RESEARCH PROPOSAL

Title: Stearic Acid and Oleic Acid in the Diet and Blood Lipid Concentrations. (Experiment FF).

Responsible Investigators: Dr. Joseph T. Anderson, Dr. Francisco Grande and Dr. Ancel Keys, Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis.

Location and Dates of Research Project: Springdale Metabolic Unit, Faribault State School and Hospital. On September 8, 1965 the project staff will assume the food service for all the patients who eat in Springdale. The count is about 65 to 70 plus about 3 to take care of the fact that 15 men eat at Springdale only

weeks until December 14.

Research Objectives: In previous experiments, diets in which cocoa butter was used produced lower serum cholesterol concentrations in the subjects than would be predicted from the fatty acid composition of cocoa butter. The data indicate strongly that stearic acid, although it is a saturated fatty acid, is completely different from the other common saturated fatty acids of food fats, such as palmitic acid, with respect to its serum cholesterol raising power. The hypotheses to be tested in the proposed experiment are:

(1) That dietary stearic acid has a much lower serum cholesterol raising effect than dietary palmitic acid, and

{?.) That the fatty acids of cocoa butter are the only constituents which have a significant effect on serum lipid concentrations.

Staffing: The Faribault State School and Hospital will continue to provide one food service person as in the past. This person, if possible, will work the regular hours of one of the usual shifts, probably Sunday through Thursday from 10 A.M. to 6:30 P.M. Her duties will be integrated into the program of

the project and she will, in most respects, work under the supervision of the dietitian, the senior food service supervisor and the cook. As at present, about nine patient helpers will be assigned to work in the food service unit, about 4 to 6 hours daily, 6 days per week.

The Laboratory of Physiological Hygiene, University of Minnesota, will provide the following staff:

Dietitian, part time, Mrs. Carol Thera

Senior Food Service Supervisor, Mrs. Florence DeCoux

Cook, Mrs. Gladys Neseth

Food Service Worker, Mrs. Mary Underdahl

Research Helpers, John Wilson and Steven Cripe

Outline of Program: The experimental subjects will be 32 men chosen from the population of the Springdale unit who customarily eat all their meals at Springdale. The rest of the men served at Springdale will be given the same food as those in the special diet except that the portions will not be weighed and the type of oils and fats used in the recipes will be chosen for convenience. The criteria for acceptance as an experimental subject will be age 35 or more years, relative body weight (as percent of normal for height) between 80 and 120%, freedom from metabolic disease and from acute disease, and ability to eat food in a normal way and to refrain from taking food which is not intended for him. The men will be divided into 4 matched groups on the basis of age, relative body weight and serum cholesterol level.

The standard serving will be set at 2600 Cal. /day and adjustments above and below this will be made as required to keep each man's body weight constant.

The adjustments will be made chiefly by adding or deducting bread and jelly. The supplement will consist of 90 grams of fat or oil providing 810 Cal. daily. The remaining 1790 Cal. will be provided by the basic diet. This will be a mixture of ordinary foods making up the usual American diet pattern and containing all the needed protein, vitamins and minerals. The basic diet will contain about 25 grams daily of fat which will make the total fat in the 2600 Cal. standard serving about 115 grams or 40% of calories. The diet will contain meat, poultry of fish twice daily, a 3 oz. portion at noon and a 1 *oz,* portion in the evening. Beef, pork cured ham, chicken, turkey, tuna and frozen fish fillets will be served.

The schedule of diets is given in Table 1 and the composition of the oil mixtures is given in Table 2.

TABLE 1

Revised Schedule of Diets for Exp. FF (Sept. 10, 1965)

-riod	Weeks	Group W	Group X	Group Y	Group 2
1	1-2	Butter	Butter	Butter	Butter
2	3-5	NPO	IPO	NCB	ICB
3	6-8	IPO	NPO	ICB	NCB
4	9-11	ICB	NCB	IPO	NPO
5	12-14	NCB	ICB	NPO	IPO

Each standard daily serving will contain 90 grams of one of the oil mixtures or of butter fat.

Key to abbreviations used in naming the **oil** mixtures:

NPO = natural palm oil
IPO = imitation palm oil
NCB = natural cocoa butter
ICB = imitation cocoa butter

TABLE 2

Oil Mixtures for Experiment FF

	Composition, g/100 g oil				
	< 16:0	> 18:0	monoene	polyene	Percent to be used in mixture
Mixture NPO					
Palm Oil, 8-10-65	46.2	6.3	36.8	10.7	100.0%
Mixture IPO (Interesterified)					
THSBO, UEF-V-1933-177	10.2	86.7	3.1		47.3%
Olive Oil, 8-9-65	9.8	3.2	75.6	11.4	45.5
Safflower Oil, 51 m 64	6.6	3.4	12.6	77.4	7.2
Mixture	9.8	42.7	36.8	10.7	100.0
Mixture NCB					
Cocoa Butter, 8-9-65	26.4	35.1	35.4	3.1	94.0%
Safflower Oil, 8-9-65	6.6	3.4	12.6	77.4	6.0
Mixture	25.2	33.2	34.0	7.6	100.0
Mixture IC3 (Interesterified)					
Palm Oil, 8-10-65	46.	6.3	36.8	10.7	42.0%
Olive Oil, 8-9-65	9.8	3.2	75.6	11.4	23.0
THSBO, UEF-V-1933-177	10.2	86.7	3.1	---	34.4
Safflower Oil	6.6	3.4	12.6	77.4	0.6
Mixture	25.2	33.2	34.0	7.6	100.0

Oil mixtures NPO and IPO are designed to have the greatest possible difference between palmitic acid (16:0) and stearic acid (18:0). The difference is 36.4 grams (as glycerides) per 100 grams of oil. Judging by previous results this is a large enough difference in palmitic acid to produce a serum cholesterol response of about 33 mg/100 ml under the assumption that stearic acid has no effect at all. These two oil mixtures are equal in monoene and in polyene content. No serum cholesterol effect due to either of these components will be caused by exchanging these oils.

