Physico-Chemical Changes in Artificial Fat Emulsions during Storage

Studies of the Hydrolysis and its Physiological Effects

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Artificial soybean oil emulsions intended for intravenous administration have been studied, with particular reference to their spontaneous hydrolysis. In kinetic studies in buffered systems, the reaction-rate constant has been determined at varying temperature and pH. In the pH range studied, a hydrolysis minimum is found at about pH 7. Since the addition of electrolytes impairs the stability of the emulsion, buffered emulsions cannot be prepared commercially. Consequently, the course of hydrolysis has also been studied in unbuffered systems, in which the liberation of free fatty acids (FFA) is found to be rapidly accelerated at high temperatures, due to the catalytic action of the hydronium ions.

Since the concentration of FFA liberated on hydrolysis is hitherto the only certain parameter of the toxicity, toxicity studies have been made with emulsions stored for long periods at high temperatures. LD_{50} in the rabbit is determined, and found to be 0.21 mequiv. FFA/kg body weight.

Long-term tolerance tests have been made in the dog, with administration of 9 g of fat/kg body weight and day, representing the total caloric requirement, for 28 consecutive days. Emulsions (Intralipid 20 %) stored up to 3 years are found to be well tolerated.

Clinical studies have been made with emulsions (Intralipid 10 % and 20 %) stored up to 24 months at temperatures ranging from + 4 to + 20°C. The low incidence of toxic reactions noted is in good agreement with corresponding results on infusion of newly prepared emulsions.

The possibility of supplying the organism with large quantities of calories by the parenteral route has been of considerable clinical interest for several decades. Consequently, numerous fat emulsions for intravenous use have been produced and tested.

One of these, a soybean oil emulsion,* has been subjected to comprehensive studies in both animals and man.[1-3] This emulsion has, for example, proved to have low toxicity in long-term tolerance tests in the dog,[4-6] when up to 9 g of fat/kg body weight and day were given. Moreover, its rate of elimination from the blood stream of the dog was shown to be similar to that of the chylomicrons.[7] The emulsion has also been used as a substrate in lipoprotein-lipase reactions.[8]

Certain studies of the stability of the artificial soybean oil emulsion have been reported previously.[9] An account is given in the present paper of studies of some physico-chemical changes in the emulsion, which occur as a result of long-term storage at varying temperature. It is demonstrated, *e.g.*, that buffered emulsions have a hydrolysis minimum at about pH 7. In connexion with this finding, the physico-chemical changes in the emulsion have been studied with respect to their physiological effects when infused intravenously in the rabbit, dog and man.

MATERIAL AND METHODS

Composition of emulsions used and physico-chemical analyses

Fat emulsions. 1. Commercial soybean oil emulsions (Intralipid 10 % and Intralipid 20 %); the compositions are shown in Table 1. They were stored at + 4, + 12, + 20, and + 40°C for up to 3 years.

Table 1. Composition of fat emulsions used.

	Intralipid 10 %	Intralipid 20 %
Soybean oil	100 g	200 g
Egg-yolk phospholipids	12 g	12 g
Glycerol (85−89 % anhydrous)	25 g	25 g
Sterile water to	1000 ml	1000 ml

pH adjusted to 6.0−6.5

2. Nine emulsions with 20 % soybean oil and otherwise the same composition as in Table 1, except for pH. They were prepared from the same stock emulsion in such a way that all emulsions, before sterilization, had a pH within the range 4−11 and with a pH difference of 0.8−1 unit. These emulsions were stored at + 80°C for 11 days and at + 40°C for 240 days, respectively.

3. Four emulsions with 20 % soybean oil and otherwise the same composition as in Table 1 were buffered by the addition of a sodium phosphate buffer corresponding to a final concentration of 0.05 M. Their pH was then adjusted to 5.8, 6.3, 6.8, and 7.3, respectively. pH was adjusted with a 0.1 M solution of either sodium hydroxide or hydrochloric acid.

All emulsions were filled into 100 or 500 ml bottles, which were sterilized by autoclaving. They were then stored in the dark at varying temperatures. Samples for testing were withdrawn at fixed intervals.

Determination of free fatty acids (FFA). Dole's method [10] was used, in the modification of Trout *et al.*[11] pH was determined with a glass-electrode pH meter (Radiometer, Copenhagen, Denmark, Model 25 SE).

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* Intralipid ®, Vitrum AB, Stockholm, Sweden.

Determination of particle size. A Zeiss Standard microscope with phase-contrast equipment was used. The magnification of the objective was 100 × and that of the eyepiece 20 ×. The eyepiece was provided with a micrometer whose smallest scale division corresponded to 0.5 μ. The emulsion was diluted 1:50 (20 % emulsion) or 1:25 (10 % emulsion) with a 50 % aqueous solution of propylene glycol according to Levius and Drommond.[12] One drop of the mixture was placed on a slide under a cover-glass. The number of particles with a diameter of $\geq 0.5\ \mu$ was counted in the rectangle formed by the micrometer scale of the eyepiece (35—40 particles). Ten such rectangles were counted in each analysis.

