APPENDIX October 1, 1962
RESEARCH PAPER retyped Dec. 31, 1965

Title:

Dietary factors and blood lipids related to heart disease.

Institutions;

Faribault State School and Hospital and Laboratory of Physiological Hygiene, University of Minnesota

Dates:

Beginning Oct. 1, 1962 and continuing indefinitely with annual review on Oct. 1 each year.

Investigators:

The research program will be under the supervision of Dr. Ancel Keys, Director of the Laboratory of Physiological Hygiene, University of Minnesota, and direct responsibility for the operation will be assumed by Dr. Joseph T. Anderson, Dr. Francisco Grande and other members of the staff of the Laboratory of Physiological Hygiene.

Purpose:

This program is intended to contribute to the understanding and eventual control of some forms of heart disease, particularly coronary heart disease, by a series of controlled dietary experiments in which blood lipids are observed in patients of the Faribault State School and Hospital.

Procedure:

A Metabolic Research Unit will be established in the Grandview unit of Faribault State School. This is a unit (about 3 miles removed from the Main Campus of the School) housing 50 to 60 patients and 2 to 6 staff members and

containing kitchen facilities. In this unit 50 patients will be fed carefully controlled diets in which certain components, usually fats, will be changed from time to time. The responses of blood constituents to the dietary changes will be observed. On occasion collections of feces and urine will also be made for analysis.

For the immediate future the analyses of blood, feces and urine will be done in the Minneapolis laboratories of the Laboratory of Physiological Hygiene. but eventually it is planned to set up a chemical laboratory in the basement of the Hospital on the Main Campus of the Faribault State School. Much laboratory equipment previously used at Hastings State Hospital has already been moved and placed in storage in the Hospital basement, in Faribault.

Staff:

The staff required to operate the Grandview Unit as a Metabolic Research Unit will include all the present staff of the unit including a full-time cook and a half-time cook who will remain on the Faribault State School payroll. In addition there will be a dietitian (or one or more food service supervisors) and three volunteers, young men who will assist with food preparation and serving and with care and management of the patients. These volunteers will, if space permits, live in the Grandview unit and their room, board and laundry will be provided by Faribault State School.

In matters relating to the diet and other factors important to the research program Drs. Anderson and Grande will supervise the dietitian who in turn will supervise the cooks and volunteers. The Laboratory of Physiological Hygiene will pay the salary of the dietitian and the contract fee for the services of the

volunteers.

In all other matters the operation of the unit and care of the patients will remain the responsibility of the staff of the Faribault State School. Special equipment and scientific apparatus not ordinarily used in feeding or office procedures will be supplied by the Laboratory of Physiological Hygiene. Certain items of kitchen equipment, office equipment and furniture have already been transferred from Hastings State Hospital to Faribault State School for use in the Grandview unit in addition to items mentioned above intended for use in a laboratory which will be established in the future.

In order to expedite the beginning of operations the Laboratory of Physiological Hygiene will provide the following materials needed to make the Granview unit suitable for use in the metabolic research program,

Item		
1.	Dishwasher, accessories, hood, installation, Approximate cost	$3, 300
Z.	Deep freeze cabinet 48 in. wide Approximate cost	400
3.	Materials for new cabinets in kitchen and storage area in basement	$l5
4.	Lighting fixtures and supplies	330

The above estimates include the installation of the dishwasher but no allowance for labor for the other items since this labor will be supplied by the Faribault State School.

Administrative Interrelations:

The volunteers and dietitian or food service supervisors will be paid by the Laboratory of Physiological Hygiene and will be supervised by the Laboratory of Physiological Hygiene staff.

Since the volunteers will be living in quarters of the Faribault State School they will be subject to the same rules as any employee living in similar quarters. It is understood that the dietitian will receive from the Faribault State School and Hospital and its staff members the cooperation necessary to perform her duties and to obtain the necessary materials and services with respect to functions which are part of her job including procuring food and maintaining and repairing the quarters.

Specific Research Program, 1962-3:

The main emphasis of the program will be on evaluation of the effects of specific foodstuffs on the levels of the three important lipid components in human serum, cholesterol, phospholipids and triglycerides. Many food stuffs have already been tested in this and other laboratories and the results are being applied by physicians and by the food industry in diets designed to protect against arterio-sclerotic heart disease by lowering serum cholesterol levels. The effects on margarines and other partially hydrogenated oil products have not been properly evaluated and studies in this area will be carried out.

Other studies will be made to discover the effect of environmental factors and of metabolic abnormalities on serum lipid levels.

Hydrogenated fat experiments

In commercially hydrogenated vegetable oils there are at least 4 kinds (isomer of monoene fatty acids, that is fatty acid molecules with one double bond. The most common of these forms of molecule is that with *the* double bond in the center of the 18 carbon chain (delta 9) and with the cis configuration of the groups attached to the double bond. This delta - 9-cis monoene fatty acid (the predominant constituent of olive oil) when fed to man has been found to be "neutral" in its effect on

serum cholesterol level, that is it has the same effect as an equicaloric quantity of carbohydrate. The effects of the other three common isomers, namely delta-9-trans, delta-12-cis, and delta-12-trans are unknown. Margarines, shortenings and other hydrogenated food products such as creamy peanut butter contain these isomers of unknown action.

