Intravenous Infusions Into Human Subjects of Fractionated Coconut Oil Emulsions. (19498)

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The purification of natural coconut oil is of major importance in the proper development of a fat emulsion for intravenous use in man. Although coconut oil is sufficiently purified for edible purposes, it nevertheless contains a variety of non-essential nonglyceride substances which must be considered as undesirable impurities for inclusion in an intravenous preparation. These latter substances are the hydrocarbons, free fatty acids, phytosterols, carotenoid pigments etc. By means of special filtration and distillation technics natural coconut oil can be refined to yield three fractions of relatively pure fatty acid triglycerides. The present report is devoted to a clinical study in which each of these fractions of coconut oil was emulsified and injected intravenously into human subjects.

Method of Study. Emulsions containing 10% of fraction A or fraction B or fraction C were prepared by homogenization and stabilization with 2% gelatin and 5% glucose in the manner previously described(1). In addition, combinations of fractions A and B, 5% of each, fractions A and C in the same proportion and also fractions B and C were made into emulsions. A total of 24 hospital patients were divided into 6 groups corresponding to the type of emulsion administered. In addition, 8 patients of this series received infusions of all 3 fractions or of their combinations. Each infusion was started in the morning with the patient in the post-absorptive state. During the study period which lasted 24 hours, temperature, pulse, respirations and blood pressure were recorded hourly. Four samples of blood were drawn; an initial sample at the start of the infusion, a sample at the termination of the infusion and samples at 12 and 24 hours. The following determinations were made on the blood; hemoglobin(2), hematocrit cell volume, total lipids (3), and cholesterol(4). Hemolysis *in vivo* was determined by the quantitative estimation of free hemoglobin in plasma by a modification of Flink's method(5). Complete blood counts were also made. Catheterized urine specimens obtained at the same intervals as noted above were examined for ketone products, urinary fat and blood.

Results. Group I. Four patients were each infused with one liter of the emulsion containing 10% fraction A coconut oil. In every case there was a severe reaction during the course of the infusion necessitating its discontinuance. Toleration for this emulsion did not exceed 400 ml. The rate of administration did not appear to alter reactivity. Symptoms were similar in all cases. These were dyspnea, cough with expectoration of frothy sputum, retching and vomiting of gastric contents tinged with blood and considerable bile. The patients complained of severe pain which was localized in the mid-back region corresponding to the site of the kidneys or pancreas. The temperature rose from 4 to 6 degrees even before the infusion was discontinued, persisted for a period of 2 to 3 hours and then subsided rapidly to the initial level. The blood studies showed a significant fall in hemoglobin, hematocrit cell volume and number of red blood cells, particularly in sample 2 and sample 3. Leukocytosis was most marked at the completion of the infusion. The values for total lipids and cholesterol rose above the initial values but declined subsequently to values even below the initial level. Free hemoglobin in plasma was markedly elevated in samples 2, 3, and 4. The intermediate urine samples were positive for acetone. All the urine samples after the infusion were strongly positive for blood.

Group II. Three patients from the previous group and 4 new subjects were infused with the emulsion containing fraction B coconut oil. This emulsion was well tolerated without reaction and with little or no temperature elevation except in 3 subjects who showed a rise which averaged 1.5 degrees in

TABLE I. Data Showing Effects of Each of Three Fractions of Coconut Oil Emulsion Infused into the Same Subject.

Fraction	Sample	Hemoglobin, g %	Hematocrit, Mm	Free hemoglobin, mg %	R. B. C. × million	W. B. C. × thousand	Total lipid, mg %	Cholesterol, mg %	Urine acetone	Urine blood	Temp., °F
A	1	13.5	46	4	4.8	6.5	412	120	0	0	98.6
	2	11.6	40	108	3.2	20.3	680	160	2+	4+	98.6-102
	3	10.8	40	60	3.6	17.1	520	120	2+	4+	104 -100.2
	4	11	41	30	3.8	8.6	480	90	0	2+	100 - 98.6
B	1	14	48	2	5.1	5.8	334	106	0	0	98.6
	2	13.8	47	2	4.8	6	600	122	2+	0	98.6- 99.2
	3	13.8	46	2	4.6	5.6	522	120	0	0	99.2- 98.6
	4	13.9	46	2	4.9	5.3	420	120	0	0	98.6- 98.6
C	1	14.6	50	2	4.6	7.1	572	142	0	0	98.6
	2	14.4	48	2	4.7	7	737	168	2+	0	98.6- 99.4
	3	14	48	2	4.2	6.5	510	118	1+	0	99.6- 98.4
	4	14	49	4	4.4	6.4	430	90	0	0	98.4- 98.8

the last 2 hours of the infusion period. Except for a mild dilution effect, there were no significant changes of any of the constituents of the blood studied in this investigation. Leukocytosis did not occur. Hemoglobinemia was not demonstrable. Urinary findings were within normal limits.