Kinetic studies of hydrolysis. These were made on emulsions buffered with sodium phosphate. To determine the pH range within which the hydrolysis minimum in this system was situated, four emulsions with different pH were used, *i.e.*, pH 5.8, 6.3, 6.8, and 7.3. Samples were taken at fixed intervals from the emulsions stored at + 4, + 20, + 40, and + 60°C for long periods, for the determination of the FFA concentration.

Animal experiments

Acute toxicity. This was studied in white rabbits of both sexes, weighing about 2.5 kg. The emulsion (8 ml/kg body weight) was injected into the marginal vein of the ear. The animals were observed for 24 h. However, those that did not survive generally died immediately, or within less than 5 min after the injection.

Chronic toxicity. These studies were made in mongrel dogs. After a control period of at least 4 weeks, daily infusions were given for 28 consecutive days. During the infusion, each dog was placed in a Pavlov stand. The amount of fat administered was 9 g/kg and day, representing the total caloric requirement in the dog (about 80 cal/kg and day). The infusion rate was adjusted so that the infusion lasted for 4 h. During the whole experimental period, the dogs were allowed to eat and to drink water *ad libitum*.

To study the chemical and physiological changes in the blood, two samples of venous blood were taken before starting the infusion period, and thereafter once a week. All the samples were taken in the morning, directly before starting the infusion, with the exception of the first one, which was taken a few days before. The haemoglobin and haematocrit were determined, as well as the serum triglycerides, phospholipids, cholesterol, total protein, albumin, and globulin. The serum triglycerides and phospholipids were determined with the methods described by Carlson,[13,14] and serum cholesterol according to Sperry and Webb.[15] Total protein in serum was determined by Kjeldahl's method, and electrophoretic separation of the serum proteins was performed according to Köiw *et al.*[16]

Furthermore, the dogs were weighed once a week, and their rectal temperature was measured 15 min before starting the infusion, as well as 2, 3, 4, and 5 h after its beginning. The urine was also analyzed once a week for the presence of glucose, protein, and ketone bodies. The dogs' appetite, general condition, and behaviour were noted.

On the morning of the day after the 28th infusion, 3 of the 4 dogs were killed by injection of Veterinary Nembutal ® * (1.5 ml/kg body weight). Autopsy was then performed and specimens of different organs were taken for sectioning. Special staining procedures, as described by Meyer *et al.*[17] and Thompson and co-workers,[18-20] were used to demonstrate intravenous fat pigment in unstained and paraffin-embedded sections of the liver and spleen. The histopathological studies were carried out under supervision of Professor Anna-Lisa Obel, Department of Anatomy and Pathology, State Veterinary Medical Institute, Stockholm.

Clinical studies in man

Emulsions (Intralipid 10 % and 20 %) subjected to physico-chemical analyses, as well as to tests in animals, were used in clinical studies in man. They were stored at + 4, + 12, and + 20°C, and were tested every 6 months. The emulsions were administered as intravenous drip infusions to patients in whom indications for parenteral nutrition existed. Each infusion lasted for about 4 h. The occurrence of any side reactions — such as nausea, vomiting and rigors — was studied, as well as any rise in temperature in

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* Abbot Laboratories Ltd., Queenborough, Great Britain.

connexion with infusion. The temperature was recorded once an hour before, during and after each infusion, in all for 10 — 14 h. These infusions were carried out under the supervision of Assistant Professor Oscar Schuberth, Department of Surgery, S:t Görans Sjukhus, Stockholm.

RESULTS

In the following, the results refer to the commercially available soybean oil emulsion (Intralipid 20 %), unless it is stated that specially prepared emulsions were used.

Changes in FFA concentration and pH are demonstrated in Fig. 1. After 11 weeks' storage at + 4°C, the FFA concentration was 1.4 times the initial value (0.6 mequiv./1000 ml). After the same period and storage at + 20°C, the concentration had increased to 2.4 times the initial value; at + 40°C the value was 10-fold and at + 60°C 90-fold. The liberation of FFA during storage for 24 months at + 4, + 12, + 20, and + 40°C is illustrated in Fig. 2.

As a result of the liberation of FFA with temperature and time, there was a marked fall in pH. The changes in pH occurring during storage for 11 weeks at the four specified temperatures were most marked at the beginning of the period (Fig. 1). It is also evident from Fig. 1 that the fall in pH was remarkably large during the processes of homogenization and sterilization.

