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ESSENTIAL FATTY ACID STATUS OF CYSTIC FIBROSIS CHILDREN - EVALUATION AND TREATMENT

by

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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A B S T R A C T

Seven children with previously diagnosed cystic fibrosis, participated in a twenty-eight day study designed to evaluate the efficacy of two treatment methods on essential fatty acid status. Seven day food records were calculated for protein, fat, carbohydrates, linoleic acid and caloric content. Children were randomly assigned to either the topical or oral treatment group, where the amount of oil to be used was determined to be 1 or 2 percent of daily calories, respectively. Blood samples taken on day 1, 15 and 29, were analyzed for total plasma, plasma phospholipid and total red cell fatty acids, using gas-liquid chromatography. Children receiving enzyme replacement therapy had fatty acid patterns indicative of EFA deficiency; a significant depression in 18:2 (20 - 28%), and increased concentrations of 16:1 and 18:1. Little alteration occurred in 20:4. The non-essential fatty acid 20:3n9 was observed in all subjects and controls; the highest concentration was 2.3%. The oral treatment improved the fatty acid status of one

subject; 18:2 increased 20% in plasma phospholipids, 20:4 increased 8.5% in total plasma lipids. In the one subject who showed some response to topical treatment, the phospholipid concentration of 20:3n9 declined by 57%, while changes in other fatty acids (16:1, 18:1, 18:2) indicated an exacerbation of the deficiency. Children not receiving enzyme therapy had fatty acid patterns comparable to control values, and demonstrated little response to either form of treatment. The results showed that despite intakes of linoleic acid within recommended amounts (mean intake 4.7%), only children receiving enzyme therapy had fatty acid patterns suggestive of EFA deficiency. This further implies that EFA deficiency is secondary to malabsorption in cystic fibrosis.

The topical treatment was poorly accepted, and limiting in the amount of oil that could be used. Oral treatment was the preferred method and resulted in improvement in fatty acid status when compliance was assurred.

A C K N O W L E D G E M E N T S

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L I S T O F T A B L E S

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REVIEW OF LITERATURE

Originally considered to be an experimental condition inducible in rats, essential fatty acid (EFA) deficiency has been observed in humans with increasing frequency (Connor 1975). While a naturally occurring deficiency has not yet been reported, EFA deficiency can develop secondarily to dietary modification and surgical intervention (Prottey 1976). It has been reported in infants fed low-fat formulas, during prolonged intravenous hyperalimentation with a fat-free infusate (Collins et al 1971) and in cases of continual fecal loss of fat due to malabsorption (Rosenlund et al 1974). A deficiency in the adult may be difficult to induce, particularly if body stores of essential fatty acids are adequate. However, the onset may be fairly rapid when there is an increase in body catabolism. The ensuing complications from EFA deficiency are widespread, and without careful monitoring and treatment, can be fatal.

Elucidation of the function of essential fatty acids in human nutrition began with the observations of Burr and Burr, in 1929, that rats required unsaturated fats in the

diet for normal growth. From that point, numerous studies with animals and humans have extended the knowledge about the significance of essential fatty acids (Holman 1975). Of major importance, is the structural role which EFA plays in imparting desirable physical properties to membranes. Esterification of polyunsaturated fatty acids in the β-position of the phospholipid molecule, ensures the correct physiochemical properties for optimal functioning of the biomembranes. This vital role is essential for the integrity of all living animal tissue (Vergroesen et al 1975).

Trienoic fatty acids with chain lengths of 19, 20, 21 carbon atoms and with cis double bonds in positions 8, 11 and 14, are capable of acting as precursors for the synthesis of prostaglandins with high biological activity (van Dorp 1976). Only those fatty acids capable of this function are considered to have essential fatty acid status. Prostaglandins have many pharmacological properties; smooth muscle stimulation, constriction and dilation of blood vessels (Gurr and James 1975), and bronchial passages, (Friedman and Demers 1978) and an influence on blood platelet aggregation induced by ADP. This last effect

suggests the possibility of a regulatory function in the formation of arterial thrombi (Vergroesen et al 1975).

It is not surprising on the basis of the two major functions of essential fatty acids that clinical symptoms are extensive in a deficiency state. Essential fatty acid deficiency in man is characterized by dermatitis, growth failure, increased susceptibility to infection, increased permeability of the skin to water, and a decreased synthesis of prostaglandins (Vergroesen et al 1975) which have been implicated in the pathophysiology of pulmonary function (Friedman and Demers 1978), and in abnormal thrombocyte aggregation. However, prior to the onset of clinical signs of deficiency, abnormalities can be identified at a biochemical level. A characteristic series of changes in the fatty acid pattern of serum and tissues occurs in individuals with EFA deficiency. The concentration of the essential fatty acids, linoleic (18:2) and arachidonic acid (20:4) decrease, while there is a concurrent increase in the endogenous monoenoic fatty acids, palmitoleic (16:1) and oleic (18:1). The presence of a non-essential fatty acid 5, 8, 11 - eicosatrienoic acid (20:3n9) is considered indicative of a deficiency (Rivers and Hassam 1975). This

fatty acid arises from the desaturation of oleic acid, utilizing the same enzyme system as is required for the conversion of linoleic acid to arachidonic acid (Sprecher 1975). Linoleic acid acts to competitively inhibit the formation of 20:3n9. In a deficiency state, 20:3n9 is substituted for arachidonic acid in the phospholipid structure to maintain the required degree of unsaturation. This substitution however, does not allow for normal cell functioning. As the level of 20:4 decreases in EFA deficiency, the production of 20:3n9 increases. Holman (1960) described a triene : tetraene ratio (20:3n9 : 20:4) as a criterion to explain adequacy of EFA in the diet. A normal value of 0.4 or less indicates that the minimum requirement for 18:2 has been met, while in a deficiency state, the ratio may be elevated to a value of 5 to 6.

The actual requirement for essential fatty acids has been estimated from results of experimental work with animals and humans. It is often expressed as a percentage of calories and refers to an amount of linoleic acid (18:2). In man, this fatty acid can be converted to arachidonic acid (20:4) in vivo. Long-chain saturated triglycerides

increase the requirement for linoleic acid (Kaunitz et al 1960) as do medium chain triglycerides (Hirono et al 1977). In human subjects, 1% of total kilocalories was found to prevent dermal lesions in infants (Vergroesen et al 1975), while 4% was necessary for optimal caloric efficiency of infants (Adam et al 1958). When body catabolism is increased, an intake of 1% of kcals as linoleic acid may be inadequate for new tissue synthesis (Connor 1975). Vergroesen et al (1975) recommended a dietary linoleic acid intake of 13 - 16% of total kcals. At these levels, platelet aggregation was significantly decreased, coronary blood flow was increased and serum cholesterol levels were lowered. All of which may prove to be significant for patients with cardiovascular complications.

Because of the absence of a naturally occurring EFA deficiency in man, it is assumed that a mixed diet contains an adequate amount of linoleic acid. The concern therefore, is for the optimal intake necessary to correct or prevent EFA deficiency in those individuals considered to be at risk. Attention has recently been focused on cystic fibrosis patients in whom essential fatty acid deficiency has been routinely identified (Caren and Corbo 1966; Kuo et al 1962; Kuo and Huang 1965; Robinson 1975; Rosenlund et al 1974; Watts et al 1975).

Cystic fibrosis (CF) is a lethal hereditary disease of unknown etiology. Clinically, its main features include elevation of sweat electrolytes, involvement of the respiratory system and pancreatic insufficiency (Crozier 1974). Of concern nutritionally, is the presence and severity of the pancreatic insufficiency, which occurs in 80% of all patients resulting in steatorrhea and generalized malabsorption. These features either separately, or in combination with other factors, are being implicated in the etiology of the EFA deficiency in CF children.

While EFA deficiency has been observed primarily at the biochemical level in cystic fibrosis (i.e., a decrease in 18:2, 20:4; an increase in 16:1, 18:1) the concern is for the effects of a lifelong subclinical depletion and the possible consequence on the course of the CF. The exact etiology of EFA deficiency in cystic fibrosis has not been clearly defined, though most researchers agree that the intake of fat and the efficiency of absorption of each child are intimately involved in the onset of the deficiency. There is little support for the hypothesis of Rivers and Hassam (1975) that EFA deficiency may be the primary defect in cystic fibrosis.

Traditional diet therapy for CF patients involved restriction of fat intake to reduce persistent steatorrhea and its associated nutrient loss, particularly nitrogen. The validity of this practise was questioned by Chung et al (1951). These investigators were able to demonstrate that fat absorption improved in proportion to a three to four fold increase in fat intake. Although steatorrhea also increased, there was no change in the excretion of nitrogen. The restricted fat diet is still often used because of individual intolerance.

It is imperative that one understand the mechanism of effective absorption of fat, to comprehend the malabsorption syndrome of CF and the implications for EFA deficiency. Physical characteristics of the fatty acid molecule play an important role in absorption. Saturated fatty acids are less well absorbed than the unsaturated homologue, and the polyunsaturated molecule is more completely absorbed than the monounsaturated compound. With an increase in molecular weight, absorption decreases. The absorption of any one fatty acid can be affected by the fat with which it is simultaneously ingested (Fernandes et al 1962). From these observations, it has been concluded that the feeding of

unsaturated fatty acids would be beneficial to patients with steatorrhea. Similar findings were reported by Pinter et al (1964) in a study designed to evaluate the effectiveness of different fats on decreasing steatorrhea. Steatorrhea was described as "marked" for subjects receiving butter or glyceryl monostearate; "less severe" for those receiving cottonseed oil or glyceryl monolinoleate; and "much improved" to "no steatorrhea" for those receiving medium chain triglycerides (MCT). All subjects were given known amounts of fat (approximately 40% of kcals) based on an isocaloric substitution of previously established intakes.

