serum cholesterol for at least 2 years(2). This suggests that the injected DNA altering serum lipid regulation may be in some form of a "depot"; utilized slowly (years) under normal dietary conditions and utilized more rapidly (months) when the animal is fed a saturated fat. It appears, for the first time in these studies, that the prolonged, "non-genetic" alteration of serum lipids induced by DNA may not be permanent.

Summary. Three groups of normal rabbits were injected with varying amounts of DNA along with a control group which received saline. One year after the DNA injections, the animals were fed a standard feed supplemented with 15% hydrogenated coconut oil for 4 months. All the animals, experimental and control, developed a mild hypercholesterolemia. At the end of one month of fat feed, all 3 DNA-injected groups had significantly lower serum lipid levels than the controls. However, the continued fat feeding resulted in a quantitative, direct relationship between amount of DNA injected and length of time the rabbits maintained lower serum lipids.

I wish to thank Mr. Ben Weinberg for excellent technical assistance.

1. Savitsky, J. P., *Am. J. Physiol.,* 1959, v197, 1359.
2. ———, *ibid.,* 1961, v200, 541.
2. ———, *ibid.,* 1962, v203, 929.
4. ———, *ibid.,* 1960, v199, 143.
5. Lambert, G. F., Miller, J. P., Olsen, R. T., Frost, D. V., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 544.
6. Wigand, G., *Acta Med. Scand.,* 1959, v166, supp. 351.
7. Steiner, A., Varsos, A., Samuel, P., *Circ. Res.,* 1959, v7, 448.
8. Abell, L. L., Levy, B. B., Brodie, B. B., Kendall, F. E., *J. Biol. Chem.,* 1952, v195, 357.
9. Fiske, B. I., Subbarow, Y., *ibid.,* 1925, v66, 375.
10. Lamonthezie, N., Piot, M., Guerineau, M., PROC. SOC. EXP. BIOL. AND MED., 1963, v113, 27.

Received February 7, 1964. P.S.E.B.M., 1964, v116.

Iron Deficiency in Monkeys Fed Diets Containing Soybean Protein.* (29180)

COY D. FITCH, WILLIAM E. HARVILLE,† JAMES S. DINNING AND F. STANLEY PORTER
Departments of Biochemistry, Medicine, Pathology, and Pediatrics, University of Arkansas School of Medicine, Little Rock

Certain foods reduce iron absorption from the gastrointestinal tract(1). In comparison to many foods the iron of soybeans and of diets containing isolated soybean protein has been found to be well absorbed(2-4). However, in the experiments to be described we observed reduced gastrointestinal absorption of iron and iron deficiency anemia in monkeys receiving a diet containing isolated soybean protein.

Methods. Young Rhesus monkeys *(Macaca mulatta)* of both sexes initially weighing 1.5 to 2.5 kg were used. Different groups received basal diet alone (Table I), basal diet supplemented with 100 mg of choline per 100 g diet, or basal diet supplemented with 100 mg of choline, 630 mg of methionine and 73 mg of cystine per 100 g of diet. Preparation of the diets including heating in an oven to form a cake has been described previously(5). Some of the animals in each group received on their food 80 mg of DL alpha-tocopheryl acetate dissolved in ethanol 3 times weekly. Food and water were given once daily.

Hemoglobin concentrations and reticulocyte counts were obtained by standard hematologic techniques. Bone marrow examinations were done on direct smears of material obtained by tibial aspiration. Serum iron concentration was determined by the method of Trinder(6) and serum iron binding capac-

* Supported by research grant from Nat. Inst. Health, U.S.P.H.S.

† Present address: Dept. of Pathology, New England Deaconess Hospital, Boston, Mass.

TABLE I. Composition of Diet.

Component	%
Archer Daniels Midland assay protein C-1	18.0
Sucrose	22.0
Corn starch	43.9
Lard*	8.0
Inositol	.1
Salts (HMW)‡	4.0
Linoleic acid†	3.0
Vitamins§	.5
Baking powder	.5

* "stripped lard" from Distillation Products, Inc., Rochester, N. Y.

