the walls of sensitive organisms and in the case of resistant organisms there was relatively less penetration of polymyxin through the cell wall to the underlying membrane or osmotic barrier.

Summary. While normal cells of polymyxin-sensitive *V. cholerae,* polymyxin-resistant *V. cholerae* and *V. eltor* were found to exhibit a degree of resistance to polymyxin, increasing in that order, protoplasts of all 3 strains were sensitive to a polymyxin concentration insufficient to cause irreversible inhibition of growth of the normal vibrio cells. From the results it appears that the cell wall of El Tor vibrios differs in character from that of *V. cholerae* and this difference may be an important factor responsible for the resistance of *V. eltor* to polymyxin. Further, some other factor(s) in addition to the cell wall structure, such as a difference in the cell membrane of *V. eltor* from that of *V. cholerae,* may contribute to the difference in the sensitivity of the two types of vibrios to polymyxin.

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Cholesterol-Lowering Effects of Certain Grains and of Oat Fractions in the Chick.* (32378)

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An anti-hypercholesterolemic effect has recently been demonstrated with chicks for complex carbohydrates, including pectin, various gums, and scleroglucan(1-3). In a continuing effort to explore the relationship between certain complex carbohydrates and cholesterol metabolism, we have examined a number of common cereals in relation to their possible cholesterol-lowering activity, and now wish to report on the efficacy of oats in this regard. An earlier report by De Groot *et al*(4) suggested a cholesterol-lowering effect from rolled oats with rats and humans that the authors believed to be, at least in part, due to the polyunsaturated fat contained in this cereal.

Materials and methods. With one exception, one-week-old male chicks, in duplicate groups of from 6 to 10 chicks per group, were fed *ad lib* a hypercholesterolemic diet after one week on a standard chick ration. In one experiment they were placed on the experimental diet when one day old. The experimental diet consisted of 25% whole egg powder, 25% soybean meal, 3% cellulose, 2% dicalcium phosphate, 1% trace mineral concentrate,† 0.5% NaCl, 0.2% vitamin mixture,‡ 0.2

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† Mico concentrate, Limestone Products Corp. of America, Newton, N. J. Composition, in %: calcium, 32.0; magnesium, 2.6; manganese, 1.0; iodine, 0.225; iron, 0.175; copper, 0.125; fluorine, 0.01; cobalt, 0.01; zinc, 0.009.

‡ Provides, in mg/kg diet: thiamine HCl, 25; riboflavin, 16; Ca-pantothenate, 20; pyridoxine HCl, 6; biotin, 0.6; folic acid, 4; menadione sodium bisulfite complex, 5; cyanocobalamine, 0.02; nicotinic acid, 150; ascorbic acid, 250, In I.U./kg diet: vitamin A, 10,000; vitamin D_3, 600; vitamin E, 5.

choline-Cl (70%), 0.1% DL-methionine, and 43% of variable carbohydrate sources. The diet supplied 23% protein, 12.7% fat, and 0.65% cholesterol, the last two derived to a large extent from the egg powder.

The oat oil and oat starch were specially prepared for this study. The oil was obtained by extraction of dried, ground, dehulled oats with anhydrous ethyl ether, followed by removal of the solvent in an evaporator. Gas chromatographic analysis showed the following fatty acid composition for the oat oil (in %): myristate, 0.2; palmitate, 19.1; stearate, 1.4; oleate, 29.8; linoleate, 48.1; linolenate, 1.4. The oat starch was obtained by soaking in water dried, ground, dehulled oats, sieving through a 200 mesh screen, washing in dilute alkali (pH 9.0-9.5) and isolating from an aqueous neutral wash. The large starch chunks were reduced in size by gentle crushing with mortar and pestle. The material had a crude protein content of 0.74%.

During the final week of the experiment excreta were collected for two 48-hour periods and dried at 80°C to constant weight; total lipids and Lieberman-Burchard-positive sterols were determined as previously described(3). At the termination of each experiment (3rd or 4th week) the chicks were bled by heart puncture for plasma cholesterol determinations. In the last experiment, following the heart puncture, the birds were sacrificed with chloroform and the livers removed. The livers were blotted dry and wet weighed; a portion was taken for homogenization and lipid and cholesterol determinations by the methods employed for determination of these components in the excreta.

TABLE I. Effect of Different Carbohydrates and Cereals on Plasma Cholesterol Level.

Dietary carbohydrate*	Body wt (g)†	Plasma cholesterol (mg %)†
Corn starch	350 ± 13‡	255 ± 18
Glucose monohydrate	396 ± 9	237 ± 6
Sucrose	373 ± 10	229 ± 14
Ground corn	364 ± 12	230 ± 9
" barley	347 ± 13	201 ± 8
" wheat	346 ± 7	191 ± 15
" oats	348 ± 10	196 ± 16
Glucose + 3% pectin N.F.	274 ± 24	181 ± 10

* 43% of diet.
† At 3 weeks; chicks started at one week of age on experimental diets; 10 chicks per group.
‡ Mean ± S.E.