Because of the relatively short fatty acid chain lengths in MCT (C8:0, C10:0), it appears to be absorbed intact and transported directly to the portal vein without prior hydrolysis. While the effectiveness in treating steatorrhea with MCT is well noted, the use of MCT results in fatty acid changes characteristic of essential fatty acid deficiency. Kuo and Huang (1965) observed changes in the fatty acid composition of plasma and depot fats after feeding MCT to CF children for a three to five month period, at amounts comparable to a normal fat intake. Prior to treatment, fatty

acid patterns were characteristic of endogenous lipogenesis which resulted from impaired fat digestion and absorption, i.e., increased proportions of saturated and monoenoic fatty acids of 16 and 18 carbons and depressed levels of linoleic and arachidonic acids. Feeding with MCT magnified these changes and produced sharp depressions of the polyenoic fatty acids. Despite the biochemical appearance of EFA deficiency, subjects did not demonstrate any clinical signs. Similar results have been reported by Hirono et al (1977) in infants fed MCT. Thus it can be seen that the type of fat ingested can affect the efficiency of absorption by the intestine.

The presence of fat together with pancreatic lipase in the small intestine, is not sufficient to ensure optimal absorption. The action of lipase itself can be altered by changes in the pH of the gut contents. Investigations related to the efficacy of pancreatic extracts, have shown that lipase is inactive at pH 4 or less. This is of major importance for patients with pancreatic insufficiency because the intragastric and intraduodenal pH have been reported as consistently less than 4, possibly due to reduced bicarbonate secretion (Di Magno et al 1977). The

routine use of enzyme supplementation for cystic fibrosis patients does not totally relieve steatorrhea. Graham's (1977) investigation of commercially available extracts demonstrated that a wide range of enzyme activity could be found in commercial preparations. Again, the persistently low gastric pH greatly reduced the effectiveness of all compounds.

After cleavage of the triglyceride by pancreatic lipase, the resulting monoglyceride and free fatty acids are absorbed into the mucosa in the form of a micelle, containing bile salts (Desnuelle 1973). It has been shown that excretion of bile acids in the feces of children with cystic fibrosis may be seven times greater than losses in normal control subjects. Excretion was substantially higher in those not taking pancreatic supplements (Weber et al 1973). While there is no explanation for this finding, it is postulated that the presence of unhydrolyzed fat and other nutrients may impair bile acid reabsorption. The concern over such continual losses is for depletion of the bile acid pool, thereby further compounding the problems of fat malabsorption because of intraluminal bile acid deficiency.

The long-term implications of a sub-clinical deficiency of essential fatty acids in CF are related to the functions of EFA, but at best, any discussion in this area is strictly speculative when based on animal and human studies where EFA deficiency has developed to life-threatening severity. Of particular interest, is the possible effect of an EFA deficiency on the severity of pulmonary complications. In essential fatty acid deficiency, the proportion of oleic acid to linoleic acid has been altered. Under these conditions, Campbell et al (1976) reported an interference in oxygen uptake by hemoglobin in erythrocyte membranes. They hypothesized that this could account for the digital clubbing observed in cystic fibrosis and for the severity of the respiratory problems. Investigations by Godinez and Longmore (1973) using perfused rat lung, demonstrated a direct correlation between the concentration of palmitic acid in the blood and the rate of surfactant formation. At a stable concentration of palmitic acid, an increase in oleic acid reduced the incorporation of the former into surfactant. A decrease (of approximately 30%) in palmitic acid concurrent with an increase in oleic acid (about 14%) has been observed in the lecithin fraction of mucus in CF children, and may

relate to severity of respiratory problems (Sanjurjo et al 1977). A respiratory distress syndrome has also been identified in EFA deficient chickens (Hopkins et al 1963).

To further substantiate the evidence implicating EFA deficiency in respiratory function, one must examine the metabolic behaviour of the prostaglandins. The lungs have been identified as a major site for the synthesis, release and degradation of prostaglandins. Prostaglandins of the E and F series exert potent physiological effects on smooth muscle of blood vessels and the tracheobronchial tree; the E series facilitates dilation while the F series facilitates constriction. Plasma samples analyzed by radioimmunoassay, showed significantly increased levels of both E and F series prostaglandins in newborns with respiratory distress syndrome (Friedman and Demers 1978). It is not known whether synthesis and release of the prostaglandins are enhanced during the acute phase of the disease or if there is merely an interference in the degradation by the lungs because of the disease. The elevated levels of both E and F series declined to normal values as the respiratory status of the children improved.

Prostaglandin synthesis is determined by the relative amounts of 20:4, 18:2 and 20:3n9 available. The non-essential

fatty acid 20:3n9 has been shown to be inhibitory to the synthesis of the E series, but can enhance synthesis of the F_{α} series (Dodge and Hamdi 1978). A study by Chase and Dupont (1978) revealed that levels of the $F_{2\alpha}$ prostaglandin were elevated above normal in children with cystic fibrosis who demonstrated biochemical signs of essential fatty acid deficiency. Correction of the EFA deficiency resulted in a decline in the $F_{2\alpha}$ levels towards normal values.

Results from investigations by Hubbard et al (1977) do not support the hypothesized involvement of essential fatty acid deficiency in respiratory status. They examined the fatty acid composition of plasma lipids in CF children with and without pancreatic insufficiency, and found the characteristic alterations only in those children with malabsorption requiring enzyme replacement therapy. However, progressive chronic pulmonary disease is found in CF children regardless of pancreatic function. These results suggest EFA deficiency is associated with pancreatic function, not respiratory status. The work of Galabert et al (1978) substantiates these observations. It is obvious that further research is required to clarify the relationship between

prostaglandins and EFA in the etiology of lung disease. Similarly, the extent to which the abnormal substitution of 20:3n9 in the phospholipids will affect membrane integrity, growth and infection, is not known and requires further investigation.

Intravenous administration of a soya oil emulsion (Intralipid) has been used successfully in the treatment of essential fatty acid deficiency in surgical patients. Collins et al (1971) found that administration of 22.5 grams of linoleic acid daily (6.4% of kcals) improved the 20:4 level of serum phospholipids and decreased the proportion of 20:3n9, suggesting a requirement of at least this amount. Fatty acid supplementation by regular intravenous infusions of Intralipid improved the fatty acid status of seven children with cystic fibrosis (Elliott 1976) though details of the lipid profiles were not given. The authors also claim improvement in the clinical course of CF; decreased sweat sodium concentrations, weight gain and improvement in pancreatic function (in two subjects). The small sample size plus the possibility of a placebo effect must be considered as influences on these findings.

Ideally, a treatment for EFA deficiency in CF should be simple, convenient and readily adaptable to the lifestyle of a child. Intravenous administration of fat does not equate with any of these criteria. While the effectiveness of Intralipid has been established, use should be restricted to the critical cases not responding to other therapeutic measures.

Press et al (1974) demonstrated successful treatment of essential fatty acid deficiency in adult men using cutaneously applied sunflower oil. Approximately 120 mg of linoleic acid (2-3 mg/kg/day) applied daily to one forearm, resulted in improvement in the fatty acid profiles of the serum lecithin fraction within one to two weeks. Similar results are reported in newborn infants, using 1-4 gm/kg/day of sunflower oil (approximately 0.9 gm/kg/day of 18:2) (Friedman et al 1976). It has been suggested that linoleic acid might be directly incorporated into circulating lipoproteins without first passing through the liver. This hypothesis would explain the greater efficiency observed using cutaneous oil versus one orally administered (Press et al 1974). In 1977, Rosenlund et al investigated the effects of orally

administered corn oil on the fatty acid status of cystic fibrosis children, using 1 gm/kg/day of oil over a one year period (approximately 0.5 gm/kg/day 18:2). Lipid profile data are given for the initial and final blood samples. After a one year period, the concentration of linoleic and arachidonic acids in the phospholipid fraction were shown to have risen by 106% and 96% respectively. These results suggest that oral administration of a source of EFA can be used to treat EFA deficiency in cystic fibrosis.

O B J E C T I V E S

The primary objectives of the study were:

1. to assess the fatty acid status of children with cystic fibrosis, particularly noting the presence of essential fatty acid deficiency
2. to assess the efficacy and acceptability of two treatment methods on the fatty acid status of the children in a free-living situation
3. to make recommendations regarding an acceptable method of treatment.

Approval

Approval was received by the University of Manitoba Faculty of Medicine Committee on the Use of Human Subjects in Research, and the Research Co-ordinating Sub-Committee of the Children's Centre, Winnipeg.

E X P E R I M E N T A L M E T H O D S

A. Subjects

A list of prospective subjects attending the Cystic Fibrosis Clinic was compiled by the Home Care Department, Children's Centre, Winnipeg, Manitoba. Inclusion in the study was determined by expected compliance, with no regard being given to the pancreatic function of the children involved. Families were contacted by letter with a follow-up telephone call. Those expressing an interest in the study were interviewed at the Clinic or in their homes, to ensure total comprehension of the project by both parents and children. Written parental consents were obtained (Appendix 1). Personal data of the subjects is summarized in Table 1.

