reached a maximum in the dog within 30–40 minutes. The relatively slow appearance of both clearing factor and radioactivity in the thoracic duct lymph of the dog indicated that absorption was proceeding via the portal circulation. Approximately 2% of the total dose was absorbed, 50% of the absorbed heparin accumulating in the liver. Negligible radioactivity was found in the muscle and perirenal fat.

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1. Engel, R. H. and Fahrenbach, M. J., Proc. Soc. Exptl. Biol. Med. **129**, 772 (1968).
2. Engel, R. H., Riggi S. J., and Fahrenbach, M. J., Nature **219**, 856 (1968).
3. Korn, E. D., Methods Biochem. Analy. **7**, 145 (1959).
4. Constantinides, P., Cairns, A., and Werner, A., Arch. Intern. Pharmacodyn. **99**, 334 (1954).
5. Bragdon, J. H. and Havel, R. J., Science **120**, 113 (1954).
6. Conner, W. E. and Eckstein, J. W., J. Clin. Invest. **38**, 1746 (1959).
7. Spitzer, J. A. and Spitzer, J. J., Am. J. Physiol. **185**, 18 (1956).
8. Kakiuchi, K., Hattori, K., and Isemura, T., Bull. Chem. Soc. Japan **36**, 1250 (1963).
9. Reynolds. J. A., Herbert, S., Polet, H., and Steinhardt, J., Biochemistry **6**, 937 (1967).
10. Fisher, A., Biochem. Z. **278**, 133 (1935).
11. Jaques, L. B. *in* "Anticoagulant Therapy," p. 35. Thomas, Springfield, Illinois (1965).
12. Constantinides, P., Saunders, P., and Wood, A., Arch. Pathol. **62**, 369 (1956).
13. Meng, H. C. and Davis, W. S., Arch. Pathol. **60**, 276 (1955).
14. Constantinides, P., Syasz, G., and Harder, F., Arch. Pathol. **56**, 36 (1953).
15. Engelberg, H., Circulation **10**, 604 (1954).
16. Engelberg, H., Kuhn, R., and Steinman, M., Circulation **13**, 489 (1956).

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Effect of Carbohydrate Source on Serum and Hepatic Cholesterol Levels in the Cholesterol-Fed Rat (33679)

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Modified food starches are used widely in the food industry because of their special cold stability and resistance to syneresis. This stability is achieved through the selection of starches high in amylopectin content (usually waxy maize or sorghum) which are then chemically treated to cross-link the starch polymer.

Several review articles emphasized that the consumption of complex carbohydrates in both man and experimental animals is usually accompanied by a slight but statistically significant decrease in the concentrations of cholesterol and lipid in the blood whereas a high intake of simple sugars has the opposite effect (1–3). There is not complete agreement, however, on the comparative effects of starch vs sucrose feeding since there are reports that starch feeding is not followed by reductions in serum cholesterol in man (4) and that starch-fed rats actually had higher serum and hepatic cholesterol levels than sucrose-fed controls (5). No published information is available on the influence of modified food starches on serum or hepatic lipid levels in man or experimental animals. The purpose of the present study was to evaluate the comparative effects of sucrose and complex carbohydrates in the form of modified food starches on serum and hepatic lipids in the cholesterol-fed rat.

Methods. Six groups of 10 male weanling rats each of the Harlan-Wistar strain were fed the basal ration shown in Table I. The carbohydrate portion of the various rations was supplied from the following sources:

TABLE I. Basal Ration.

Item	(%)
Carbohydrate	67.8
Vitamin-free casein	18.0
Soybean oil	7.0
Salt Mixture USP XIV	4.0
Vitamin mix[a]	2.2
Cholesterol USP	1.0
Total	100.0

[a] Vitamin diet fortification mixture, Nutritional Biochemicals Corporation.

Ration 1, sucrose; Ration 2, corn starch; Ration 3, a modified waxy maize starch;[1] Ration 4, a modified corn starch;[2] Ration 5, a modified tapioca starch;[3] and Ration 6, an experimental modified white milo starch.[4]

Two separate experiments were conducted, the first fed cooked starches and the second fed starch in the raw form. The cooked starches were prepared by making a 10% slurry of raw starch and water, then steam-injecting this suspension at 208°F. The gelatinized starch was drum dried at 245°F and 20 lb of steam pressure to an average moisture content of approximately 5%. The cooked, dried starches were milled through an 0.033-in. screen to obtain a particle size comparable to sucrose.

The rats were individually caged and fed the assigned rations *ad libitum* for 4 weeks. At the end of the 28-day feeding period all rats were quickly killed in random order with a Harvard model 130RM decapitator. Blood serum and livers were collected for further analysis. Total serum and hepatic cholesterol were determined according to the method of Mann (6). Total hepatic lipid was determined gravimetrically by evaporating a 2:1 chloroform–methanol extract of liver tissue to dryness after Folch washing the extract (7). The data were subjected to an analysis of variance as outlined by Snedecor (8).