Changes in particle size. In freshly prepared emulsions, the optical diameter of all particles was less than 1 μ, and most of them were smaller than 0.5 μ. Fig. 2 shows the changes in size of the particles as a result of storage of the emulsions for 24 months at + 4, + 12, and + 20°C. In each case, the results represent the mean value of 8 batches. It can be seen that the increase in
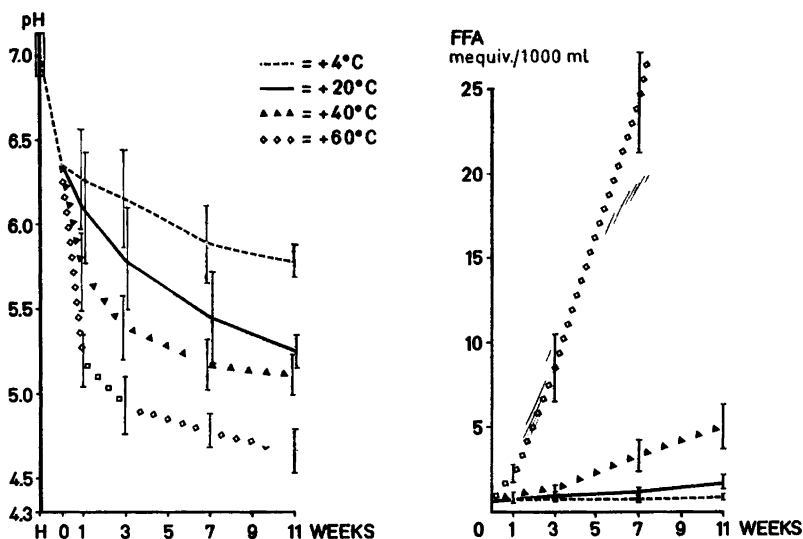

Fig. 1. Changes in pH and release of FFA during storage of soybean oil emulsion (Intralipid 20 %) at four different temperatures. H = pH during homogenization. Mean of 4 batches.
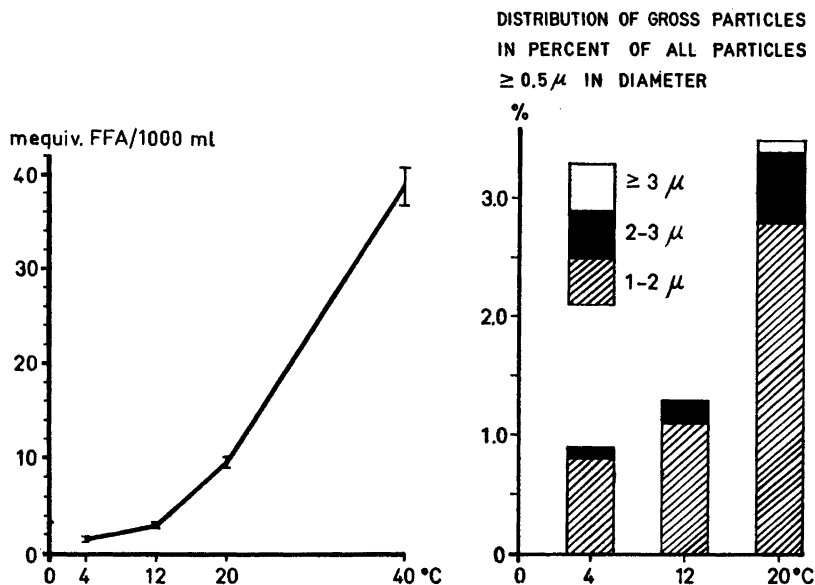
DISTRIBUTION OF GROSS PARTICLES
IN PERCENT OF ALL PARTICLES
≥ 0.5 μ IN DIAMETER

Fig. 2. Changes in FFA and particle size in soybean oil emulsion (Intralipid 20 %) after storage for 24 months at various temperatures. All particles in fresh emulsions have a diameter ≤ 1 μ. Mean of 8 batches.

particle size was dependent on the temperature. Thus, particles with a diameter exceeding 3 μ were present only in emulsions stored at + 20°C.

pH-dependence of hydrolysis. Nine emulsions with varying pH, prepared from the same stock emulsion, were used for this study. In Fig. 3, the FFA concentration is plotted against the final pH of the emulsions. After storage at + 80°C for 11 days and at + 40°C for 240 days, the emulsions whose pH was between 6 and 7 showed a minimum of hydrolysis.

It can be seen in Fig. 3 that hydrolysis was catalyzed by both hydronium and hydroxyl ions. Another matter of interest in this connexion was demonstration of the toxicity of the free fatty acids in the rabbit. Thus, at FFA concentrations exceeding 20 mequiv./1000 ml emulsion (representing a FFA dose of 0.16 mequiv./kg body weight), injection had a lethal effect in the majority of cases.