B. Control Subjects

Siblings of the cystic fibrosis subjects were used as controls. Previous screening of this group ensured the absence of malabsorption problems. Three children agreed to participate in the study. Written parental consents were

Table 1

Personal Data of Subjects

Subject	Age Year - Month	Sex	Height Cm	Height Percentile	Weight Kg	Weight Percentile
DJ	5 yr - 6 m	M	111	50 - 75	17.6	25 - 50
SM	9 yr - 5 m	M	132	50 - 75	31.5	75 - 90
MF	9 yr - 8 m	M	126	10	24.7	10
DD	10 yr	F	137	50	26.5	3 - 10
DB	12 yr - 8 m	M	147	10 - 25	41.0	50 - 75
KD	14 yr - 6 m	F	151	3 - 10	35.4	3
RB	16 yr - 2 m	M	170	25 - 50	59.5	50 - 75

Controls	Age	Sex
CJ	4 yr	M
RF	6 yr	M
KM	7 yr	F

obtained (Appendix 2). Because of the limited sample group available, it was not possible to match for age and sex.

C. Food Records

Prior to commencing treatment, each subject (or parent, depending on age of subject) was required to keep a continuous seven-day record of all food and drink consumed. A detailed list of instructions (Appendix 3) was compiled to help subjects accurately record their intakes on the food intake sheets provided (Appendix 4). Subjects were asked to include daily intakes of vitamin and mineral supplements, and enzyme preparations. Total calorie, protein, fat, carbohydrate and linoleic acid content was calculated, using food composition tables and current data from the literature. [1] Approximations were made if the intake data were unclear.

1 J. Am. Dietet. Assoc. 66:482, 1975; 67:35, 111, 351, 1975; 68:224, 335, 1976; 69:44, 243, 517, 1976; 70:53, 1977; 71:412, 518, 1977; 72:48, 1978.

Adams, C.F., Nutritive Value of American Foods in Common Units. Agriculture Handbook No. 456. USDA, Washington, D.C., 1975.

D. Sunflower Oil

Commercially available sunflower oil (Safflo) [2] obtained from the processor, was used throughout the study.

(i) Fatty Acid Pattern

A 300 mg aliquot of oil was analyzed in duplicate for fatty acid composition by the method of Metcalfe et al (1966). Methyl esters were injected directly into the gas liquid chromotograph and resolved using conditions described elsewhere (Section G (i)). Results are shown in Table 2.

(ii) Determination of Oil For Use in Treatment

The amount of oil to be used was determined for each child as 2% of his/her average daily caloric intake, based on food intake data. The following equation illustrates the calculation used:

$$\text{Calorie Level} \times .02 \div 9 \times \frac{1}{b} \times \frac{1}{.95_c} \times \frac{1}{.71_d} = \text{ml of sunflower oil}$$

[2] Safflo, Gardenland Packers, Altona, Manitoba.

Table 2

Fatty Acid Analysis of Sunflower Oil [1]

Fatty Acid		% of Total Fatty Acids
Palmitic	C16:0 [2]	6.3
Stearic	C18:0	4.5
Oleic	C18:1	16.9
Linoleic	C18:2	70.7
Linolenic	C18:3	0.7
Arachidic	C20:0	0.3
Behenic	C22:0	0.5

1 As determined by gas-liquid chromatography.

2 Carbon number: number of double bonds.

For discussion regarding variability of fatty acid content of sunflower seeds, see Robertson (1972), Zimmerman and Fick (1975).

Where

a = 2% of total kcals

b = kcals/gm of fat

c = conversion factor for mg to ml

d = % of 18:2 in sunflower oil

E. Treatment

Subjects were arbitrarily assigned to one of two treatment groups, oral or topical, with one exception, two sisters, KD, DD were placed on the same treatment in the hope they would encourage each other to comply with the procedure. Duration of the study was twenty-eight days for both groups. Each subject was provided with a 30 cc disposable plastic syringe [3] for measuring the oil, one 24 ounce tin of sunflower oil and a standardized procedure sheet giving instructions for use of the oil, and times and dates of all blood collections (Appendix 5 and 6). More specifically, the topical group was instructed to rub the oil on their skin

3 Plasti-Pak Disposable Syringe #5662, Becton-Dickinson and Co., Mississauga, Ontario.

using their own hand. The oil could be used anywhere on the body. Dividing the quantity into several applications was permitted, providing the total volume of oil remained constant. The oral group was instructed to take the oil either in combination with other foods or alone. Again, division of the oil was permitted. Both groups were requested to record days in which the total allottment of oil was not used, with an explanation where possible. It was emphasized repeatedly that subjects should continue to eat and drink as usual, without changing their intake of vitamins, minerals or enzyme preparations.

F. Blood Collection

Fasting venous blood samples (10 ml) were drawn from all subjects on days 1, 15 and 29 of the study, with one exception. Subject DD did not allow the first blood sample to be taken. Control subjects provided only one 10 ml sample at the beginning of the study. Blood was drawn into two - 7 ml BD vacutainer tubes (#4759) containing EDTA, [4]

[4] Becton-Dickinson, Mississauga, Ontario.

and packed on ice until ready to process. Specimens were transferred into one 10 ml siliconized BD vacutainer (#4710) and centrifuged [5] at 4°C, at 1000 x g for fifteen minutes. Plasma was carefully removed and pipetted into two glass vials, flushed with nitrogen and frozen for later analysis. Extraction of lipid from the packed red cells was done immediately. The extract was then flushed with nitrogen and frozen for completion at a later date.

G. Blood Analysis

Plasma from both subjects and controls was analyzed by gas-liquid chromatography (GLC) for plasma phospholipid, and total plasma fatty acid content. Red cells were only analyzed for total fatty acids. Because the study involved children, the volume of blood drawn from each child was restricted to 10 ml. This did not allow for duplication if technical error occurred. Therefore, duplicates were randomly performed as a procedural check.

All analyses were performed without the use of antioxidants. Severe tailing of the solvent peak and

5 Model #B-20, Centrifuge, International Equipment Co., Needham Heights, Massachusetts.

interference in the resolution of myristic (C14:0) and palmitic (C16:0) acids were undesirable side effects. In preliminary experimentation, results were satisfactory if care was taken not to expose the sample to the air.

(i) Total Fatty Acid Pattern of Red Cells

Lipid was extracted from 1 ml of packed red cells using a chloroform - isopropanol [6] 7:11 (v/v) solution, as described by Rose and Oklander (1965). Centrifugation of the whole blood was modified to 1000 x g for fifteen minutes at 4°C. Cells were washed three times with two volumes of 0.89% NaCl [7] and centrifuged for ten minutes between washings. A vacuum pipette was employed to facilitate removal of saline and the buffy coat.

Saponification and methylation of the fatty acids were performed according to the method of Morrison and Smith (1964). Lipid extracts were evaporated to dryness under a stream of nitrogen and transferred to a 10 ml tube with a

6 Solvents, Glass distilled, Caledon Laboratories, Georgetown, Ontario.

7 NaCl, Reagent Grade, Fischer Scientific Co., Fairlawn, New York.

teflon-lined screw cap. One milliliter of boron fluoride-methanol (BF_3)[8] was added under nitrogen. The tube was tightly sealed and placed in a boiling water bath for thirty minutes. The sample was cooled and the tube opened. Esters were extracted using two volumes of pentane,[9] and one volume of distilled water. After shaking briefly and centrifuging[10] until both layers were clear, the top layer was removed and evaporated to a suitable volume in a conical vial.[11] Analyses were performed on a Varian Aerograph gas chromatograph (Model 1740-1),[12] equipped with dual columns, flame ionization detectors, a Varian Aerograph single pen recorder (Model 20)[12] and a Varian Aerograph digital integrator (Model 477).[12] Samples were resolved on a

8 Boron-fluoride Methanol, Eastman-Kodak, Rochester, New York.

9 Solvents: pentane, methanol, acetone. Reagent Grade (re-distilled for use). Fischer Scientific Co., Fairlawn, New York.

10 Model #CS, Centrifuge, International Equipment Co., Boston, Massachusetts.

11 Reacti-Vial #13221, Pierce, Rockford, Illinois.

12 Varian Aerograph, Malton, Ontario.

2.7 m x 3.2 mm stainless steel column packed with 10% EGSS-Y on 100/120 mesh GAS CHROM Q.[13] The flow rates were 30 ml/min for helium, 25 ml/min for hydrogen[14] and 250 ml/min for air. The column was operated isothermically at 190°C with the injector and detector temperatures at 230°C and 250°C respectively. Individual fatty acid methyl esters were identified by comparing them with linear log plots of carbon number versus retention time of reference standards.[15]

(ii) Plasma Phospholipid Fatty Acid Pattern

Total lipid was extracted from 1 ml of plasma by the method of Folch et al (1957). Phospholipids were precipitated in acetone according to Beare-Rogers (1969). Methyl esters of the fatty acids were prepared for analysis by the procedure of Morrison and Smith (1964), using 1 ml BF_3 and heating in a boiling water bath for thirty minutes. Methyl esters were

13 Applied Science Laboratories, Inc., State College, Pennsylvania.

14 Welder's Supplies, 25 McPhillips St., Winnipeg, Manitoba.

15 Hormel Institute, Lipids Preparation Lab., Austin, Minnesota.

extracted and concentrated as previously described (Section G (i)). Samples were injected directly into the GLC and allowed to separate for sixty minutes. The injection port temperature was increased to 240 - 245°C to allow for better resolution of the longer chain fatty acids.

