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Emulsification of Fat for Intravenous Administration.

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An emulsion of fat that could safely be given in quantity by vein was first developed by Yamakawa and his collaborators[1] in Japan. Recent observations in Latin America and in this country[2] have confirmed and extended their work and have furnished very suggestive clinical and experimental evidence of the value of such preparations. The technique used by Japanese and American workers is essentially similar: the fat or oil is mixed with purified egg lecithin (Merck's or Kahlbaum's) and water and passed through a 2-stage dairy homogenizer operating at 3000 to 4000 lb. pressure, after which the product is sterilized by heat in sealed containers. When suitable quantities are used, it is possible to obtain an emulsion in which practically all the lecithin-coated fat globules are less than 2μ in diameter with only an occasional one as large as 3 or 3.5μ. Such an emulsion passes through the lung capillaries with ease,[3] avoiding the danger of fat embolism, which may occur when the particles exceed 4μ in diameter.[4]

A difficulty that has been experienced—both with Japanese and American preparations—is the limited time during which these emulsions are stable. In the course of a few weeks, more or less, the larger fat particles which are in less active Brownian movement tend to rise to the surface, forming a visible cream layer. Although the formation of the cream does not necessarily indicate an increased particle size, nevertheless the large fat particles are in close apposition, and there is danger of their coalescing to form larger aggregates. The present studies were undertaken in an attempt to pro-

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1 Yamakawa, *Nippon Naika Gakkwai Zasshi*, 1920, **17**, 1, 22; Yamakawa and Nomura, *Jikken Iho*, 1928, p. 523; a series of papers in *Tohoku J. Exp. Med.*, 1928-1932, by Nomura, Baba, Sato, Hotta and others.

2 Valledor, Casas, and Gomez del Rio, *Vida Nueva*, 1928, **23**, 156; Valledor, *Arch. de Med. des Enf.*, 1933, **36**, 276; Holt, Tidwell and Scott, *Am. J. Dis. Child.*, 1934, **48**, 926; *J. Pediatrics*, 1935, **6**, 151; Gordon and Levine, *Am. J. Dis. Child.*, 1935, **50**, 894.

3 Sato, *Tohoku J. Exp. Med.*, 1931, **18**, 120.

4 Markowitz and Mann, *Am. J. Physiol.*, 1931, **98**, 521.

duce emulsions which would be more stable, over a longer period of time, and hence more practical for intravenous administration.

It was found that a part of the difficulty was due to decomposition of lecithin under the influence of light and oxygen to form darker compounds; this change was also accompanied by the development of hemolytic properties. By using strictly fresh lecithin, sealing the material in nitrogen and keeping the preparations in the dark, some delay in creaming could be obtained.

The hydrogen ion concentration of the emulsion was next studied,† since it had been noted that the acidity increased on standing, and an attempt was made to prevent this by means of buffers. The introduction of a small amount of sodium bicarbonate sufficed to keep the pH above 6.0, and delayed the creaming but did not prevent it altogether. One might have feared that such addition of electrolyte would neutralize the charge on the fat particles and favor their aggregation, but such an effect was not observed at the concentrations employed. However, the addition of the sodium bicarbonate seemed to cause unfavorable reactions when the emulsion was injected into infants and it was therefore abandoned.

We then investigated the possibility that the interfacial tension between the lecithin and the fat might be a factor of significance in determining the stability of the emulsion. Measurements were made of the interfacial tensions between lecithin and olive oil mixtures, and we soon found, as had Okuneff,[5] that a very small concentration of lecithin was sufficient to reduce the interfacial tension to an exceedingly low value. It was therefore felt that this was not a factor of significance in influencing the creaming. Our next step was to study the effect of varying the lecithin-fat ratio. The Japanese investigators had employed a ratio of 1:1.65, whereas Holt, Tidwell and Scott[2] had used a ratio of 1:2; we were unable to find any data as to the optimum ratio for purposes of emulsification. The virtue of lecithin as an emulsifying agent is supposed to lie in the fact that it contains both polar and non-polar groups, and theoretical considerations would lead one to believe that in such emulsions the lecithin forms a surface film bounding the fat globule. That the lecithin is indeed located at the surface of the particles was suggested by experiments in which emulsions were allowed to cream, the cream layer separated, and determinations of the lecithin-fat ratio made both in the cream layer containing larger particles and the underlying layer containing the smaller particles. In the latter,

† We wish to thank Dr. J. A. Pierce for these measurements, which were made with a quinhydrone electrode.