† Linoleic acid, 75%, from Nutritional Biochemicals Corp., Cleveland, Ohio.

‡ Reference 11.

§ The vitamin mixture consisted of riboflavin, 105 mg; pyridoxine, 105 mg; folic acid, 105 mg; calcium pantothenate, 420 mg; nicotinic acid, 420 mg; menadione, 92.4 mg; and sucrose, 98.75 g. In addition each monkey received daily on his food 0.5 mg thiamine hydrochloride, 1 μg vit B_{12}, 20 mg ascorbic acid, 3000 I.U. vit A acetate, and 400 I.U. of vit D.

ity was estimated by the method of Rath and Finch(7). For comparison, hematologic studies were done on a group of normal Rhesus zoo monkeys of approximately the same age as the animals used in these experiments.

To measure the absorption of iron from the gastrointestinal tract of anemic monkeys, 10 μc of Fe^{59} as ferrous citrate (approximately 1 μg of iron) was mixed in 50 ml of distilled water with 3 g of one of the following: casein, isolated soybean protein, basal diet or cellulose. One hundred milliliters of distilled water were then used to wash the mixture through a nasogastric tube into the monkey's stomach. Thereafter, at varying intervals, the radioactivity of 0.1 or 1 ml samples of whole blood was measured in a well-type sodium iodide crystal scintillation counter.

Results and discussion. Monkeys in each group, including those receiving Vit. E, became anemic within 2 to 7 months after the diets were begun. At this time none of the monkeys showed the usual signs of Vit. E deficiency(5). There was no creatinuria; the bone marrow showed erythroid hyperplasia, without multinucleated erythroid cells of the type that characterize the bone marrow of Vit. E-deficient monkeys(8); and direct smears of peripheral blood showed microcytic hypochromic erythrocytes.

The total serum iron binding capacity of the monkeys was generally high, in comparison to values found in man, but the highest values were observed in anemic monkeys (Table II). Serum iron concentrations were reduced in the anemic monkeys with one exception (Table II). However, in this monkey the per cent saturation of the total serum iron binding capacity was low, as it was in the other anemic animals.

Iron therapy reversed the hematologic abnormalities (Table II) with either parenteral (Fig.1) or oral iron (Fig. 2) being effective.

TABLE II. Hematologic Abnormalities* and Effect of Iron Therapy.

Animal or group	Diet	Hemoglobin (g/100 ml)	Serum iron (μg/100 ml)	Total serum iron binding capacity (μg/100 ml)	% saturation of binding capacity
Zoo monkeys	Normal zoo diet	13.0-15.0 (4)†	150-300 (4)	350-471 (3)	36.4-75.0 (3)
241	Basal	9.2			
240	*Idem* + choline	8.2	150	600	25.0
246	" + "	5.0	112	612	18.3
237	" + "	6.1	40		
233	Basal + choline and vitamin E	7.9	94	294	32.0
243	*Idem*	7.4	94	334	28.1
231	" + cystine and methionine	7.0	252	702	35.9
Treated monkeys					
231-246	Previous diets + iron	10.6-14.0 (7)			

* Values obtained just prior to iron therapy.

† Range of values for zoo monkeys and for experimental monkeys after iron therapy is shown. Number of animals in these groups is in parentheses.

Initially during oral therapy the iron was given between feedings. Subsequently the diet was modified by addition of 33 mg of $FeSO_4$ and 2 g of $CaCO_3$ per 100 g diet. Thereafter, hemoglobin concentrations remained normal until Vit. E deficiency developed in the monkeys receiving no Vit. E supplement.

