J. Pharm. Pharmacol., 1964, **16**, 641–646 Received April 13, 1964

Research Papers

Physical and biological changes in an artificial fat emulsion during storage

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A soybean oil emulsion for parenteral nutrition has been stored for about 2 years at 4, 20 and 40°. During storage, the pH fell and the FFA concentration increased. The Michaelis-Menten constant (K_m) and the maximum velocity of the lipoprotein-lipase reaction (V_{max}) also increased. Gross particles were formed in the fat emulsion, and its toxicity increased with time. All these changes were most pronounced during storage at 40°.

A SOYBEAN oil emulsion for parenteral nutrition (Schuberth & Wretlind, 1961) has been investigated in dogs and rabbits by several authors. The freshly prepared emulsion has a low toxicity in dogs at doses up to 9 g/kg weight for periods of 4 weeks (Edgren, Hallberg, Håkansson, Meng & Wretlind, 1964). Furthermore, the elimination of this emulsion from the blood stream in dogs is similar to that of dog chylomicrons (Carlson & Hallberg, 1963). The emulsion has also proved to be a suitable substrate in the lipoprotein-lipase reaction (Boberg & Carlson, 1964).

During the past 3 years this emulsion has been used in clinical practice (Schuberth & Wretlind, 1963) but it has not been subjected to systematic studies of the physico-chemical and biological changes occurring as a result of lengthy storage under various conditions. We now describe some physico-chemical changes observed during long-term storage at different temperatures, and also the physiological effect of these changes.

Experimental

Emulsion. The emulsion* contains 10% soybean oil, 1·2% egg phosphatides and 2·5% glycerol in the aqueous phase. One batch was transferred to bottles containing 100 ml. The bottles were sterilised by autoclaving, and stored in the dark at 4, 20 and 40°. The longest storage period was more than 2 years. After various intervals, the required number of bottles, were withdrawn and tested.

pH determination. Measurements were made with a glass electrode.

Determination of free fatty acids. Free fatty acids (FFA) were assayed in duplicate by the method of Dole (1956), as modified by Traut, Estes & Friedberg (1960).

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Supported by grants from Reservationsanslaget, Karolinska Institutet, Stockholm 60, Sweden.

* Intralipid, kindly supplied by Vitrum AB, Stockholm.

Estimation of lipoprotein-lipase activity. FFA released during incubation of enzyme plasma and substrate *in vitro* was titrated according to Boberg & Carlson (1964). Plasma containing lipoprotein lipase was obtained by withdrawing blood from a fasting, healthy human donor, 1 hr after intravenous injection of 50 mg of heparin. The blood was centrifuged at 1000 *g* for 15 min. Plasma was drawn off, and frozen in tubes at −20°. The emulsion was used as substrate in the lipoprotein-lipase reaction. The lipoprotein-lipase activity was determined at three different substrate concentrations, and plotted according to Lineweaver & Burke (1934).

Particle-size determination. The particle size was measured under a microscope. The emulsion was diluted 1:25 with a 50% solution of propylene glycol in water, as described by Levius & Drommond (1953). The number of particles with a diameter of more than 0·5 μ was counted within one square of a ruled eyepiece scale. Ten squares were examined in each slide.

Animal experiments. Albino rabbits of either sex, weighing about 2·5 kg, were used. In each experiment, 20 ml of the emulsion, warmed to body temperature, was infused into the marginal ear vein over 25 sec. Six rabbits were injected with each emulsion in every test, and observed for one day. In those that did not survive, death occurred within less than 5 min.

Results

pH changes. Fig. 1 shows that pH fell during storage. The fall was most pronounced during the first 3 months. There was a significant difference between the pH of the emulsion stored at 4° and that stored at 40°.

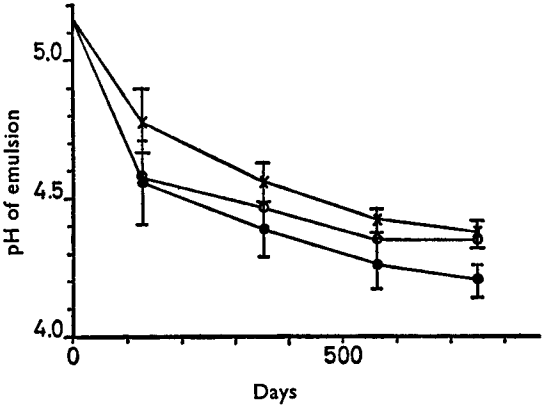

FIG. 1. Changes in pH during storage of emulsion at ×, 4°; ○, 20°; ●, 40° (mean ± s.e.).

