–2.0	5.69	21.4	18.08	37.24	11.91	12.24	17.2	11.6	15.6	9.2
2.0–2.5	0.5	3.8	2.2	9.6	7.0	15.5	9.8	14.3	10.8	13.7
2.5–3.0	0.1	1.6	1.4	11.2	4.5	18.2	6.3	16.7	6.2	14.5
3.0–4.0	—	—	0.3	5.1	3.4	28.4	5.4	29.3	6.9	32.3
4.0–5.0	—	—	0.03	1.1	0.5	9.0	1.4	16.2	1.9	19.6
5.0–7.0	—	—	—	—	0.03	1.6	0.08	2.4	0.12	2.7
Relative density of liver scans[a]	<40		47		57		56		50	

[a] In rabbits at a dose of 0.5 ml/kg using computerized tomography; density of control animal was 32. Numbers expressed in EMI Units (EU) on the 500 scale.

All batches were made using aseptic techniques and were found to be sterile and pyrogen free.

Correlation of Globule Size and Density of Liver Scans—Table II shows the globule size distribution, both by number and volume (mass), of various emulsions along with the results of their *in vivo* testing. Since only the volume of the oil absorbed in the liver affects the degree of opacification, the density of liver scans was correlated with the volume distribution and not the number distribution of the globules. The size range of less than 1.5 μm was ruled out completely because Emulsion III, which contained the highest amount of oil in this range, was relatively inactive. Likewise, the size range of 1.5–2.0 μm was ruled out because, although Emulsion IV contained 37.2% oil of this size, it opacified the liver poorly as compared to Emulsions V, VI, and II*b*, which all contained far less oil in this range. Size above 3.0 μm was also ruled out because II*b*, which contained the highest amount of oil in this range, opacified the liver less than Emulsions V and VI.

It appeared, therefore, that opacification of the liver was related to the amount of the oil in the 2.0–3.0-μm range. Also, there was a direct rank-order correlation between the volume of the oil in the 2.0–3.0-μm size range and the density of the liver scans (Table II). Another indication of the importance of the 2–3-μm size range was observed when Emulsion IV, after being stored at room temperature for several months, opacified the liver better than when freshly prepared and had a potency comparable to Emulsions V and VI. A globule size analysis of the aged Emulsion IV showed that globules had generally increased in size and that 32% (v/v) of the oil was now in the 2.0–3.0-μm range (compared to 20.7% in fresh Emulsion IV, Table II). Therefore, it can be assumed that the increased potency of the aged Emulsion IV was due to an increase in the volume of the oil in the 2.0–3.0-μm range.

A correlation of the density of the liver scans with the volume fraction of the oil in a particular size range has been shown to be very important. Therefore, to prepare the best emulsion, the formulator should attempt to prepare emulsions such that most of the oil globules are in the specific size range needed, rather than homogenizing the emulsion to a globule size less the 1 μm as has often been done (7, 17, 18). As seen from Table II, only about a third of the oil in Emulsion V was in the 2.0–3.0-μm range. Since the liver appears to take up oil globules preferentially between 2.0 and 3.0 μm, if an emulsion could be prepared in which all the oil was in this range, it might be possible to reduce the emulsion dose to perhaps one-third and still deposit the same amount of iodinated oil in the liver. Such a preparation would have an added advantage of sparing other tissues from unnecessary exposure to iodinated oil.

The results of the present investigation indicate that at least one organ, the liver, has preference regarding the size of the oil globules it absorbs. If similar specificity exists for other tissues and organs, it would be possible to prepare emulsions of different globule size that preferentially opacify a particular organ or tissue. Work is in progress to prepare emulsions with a more uniform globule size and to test this hypothesis.

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