(iii) Total Plasma Fatty Acid Pattern

Total lipid was extracted from 1 ml of plasma according to the method described by Folch et al (1957). Saponification and methylation were conducted by the method of Morrison and Smith (1964), using pentane in place of benzene in the reagent. Samples were heated in a boiling water bath for forty-five minutes. Extraction and concentration of methyl esters has been previously described (Section G (i)). Samples were injected directly into the GLC and allowed to separate for forty-five minutes under conditions described elsewhere (Section (G (ii)).

H. Statistical Analysis

The two-sample student's t-test was used to determine significant differences in fatty acid patterns between controls and CF children before treatment.

R E S U L T S

A. Subjects

Participation in the study was a result of expressed interest by the parents, in consultation with their children. At the time the project was conducted, no information had been published which documented the fatty acid pattern of CF children who were not receiving enzyme replacement therapy. It was therefore assumed that these children did not differ in essential fatty acid status from those receiving therapy, and no attempt was made to exclude them from the study.

The validity of using siblings of children with an inherited disease as normal controls can be questioned. The possibility of an unknown bias in this situation is acknowledged. However, it is believed that no alteration occurs in the gastrointestinal function of children who are carriers for cystic fibrosis.

B. Nutrient Intake Data

Table 3 shows the mean daily intake of each subject for protein, fat, carbohydrate, linoleic acid and kcals. Nutrients are also expressed as a percentage of caloric intake. Only five of seven subjects completed the food records satisfactorily. The remaining two found the task too difficult without parental assistance. Their records could not be accurately calculated and served only as a guide to approximate caloric intake.

The actual distribution of calories differs from the results of Bennett and Medwadowski (1967) who found intakes of CF children to be comprised of 18% protein, 28% fat and 55% carbohydrate. The higher intake of fat found in the present study may reflect the more liberal diet therapy of recent years, and is approaching the average Canadian intake of 12% protein, 41% fat and 47% carbohydrate (Health and Welfare, 1976). It is interesting to note that although fat intake was low in these subjects, the linoleic acid content of their diet was within the suggested requirement of 1 - 4% of kcals. The mean caloric intake for each child is expressed as a percentage

Table 3

Daily Nutrient Intake of Each Subject [1]

Subject	Caloric Level		Protein		Fat		Carbohydrate		Linoleic Acid	
	Cals	% CDS [2]	Gm	% of Cals	Gm	% of Cals	Gm	% of Cals	Gm	% of Cals
DJ	1807 ± 359	100	78.9 ± 26.1	17.5	50.9 ± 17.8	25.4	258.0 ± 61.1	57.2	11.3 ± 8.3	4.2
SM	2058 ± 341	93	62.4 ± 17.1	12.1	79.1 ± 26.8	34.6	273.0 ± 30.3	53.1	9.9 ± 5.4	4.3
MF	2733 ± 289	119	92.8 ± 14.1	13.6	102.4 ± 16.3	33.7	360.0 ± 72.3	52.7	8.8 ± 4.7	2.9
DB	2021 ± 555	81	87.9 ± 19.2	17.4	89.3 ± 27.1	39.8	216.4 ± 76.9	42.8	15.5 ± 7.1	6.9
RB	2783 ± 735	87	122.2 ± 35.5	17.6	119.7 ± 39.6	38.7	304.2 ± 70.7	43.7	15.9 ± 6.9	5.1

1 Results are expressed as mean ± S.D.
2 Canadian Dietary Standard (1974).

of the recommendation for age as stated in the Canadian Dietary Standard (1974). All children received at least 80% of the recommended caloric intake. While this appears satisfactory, the recommendations are stated for healthy children. The actual caloric requirement for children with CF is not known. If malabsorption is present, requirements may be increased 25 - 50% above normal (Holvey 1972). Table 1 documents the height and weight percentiles for all subjects. Four children fall below the fiftieth percentile in height and/or weight categories. Sproul and Huang (1964) reported growth patterns in fifty children with CF and found that the median height/weight never exceeded the normal tenth percentile. No correlation was seen in their results between pancreatic deficiency and the degree of growth retardation, though excellent correlation existed between the severity of respiratory disease and growth. Such a wide discrepancy of findings between the present study and that in 1964, suggests that the subjects in this study may have less severe respiratory disease or a more adequate nutritional intake.

The total nutritional adequacy of the subjects' diets was not determined. The food records were intended to be used only to calculate linoleic acid requirements. The amount of oil and linoleic acid used by each subject is shown in Table 4. Details of the supplements taken by each subject, including vitamins, minerals and enzyme preparations, are shown in Appendix 7.

C. Acceptability of Treatment

Children receiving the sunflower oil orally accepted the treatment satisfactorily. The oil was usually taken in one dose with meals, though two children drank it in tea. More problems were apparent with the topical use of oil. With the first subject to start this treatment (SM), it was obvious that 7 ml of oil (corresponding to 2% of kcals) was too large an amount to be rubbed into the skin. Therefore, the oil was reduced to 1% for the remaining two weeks of the study for SM. The smaller amount was used for all subsequent participants in the topical treatment group. However, the topical treatment was still not well accepted.

Table 4

Amount of Sunflower Oil and Linoleic Acid

Used by Each Subject

Subject	Treatment [1]	Oil (ml/day)	Linoleic Acid (gm/day)	Linoleic Acid (mg/kg/day)
DJ	0	6	4.05	230
SM	T	7-3.5 [2]	4.72-2.36	150-75
MF	T	4.5	3.04	123
DD	0	8	5.4	204
DB	T	3.5	2.36	58
KD	0	8	5.4	153
RB	0	9	6.07	102

1

T = Topical
0 = Oral

2

SM used 7 ml for two weeks. This was reduced to 3.5 ml for remaining period.

Complaints were expressed regarding the messiness and inconvenience of the procedure. Family encouragement and assistance was essential. It is impossible to estimate the amount of oil absorbed by bedding, clothing or lost by the washing of hands. Subject DB secretly withdrew from the topical treatment sometime after the third week, complaining of wheezing and diarrhea.

D. Evaluation of Essential Fatty Acid Status Prior to Treatment

Preliminary examination of the fatty acid patterns of the blood samples taken on day 1, showed a difference in results between children who received enzyme replacement therapy, and those who did not. This finding necessitated the division of the subjects into two categories, Group A: children receiving enzyme therapy; Group B: children not receiving enzyme therapy. This distinction will be used in all discussions regarding the fatty acid patterns of CF children. The possibility of some degree of pancreatic insufficiency and malabsorption occurring in the Group B subjects is recognized. However, in the

absence of fat balance data, enzyme replacement therapy provides a simplistic method of categorization.

Identification of 5, 8, 11 - eicosatrienoic acid (20:3n9) is considered mandatory for verification of essential fatty acid deficiency. The problems encountered in the resolution and identification of this fatty acid have been noted by other investigators (Watts et al 1975). Gas chromatography of the methyl esters in this study, showed the presence of three peaks with equivalent chain lengths, using the "carbon-number" system for identification (Woodford 1960). Using reference standards, the second and third peaks were identified as 20:3n6 (di-homo-γ-linolenic) and 20:4n6 (arachidonic acid). In the absence of a standard for the 20:3n9, the first peak was tentatively identified as the 5, 8, 11 - eicosatrienoic acid, based on the findings of Collins et al (1971). Because of extremely small amounts of 20:3n9, it was difficult to determine the exact percentage present in any sample.

The fatty acid composition of total plasma lipids, plasma phospholipids and red cells for children with

cystic fibrosis (before treatment with oil) are shown in Tables 5, 6 and 7, respectively. In each table, data are expressed as a percentage of total fatty acids, and are tabulated with data from controls for comparative purposes. Unless otherwise noted, all values represent single determinations. For subjects in Group A, the linoleic acid (18:2) content was significantly lower in all three fractions when compared with controls. The decreases ranged from 29% in the total plasma lipids to 20% in phospholipids and 25% in red cells. Elevation of the monoenoic fatty acids (palmitoleic 16:1, oleic 18:1), was again noted in all fractions, but was significantly elevated only in the phospholipids. No decrease was observed in arachidonic acid (20:4). The content of myristic acid (14:0) in Group A was significantly elevated only in total plasma lipids. Little is known regarding the effect of EFA deficiency on this fatty acid since it is poorly resolved when BHT is used as an antioxidant. The differences obtained in the present study may be due to subject variability. In Group B subjects, no significant alterations were observed in any of the fatty acid

Table 5

Fatty Acid Composition (%) [1] of Total Plasma Lipids in Children With Cystic Fibrosis Before Treatment With Sunflower Oil

Fatty Acid	Control Group (n = 3)	A [2] (n = 3)	B [3] (n = 3)	Significance
14:0	1.2 ± 0.4	1.8 ± 0.3	1.6 ± 0.2	Control vs A**
16:0	21.2 ± 1.2	22.5 ± 1.2	21.4 ± 0.8	
16:1	2.4 ± 0.4	5.1 ± 2.0	2.4 ± 0.4	
18:0	8.1 ± 0.6	7.8 ± 1.0	7.8 ± 0.5	
18:1	24.9 ± 0.8	27.5 ± 2.2	25.3 ± 3.9	
18:2	33.7 ± 0.6	24.2 ± 4.6	32.8 ± 4.3	Control vs A**
20:3n9	N.D. [4]	0.5 ± 0.2	0.1 ± 0.1	Control vs A***
20:3n6	1.2 ± 0.1	1.8 ± 0.4	1.3 ± 0.2	Control vs A*
20:4	6.2 ± 0.8	5.8 ± 1.5	5.9 ± 0.6	
20:3n9 / 20:4	-	.09	.02	

[1] Results are expressed as a percentage of total fatty acids ($\bar{x} \pm$ S.D.)
[2] A: Children receiving enzyme replacement therapy.
[3] B: Children not receiving enzyme replacement therapy.
[4] Not detectable.