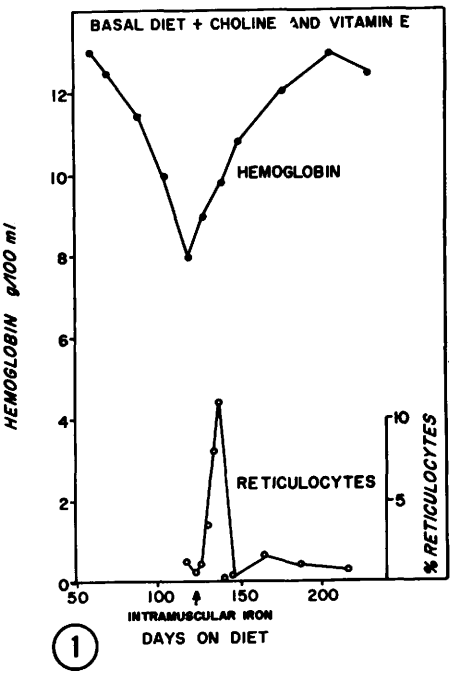
We have not previously observed iron deficiency in monkeys receiving casein-containing diets having the same amounts of dietary iron as the initial diets in these experiments (21 mg of iron per 100 g of diet). Substitution of isolated soybean protein for an equal weight of casein is the major difference between the present diets and those previously used. Isolated soybean protein is known to impair the utilization of zinc(3,9), manganese and copper(3). In the case of zinc it has been suggested that phytins, which are present in isolated soybean protein,‡ bind the zinc and prevent absorption(10).

In 3 anemic monkeys, gastrointestinal absorption of Fe^{59} from mixtures containing either basal diet or isolated soybean protein alone was negligible during a 4-hour period of observation. By contrast, in the same monkeys, significant absorption occurred during similar studies using an Fe^{59}-cellulose mixture. To quantitate the effect of isolated soybean protein on iron absorption, incorporation of Fe^{59} into hemoglobin was measured (Fig. 3). In the same anemic monkey absorption of the iron from a soybean protein Fe^{59} mixture was approximately 50% as great as from a casein Fe^{59} mixture.

These studies show that, in the Rhesus monkey, diets containing isolated soybean protein decrease the absorption of iron and may induce iron deficiency anemia. Since isolated soybean protein contains appreciable quantities of phytins, binding of iron by phytins is a possible explanation for our data. However, further work is required to identify the binding material.

Summary. After receiving purified diets containing isolated soybean protein for 2 to 7 months, young Rhesus monkeys *(Macaca mulatta)* became anemic. The erythrocytes

‡ Specifications for ADM assay protein, Archer Daniels Midland Co., Cincinnati, Ohio.



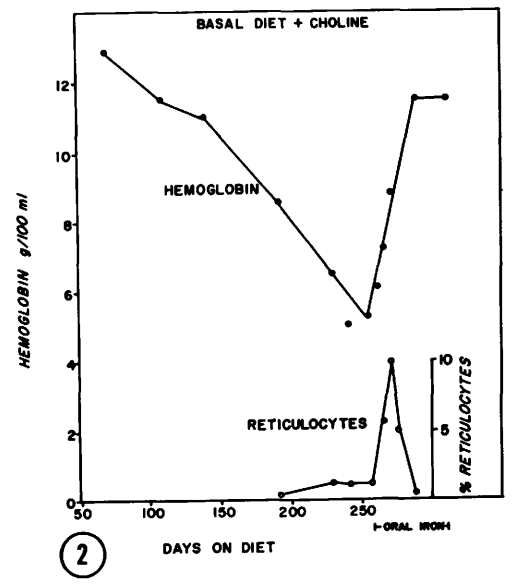


FIG. 1. Response of an anemic monkey (233) to parenteral iron. An iron-dextran complex (Imferon, Lakeside Laboratories, Inc.) equivalent to 400 mg of elemental iron was given intramuscularly.

FIG. 2. Response of an anemic monkey (246) to oral iron. Iron was given daily before feeding as 100 mg ferrous sulfate mixed with 40 mg of ascorbic acid.