Kinetic mechanism of hydrolysis. Since the hydronium ion concentration in the buffered emulsions was practically constant, the reaction could be regarded as a pseudo first-order reaction in the pH range studied. In view of the fact that hydrolysis was followed in only a small part of its course, Guggenheim's method [21] was used to determine the reaction-rate constants. According to this method, the rate constants can be determined without knowledge of the total course of hydrolysis, using the following equations:

$$v' - v = (v_\infty - v_0) \ (1 - e^{-h\tau}) \ e^{-ht} \tag{1}$$
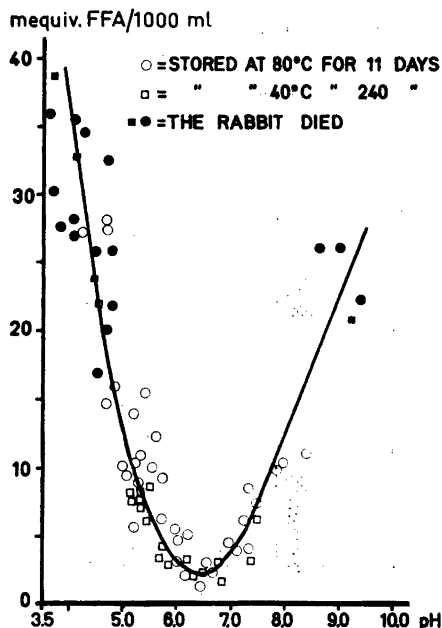
mequiv. FFA/1000 ml

Fig. 3. Effect of pH on FFA liberation in soybean oil emulsions (20 %) at two different temperatures. Nine emulsions with different pH were used. The concentration of FFA is plotted against the final pH. The lethal effect of the FFA in the emulsions injected in rabbits is denoted by black circles and squares.

which is logarithmized and transformed into

$$\ln (v'-v) + kt = \ln (v_\infty - v_0) (1-e^{-k\tau}) \qquad (2)$$

in which k = the reaction-rate constant, t = the time of the determinations, τ = a constant time interval, and v and v' = the concentration of FFA at time t and $t + \tau$, respectively. v_0 and v_∞ represent the FFA concentration at zero time and infinite time, respectively. Log $(v'-v)$ was plotted against time, after which k was calculated from the graphic representation, as seen in Fig. 4. The reaction-rate constants calculated from Fig. 4 with Briggsian logarithms are listed in Table 2.

Table 2. Rate constants for the hydrolysis of buffered fat emulsions at various temperatures and pH, calculated on the basis of the curves in Fig. 4.

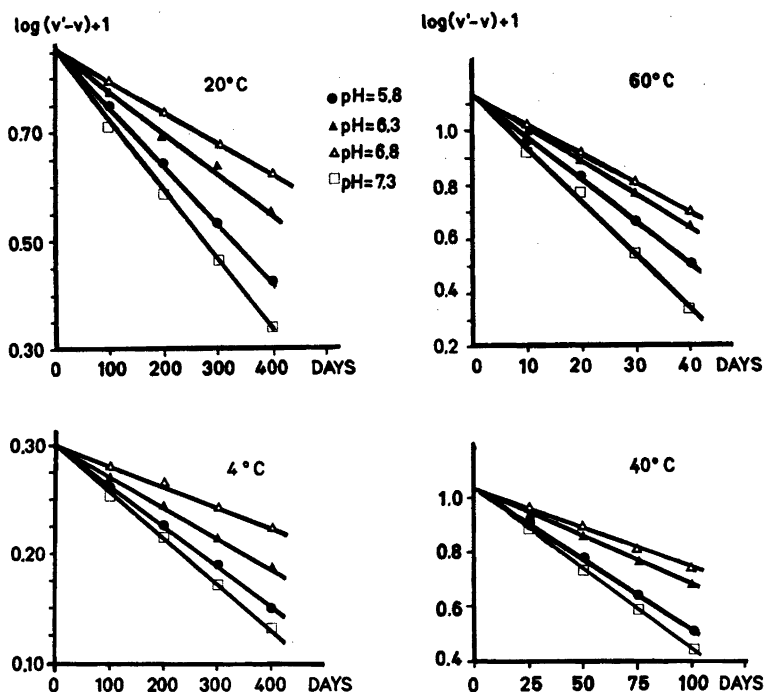| °C	pH = 5.8	pH = 6.3	pH = 6.8	pH = 7.3
	$k \times 10^3$ day^{-1}	$k \times 10^3$ day^{-1}	$k \times 10^3$ day^{-1}	$k \times 10^3$ day^{-1}
4	0.78	0.60	0.41	0.96
20	2.41	1.73	1.33	2.82
40	11.9	8.10	6.50	14.1
60	36.3	27.7	24.1	45.8

Fig. 4. Hydrolysis of buffered soybean oil emulsions (20 %) at four different temperatures and pH.

In Fig. 5, the reaction-rate constants expressed as p*k* are plotted against pH. The treatment of the course of hydrolysis as a pseudo first-order reaction seems to be valid within the experimental accuracy.