* Significance $p \leq 0.05$
** Significance $p \leq 0.025$
*** Significance $p \leq 0.01$

Table 6

Fatty Acid Composition (%) [1] of Plasma Phospholipids in Children With Cystic Fibrosis Before Treatment With Sunflower Oil

Fatty Acid	Control Group [4]	A [2]	B [3, 4]	Significance
	(n = 3)	(n = 3)	(n = 3)	
14:0	0.9 ± 0.2	1.4 ± 0.7	0.9 ± 0.2	
16:0	28.2 ± 1.3	29.6 ± 1.3	29.4 ± 0.9	
16:1	1.1 ± 0.4	2.1 ± 0.7	0.5 ± 0.6	Control vs A*
18:0	16.7 ± 1.0	15.4 ± 1.1	16.0 ± 0.4	
18:1	15.1 ± 0.6	17.4 ± 1.4	14.7 ± 2.0	Control vs A*
18:2	24.7 ± 1.8	19.7 ± 3.3	25.9 ± 4.0	Control vs A*
20:3n9	0.3 ± 0.1	1.2 ± 1.1	0.3 ± 0.2	
20:3n6	2.6 ± 0.4	3.5 ± 0.1	2.6 ± 0.8	Control vs A**
20:4	8.6 ± 0.6	8.1 ± 0.4	8.7 ± 0.4	
$\frac{20:3n9}{20:4}$	.03	.15	.03	

[1] Results are expressed as a percentage of total fatty acids ($\bar{x} \pm$ S.D.)
[2] A: Children receiving enzyme replacement therapy.
[3] B: Children not receiving enzyme replacement therapy.
[4] Represents mean of duplicate analysis for one subject in this group.

* Significance $p \leq .05$
** Significance $p \leq .025$

Table 7

Fatty Acid Composition (%) [1] of Total Red Cell Lipid in Children With Cystic Fibrosis Before Treatment With Sunflower Oil

Fatty Acid	Control Group (n = 3)	A [2] (n = 3)	B [3] (n = 3)	Significance
14:0	1.4 ± 0.1	1.7 ± 0.6	1.4 ± 0.2	
16:0 [4]	27.4 ± 0.9	29.8 ± 2.1	27.0 ± 0.7	
18:0	19.4 ± 0.7	18.1 ± 1.0	18.8 ± 0.3	
18:1	18.3 ± 1.7	19.7 ± 0.3	19.5 ± 0.4	
18:2	13.6 ± 0.7	10.2 ± 1.4	13.5 ± 0.7	Control vs A***
20:3n6 [5]	1.5 ± 0.6	1.7 ± 0.3	1.5 ± 0.7	
20:4	17.6 ± 1.2	17.8 ± 0.7	17.4 ± 0.7	

[1] Results are expressed as a percentage of total fatty acids ($\bar{x} \pm$ S.D.)
[2] A: Children receiving enzyme replacement therapy.
[3] B: Children not receiving enzyme replacement therapy.
[4] Includes 16:1.
[5] Includes 20:3n9.

*** Significance $p \leq .010$.

patterns. All results were comparable to control values.

Of particular interest, was the presence of 20:3n9 found not only in Group A and B subjects, but also in the phospholipid fraction of the control children. This observation supports the work of Watts et al (1975) who noted 20:3n9 in the phospholipids of healthy young children. Since this fatty acid is preferentially retained by the phospholipids, the higher value observed in this fraction of Group A children was expected. However, 20:3n9 was not present in sufficient quantities to significantly alter the triene: tetraene ratio of either group. Individually, MF had the highest ratio (0.28) in the plasma phospholipids. This value is still below 0.4, suggesting that the minimum dietary requirement for linoleic acid has been met (Holman 1960). In children from Group A, an increased concentration of 20:3n6 was observed in all fractions, and was significant in both total plasma and phospholipid fractions.

E. Effect of Treatment on Fatty Acid Status

The variation observed in the fatty acid patterns of Group A and Group B subjects, necessitated grouping the children in order to discuss results of the treatments. The imbalanced distribution of subjects illustrated in the following figure, is due to the random placement of the children without regard to pancreatic function.

Treatment

Enzyme Therapy	Oral	Topical
Group A: Receiving Therapy	DJ, KD, DD	MF
Group B: Not Receiving Therapy	RB	DB, SM

(i) Group A Subjects

Because the blood sample taken on day 1 was suggestive of EFA deficiency, it was anticipated that definite changes would occur in these subjects with either form of treatment. The response of total plasma, plasma phospholipids and total red cell fatty acids to oral administration of sunflower oil (subjects DJ, KD, DD), is shown in Tables 8, 9 and 10, respectively. All results are expressed as a percentage of total fatty acids. Each subject will be discussed individually because of the wide variations noted in the response of each child to the same treatment.

Subject DJ showed increased concentrations of linoleic (18:2) and arachidonic acids (20:4) in all three fractions by the end of the twenty-eight day period. Of the monoenoic fatty acids, palmitoleic (16:1) decreased, while little change was seen in oleic acid (18:1). The variations that occurred in the 20:3n9 could be due to technical problems of analysis. The response of KD was not as remarkable. Linoleic acid increased in the red cell fraction and decreased in the plasma fatty acids and phospholipids. The initial increase in 20:4 seen by

Table 8

Total Plasma Fatty Acids (%) [1] of Subjects [2] DJ, KD, DD in Response to Oral Administration of Sunflower Oil

	Days From Start of Treatment							
	DJ			KD			DD	
Fatty Acid	1	15	29	1	15	29	15	29
14:0	1.5	1.8	1.3	1.9	2.7	1.9	2.0	2.0
16:0	21.3	20.9	19.8	23.6	25.9	23.7	23.2	24.1
16:1	2.8	3.0	N.D. [3]	6.4	7.5	6.1	6.1	6.1
18:0	8.1	7.7	8.2	6.7	8.1	7.9	7.8	7.1
18:1	25.0	24.6	25.7	29.0	29.2	29.3	25.5	26.8
18:2	28.8	31.2	34.3	24.0	17.2	19.8	22.8	20.4
20:3n9	0.4	0.3	0.2	0.4	0.4	0.7	0.2	0.3
20:3n6	2.1	1.8	1.7	1.3	1.2	1.8	1.4	1.4
20:4	7.1	6.5	7.7	4.2	3.9	6.9	9.1	9.8

1 Results are expressed as a percentage of total fatty acids.
2 Group A subjects: received enzyme replacement therapy.
3 Not detectable.

Table 9

Plasma Phospholipid Fatty Acids (%) [1] of Subjects [2] DJ, KD, DD in Response to Oral Administration of Sunflower Oil

	Days From Start of Treatment							
	DJ			KD			DD	
Fatty Acid	1	15	29	1	15	29	15	29
14:0	0.9	1.1	0.8	1.0	1.0	1.4	0.9	0.8
16:0	30.4	26.7	25.5	30.4	31.0	32.4	30.9	31.8
16:1	1.3	1.4	1.0	2.3	2.5	2.1	2.1	1.7
18:0	16.2	16.1	15.9	14.1	16.5	16.5	16.0	15.9
18:1	16.4	15.8	17.2	16.8	18.2	18.2	14.8	15.7
18:2	21.6	24.5	26.0	21.6	16.8	17.5	17.5	17.2
20:3n9	0.2	0.5	0.5	1.0	0.9	1.0	0.6	0.6
20:3n6	3.4	3.5	2.9	3.6	3.3	2.6	3.2	3.2
20:4	8.4	8.8	8.6	7.7	8.2	7.3	13.6	12.4

1 Results are expressed as a percentage of total fatty acids.
2 Group A subjects.

Table 10

Total Red Cell Fatty Acids (%) [1] of Subjects [2] DJ, KD, DD in Response to Oral Administration of Sunflower Oil

	Days From Start of Treatment							
	DJ			KD			DD	
Fatty Acid	1	15	29	1	15	29	15	29
14:0 [3]	1.1	1.3	1.7	2.3	1.9	1.7	1.6	1.7
16:0	28.0	27.5	28.7	32.1	29.3	28.9	30.3	29.7
18:0	18.2	18.4	18.4	17.1	17.1	17.6	17.2	17.5
18:1	19.9	19.3	19.0	19.9	21.0	20.5	19.4	18.3
18:2	11.8	12.8	12.8	9.4	9.9	11.3	8.7	8.5
20:3n6 [4]	1.7	1.8	1.6	1.5	1.7	1.5	1.3	2.0
20:4	17.4	17.9	17.6	17.5	18.7	18.1	21.2	21.6

1 Results are expressed as a percentage of total fatty acids.
2 Group A subjects.
3 Includes 16:1.
4 Includes 20:3n9.

day 15 in both phospholipids and red cells decreased slightly by day 29. The monoenoic fatty acids demonstrated variable responses; 16:1 decreased while 18:1 increased in the phospholipid and red cells. Total plasma fatty acids remained unchanged. The third subject DD, did not provide a blood sample on day 1, although she claimed to have participated in the treatment from the beginning of the study. Results are presented for days 15 to 29. In general, the 18:2 content was relatively constant in all fractions, with the largest change occurring in the plasma fatty acids. Arachidonic acid also remained fairly stable. The monoenoic fatty acids showed an inconsistent response.