Changes in FFA concentration. A spontaneous release of FFA occurred during storage which was practically linear with time (Fig. 2). Up to

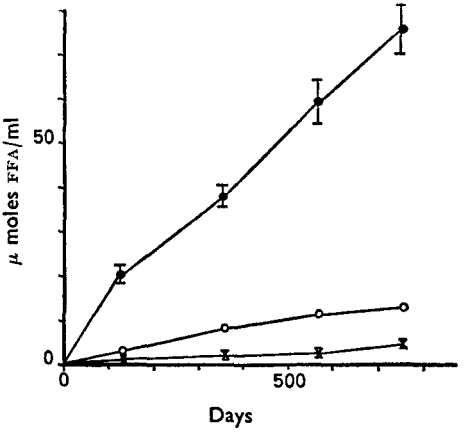

FIG. 2. Release of FFA during storage at ×, 4°; ○, 20°; ●, 40° (mean ± s.e.).

20° the FFA concentration was not high but in emulsions stored at 40° the release was much increased, for example, after 700 days storage the the FFA content of this emulsion was about 15 times greater than that of emulsions stored at 4°.

Properties of the emulsion as a substrate. Fig. 3 shows graphs of the lipoprotein-lipase reaction plotted according to Lineweaver & Burk (1934). The Michaelis-Menten constant (K_m) and the maximum velocity of the reaction (V_{max}) constant are listed in Table 1. In the emulsions stored at 4° and 20°, there were no significant changes with time. In those stored at 40°, both K_m and V_{max} increased significantly during the period studied.

TABLE 1. THE CONSTANTS K_m AND V_{max} CALCULATED ON THE BASIS OF THE CURVES IN FIG. 3

Test time, days	0		42		128		400	
Storage temp °C	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
4	0·84	0·178	0·52	0·206	0·37	0·140	1·00	0·135
20	0·87	0·180	0·16	0·144	0·74	0·080	2·54	0·285
40	0·91	0·192	0·44	0·148	5·56	0·572	20·03	1·012

K_m = Michaelis-Menten constant expressed in μ moles triglycerides/ml.
V_{max} = Maximum velocity of the reaction expressed in μ moles/min and ml.

Changes in particle size. At zero time the optical diameter of all particles was less than 0·5 μ, i.e. they could not be measured with the method used. During storage a few particles of 3 μ diameter were formed, but by far the greatest number counted were between 0·5–1 μ. The grossest particles were found in the emulsion stored at 40°.

Animal experiments. All the rabbits survived emulsion stored at 4° and 20°, whereas that stored at 40° showed an increasing lethal effect with time (Fig. 4).