Acute toxicity. Emulsions with the composition shown in Table 1 (Intralipid 20 %) kept for long periods at + 4, + 20, and + 40°C, were used to study the
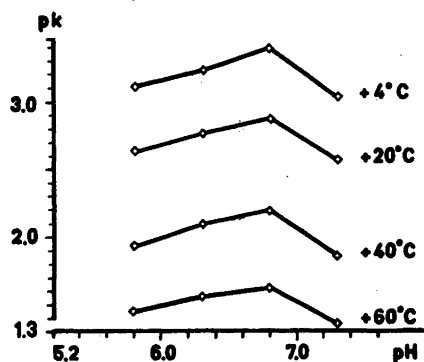

Fig. 5. Rate constants of the hydrolysis expressed as p*k* of buffered soybean oil emulsions (20 %) plotted against pH.

acute toxicity in rabbits with regard to lethal effects. Samples were taken after storage up to 24 months. Before the toxicity studies the amount of FFA was determined. The relation between the FFA concentration in the emulsion and the lethal effect is shown in Fig. 6. At a concentration of 15—20 mequiv./ 1000 ml emulsion (0.12—0.16 mequiv. FFA/kg body weight), the mortality exceeded 30 %. At a FFA concentration below 15 mequiv./1000 ml, on the other hand, the mortality was nil.

In order to determine the LD_{50} value of free fatty acids in the emulsion, 114 rabbits were divided into 5 groups, with at least 20 rabbits in each. The

AMOUNT GIVEN: 8 ml / kg B.W.

☐ = SURVIVAL
▨ = MORTALITY

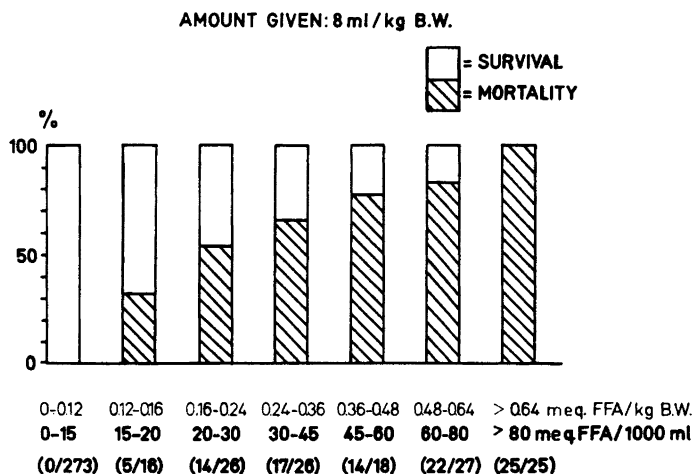| | 0-0.12 | 0.12-0.16 | 0.16-0.24 | 0.24-0.36 | 0.36-0.48 | 0.48-0.64 | > 0.64 meq. FFA/kg B.W. |
| | 0-15 | 15-20 | 20-30 | 30-45 | 45-60 | 60-80 | > 80 meq FFA/1000 ml |
| | (0/273) | (5/16) | (14/26) | (17/26) | (14/18) | (22/27) | (25/25) |

Fig. 6. Toxicity of aged soybean oil emulsions in the rabbit. Abscissa: Range of FFA concentration in mequiv./kg body weight and in mequiv./1000 ml emulsion. Bracketed numerals: mortality rate. Ordinate: % mortality.
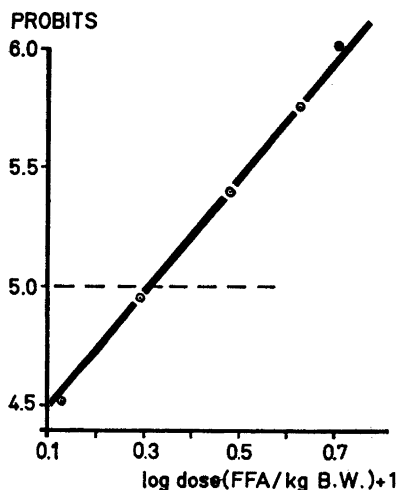
PROBITS

log dose(FFA/ kg B.W.)+1

Fig. 7. Dose-mortality curve in the rabbit of aged soybean oil emulsion. Probits are plotted against log dose (mequiv. FFA/kg body weight) after injection of emulsion in a dose of 8 ml/kg body weight.

FFA concentration in the stored emulsions given to the respective groups was 17.3, 24.7, 38.1, 53.3, and 66.5 mequiv./1000 ml. LD_{50} was determined graphically, after the percentage mortality had been converted into probits using the method of Bliss.[22] In Fig. 7 the probits are plotted against log dose (mequiv. FFA/kg body weight). With a dose of emulsion amounting to 8 ml/kg body weight, LD_{50} for FFA in the emulsion was found to be 0.21 mequiv./kg body weight, corresponding to a FFA concentration in the emulsion of 26.3 mequiv./1000 ml.

Long-term tolerance tests. The FFA concentration, pH and particle size in the tested emulsions (Intralipid 20 %) stored under varying conditions are shown in Table 3. The results of the long-term tolerance test in dogs with

Table 3. FFA concentration and pH of aged emulsions (Intralipid 20 %) stored under various conditions. 9 g fat (45 ml)/kg body weight and day given for 28 consecutive days to 4 dogs.