Results and Discussion. Every effort was made to standardize as closely as possible the conditions under which the two experiments were conducted. The various treatments were assigned the same cage positions and feeding, lighting, watering, and temperature conditions were identical in each experiment.

The effect of carbohydrate source on serum and hepatic lipids is presented in Table II. It was postulated before this study was undertaken that modified food starches being cross-linked polymers might be less easily absorbed than unmodified starch from the same source, therefore might act similarly to mucilaginous polysaccharides by inhibiting cholesterol absorption (10). The data show that the feeding of complex carbohydrates did not result in a lowered serum or hepatic cholesterol level. The findings reported here are in close agreement with those of Guggenheim *et al.* (5) who observed increases in serum and hepatic cholesterol levels in starch-fed rats compared with sucrose-fed control animals.

When the data from Expt. I were analyzed and it was found that serum and hepatic cholesterol were significantly elevated by the feeding of complex carbohydrates in the form

[1] National Starch and Chemical Corporation, Plainfield, New Jersey.

[2] A. E. Staley Co., Decatur, Illinois.

[3] National Starch and Chemical Corporation, Plainfield, New Jersey.

[4] Corn Products Company, Argo, Illinois.

TABLE II. Effect of Carbohydrate Sources on Serum and Hepatic Lipids in the Cholesterol-Fed Rat.

	Serum cholesterol (mg/100 ml)		Total hepatic lipid (%)		Hepatic cholesterol (mg/g)	
Ration	I[e,g]	II[f]	I	II	I	II
1	83^{ab}	89^{a}	7.2	7.8	11.8^{a}	10.3^{a}
2	84^{ab}	126^{abc}	9.2	12.0	16.0^{a}	24.5^{b}
3	94^{bc}	165^{c}	8.2	12.7	28.6^{bc}	41.4^{cd}
4	104^{bc}	144^{bc}	9.3	15.0	23.3^{bc}	41.7^{d}
5	114^{c}	163^{bc}	9.5	13.6	28.7^{c}	39.2^{cd}
6	101^{bc}	156^{bc}	9.3	15.1	22.5^{bc}	38.5^{cd}
Mean	97	140	8.8	12.7	21.8	32.6
SE of mean	±7.0	±13.2			±3.24	±4.10

[e] Expt. I. Steam-injected, drum-dried starch.

[f] Expt. II. Raw starch.

[g] Vertical values not followed by the same letter are significantly different ($p < 0.01$) using Duncan's Multiple Range Test (9).

of cooked, modified food starches, the second experiment was undertaken to establish whether or not the anomalous results of the first experiment could be explained as due to the physical form in which the various starches were fed. This possibility was suggested by Lees (4) who found that the response of plasma cholesterol concentrations to high carbohydrate diets was identical whether his patients were fed sugar or cooked starch of wheat or rice origin. The results of the second experiment do not support the theory advanced by Lees (4) that reports showing a cholesterol lowering effect with starch may be due to the fact that the starch used was uncooked and therefore poorly absorbed.

Under the specific experimental conditions described herein, it was demonstrated that feeding complex carbohydrates in the form of modified food starches, whether raw or cooked, results in a statistically significant elevation in serum and hepatic cholesterol levels, compared either with the sucrose-fed controls or rats fed unmodified corn starch. Serum cholesterol levels of rats fed unmodified corn starch were not significantly different from the sucrose-fed controls in either experiment although the elevation in serum cholesterol noted in rats fed Ration 2 closely approached statistical significance in the second experiment.

Total hepatic lipid concentrations were also markedly increased in all the starch-fed rats but statistical significance could not be assigned to this observation since these determinations were made on pooled liver samples from each treatment group.

The increases in serum and hepatic cholesterol and total hepatic lipid in the rats fed complex carbohydrates were much more pronounced when the ration was composed of raw starch. The reason for this finding is not clear. If raw modified starches are not digested as easily as cooked starches one would assume that this might in turn have an inhibitory effect on cholesterol absorption. Changes in intestinal flora or pH could have a bearing on the different levels of hepatic or serum cholesterol noted between Expts. I and II.

The observed elevation in serum and hepatic cholesterol levels associated with starch feeding is markedly at variance with the studies of Portman *et al.* (11) who fed 250-g rats rations containing (in percent) casein, 20; corn oil, 8; carbohydrate, 56; cholesterol, 5; salts, 4; cholic acid, 1.5; and vitamins and trace minerals. No body weight gain data were included in Portman's paper, but rations containing these high levels of cholesterol and cholic acid, in our experience, have had a very depressing effect on performance and for this reason are not considered to be justified when evaluating the physiological significance of a particular ration ingredient.