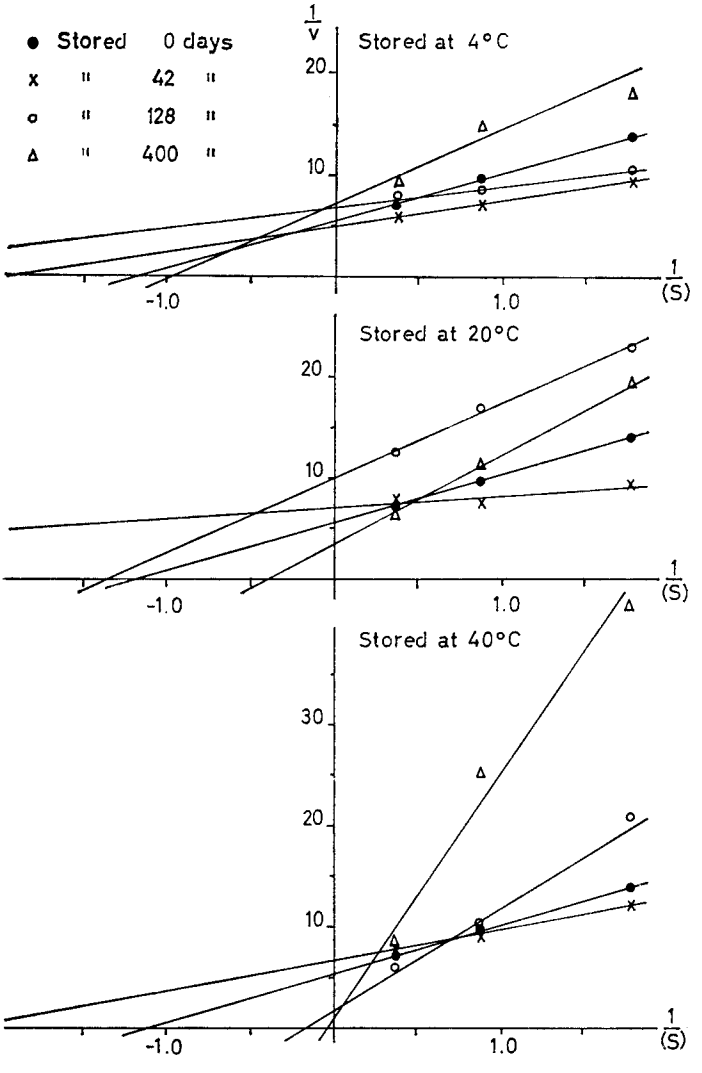

FIG. 3. Lipoprotein-lipase activity (LLA) tested on the emulsions plotted as straight lines according to Lineweaver & Burk. The equation of the straight line is:

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{(S)}$$

where K_m is the Michaelis-Menten constant and V_{max} the maximum velocity of the reaction. y intercept $= \frac{1}{V_{max}}$ and x intercept $= -\frac{1}{K_m}$. The reaction rate (v) is μ moles FFA formed per min and ml post-heparin plasma. Substrate concentration (S) is μ moles triglycerides per ml test solution.

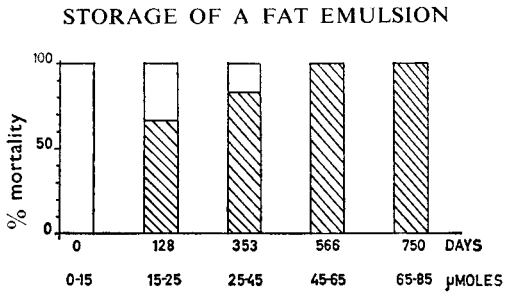

FIG. 4. Toxicity of the emulsion stored at 40° tested in the rabbit. Abscissa: Storage time in days and range of FFA concentration in μ moles/ml emulsion. Each column represents emulsion tested in 6 rabbits. Hatched area = mortality.

Discussion

It may be concluded that the pH falls with time, the FFA concentration increases with time, as do the constants K_m and V_{max}, and gross particles are formed. All these changes are more pronounced at 40°, when after 400 days, the pH falls from 5·2 to 4·4. The FFA concentration shows a five-fold increase from 0·8 to about 40 μ moles/ml. The K_m value is about 20 times higher than in the fresh emulsion, and the V_{max} value about 5 times higher. Moreover, this emulsion becomes toxic, and causes more than 80% mortality when injected into rabbits.

When the logarithm of the FFA concentration was plotted against the pH of the emulsion at each temperature, an almost linear correlation was present between these two variables. This suggests that as the fall in pH is almost linear to some extent it might be due to the increased concentration of FFA.

It is likely that the lipoprotein-lipase attacks the substrate on the surface of the particles. The increased K_m value in the lipoprotein-lipase reaction during storage may be due to the loss of affinity for the enzyme at this site. The formation of gross particles in the emulsion is probably of some importance in this respect, because, in relation to their fat mass, the surface of the gross particles is less. This necessitates the use of a higher substrate concentration to reach a zero order reaction of the lipoprotein-lipase reaction.

The only parameter of the emulsions studied, that can be correlated to the increased toxicity of the emulsion stored at 40° is the FFA concentration. This is in agreement with the findings in mice reported earlier by Orö & Wretlind (1961).

The cause of death in the rabbits is unknown but it may be of relevance in this context that injection of small amounts of saturated FFA causes massive thrombosis in dogs (Connor, Hoak & Warner, 1963).

Later unpublished investigations have shown that the liberation of FFA from soybean oil emulsion is minimum when the pH is between 6 and 7.

To conclude, our results indicate that the fat emulsion can be stored satisfactorily at a temperature of 4° for up to 2 years.

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