Dog No.	Storage		FFA mequiv./1000 ml	pH
	Time months	Temp. °C		
34	6	20	1.65	5.19
41	12	20	4.60	4.97
68	36	4	2.90	5.14
71	36	12	6.10	4.90

9 g of fat/kg body weight and day for 28 consecutive days are depicted in Fig. 8. Slight anaemia appeared in all the dogs, with a fall in both haemoglobin and haematocrit to about 80 % of the initial value. The two dogs given emulsion stored for 36 months at + 4 and + 12°C showed a somewhat greater decrease in haemoglobin concentration than that in the other two, which had got emulsions stored at + 20°C for 6 and 12 months, respectively.

Apart from the triglyceride concentration in dog 34 (emulsion stored for 6 months at + 20°C), the concentration of all serum lipids determined rose during the infusion period. This rise showed an increase with storage temperature and time. For example, dog 71 (emulsion stored for 36 months at + 12°C) showed an almost 6-fold increase in both triglycerides and phospholipids during the 4-week period, and the cholesterol level increased 4.5 times.

As far as total serum protein was concerned, a moderate increase was recorded in 3 of the 4 dogs. The exception was dog 41 (emulsion stored for 12 months at + 20°C); a decrease of 10 % occurred during the infusion period. An increase in serum albumin was found only in dog 34 (emulsion stored for 6 months at + 20°C). In the other three, the serum albumin concentration fell by 7 % (dog 41), 14 % (dog 68) and 7 % (dog 71). A distinct increase in serum globulin concentration was noted in the two dogs (68 and 71) given emulsion stored for 36 months at + 4 and + 12°C, respectively.

During the whole experimental period, all the dogs were in good condition, had a normal coat and exhibited normal reactions. On no occasion did the
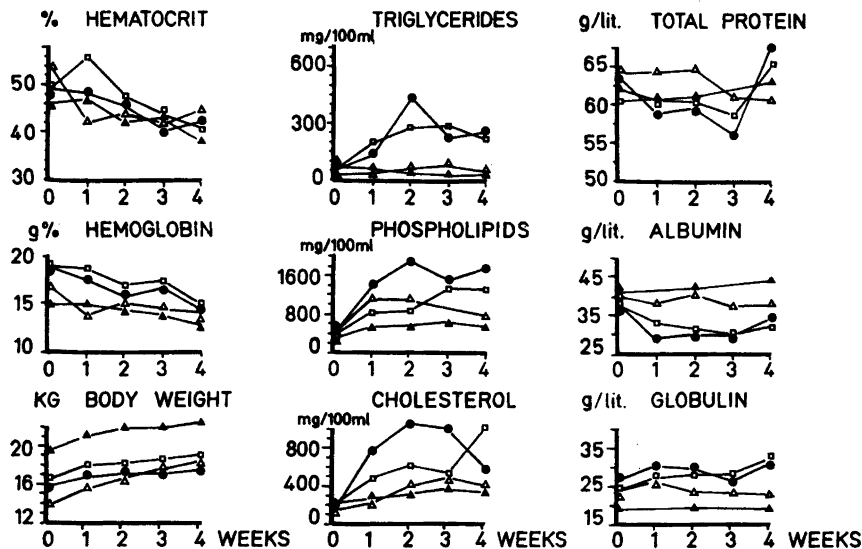
Fig. 8. Effects of aged Intralipid 20 % on the haematocrit, haemoglobin, body weight, serum lipids and serum proteins in the dog.

urine of any of the dogs contain glucose, protein, or ketone bodies. A rise in temperature exceeding 0.5°C during the infusion was recorded on only one occasion (20th infusion in dog 41 with a rise of 0.6°C). The dogs' appetite was good, and all gained in weight during the experimental period. The gain amounted to 15 %, 35 %, 13 %, and 10 % of the initial weight (dogs in the order shown in Fig. 8). All the changes in the blood constituents are also recorded in Fig. 8.

Autopsy was performed on three of the four dogs used. Table 4 lists the histopathological changes that can be attributed to the administration of Intralipid 20 % after lengthy storage. Intravenous fat pigment was observed in the cells of the reticuloendothelial system of the liver, spleen, lymph nodes, and bone marrow. Slight erythrophagia and centrilobular necrosis of the liver were observed in one dog. Generalized jaundice was present in two dogs and fat in degenerated liver cells in one.

Clinical studies in man. Table 5 shows the results from 8 series of infusion of Intralipid 10 % and Intralipid 20 % after 18 months' storage at + 12 and + 20°C, as well as after 24 months' storage at + 4, + 12, and 20°C. On each occasion of infusion recorded in the Table, 4 batches each of Intralipid 10 % and Intralipid 20 % were used. The number of infusions of emulsions stored for the

Table 4. Histopathological changes in dogs given aged fat emulsions (Intralipid 20 %) in doses of 9 g fat/kg and day for 28 consecutive days.