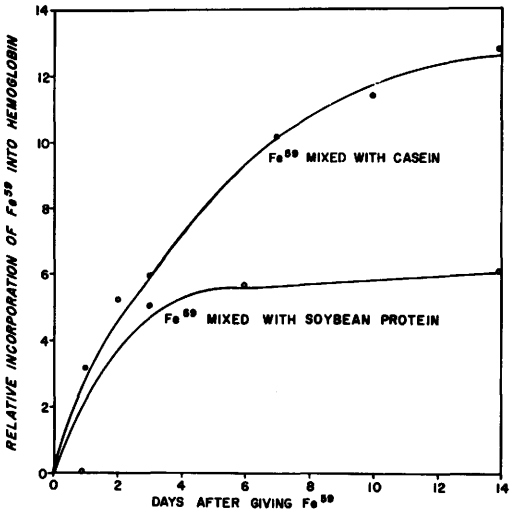


FIG. 3. Effect of isolated soybean protein on gastrointestinal absorption of iron. Incorporation of Fe^{59} into hemoglobin is expressed as cpm in 1 ml blood $\times 10^4$ divided by the cpm in the initial dose. During these studies hemoglobin decreased from 7.1 to 5.2 g per 100 ml. The study using soybean protein was done 36 days after the one using casein and appropriate corrections were made for residual radioactivity.

were microcytic and hypochromic, serum iron concentrations were low and serum iron binding capacities were high. Iron therapy, either parenteral or oral, produced a prompt remission of anemia. Since these diets contain as much iron as other diets that are adequate in this respect, gastrointestinal absorption of Fe^{59} was evaluated in the presence of soybean protein and compared to Fe^{59} absorption in the presence of casein. Soybean protein caused a significant reduction in gastrointestinal absorption of Fe^{59}.

We are indebted to Mr. Raymond A. Squires and the Little Rock Municipal Zoo for permitting us to study the Zoo monkeys and to Mr. Ralph S. Izard for technical assistance.

1. Sharpe, L. M., Peacock, W. C., Cooke, R., Harris, R. S., *J. Nutrition,* 1950, v41, 433.
2. Miller, C. D., Louis, L., *ibid.,* 1945, v30, 485.
3. Davis, P. N., Norris, L. C., Kratzer, F. H., *ibid.,* 1962, v77, 217.
4. Subrahmanyan, K., Sathe, V., De, S. S., *Ann. Biochem. and Exp. Med.,* 1950, v10, 13.
5. Dinning, J. S., Day, P. L., *J. Exp. Med.,* 1957, v105, 395.
6. Trinder, P., *J. Clin. Path.,* 1956, v9, 170.
7. Rath. C. E., Finch, C. A., *J. Clin. Invest.,* 1949, v28, 79.
8. Porter, F. S., Fitch, C. D., Dinning, J. S., *Blood,* 1962, v20, 471.
9. Morrison, A. B., Sarett, H. P., *J. Nutrition,* 1958, v65, 267.
10. O'Dell, B. L., Savage, J. E., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 304.
11. Hubbell, R. B., Mendel, L. B., Wakeman, A. J., *J. Nutrition,* 1937, v14, 273.

Received February 7, 1964. P.S.E.B.M., 1964, v116.

Destruction of Enteroviral Ribonucleic Acid Infectivity by Phosphodiesterase.* (29181)

H. ROUHANDEH AND G. R. DUBES (Introduced by H. A. Wenner)
Section of Virus Research, Department of Pediatrics, and Department of Microbiology, School of Medicine, University of Kansas, Kansas City, Kansas.

Colter *et al*(1) showed that poliovirus preparations after treatment with phenol contained ribonuclease-labile infective units. Infection of primary monkey kidney cultures with such units was found to be facilitated by inoculating calcium-depleted cultures with the infective ribonucleic acid (RNA) adsorbed to a facilitator (*e.g.,* talc)(2). The infection of such cultures with RNA from Coxsackie A23 (Vispo)(3), Coxsackie A7, A9, and B3, and enteric cytopathogenic human orphan (ECHO) 5, 7, 8, 11, 12, 19, and 26 (Rouhandeh, manuscript) viruses has since been found to be facilitated by this method, as also has the infection of mouse L-cells with encephalomyocarditis virus RNA (4).

* This research was supported by a grant from the National Foundation and from U. S. Public Health Service training grant to Dept. of Microbiology.