Staub and Thiessen (12) recently reported that rats the same age as those used by Portman *et al.* (11) and fed diets containing 1% cholesterol, 0.5% cholic acid, and pregelatinized starch as the carbohydrate source had serum cholesterol levels significantly lower than the sucrose-fed control animals. No hepatic lipid or cholesterol data were included in their paper. These authors also fed a basal ration with 24% hydrogenated vegetable oil, considerably higher than the 7% level used in the modified starch diets.

Guggenheim *et al.* (5) fed 50-g rats rations which were very similar to those chosen for the study reported here (in percent) casein, 18; carbohydrate, 73; soya oil, 5; salt mixture, 4; and vitamins. One and one-half percent cholesterol and/or 0.5% cholic acid were added to this basal ration. Serum and hepatic cholesterol values were reported for each treatment group. The addition of cholic acid to the ration was necessary before even a moderate depression in serum cholesterol could be demonstrated with starch feeding. Hepatic cholesterol levels were consistently higher in the starch-fed rats regardless of whether cholesterol or cholic acid were fed alone or in combination. Total hepatic lipid was also significantly elevated in all starch-fed animals.

It is apparent that the experimental design will have a considerable influence on the comparative effects of sucrose and complex carbohydrates on serum and hepatic lipid levels. The age, species, and particular strain of experimental animal chosen, whether or not the ration contains cholesterol and/or cholic acid, the level and physical form of carbohydrate in the ration, and even the duration of the feeding study as brought out by Fillios *et al.* (13) can all have a bearing on the experimental results.

It is suggested that the addition of cholic acid to diets used in evaluating the comparative effects of simple vs complex carbohydrates on cholesterol metabolism places an unwarranted physiological stress on the experimental animal which makes extrapolation of the data to the "normal" situation particularly hazardous. Even the addition of 1% cholesterol to the diet represents stress of unknown magnitude. Certainly no one would advocate that human subjects consume a diet consisting, in part, of 5% cholesterol and 1.5% cholic acid to evaluate the effect of carbohydrate source on lipid metabolism, yet many animal feeding studies include variables which make it difficult to ascertain the practical significance of the data obtained. Hence, the generally accepted opinion that starch per se has a cholesterol-lowering effect in rats without first defining carefully the experimental protocol under which this effect was observed.

Under the specific experimental conditions imposed in this study, a marked increase in serum and hepatic cholesterol levels was observed in rats fed modified starches. This effect was more pronounced when these starches were fed in raw form. Obviously complex carbohydrates which have been modified by various chemical processes are not metabolized in a manner identical to their unmodified counterparts. It also follows that two starches from the same source (i.e., waxy maize) which have been modified by different chemical treatments may have markedly differing physiological effects upon lipid metabolism. It is not possible to make any inferences from this study as to what effect modified food starches would have on cholesterol metabolism in man but the question is an intriguing one.

Summary. The comparative effect of sucrose and complex carbohydrates in the form of corn starch and four different modified food starches was studied in the cholesterol-fed rat. Two separate experiments were conducted, one in which the starch was fed in the cooked form, the second in which the starch was fed raw. Sucrose-fed rats had significantly lower serum and hepatic cholesterol levels ($p<0.01$) and total hepatic lipid than any of the animals fed modified food starches. This finding held true regardless of whether the starch was cooked or raw; however, cholesterol and lipid levels were markedly higher when raw starch was fed. Feeding unmodified corn starch resulted in serum and hepatic cholesterol levels which were significantly lower than those observed with modified food starch, but not significantly higher than the sucrose-fed controls with the exception of hepatic cholesterol which was significantly elevated when raw corn starch was fed.

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1. Hodges, R. E. and Krehl, W. A., Am. J. Clin. Nutr. 17, 334 (1965).

2. Grande, F., Am. J. Clin. Nutr. 20, 176 (1967).

3. MacDonald, I., Nutr. Rev. 22, 257, (1964).

4. Lees, R. S., Clin. Res. 13, 549 (1965).

5. Guggenheim, K., Dan, J., and Peretz, E., J. Nutr. 72, 93 (1960).

6. Mann, G. V., Clin. Chem. 7, 275 (1961).

7. Folch, J., Lees, M., Sloane Stanley, G. H., J. Biol. Chem. 226, 497 (1957).

8. Snedecor, G. W., Iowa State Univ. Press, Ames, Iowa (1956).

9. Duncan, D. B., Biometrics 11, 1 (1955).

10. Fahrenbach, M. J., Riccardi, B. A., and Grant, W. C., Proc. Soc. Exptl. Biol. Med. 91, 321 (1956).

11. Portman, O. W., Lowry, E. Y., and Bruno, D., Proc. Soc. Exptl. Biol. Med. 91, 321 (1956).

12. Staub, H. W. and Thiesson, R., Jr., J. Nutr. 95, 633 (1968).

13. Fillios, L. C., Naito, C., Andrus, S. B., Portman, O. W., and Martin, R. S., Am. J. Physiol. 194, 275 (1958).

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