Oil mixtures NC3 and ICB are equal in all 4 of the parameters which are known to influence serum cholesterol concentration, i.e. palmitic acid (16:0), stearic acid (18:0). monoene fatty acids and polyene fatty acids. They are expected to produce equal serum cholesterol concentrations. If interchanging these oils causes a change in serum cholesterol the inference is that cocoa butter contains some special component, apart from stearic acid, which has a powerful effect on serum cholesterol concentration. Our hypothesis number two will be sustained if the serum cholesterol concentrations of the men are equal when they are receiving oil mixtures NC3 and ICB.

Fasting blood samples will be taken at the end of each diet period and the serum will be analyzed for total cholesterol and for triglycerides.

The servings of food which contain the supplementary fat will be weighed for each man. Other servings will be measured either by weight or volume. The food rejected by each man at each meal will be recorded by estimating the fraction of the serving rejected. By use of a computer program recently developed the values of food rejection and of food adjustments will be translated into mean deviations of nutrients from the nutrients of a standard serving, There will be a deviation value for each man, each nutrient and each period. The actual composition of the standard serving will be determined by chemical analysis of 14-day composites of duplicate servings, identical to the food given to a typical subject and by analysis of the oil mixtures. The actual mean consumption of calories by a specific man in a specific period will be obtained by adding (or subtracting) the deviation of calories to the calories in the standard serving. Other nutrients will be calculated in a similar way.

Body weights of the men in night clothing will be measured weekly in the morning after urinating and before breakfast. Constancy of body weight within 3 kg. per 10 week period will be a criterion of good performance.

It is known that fats with high melting points tend to be poorly digestible and that long chain saturated fatty acids such as stearic acid tend to produce high melting, hard fats. The totally hydrogenated soybean oil (THSBO) incorporated into mixtures IPO and ICB is a material harder and higher melting than candle wax. Such fats are known to be poorly absorbed and to appear in the stool. Mixture IPO and to a lesser degree mixture ICB is rather high in stearic acid for good digestibility. Fats are composed of triglyceride molecules each containing three fatty acid residues attached to one glycerol residue. Those molecules with 2 or 3 stearic acid residues are the type which are high melting and tend to crystallize from a mixture of triglycerides. Molecules which contain only one stearic acid residue are lower melting particularly if the other fatty acid residues in the same molecule are monounsaturated (monoene) or, better yet, polyunsaturated (polyene).

Oil mixtures IPO and ICE, which contain large proportions of the totally hydrogenated soybean oil will be subjected to a process known as interesterification and are expected to be well digested and absorbed. In order to have evidence on this point eight subjects will be chosen, 2 from each group, and stool samples will be collected from them during the 7 days before the blood sample for determination of unabsorbed fat. Each subject from whom stools are collected will be given weighed tablets containing insoluble chromium oxide,

3 per day, one with each meal 7 days before the first stool collection day and continuing without interruption to the end of the experiment. The greatest possible care will be taken to insure that each of these men actually swallows the 3 tablets daily. By this method it will be possible to calculate an accurate value for fat excretion even though the subjects may fail to save all the stools. The stools passed in seven days will be combined and homogenized with the addition of water. This homogenate will be weighed and samples will be analysed for chromium and for fat. From the chromium found the effective duration of the collection period will be calculated. In this way the fat excretion per day will be obtained.

RESEARCH PROPOSAL: Addendum, September 10, 1965

Title: Stearic Acid and Oleic Acid in the Diet and Blood Lipid Concentrations. (Experiment FF)

On September 9, the feeding of the special diet was started. The 32 patients who were selected as experimental subjects were seated in order of assigned number in the north end of the dining room and the remainder of the patients filled the rest of the places. Every man had a tray with his name on an embossed plastic strip label. Every man got the same food with butter added. The quantity of bread and jelly was adjusted for each man based on previous records of requirements. The only obvious difference between the subjects and the non-subjects was the fact that the subjects sat in the north end of the room and the non-subjects in the south end. Less obvious differences were the facts that the name strip for each subject contained a number (from 1 to 8) and the strips were red, yellow, blue or green. For the non-subjects the name-strips were gold in color and lacked a number.

After the meal some of the non-subjects complained that they did not like the diet because there was no butter for the bread and no gravy for the potatoes. (The first idea was true, the second was anticipation based on previous experience.)

Several staff members made suggestions for eliminating the discontent among the patients. After considerable discussion a decision was reached to treat all the patients as if they were subjects and to tell the patients (and their relatives) that all of them are subjects. In order to accomplish this the seating arrangement in the dining room will be changed so as to intersperse non-subjects with subjects. Blood samples will be taken from both groups alike. Body weighing and adjustment of the quantity of bread and jelly will be carried out with all. Recording of food rejected and coaching with respect to proper eating and cleaning up the plate will be impartially carried out for all the patients.

These changes in procedure will, it is hoped, overcome most of the dissatisfaction of the patients.

Joseph T. Anderson