5 Okuneff, *Biochem. Z.*, 1928, **198**, 296.

a relatively larger surface would exist per unit volume of fat, and if the lecithin were at the surface, the lecithin-fat ratio should be higher in this portion of the emulsion than in the cream layer. This was actually found to be the case. Analysis of an emulsion which had stood for some time showed that the underlying unseparated layer had an L/F ratio of approximately 1:1 while the cream layer showed a ratio of approximately 1:2. Although the L/F ratio in the more stable part of this emulsion was of the order of 1:1, and although theoretical calculations of the ideal ratio based on rather crude estimates of average particle size pointed to this ratio as the ideal one, nevertheless we were not able to establish by experiment that such was the case. In general, stability seemed to improve with an increased proportion of lecithin, but no sharp limits were found. Even when the L/F ratio was carried beyond 1:1 the creaming difficulty was not obviated.

It seemed likely that the phenomenon of creaming could be obviated if the size of the particles could be sufficiently reduced, for as the size of the particles diminishes and the Brownian movement becomes more active, the influence of specific gravity is less effective in causing the particles to rise. With a homogenizer or a colloid mill it is not possible to reduce the size of particles beyond the dimensions described except by extreme dilution, which would have vitiated our purpose; we therefore turned our attention to another means of emulsification—namely the use of sonic and supersonic waves, first reported by Wood and Loomis.[6] Our first attempts were made with audible sound of a frequency of 9,000 per second, generated by a magneto-striction apparatus‡ as used by Chambers and Flosdorf.[7]

The materials to be emulsified were brought into contact with a vibrating nickel rod. Although considerable emulsification took place through the use of this method, it had to be abandoned because of the quantity of nickel that was suspended.

We then attempted to emulsify our materials by means of high frequency supersonic waves, generated from quartz crystals by means of the piezo-electric effect. Daniewski[8] had demonstrated that the optimum frequency for emulsifying kerosene in water lay between 150,000 and 400,000 cycles per second. When Daniewski's curves are recalculated with power input as one of the variables it

[6] Wood and Loomis, *Phil. Mag.*, 1929, **4**, 418.

‡ This apparatus was kindly placed at our disposal by Drs. Leslie A. Chambers and Earl W. Flosdorf of the University of Pennsylvania.

[7] Chambers and Flosdorf, *J. Biol. Chem.*, 1936, **114**, 75.

[8] Daniewski, *Acta Physica Polonica*, 1933, **2**, 45.

is found that the rate of emulsification is proportional to power input. It therefore seemed desirable to use a frequency between these two figures with the maximum possible power input.

By means of such an apparatus, generating waves with a frequency of 300,000 cycles per second, it was found that fine emulsions of a high degree of stability could be obtained after rather prolonged radiation.§ When an emulsion with a lecithin-olive oil ratio of 1:2 (total lipids 7%) was homogenized and sealed under nitrogen in 20 cc. thin-walled glass ampoules, very short periods of radiation did not appreciably retard the creaming. However when the ampoules were radiated for a total of 80-120 minutes,|| a product was obtained which kept for 2 months and often more without the slightest evidence of creaming. The emulsion appeared slightly opalescent, rather than entirely opaque, and inspection under the darkfield microscope showed that practically all of the visible particles were 0.5μ in diameter, and none was larger than 1μ, even after 2 months.

An interesting observation was made in regard to the effect of the lecithin-olive oil ratio upon the result of the supersonic radiation. When a homogenized 6% water suspension of purified lecithin (containing very little egg oil) was used, 30 to 60 minutes of radiation produced a completely transparent solution in which no particles were visible in the darkfield. The gradual substitution of olive oil for part of the lecithin and the resulting gradual increase of the lecithin-olive oil ratio caused a progressive change from transparency to opalescence and then to the fairly opaque emulsion, observed at a 1:2 ratio, in which particles were visible in the darkfield.

We also studied the effect of omitting the preliminary homogenization in the dairy homogenizer before the supersonic treatment, and came to the conclusion that such preliminary treatment is necessary unless the proportion of lecithin is greatly increased. If the lecithin-fat ratio was greater than 2:1, a fine emulsion could be prepared by prolonged radiation of the crude (unhomogenized) mixture. However, when the standard 1:2 emulsion was radiated without homogenization, the supersonic waves failed to produce a stable emulsion. In fact, the occasional appearance of small oil droplets indicated that some of the coarse particles had been de-emulsified.