Of the three children taking sunflower oil orally, a substantial improvement in fatty acid status was observed only in DJ. Compliance may be an important factor. Family encouragement and assistance was given to DJ, while DD and KD (sisters) obtained little support.

MF was the only child taking enzyme supplementation who participated in the topical treatment group. The response of plasma, phospholipid and red cell fatty acids

to topical administration of sunflower oil is shown in Table 11. The highest percentage of 20:3n9 of all subjects was seen in the phospholipid fraction of MF. After twenty-eight days of treatment, a 57% decrease was noted in 20:3n9. A similar but less pronounced decline was observed in the total plasma fraction. Despite this significant alteration in the 20:3n9, the monoenoic fatty acids 16:1, 18:1 increased in all fractions. Although the increase was small (3 - 18%), it is contrary to an improvement in fatty acid status. No dramatic change was seen in the concentrations of 18:2. In both the plasma phospholipid and total plasma fatty acids, 18:2 peaked by day 15, but declined to a value lower than the initial day 1 sample. A similar response was observed for arachidonic acid.

All four children in Group A were receiving enzyme replacement therapy throughout the study, yet the response to both forms of treatment was diverse. Despite the use of 50% less oil for the topical treatment, MF demonstrated a significant improvement as shown by the decline in concentration of 20:3n9. Changes in other fatty acids

Table 11

Total Plasma, Phospholipid and Red Cell Fatty Acids (%) [1] of Subject [2]

MF in Response to Topical Administration of Sunflower Oil

Days From Start of Therapy

Fatty Acid	Total Plasma			Plasma Phospholipid [3]			Total Red Cell		
	1	15	29	1	15	29	1	15	29
14:0	2.0	1.9	2.5	2.2	1.4	0.9	1.7	2.2	2.1
16:0	22.5	24.0	26.1	28.1	29.2	29.0	29.4	30.6	30.3
16:1	6.0	5.7	7.1	2.7	2.4	2.8	N.D. [4]	N.D.	N.D.
18:0	8.6	8.1	8.3	15.9	16.2	16.8	19.0	17.9	18.2
18:1	28.4	28.2	31.2	19.0	17.7	20.3	19.4	20.0	19.6
18:2	19.7	21.9	16.1	15.9	18.1	15.5	9.4	8.9	8.8
20:3n9	0.7	0.6	0.3	2.3	1.0	1.2	N.D.	N.D.	N.D.
20:3n6	1.9	1.6	1.2	3.4	3.6	3.0	2.0	1.6	1.7
20:4	6.1	6.2	5.2	8.1	9.0	8.7	18.6	18.0	17.6

1 Results are expressed as a percentage of total fatty acids.
2 Group A subject.
3 Represents mean of duplicate analysis.
4 Not detectable.

were indicative of an exacerbation of EFA deficiency. In the oral treatment group, DJ showed the most pronounced improvement. Subjects KD and DD did not respond to therapy.

(ii) Group B Subjects

The response of total plasma, plasma phospholipid and total red cell fatty acids to oral administration of sunflower oil is shown in Table 12. The only subject in this treatment group was RB. Treatment resulted in a further increase in the total plasma and plasma phospholipid concentrations of 18:2, despite the level seen in day 1. Little change was noted in 16:1, 18:1, 20:3n9 or 20:4, except for a slight increase (12%) in the red cell concentration of 20:4. The fatty acid status of RB appeared to be optimal initially, a lack of response to the treatment was therefore expected. The response of subjects SM and DB is averaged due to their similarity. Table 13 illustrates the response to topical treatment of these subjects. Very little alteration occurred in the concentration of 18:2 for any fraction. Arachidonic acid showed a slight overall increase after

Table 12

Total Plasma, Phospholipid and Red Cell Fatty Acids (%) [1] of Subject RB [2] in Response to Oral Administration of Sunflower Oil

Days From Start of Therapy

Fatty Acid	Total Plasma			Plasma Phospholipid			Total Red Cell		
	1	15	29	1	15	29	1	15	29
14:0	1.7	1.8	1.5	1.0	1.0	0.6	1.2	1.5	1.5
16:0	22.2	20.2	20.5	28.4	27.6	27.1	26.4	26.7	26.6
16:1	2.0	2.1	1.9	0.5	0.8	0.9	N.D.	N.D.	N.D.
18:0	8.3	7.2	8.6	16.0	15.1	16.3	18.9	18.4	18.0
18:1	25.1	23.4	25.1	13.7	13.0	14.3	19.0	19.1	18.0
18:2	32.7	36.5	34.8	28.7	28.9	29.0	14.2	14.2	13.4
20:3n9	N.D. [3]	0.2	N.D.	0.4	0.5	0.4	N.D.	N.D.	N.D.
20:3n6	1.1	1.1	0.7	1.9	2.6	1.8	1.4	1.0	1.5
20:4	5.3	5.3	5.1	8.4	8.8	7.9	18.1	18.6	20.4

1 Results are expressed as a percentage of total fatty acids.
2 Group B subjects: did not receive enzyme replacement therapy.
3 Not detectable.

Table 13

Total Plasma, Phospholipid and Red Cell Fatty Acids (%) [1] of Subjects [2]

SM, DB in Response to Topical Administration of Sunflower Oil

Days From Start of Therapy

Fatty Acid	Total Plasma			Plasma Phospholipid			Total Red Cell		
	1	15	29	1	15	29	1	15	29
14:0	1.5 ± 0.3 [3]	1.5 ± 0.1	1.9 ± 0.9	0.9 ± 0.3	0.9 ± 0.4	0.7 ± 0.4	1.5 ± 0.1	1.5 ± 0.4	1.4 ± .07
16:0	21.1 ± 0.6	19.5 ± 0.8	19.8 ± 0.3	29.9 ± 0.1	26.3 ± 0.5	25.9 ± 0.7	27.3 ± 0.6	26.7 ± 1.1	26.7 ± 0.1
16:1	2.6 ± 0.3	2.4 ± 0.5	2.2 ± 0.1	0.6 ± 0.8	0.8 ± 0.3	0.9 ± 0.0	N.D. [4]	N.D.	N.D.
18:0	7.6 ± 0.4	7.7 ± 0.4	8.0 ± 0.6	16.5 ± 0.5	16.5 ± 0.2	16.7 ± 0.6	18.7 ± 0.4	18.5 ± 0.8	19.3 ± 0.6
18:1	25.4 ± 5.5	28.0 ± 3.4	25.1 ± 1.4	15.2 ± 2.6	16.4 ± 1.0	15.2 ± 0.9	19.8 ± .07	19.4 ± .07	19.6 ± 0.7
18:2	32.9 ± 6.0	31.8 ± 4.3	32.7 ± 3.5	24.5 ± 4.5	26.3 ± 2.1	25.6 ± 2.5	13.1 ± 0.3	13.6 ± 0.5	13.9 ± 0.1
20:3n9	0.2 ± .07	0.2 ± 0.2	0.2 ± .07	0.2 ± 0.3	0.3 ± 0.0	0.4 ± .07	N.D.	N.D.	N.D.
20:3n6	1.4 ± .07	1.2 ± 0.2	1.3 ± 0.1	2.9 ± 0.7	2.4 ± 0.4	2.7 ± 0.3	1.6 ± 0.9	1.6 ± 0.6	1.3 ± 0.4
20:4	6.3 ± .07	5.7 ± 0.2	6.6 ± 0.7	8.9 ± 0.5	9.0 ± 0.3	9.8 ± 0.6	17.1 ± 0.4	17.7 ± 1.6	17.2 ± 0.4

1 Results are expressed as a percentage of total fatty acids.
2 Group B subjects.
3 Mean ± S.D.
4 Not detectable.

twenty-eight days of treatment. The monoenoic fatty acids remained fairly constant throughout the treatment period, as did the concentration of 20:3n9.

A similar response was observed in the fatty acid patterns of Group B children, regardless of the treatment. The slight changes seen may be attributed to the initial fatty acid status of each child, rather than the treatment.

D I S C U S S I O N

In the absence of clinical signs of essential fatty acid deficiency, identification of the condition is based on the appearance of characteristic alterations in the fatty acid patterns of blood and tissue lipids. Normal healthy subjects generally provide control data because of the absence of standardized normal values. In the present study, the children who served as controls were siblings of the CF subjects and therefore, possible heterozygotes. Despite their genetic make-up, fatty acid values obtained from the three controls were within range of values reported elsewhere (Chase and Dupont 1978; Watts et al 1975) with the exception of lowered concentrations of arachidonic acid. Hubbard et al (1977) also reported the fatty acid profile of obligate heterozygotes to be comparable to normal controls. Therefore, use of siblings as control subjects may not unduly prejudice the results, while providing a convenient sample pool that may have eating patterns similar to the subjects.