Group III. Four new subjects and the same 3 patients who had been tested with the fraction A and B emulsions received infusions of the emulsion containing fraction C coconut oil. In this group infusion reactions did not occur. Temperature elevations were minimal and did not exceed 1.2 degrees. The laboratory data was similar to that obtained in the Group II studies.

Groups IV and V. The subjects in the latter 2 groups were infused with emulsions which combined fractions A and B or fractions A and C. Reactions were found to occur in every case but were less severe than those encountered when fraction A emulsion alone was administered. The limit of tolerance for either combined emulsion did not exceed 700 ml. Temperature elevations averaged 3 degrees. Leukocytosis was not as great. Free hemoglobin in plasma did not exceed 85 mg %. Clinically, vomiting, cough and back pain were observed as previously.

Group VI. Four patients comprising this group received infusions of the emulsion combining fractions B and C. These infusions were well tolerated without untoward reactions. The laboratory data showed little variation from that noted for Group II or III.

Discussion. The 3 fractions discussed above were prepared by Drs. Barsky and Zinzalian of the research division of the E. F. Drew Co. Each fraction consisted principally of the glyceride esters of a limited number of homologous saturated fatty acids. In the B and C fractions smaller amounts of the esters of the unsaturated fatty acids were also present. Fraction A consisted mainly of a mixture of the glycerides of caprilic and capric acids. Fraction B comprised the esters of lauric and myristic acids and minor concentrations of linoleic and oleic acid esters. Fraction C contained principally the glycerides and palmitic and stearic acids and lesser quantities of the esters of oleic, linoleic, myristic and lauric acids. The concentration of the saturated fatty acid esters in each fraction was 90% or greater. The esters of oleic and linoleic acids which constituted about 6% of the whole coconut oil were unequally distributed in the B and C fractions. As a result of the fractionation processes, non-fatty material and other non-glyceride substances present in natural coconut oil remained as an untested residue.

Emulsions of B and C fractions were well tolerated while serious reactions were found to occur with the intravenous infusions of the emulsions containing the A fraction. The basic patho-physiologic process associated

with the infusion of the reactive fraction appeared to be that of hemoglobinemia due to hemolysis *in vivo*. This can be accounted for on the basis of intolerance of the organism for low molecular weight fatty acids and their esters such as were contained in fraction A. The relative solubility of the latter and their ease of dissociation hastened the process of saponification in the blood and the surface action effect on the red blood cells causing hemolysis and the resultant hemoglobinemia. Better toleration obtained with the injections of the B and C fractions can be accounted for on the relative insolubility of the long chain fatty acid esters retarding in the blood any chemical interaction or hydrolysis.

Summary. 1. Emulsions of the glycerides of the short chain fatty acids were toxic when injected intravenously into human subjects. 2. The glycerides of the high molecular weight fatty acids, lauric, myristic, palmitic and stearic acids were non-toxic when injected in emulsion form. 3. Inclusion with the latter of small concentrations of the fatty acid esters of the type of oleic and linoleic acids did not affect the toxicity of the emulsions.

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α-Lipoic Acid in Oxidative Reactions of a *Corynebacterium*. (19499)

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α-Lipoic acid is a highly active growth factor for several microorganisms(1,2) and functions in the oxidative conversion of pyruvate to acetate by *Streptococcus faecalis*(1). Acetate will replace the factor for the growth of several lactic acid bacteria. On the other hand, acetate will not substitute for a lipoic acid-like material in the growth of a *Corynebacterium*(2,3). This difference prompted the present study of the *Corynebacterium*, since it suggested that α-lipoic acid possesses a function or functions in addition to its role in the formation of acetate from pyruvate.

Methods. The organism,* maintained on yeast extract-glucose-agar slants, was grown in a medium similar to that described by Stokstad, *et al.*(3). Instead of "protogen", a concentrate of lipoic acid was used; this material, potency 50,000 acetate equivalents/mg(4), gave suitably deficient cells at a level of 50 μg/liter or less. To permit aeration of the medium, 1.5 mg/liter of Dow-Corning Antifoam A were added. A 10% inoculum, 24 hours old, was used for 14 liter batches. After 24 hours at 25°C, the cells were harvested with a Sharples Super-Centrifuge, washed with 0.9% saline, suspended in water, lyophilized, and stored at −18°C. The experiments described below were performed with these dried, deficient cells, utilizing the usual manometric methods. The cells and crystalline α-lipoic acid† were mixed just before equilibrating the Warburg flasks.

Lipoic acid effects. A preliminary survey revealed that a variety of substances was oxidized by the dried cells and that the rate of oxygen uptake for many of these was stimulated by α-lipoic acid (Table I, Columns

* Our thanks are due to Dr. E. L. R. Stokstad for a culture of this organism.

† We are indebted to Dr. L. J. Reed for samples of α-lipoic acid.