Dog No.	Storage conditions	Pathological changes
41	12 months at + 20°C	Intravenous fat pigment in cells of reticuloendothelial system of liver and lymph nodes. Fat in degenerated centrilobular liver cells.
68	36 months at + 4°C	Large amount of intravenous fat pigment in cells of reticulo-endothelial system of liver, spleen, lymph nodes and bone marrow. Centrilobular necroses in the liver. Generalized jaundice. Signs of erythrophagia.
71	36 months at + 12°C	Large amount of intravenous fat pigment in cells of reticulo-endothelial system of liver, spleen, lymph nodes and bone marrow. Generalized jaundice.

relevant time (18—24 months) and temperature (+ 4, + 12, and + 20°C), the quantity of fat administered, the FFA concentration and pH of the various emulsions can also be inferred from Table 5. It also lists the rises in temperature divided into four groups, in which all infusions with a temperature rise of less than 0.6°C are collected in the first group. Increases limited by temperature rises of 0.6—1.1°C and 1.1—1.6°C, respectively, form the second and third groups, while a temperature rise exceeding 1.6°C constitutes the fourth. In addition, other reactions — such as nausea, vomiting and rigors — observed during and after infusion have also been noted. In a total of 122 infusions recorded, *ca.* 2 g of fat/kg body weight have been given on 97 occasions. A temperature rise of less than 1.1°C has been recorded at 109 infusions. Of these 65 showed an increase not exceeding 0.6°C. In 114 out of 122 infusions no "other reactions" have been reported.

In Intralipid 10 % after storage for 18 months at + 20°C, the mean FFA concentration was 8.30 ± 0.52 mequiv./1000 ml emulsion, while Intralipid 20 % stored for 24 months at + 20°C showed a mean concentration of 9.90 ± 0.36 mequiv. FFA/1000 ml. Consequently, the patients given 2 g of fat/kg body weight of Intralipid 10 % received a FFA dose of around 0.17 mequiv./kg body weight. In view of the variations in FFA concentration in the four batches of Intralipid 10 % used, and the fact that the amount infused exceeded 20 ml/kg body weight in certain cases, a dose of 0.20 mequiv./kg body weight was given in a few infusions.

DISCUSSION

As a result of the spontaneous hydrolysis occurring in the commercial fat emulsions (Intralipid 10 % and 20 %), the concentration of free fatty acids (FFA) increases with time and temperature of storage. This, in turn, produces a fall in pH. Since hydrolysis is catalyzed by hydronium ions, the reaction rate gradually increases with a rising concentration of these ions. However,

Table 5. Clinical studies of aged Intralipid. Mean of four batches each of 10 % and 20 % emulsion. Other reactions include nausea, vomiting, rigors *etc.*

Emulsion: Intralipid %	Infusions (total No.)	Storage		pH	FFA mequiv./1000 ml	No. of infusions with amount of fat given (g/kg BW)			No. of infusions with rise in temperature expressed as °C				No. of infusions with other reactions recorded as		
		Temp °C	Time months			0.9—1.3	1.3—1.8	1.8—2.5	≤0.6	0.6—1.1	1.1—1.6	≥1.6	none	slight	moderate
10	13	12	18	5.11	2.72	2	7	4	3	9	0	1	12	0	1
20	23	12	18	5.12	2.57	0	5	18	8	7	5	3	22	0	1
10	12	20	18	4.78	8.30	0	0	12	7	5	0	0	12	0	0
20	23	20	18	4.81	5.08	2	4	17	13	9	0	1	23	0	0
10	10	12	24	4.84	3.14	1	1	8	7	3	0	0	10	0	0
20	12	4	24	5.15	1.24	1	0	11	6	6	0	0	11	1	0
20	16	12	24	4.86	2.78	0	0	16	11	2	2	1	13	3	0
20	13	20	24	4.72	9.90	0	2	11	10	3	0	0	11	2	0
Total	122					6	19	97	65	44	7	6	114	6	2

in alkaline hydrolysis — whose reaction rate is higher than that of the acidic hydrolysis — the reverse applies in unbuffered emulsions. The reaction rate decreases rapidly as hydrolysis proceeds, because the pH of the emulsion approaches the neutral point. In buffered emulsions, particularly at a pH around 6.8, the rate of hydrolysis has a minimum. Buffering substances other than sodium phosphate were tested with similar results.

