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Corn Oil and Hypercholesteremic Response in the Cholesterol-Fed Chick.* (22672)

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Recently Ahrens(1,2), Kinsell(3,4,5,6) and Bronte-Stewart(7) and their coworkers have reported unusual reductions of the serum cholesterol in subjects receiving a formula diet in which various vegetable oils, in rather large quantities, were the main source of fat. Ahrens, *et al.*,(2) demonstrated that corn oil was the most effective of these agents in maintaining a low level of serum cholesterol. They also have shown a correlation between the iodine number and the hypocholesteremic effect of several vegetable oils, and have postulated that the degree of unsaturation of the fatty acids is responsible for that effect. Meanwhile, we had noted a lower level of serum cholesterol in cholesterol-fed control chicks used to assay fractions of a hypocholesteremic brain extract(8). This difference could be related to the fact that we had begun to use corn oil instead of cottonseed oil as a vehicle for the administration of cholesterol in the atherogenic diet.

The following experiments were performed in an effort to test the hypothesis that it is the slightly higher level of unsaturated fatty acids in corn oil which distinguishes it from cottonseed oil in its effect on the cholesterol-fed bird.

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Methods. In each experiment, 40 8-week-old White Rock cockerels were divided into 4 dietary groups, each of which were offered equal weights of the diets. Diet consumption was estimated daily, body weight weekly. Two birds losing weight and apparently ill were excluded from the data. All of the diet offered was eaten, except as noted below. The chicks were bled from the alar vein at 2-week intervals, and the plasma analyzed for serum cholesterol by the method of Abell, *et al.*(9). At termination of the experiments, the aortae were examined for gross atherosclerotic plaques and graded as described by Horlick and Katz(10).

Experiment 1: To test the importance of the relative proportions of oleic and linoleic acids as they occur in corn oil as opposed to cottonseed oil, we used 2 "neo-fat" fatty acid products of Armour Chemical Division. Neo-Fat 105 consists of fatty acids redistilled from the cottonseed oil; Neo-Fat 110 is also derived from cottonseed oil fatty acids but fractionated so as to approximate the same relative proportions of palmitic, oleic and linoleic acids as occur in corn oil. According to the producers, these 2 distillates had the same minor difference in iodine number seen between corn oil and cottonseed oil, but neither had any myristic acid, which is normally present in corn oil. Each contained approximately 2% unsaponifiable matter derived from cottonseed oil. One group was fed 10% solvent extracted corn oil (Mazola); the second group 10% cottonseed oil (Puritan Oil, Procter and Gam-

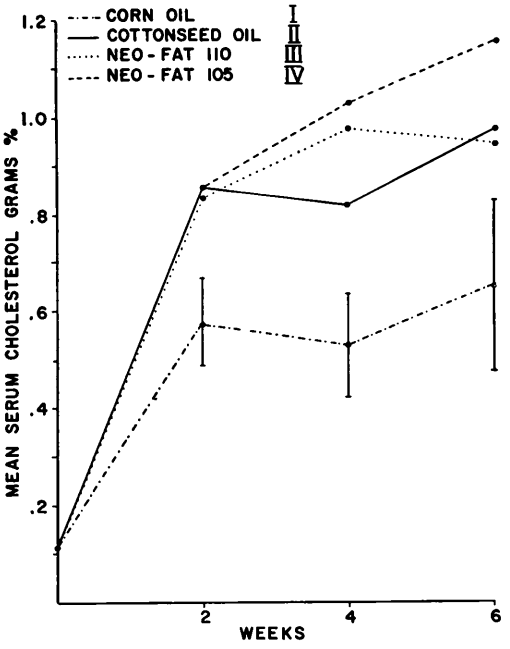

FIG. 1. Mean serum cholesterol in the 4 dietary groups indicated above. Vertical lines indicate ± one stand. dev. in the corn oil fed birds.

ble). The third group was fed 10% Neo-Fat #105, the fourth, 10% Neo-Fat #110 plus 0.1% myristic acid; each diet contained 1% cholesterol and Kay Bee chick starter mash. The latter contains ground corn, oats and wheat bran, plus alfalfa and soy bean meals which provide no less than 3% fat.