Analysis of the initial blood sample from children who received enzyme therapy, revealed a significant decrease (20 - 28%) in the linoleic acid content of all lipid fractions. However, other investigators have reported greater decreases of 41% (Hubbard et al 1977) 48%, (Caren and Corbo 1966), and 50% (Rosenlund et al 1974) in the 18:2 content of phospholipids in CF children. In extreme cases of clinically defined EFA deficiency, linoleic acid may be barely detectable. Collins et al (1971) observed a 92% reduction in 18:2 in one subject who developed EFA deficiency secondary to intestinal resection. Despite the dramatic changes observed in linoleic acid, surprisingly little variation occurred in the phospholipid or total plasma fatty acid concentration of arachidonic acid. The present study supports this observation. It has been suggested that only in a severe EFA deficiency are changes observed in the 20:4 content of these fractions (Hubbard et al 1977). Other lipid fractions appear to reflect a more rapid change in this fatty acid. Collins et al (1971) and Caren and Corbo (1966) both noted the greatest change of arachidonic acid in the triglyceride fraction.

The appearance of other parameters of EFA deficiency, notably the increased concentrations of palmitoleic and oleic acids, confirms the results of other researchers (Rosenlund et al 1974; Kuo and Huang 1965; Chase and Dupont 1978). However, as with the polyenoic fatty acids the magnitude of change was not as great in the present study as that reported elsewhere.

In the absence of an exogenous source of linoleic acid, the body will convert oleic acid to 5, 8, 11 - eicosatrienoic acid (20:3n9) in an attempt to maintain the required degree of unsaturation (Sprecher 1975). This non-essential fatty acid is considered to be a prime indicator of essential fatty acid deficiency. Eicosatrienoic acid was observed in the phospholipid and total plasma fatty acid fractions on all subjects and controls with a maximum concentration of 2.3% (Subject MF). Collins et al (1971) have reported levels up to 14.2% in the phospholipid fraction of EFA deficient adults. The concentration of 20:3n9 was not sufficient to significantly alter the triene: tetraene ratio. In other investigations of EFA deficiency in CF, the triene: tetraene ratio in serum phospholipids was also within normal limits (Galabert et al 1978, Rosenlund

et al 1977; Watts et al 1975).

It is interesting to note that 20:3n9 arises from the desaturation of oleic acid, using the same enzyme required to produce 20:4n6 from 18:2n6. The presence of linoleic acid is inhibitory to the synthesis of 20:3n9 (Sprecher 1975). Investigations of inter-conversion rates of fatty acids demonstrates a rapid conversion of 18:2 into 20:3n6, yet a much slower conversion of 20:3n6 into 20:4n6. If one considers the requirement for polyunsaturated fatty acids by the cell during essential fatty acid deficiency, it can be hypothesized that an increased concentration of 20:3n6 (like that of 20:3n9), is an attempt to maintain the required degree of unsaturation. In fact, the present study showed an increased concentration of 20:3n6 in Group A children prior to treatment. Values for the triene : tetraene ratio are elevated above 0.4 if both the n6 and n9 eicosatrienoic acids are used in the ratio. The results of Hubbard et al (1977) correspond to these findings.

Identification of 20:3n9 in normal subjects is confirmed by other researchers (Watts et al 1975; Hubbard

et al 1977), and further suggests that 20:3n9 can be isolated in the plasma phospholipid fraction of all EFA deficient children if specific analytical methods are used. The relatively low broad peaks of carbon-twenty fatty acids and the small concentration of 20:3n9, make it difficult to identify on the chromatograph (Watts et al 1975).

Only children in Group A (requiring enzyme therapy), demonstrated the fatty acid pattern indicative of EFA deficiency. Children in Group B (not receiving enzyme therapy), had fatty acid patterns comparable to normal controls. In the absence of fat balance data for these children, no information is available about their pancreatic function. However, Galabert et al (1978) documented fatty acid patterns of CF children along with fat absorption levels. As fat absorption decreased, concentrations of 18:2 and 20:4 also decreased while 20:3n9 and 18:1 increased. These results support the findings of Hubbard et al (1977) and suggest essential fatty acid deficiency in CF is a secondary complication of pancreatic insufficiency and malabsorption. It seems

logical therefore, to either increase the oral intake of essential fatty acids (assuming the normal intake is insufficient to compensate for an increase in loss or dietary requirement), or bypass the enteral route and consider cutaneous absorption as treatment for the deficiency. Regardless of the treatment employed, the optimal amount required for supplementation is unknown. Rosenlund et al (1977) treated subjects with corn oil using body weight to establish the dose, while other researchers have used caloric intake for this purpose. The net result is considerable variation in the quantity of oil used, making treatment comparisons difficult. The requirement for essential fatty acids has been generally accepted as 1 - 4% of kcals per day. Since CF children tend to be underweight despite normal caloric intakes (Sproul and Huang 1964), kcals may be more appropriate than body weight for determination of treatment doses.

In the present study, subjects received 1 or 2% of kcals as linoleic acid in addition to calculated dietary intakes of 18:2 of an average of 4% of kcals.

Yet despite a total 5 - 6% of kcals as essential fatty acids, treatment failed to produce a significant response in fatty acid status of all subjects.

Oral administration of sunflower oil for twenty-eight days resulted in an improvement in fatty acid status of only one subject (DJ). He received 6 mls of oil daily, corresponding to 230 mg/kg/day of linoleic acid. The most pronounced increases in 18:2 and 20:4 occurred in the phospholipids (20%) and total plasma fatty acids (8.5%) respectively. Using 1 gm/kg/day of corn oil (approximately 500 mg/kg/day of 18:2) over a one year period, Rosenlund et al (1977) reported an increase of 106% in 18:2 and 96% in 20:4 content of serum phospholipids. However, in contrast to the present study, children participating in that trial had lower blood lipid values prior to treatment. They received twice the amount of 18:2 over a considerably longer treatment period. The response after one month of treatment would be of interest.

In the topical treatment group, MF (the only subject receiving enzyme therapy), demonstrated a significant decrease in the concentration of 20:3n9 after twenty-eight days of treatment when 4.5 ml or oil (123 mg/kg/day of 18:2) was

used. Conversely, the increase in 16:1, 18:1 and decrease in 18:2, implied an exacerbation of EFA deficiency with treatment. The increased concentration of 18:2 and 20:4 seen on day 15 was suggestive of an improvement in fatty acid status. However, these fatty acids declined to below the baseline value by day 29. A similar, but reverse response, was noted for 16:1, 18:1, i.e., an initial decline, followed by an increase. Press et al (1974) documented successful treatment of EFA deficiency in adults through topical administration of sunflower oil. It is interesting to note that after two weeks of treatment, a temporary exacerbation of the deficiency was observed, that was corrected by further treatment. This was seen in 18:2, 16:1 and 18:1 in the phospholipids of one patient. Sunflower oil was applied to the forearm, providing 120 mg 18:2/day (2-3 mg/kg/day), a dose considerably less than that used in the present study. Hunt et al (1978) used topical sunflower oil to treat neonates with EFA deficiency. The dose of oil was selected to provide the recommended daily linoleic acid intake of approximately 100 mg/kg. After a ten day trial, patients demonstrated alterations in fatty acid composition suggestive of an exacerbation of EFA deficiency. The

authors concluded the treatment was ineffective, claiming the oil might be absorbed through the skin, but was not being utilized. The fatty acid values obtained by day 10 corresponded to the temporary stage observed in the present study and by Press et al (1974). It is conceivable that a longer duration of treatment would have provided more definitive alterations in fatty acid patterns. Despite apparent success in treatment, results from Press et al (1974) revealed excessive elevation of 18:1 after twelve weeks of treatment. No explanation is provided for the values that were 87% higher than controls. It is evident that there is a paucity of information available on the cutaneous treatment of EFA deficiency. More research is required to clarify the conflicting reports appearing in the literature.

In all subjects participating in the present study, no significant alterations occurred in the fatty acid composition of the red cells with treatment, despite an initial 25% reduction in 18:2 content of Group A. Similar alterations in red cell fatty acid pattern have been reported by others (McEvoy 1975; Underwood et al 1972). Analysis of red cells can provide a useful guide

to tissue change and severity of EFA deficiency. Farquhar and Ahrens (1963) reported a linear relationship between the linoleic acid content of the diet and red cells. Changes in this fatty acid occur more slowly in the red cells than in the plasma, but more rapidly than in adipose tissue. Since the life span of a red cell is 120 days, a slow change in fatty acid content is expected. However, it appears that some incorporation of fatty acids occurs apart from erythropoiesis. The lack of gross alteration in the red cell fatty acid profile in the present study, further supports the evidence that the subjects did not have a severe depletion of EFA.

The apparent lack of response to either form of treatment can be attributed to several factors. The characteristic fatty acid pattern of EFA deficiency was observed in the Group A children in this study. However, the alterations seen were unremarkable when compared with results from other researchers. This is supported by the minor changes found in red cell lipids and the triene : tetraene ratio. Although fat balance data were not available, it is possible these children had only a moderate degree of malabsorption. All subjects had

intakes of linoleic acid within recommended limits. It is likely that the duration of treatment was too short to show dramatic alterations in the fatty acid patterns. This is especially valid for the topical treatment group, who received only one half (1% of calories) the amount of oil given the oral group. Increasing the amount of oil may shorten the time period, but the acceptability of the treatment must be considered. The topical treatment was not well accepted by the children, even when the amount of oil was reduced. This places a strict limitation on the applicability of the treatment. Conversely, little criticism was expressed about the oral method. However, it is possible a larger dose of oil might not be well accepted. The oral treatment is recommended as a method of treating EFA deficiency in cystic fibrosis because of its flexibility and acceptability.