A gradual increase in particle size was demonstrated after long-term storage of emulsions at varying temperatures. Although this kind of instability increases with temperature, it is probably not primarily dependent on the increased concentration of FFA. Thus, buffered emulsions with a pH around the neutral point show a low FFA concentration but impaired stability, with increasing particle size and creaming as a result. Another measure of the physical stability of the emulsions was obtained by applying the same principles as those described by Singleton et al.[23] The resistance of the emulsions to mechanical shock was studied with respect to such factors as particle size and creaming, after shaking in a shaking apparatus. All emulsions containing electrolytes then proved to be highly unstable. The instability increased with rising ionic strength, appeared within a relatively large pH range, and varied somewhat with different electrolytes. In the oil/water system studied with phospholipids as emulsifiers, it is likely that valency of the ions, ionic strength and pH of the emulsion are of importance for the interaction with the phospholipids, which can be characterized as ampholytes.

It is evident from the studies described in this paper that the FFA concentration of the emulsion was, in fact, the only one of the parameters studied that could be correlated to the increased toxicity in the rabbit. The toxicity did not seem to be influenced by a final pH in the range of 3.5—9.5, despite the borderline values being somewhat extreme from the physiological point of view, nor by any unknown hydrolysis products that might have been present at these pH values, or the fact that an increase of the particle size was demonstrated at storage for a long time at higher temperatures. Injection of 8 ml of 20 % emulsion, representing 1.6 g of fat/kg body weight and a FFA concentration below 0.12 mequiv./kg body weight, proved to be non-lethal in every case.

Using the procedure described, LD_{50} in the rabbit was determined, and found to be equal to a FFA dose of 0.21 mequiv./kg body weight, corresponding to 26.3 mequiv./1000 ml emulsion. This is in agreement with the results obtained by Orö and Wretlind [24] in mice. Among the findings of these authors were that LD_{50} of stearic acid was about 0.08 mequiv./kg body weight, whereas that of palmitic acid was about 0.22 mequiv./kg. The mechanism responsible for the lethal effect of FFA on infusion is unknown, but some relevance can perhaps be ascribed to the fact that small doses of saturated FFA cause massive thrombosis in the dog.[25]

The haematological changes differed in the dogs given 9 g of fat/kg/day, in the form of Intralipid 20 % stored for long periods under varying conditions. For example, two dogs given emulsion stored for 36 months showed a marked increase in serum lipids compared to those given emulsion stored for 6 and 12 months, respectively. With respect to serum albumin concentration, a relatively marked decrease was recorded in the two former dogs. However, apart from the serum cholesterol in one of them, a normalization in the con-

centration of both serum lipids and albumin took place towards the end of the infusion period.

As far as the histopathological changes are concerned, centrilobular necrosis of the liver was observed in one of the two dogs given emulsion stored for 36 months, and generalized jaundice was present in both. Moreover, there was a larger amount of intravenous fat pigment in these dogs than in the others. Because of the large doses that have been given for a long time and in view of the few dogs involved, no definite conclusions can be drawn from these results. In addition to that, emulsions stored up to 16 months at temperatures between $+ 12$ and $+ 20°C$ have been used in similar experiments without observing the above discussed findings at sectioning.

The primary aim of the present study was to ascertain whether the emulsions could be stored for long periods at varying temperatures without the appearance of toxic effects. Another aim was to find one or several parameters that could be used, in a simple way, to determine whether such emulsions could be used clinically. Clinical tests were therefore made with emulsions stored under varying conditions. The results of infusions in man showed a low incidence of toxic reactions, of about the same order of magnitude as those reported by Schuberth [26] in his collocation of 1405 infusions of Intralipid.

Infusion of Intralipid stored for 18 months was associated with a larger number of relatively marked rises in temperature than was infusion of that stored for 24 months (Table 5). This apparent anomaly is explained by the fact that in the latter case the patients were selected, so that any reactions could be ascribed, as far as possible, to the infusions. This did not apply to the patients given Intralipid stored for 18 months.

Although the tolerance in the rabbit is sometimes regarded to correspond to that in man, it is perhaps hazardous to establish the toxicity of the FFA concentration in the emulsions in man on the basis of that in the rabbit. A comparison shows, however, that some of the emulsions tested clinically after storage at $+ 20°C$ for 18 months had a mean FFA concentration of 8.30 $\pm$ 0.52 mequiv./1000 ml. This implies that in the few infusions in man in which the dose of fat on infusion of Intralipid 10 % exceeded 2 g/kg body weight, the FFA dose was about 0.2 mequiv./kg. However, the marked differences in administration — in a few seconds in the rabbit, and in 4 h in man — have to be noted. This apparently good tolerance to FFA in man might be due to the rapid rate at which free fatty acids are eliminated from the blood stream.[27] Consequently, there will not be a high plasma concentration of FFA during the 4-h infusion period.

To sum up, the following conclusions can be drawn from the present study. The FFA concentration of the emulsion seems to be the most important parameter responsible for its toxicity on storage. Moreover, the toxicity of FFA in the rabbit under the conditions described might be used as a criterion of the clinical usefulness. Finally, the emulsion can, with a satisfactory safety margin, be stored up to 2 years at a temperature of $+ 4$ to $+ 8°C$.

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