Experiment 2: Group I was fed 17.8% whole corn germ (containing 56% lipid material); Group II was fed 10% crude corn oil, expressed mechanically from the whole germ; Group III was fed solvent (hexane) extracted corn oil; and Group IV received 8% solvent extracted germ plus 9.8% cottonseed oil.‡ Thus each group received 10% lipid material by weight. One percent cholesterol was added to all diets, mixed in the oil or, in the case of whole germ, added in crystalline form. Stammler *et al.*(11) have shown that the mode of addition of cholesterol to the diet is not crucial. Each diet was mixed for at least 20 minutes in a Hobart mixer.

‡ We are grateful to Dr. Artz of Corn Products Refining Co., Chemical Division, Argo, Ill. who supplied us with these preparations and their specifications.

Results. The results of the first experiment may be seen in Fig. 1. The birds fed corn oil had a consistently lower serum cholesterol level than those fed cottonseed oil or either of its fatty acid distillates. This difference was statistically significant ($P<0.01$) at each of the 3 bleedings. No statistical significance could be found between mean plasma cholesterol levels at any bleeding of the birds fed cottonseed oil, as compared with its 2 distilled fatty acid products, though both of the distillation products tended to be more conducive of hypercholesteremia under these conditions than the original cottonseed oil. No significant difference in incidence of atherosclerosis was observed though the average grade of severity tended to correspond with the mean level of serum cholesterol.

The second experiment was devised to investigate a cruder preparation of corn oil and the whole corn germ. The cholesterol levels for the 4 groups are plotted in Fig. 2. The whole germ fed birds were significantly lower than all other groups at each bleeding, manifesting a very minimal elevation of the cholesterol level. Statistically, the level of probability is less than 0.01% in the last 2 bleed-

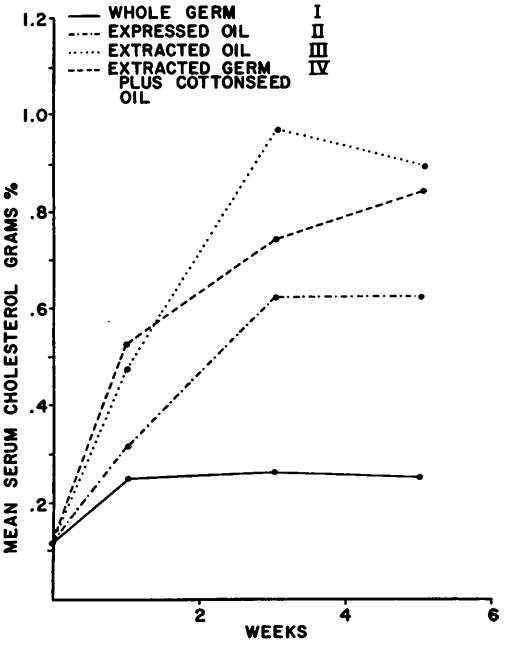

FIG. 2. Mean serum cholesterol in the 4 dietary groups indicated.

TABLE I. Estimated Proportions of Dietary Foodstuffs and Degree of Hypercholesteremia and Atherosclerosis Observed Terminally in the 4 Dietary Groups.

Source of added fat	Grams/100 g of diet estimated Fat	CHO	Protein	Terminal mean plasma cholesterol ± S.E.	Terminal mean atherosclerosis, aorta-grade (+ to 4+)
I. 17.8% whole corn germ	12.4	42.0	18.0	251 ± 29.1	.2
II. 10% crude expressed corn oil	13.0	44.5	17.8	632 ± 98.5	.6
III. 10% solvent extracted corn oil	13.0	44.5	17.8	905 ± 127	1.0
IV. 8% solvent extracted corn germ; 9.8% cottonseed oil	13.4	45.3	18.2	850 ± 112	1.0

ings. The cholesterol level is significantly lower in the expressed oil fed group than in the extracted oil group at the one and the 3-week bleedings ($P<0.05$), but not at the terminal bleeding. In this experiment the birds in Group III ate only 96%, and in Group IV, 88% of the diet offered. Groups I and II ate the full 100 g allowed per bird per day. It is doubtful that the difference between Groups II and III could be explained by the difference in cholesterol intake, though this may well explain why Group IV did not develop still higher levels of plasma cholesterol. The serum cholesterol levels achieved here are higher with corn oil than in the first experiment because of a greater dietary allowance of 100 g/day of the respective diets as opposed to 75 g/day per bird.