It is proposed to conduct a controlled dietary experiment of two 18 week periods in which these 4 isomers will be compared with each other and with a reference fat, corn oil. Fifty middle aged patients will be housed in the Grandview unit and used as dietary subjects. A set of 7 menus will be used, one for each day of the week, all providing a well balanced complete diet in which the greatest part of the fat is a 100 gram daily supplement of pure fat. This fat supplement will be incorporated by stirring small amounts into foods such as mashed potatoes, oat-meal, or hot casserole dishes. In various dietary periods the nature of the fat supplement will be changed without any other change in the diet or way of life of the subjects. The schedule of dietary periods is given in the table. (See next page)

Corn oil will be given for 3 weeks at the beginning and end of each set of comparisons in order to provide a stable reference condition for each subject. In this way the results of part 2 and part 1 can be compared and the results of this experiment can be compared with experiments done with different subjects or in other laboratories wherever a corn oil reference was used.

Supplement A will be a fat rich in delta-9-trans monoenes and free from delta-12 monoenes. Supplement B will match supplement A in all respects except delta-9-trans monoenes and will contain instead an equal amount of delta-9-cis monoenes. In this way the difference between the cis and trans form will be evident. These compositions will be obtained by using for Supplement A selectively hydrogenated olive oil (treated as oils are treated for margarine making) plus some safflower oil and for Supplement B a matching mixture of untreated olive oil plus totally hydrogenated olive oil.

Supplement C will be a fat with large amounts of all the isomeric monoenes, delta-12 as well as delta-9, made chiefly from selectively hydrogenated safflower oil with a little untreated safflower oil. Supplement D will match Supplement C in all respects except that the monoene fraction will be delta-9-cis monoene alone. The comparison of Supplements C and D in part 2 will show the effect of substituting the mixture of 3 unnatural isomeric monoenes for the delta-9-cis monoene. The previously finished comparison of Supplements A and B in part 1 will have demonstrated the effect of one of these three. The difference between comparisons will then show the effect of the mixture of the other two, the delta-12 isomeric monoenes. It will not be possible to measure the difference between delta-12-cis and delta-12-trans monoene fatty acids by this experiment.

Blood samples will be taken at 3 week intervals throughout the experiment **and** the serum analyzed for cholesterol, phospholipids and triglycerides. In a few subjects the appearance and disappearance of the isomeric fatty acids in body tissues will be followed by sampling adipose tissue (using a needle biopsy), red blood cells and the triglycerides of serum and determining fatty acids by gas liquid chromatography.

Study of Cretins

The presence of a number of cretin patients at the State School and Hospital at Faribault offers an opportunity for the study of lipid metabolism in chronic hypothyroidism. The first phase of the study will consist in comparing a group of 25 or more cretins with a similar group of euthyroid patients with other forms of mental deficiency matched as to age and sex, living in the same institution, and subsisting on the same diet. Measurements will include the serum concentration of cholesterol, phospholipids and triglycerides as well as the distribution of cholesterol in the lipoprotein fractions separated by the cold ethanol fractionation procedure of Cohn.

The second phase of the study will consist in a comparison of the responses of the serum lipids of the two groups of subjects to a change in dietary fat. This will be done essentially by the same methods which have been used in this Laboratory in similar studies on schizophrenic patients at Hastings.

The level of thyroid activity will be measured by the radioactive iodine uptake method. The methods of determination of serum lipids and lipoprotein fractions will be the same used in this Laboratory in studies of cholesterol metabolism.

It is known that in other forms, hypothyroidism is associated with high levels of serum cholesterol but little information is available about the other lipid

fractions and especially about the responses of the serum lipids and lipoproteins in this condition. We have been unable to find any useful research reports on lipid metabolism in cretins.

It is hoped that the results of this study will be helpful in understanding the physiological mechanisms involved in the serum lipid responses to dietary fat.

Screening the population for serum cholesterol level.

All the male patients of the Faribault State School and Hospital 20 years of age and older will be examined for serum cholesterol level. This information will be useful to the medical staff of the Hospital and to the research group in the selection of suitable subjects for special programs.

Significance of this research:

Diseases of the heart and circulation are involved in more than half of the deaths of Americans even when deaths of infants and children are included. Attempts to understand and control heart disease are obviously deserving of the great attention they are receiving. The proposed research is a continuation of a program pursued at Hastings State Hospital continuously since 1950. This program has been one of the three or four most productive of useful information about diet and heart disease in the U.S.A. if not the world.

In changing to Faribault larger groups of subjects and longer diet periods will be used. Both of these changes will increase the sensitivity of the methods for detecting the effects of various foodstuffs on blood lipids. The future data from the Faribault Metabolic Unit will be more accurate than previously and will undoubtedly be widely used in planning diets for coronary patients and for the general American public.

Previous publications in this field:

Selected key publications in this field are as follows:

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