As with most clinical studies, compliance is an important factor in determining the outcome of the experiment. Careful selection of subjects is invaluable to ensure compliance, especially in a home situation.

In the present study, the poor response of two subjects (KD, DD) in the oral treatment, is attributed primarily to their failure to comply totally with the procedure.

An important underlying factor in discussing essential fatty acid deficiency, is the variability present in all aspects of the condition, including biochemical diagnosis and treatment. This is magnified by the variabilities of cystic fibrosis. It is therefore mandatory that each subject be dealt with individually in terms of initial fatty acid status and response to treatment, to avoid generalizations which may be misleading.

SUMMARY AND CONCLUSIONS

The present study was designed to evaluate the essential fatty acid status of children with cystic fibrosis, and monitor their response to two treatment methods. Analyses of plasma lipids revealed alterations in the fatty acid profile suggestive of EFA deficiency. However, despite the presence of 20:3n9, the changes observed were less severe than those reported by other investigators. Only children receiving pancreatic enzyme therapy for malabsorption had depressed levels of essential fatty acids. The fatty acid pattern of children not receiving enzyme therapy was comparable to control values. Calculated results of seven day food records clearly showed that the intake of linoleic acid was within the recommended level of 1 - 4% of kcals for all children, regardless of pancreatic function. This suggests that EFA deficiency in cystic fibrosis is due to malabsorption, and not an increased requirement. Furthermore, if EFA deficiency was a biochemical defect of CF as suggested by some, (Rivers and Hassam 1974), then children without

malabsorption would also be affected.

The treatment methods investigated in this study were selected because of their simplicity and applicability to a home situation. It was intended that a level of oil corresponding to 2% of daily kcals, be used in both methods. However, with the topical method, it was discovered that the nature of the treatment placed a restriction on the amount of oil. This severely limits application of the treatment. However, both the children and parents found the procedure unacceptable. The fatty acid response to the treatment was limited. This may reflect the smaller dose of oil used, the length of the period, or failure of the fatty acids to be absorbed and utilized through the skin. Conversely, the oral procedure was well accepted and resulted in an improvement in the fatty acid status of one subject with malabsorption. As was anticipated, children not receiving enzyme supplementation demonstrated unremarkable response to both treatment methods.

The results presented strongly imply that CF children with pancreatic insufficiency are at risk of developing

essential fatty acid deficiency. While the degree of deficiency will be affected by the nutritional and health status of the individual, it is recommended that analysis of fatty acid profiles become part of the routine assessment. Oral use of an oil high in linoleic acid can be effective in reversing the biochemical alterations of essential fatty acid deficiency. It is not known whether the alteration in fatty acid composition observed in the present study would affect growth, or prostaglandin synthesis. Further long-term research is necessary to clarify the implications of essential fatty acid deficiency in cystic fibrosis.

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Appendix 1

Human Nutrition Research Project

Department of Foods and Nutrition

University of Manitoba

C O N S E N T F O R M

Cystic Fibrosis Subjects

I,_____, agree to allow my son/ daughter,_____, to participate in the research project entitled, Essential Fatty Acid Status of Cystic Fibrosis. The project has been explained to me and I fully understand the procedures involved. I am aware the blood samples will be taken at intervals throughout the study. I have been assured that the results of the study will remain the confidential property of the Department of Foods and Nutrition, University of Manitoba and the Children's Centre.

Signature_____

For (Subject's Name)_____

Relationship to Subject_____

Witness_____

Date_____

Appendix 2

Human Nutrition Research Project

Department of Foods and Nutrition

University of Manitoba

C O N S E N T F O R M

Normal Subjects

I,_____, agree to allow my son/ daughter,_____, to participate in the research project entitled, Essential Fatty Acid Status of Cystic Fibrosis. The project has been explained to me and I fully understand the procedures involved. I am aware that one blood sample will be taken at the beginning of the study. I have been assured that the results of the study will remain the confidential property of the Department of Foods and Nutrition, University of Manitoba and the Children's Centre.

Signature_____

For (Subject's Name)_____

Relationship to Subject_____

Witness_____

Date_____

Appendix 3

Instructions for Keeping a 7-Day Food Record

Inside the envelope, you will find many copies of the "7-Day Food Record". Please follow the instructions listed below to help keep an accurate record of all foods and beverages taken during the week.

1. Fill in name, day of week and date.
2. Using a new page each day, list everything you eat, drink and swallow for 24 hours. This will include food and drink taken between meals, at bedtime, away from home and watching television, and all vitamin and mineral preparations.
3. Record time of day item taken e.g., 10:30 A.M. and where you ate the item, e.g., at school, the movies, at home. Columns are provided for this information.
4. Describe the item as closely as possible by:

   methods of preparation - e.g., boiled, fried, baked

   brand names - e.g., Kellogg's Cornflakes, Monarch Margarine

   details of the food itself - skim milk, brown bread, strawberry jam

5. For combination items, each item may be listed separately.
   e.g., - Cheeseburger - meat patty
   cheese slice
   tomato slice
   bun

6. Record the amount of the item taken, weights are not necessary, household measurements can be used -
   e.g. 1 slice bread
   1 medium apple
   8 oz. milk, skim

7. Do not forget the "extras" - margarine on bread
   dressing on salad
   ketchup with fries
   filling in a sandwich
   gravy and sauces

Some Tips to Help You Collect Information About Types, Amounts and Kinds of Food Eaten

1. Items that may be counted:

   Bread - 1 slice

   Crackers - 4

   Bacon - 3 slices crisp

   Fruit - 1 medium apple

2. Items to measure in ounces/cups:

   Vegetables/cereals - 1/2 cup

   Milk - 8 oz., skim

   Casserole - 3/4 cup, macaroni and cheese

   Juice - 1/2 cup, orange, unsweetened

3. Items to measure in teaspoons/tablespoons:

   Butter - 1 tsp.

   Jam - 1 tbsp.

   Mayonnaise - tbsp.

   Sugar - 1 tsp. (for coffee, cereal)

4. Items that use a weight measurement:

   Peanuts - 15 gram bag

   Candy - 1/2 lb. hard candy

   Potato chips - 2 oz. package

   } usually weight on package

5. Items to measure serving size:

Pie - 1/8th apple pie

Cake - 2" x 3" chocolate

Meat - 3 oz. pork chop, slice of roast beef 5" x 4"

VITAMINS/MINERALS:

ENZYME PREPARATIONS:

Appendix 4

NAME:
DAY OF WEEK:
DATE:

TIME OF DAY	WHERE EATEN	FOOD OR DRINK	DESCRIPTION	AMOUNT CONSUMED

REMARKS:

Is this a usual day? Yes _____

No _____

Appendix 5

Procedure for Using Sunflower Oil (Safflo)

Dates

Start using the oil on_____, continue until _____. During this time, three blood samples will be required. Please come to the Children's Centre, Clinic #3 on these days.

1 -

2 -

3 -

Do not eat or drink anything after midnight the night before the sample is to be taken.

Oil

You will be using_____millilitres of sunflower oil daily for 28 days.

The oil is to be taken by mouth. It can be mixed in other foods or beverages, or taken alone. The amount may be divided and taken in more than one dose.

Please measure the oil in the syringe provided. Bring the bottom of the plunger up to the level indicated_____. Try to remove as much air as possible.

The syringe can be taken apart and washed after each use. PLEASE DO NOT PUT IN DISHWASHER.

While following this treatment, please do not change your intake of enzymes or vitamins and minerals. Continue to eat and drink as usual.

Appendix 6

Procedure for Using Sunflower Oil (Safflo)

Dates

Start using the oil on_____, continue until _____. During this time, three blood samples will be required. Please come to the Children's Centre, Clinic #3 on these days.

1 -

2 -

3 -

Do not eat or drink anything after midnight the night before the sample is to be taken.

Oil

You will be using_____millilitres of sunflower oil daily for 28 days.

The oil is to be rubbed on your skin using your hand. You may put the oil anywhere on your body. Try to use all the oil. It may be easier to apply a small amount of oil several times during the day. Please use it after, NOT before bathing, so it won't be washed off.

Please measure the oil in the syringe provided. Bring the bottom of the plunger up to the level indicated_____. Try to remove as much air as possible.

The syringe can be taken apart and washed after each use. PLEASE DO NOT PUT IN DISHWASHER.

While following this treatment, please do not change your intake of enzymes or vitamins and minerals. Continue to eat and drink as usual.

Appendix 7

Supplements Prescribed for Subjects

Subject	Vitamin/Minerals Brand	Dose	Pancreatic Extracts Brand	Dose
SM	Multivites [1]	3/day	-	-
	Aquasol E [2]	100 I.U. 2/day		
MF	Parametes [3]	1/day	Cotazym [4]	8/meal
DJ	Infantol [5]	1 tsp/day		
	Aquasol E	100 I.U. 2/day	Cotazym	30/day
RB	Multivites	2/day	-	-
DB	Multivites	2/day	-	-
KD	Parametes	2/day	Viokase [8]	9/meal
	Aquasol E	100 I.U. 4/day		
	Ferrous Sulphate [6]	300 mg/day		
	Vitamin K [7]	0.5 mg 3/wk		
DD	Multivites	3/day	Viokase	3 tsp/meal
	Aquasol E	100 I.U. 3/day		

1 Allen and Hanbury
2 Arlington
3 Ayerst
4 Organon
5 Horner
6 Lilly
7 Roche
8 Mead Johnson