After preliminary injection of the radiated emulsion into rabbits

§ The preliminary experiments with this type of apparatus were conducted at the Loomis Laboratory, Tuxedo Park, New York, and were made possible through the courtesy and cooperation of Mr. Alfred L. Loomis.

|| This was done in 2-minute periods with cooling in between, to avoid possible heat effects.

had demonstrated that the supersonic preparation had produced no toxic substance, clinical observations were made in normal infants, the emulsion being given intravenously as described by Holt, Tidwell and Scott.[2] No untoward effects were observed. A study of the lipemic curve** after administration of one gram of lipid per kilo, indicated that the fat was removed from the blood in a normal manner. Comparisons of the rate of removal of the finer radiated emulsion with a somewhat courser unradiated emulsion showed that the radiated fine emulsion was not quite so readily rapidly removed at first. The difference, however, did not appear to be of any practical significance. Protocols of such a comparison are shown in Figure 1.

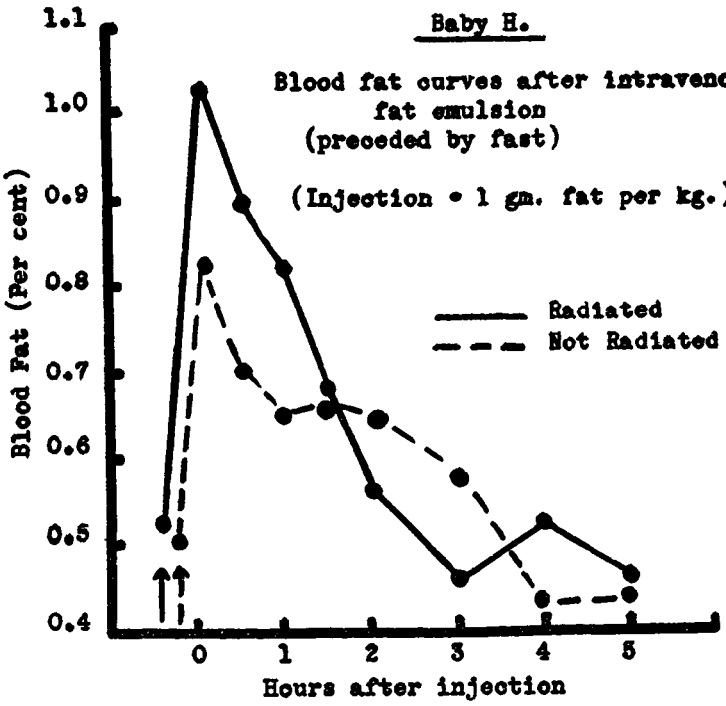

FIG. 1.

In addition to its efficiency in producing an emulsion of increased stability, the supersonic radiation method has a further advantage in that it permits autoclaving before the final step in the emulsification procedure. When emulsions are merely homogenized, they

** Blood fat determinations were made by the Gorter-Grendel micromethod (*Biochem. Z.*, 1928, **192**, 431).

must subsequently undergo a heat sterilization which usually causes some increase in particle size.

Conclusion. Supersonic radiation appears to be the most suitable method yet studied for preparing fat emulsions, suitable for intravenous use in man.

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Enlarged Tissue Cultures of European Typhus Rickettsiae for Vaccine Production.

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Our methods of producing typhus vaccines with the murine strains of Rickettsiae has depended upon the inoculation of rats in which resistance had been reduced by a variety of procedures, the most consistently successful of which has been preliminary X-ray radiation. These methods have persistently failed to give adequate results with the European virus. This fact in itself constitutes further strong evidence that the two types of Rickettsiae are distinct and biologically fixed varieties. After much effort to apply the "rat" methods to the organism of the classical European disease we were finally persuaded that other methods of approach must be sought for obtaining accumulations of the European Rickettsiae adequate for practical purposes of immunization.

In a paper, now in press, the writers have reported the results of experiments in which the active immunization of guinea pigs against the European typhus virus was accomplished, both with the use of formalinized tissue culture vaccines and by methods of sero-vaccination. While this work was going on, Kligler and Aschner[1] published observations on a method of successful animal immunization with formalinized tissue culture vaccine, similar in all important principles to the method which we are using.

Since the development of a tissue culture technique for producing vaccines in amounts adequate for practical purposes appeared the most hopeful direction of effort, we have tried for a long time to improve the technique of making such cultures, particularly in regard to increasing the volumes of the tissue cultures themselves.

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1 Kligler, I. J., and Aschner, M., *Brit. J. Exp. Path.*, 1934, **15**, 337.