The results of grading the aortae of all birds in this experiment are shown in Table I. It can be seen, as with the plasma cholesterol levels, that there is a difference of borderline significance between the oil-fed groups; but the group getting whole germ is significantly protected against the early lesions seen in the other groups.

Discussion. Ahrens and coworkers(2) were able to correlate the iodine number roughly with the serum cholesterol-lowering effect, but other possible factors were not investigated. Certainly the data of Bronte-Stewart and his coworkers(7) confirms and more strongly suggests the importance of saturation of the fatty acids for the hypercholesteremic response to cholesterol feeding in the human. Swell and Flick(12) compared the response of the serum cholesterol in rats to cholesterol feeding in conjunction with lard, oleic acid or stearic acid, and concluded that the absorption of cholesterol appears to be decreased with a highly saturated fat. Later Swell and coworkers(13) showed substantial differences in the degree of elevation of the serum cholesterol levels of rats fed equal amounts of various fat sources. Here they found the response to be greatest in the descending order: Oleic acid, stearic acid, linoleic acid, peanut oil, corn oil and linseed oil. They also demonstrated that *in vitro* esterification of cholesterol proceeded more rapidly with fatty acids than with neutral fat.

These experiments leave little doubt that the serum cholesterol level is greatly influenced by the nature of dietary fat employed. They also reach fairly general agreement that corn oil seems to promote cholesterol absorption less than many other fats or fatty acid combinations, though Lin and coworkers(14) suggested that tripalmitin or other poorly utilized fats might be used even more profitably to effect a lower serum cholesterol. Our first experiment demonstrates that corn oil does not permit as great a hypercholesteremia as cottonseed oil, and further that this difference cannot be explained by the slight differences in fatty acid composition between these two oils. While there is nothing conclusive about our second experiment, it too suggests that the degree of saturation of the fatty acids is not the sole factor in determining the response to cholesterol feeding. Virtually all of the neutral fat is obtained from the corn germ by these two methods of extraction and it is doubtful that the fatty acids occur in any different proportions in the residue than in the expressed oil. The waxes and the unsaponifiable fraction are more apt to be left behind. It is doubtful that sitosterols could explain the effect, for they occur as only approximately one percent of the oil, (or 0.1

g per chick per day).

The various oils and corn germ products were added to the mash so that the fat level was essentially equal in each group, and it can be seen from Table I that protein and carbohydrate also remained in about the same proportion. Portman, *et al.*(15) have shown that the quality of dietary carbohydrate can be important in determining the hypercholesteremic response in cholesterol-fed rats. They found that corn starch in the diet inhibited the rise usually seen with simpler sugars. Certainly this might be a partial explanation for the low levels of cholesterol seen in Group I, but Group IV received an equal supplement of corn starch in the extracted germ without any impairment of the hypercholesteremic response. It seems most likely that an unidentified fraction of the germ oil, not easily extracted, is inhibiting the expected rise in serum cholesterol.

Summary. Dietary experiments in the cholesterol-fed chick have demonstrated that a higher level of serum cholesterol is achieved when cottonseed oil is fed instead of corn oil. Reconstituting the distilled fatty acids of cottonseed oil, and adding a small amount of myristic acid so that the proportions of the major fatty acids in corn oil were observed, did not make cottonseed oil fatty acids behave like corn oil in restricting the rise in serum cholesterol. Comparison of the whole corn germ with expressed and solvent extracted corn oil revealed that it was a much more potent agent for limiting the hypercholesteremia and subsequent atherosclerosis than the more refined products. It seems unlikely that this difference between corn oil and cottonseed oil in the effect on the hypercholesteremic response of the cholesterol fed bird could be explained by a difference in fatty acid composition.

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