Results. In the first experiment (Table I) a number of cereals were compared to each other and to purified carbohydrates. With the exception of the growth retardation of the group receiving a supplement of 3% pectin, the various carbohydrates exerted no major effects on body weight. The groups fed barley, oats, wheat, and 3% pectin had plasma cholesterol levels significantly lower than the control group receiving corn starch. All subsequent experiments were restricted to oats or oat fractions as the dietary variables.

In the second experiment, whole ground oats were compared with a quantity of oat hulls equivalent to the amount supplied by whole ground oats and with an equivalent amount of dehulled oat flour (Table II). As in the first experiment, in comparison with the starch-fed group, the ground oats significantly ($P<0.01$) lowered plasma cholesterol. The oat hulls were nearly as effective as the whole ground oats, whereas the dehulled oat flour was somewhat less effective, although,

TABLE II. Effect of Different Oat Fractions on Plasma Cholesterol and Fecal Lipids.

Dietary carbohydrate	Body wt (g)*	Plasma cholesterol (mg %)	Fecal lipid (% dry wt)	Fecal sterol† (% dry wt)
Corn starch, 43%	462 ± 14‡	224 ±12	4.4	.5
Ground oats, 43%	447 ± 10	138 ± 9	3.3	.4
Oat hulls, 15% + corn starch, 28%	452 ± 11	155 ± 8	2.1	.4
Dehulled oat flour, 28% + corn starch, 15%	465 ± 8	170 ± 8	6.4	.7
Dehulled oat flour, 28% + oat hulls, 15%	464 ± 10	137 ± 7	2.5	.4

* At 4 weeks; chicks started at 1 week of age on experimental diets; 16 chicks (duplicate lots of 8) per group.
† Lieberman-Burchard-positive.
‡ Mean ± S.E.

TABLE III. Comparative Effect of Oat Hulls and Soy Sterols on Plasma Cholesterol and Fecal Lipids.

Dietary supplement	Body wt (g)*	Plasma cholesterol (mg %)	Fecal lipid (% dry wt)	Fecal sterol† (% dry wt)
Corn starch, 43%	316 ± 6‡	248 ± 16	6.7	1.3
Oat hulls, 15% + corn starch, 28%	300 ± 8	197 ± 10	5.7	.7
Soy sterols, 0.2% + corn starch, 43%	312 ± 7	211 ± 13	8.4	2.0

* At 3 weeks; chicks started at one day of age; 16 chicks (duplicate lots of 8) per group.
† Lieberman-Burchard-positive.
‡ Mean ± S.E.

in comparison with the corn starch, it still exerted a significant cholesterol-lowering effect. Results from "reconstituted" oats, a combination of dehulled oat flour and oat hulls, were essentially identical to those obtained with the whole ground oats. Birds fed the whole oats or oat hulls excreted somewhat less fecal lipid and Lieberman-Burchard-positive sterol than the corn starch controls; on the other hand, excretion of lipids was highest in the group fed dehulled oat flour.

In the third experiment, a comparison was made of the cholesterol-lowering effect of oat hulls and of soy sterols. While the experiment was in progress, analyses in our laboratory showed that the oat hulls provided less than 2% chloroform-methanol extractable lipid of which slightly less than 10%, or 0.2% of the hulls, was non-saponifiable material. Thus, the level of soy sterol fed exceeded the actual amount of oat sterol by a factor of at least 7. The results (Table III) showed a slightly greater plasma cholesterol-lowering effect from the oat hulls than from the soy sterols; the fecal lipid analysis, however, indicated a marked difference between the oat hull-fed and the sterol-fed groups, in that the former had lower lipid and Lieberman-Burchard-positive sterol levels in comparison with corn starch controls whereas the latter had much higher levels of these components than either the controls or the oat hull-fed birds.

In the last experiment of this series two additional oat components were checked for their possible cholesterol-lowering properties, oat starch and oat oil. The results (Table IV) showed a highly significant plasma cholesterol-lowering effect for ground oats, oat hulls, and dehulled oat flour. A very small but non-significant cholesterol-lowering effect was noted for oat oil. Oat starch gave values very similar to those observed with corn starch. The liver cholesterol and total lipids essentially followed the pattern indicated for plasma cholesterol, with ground oats and oat

TABLE IV. Effect of Oat Constituents on Plasma and Liver Cholesterol and on Fecal Lipid Excretion.

Dietary supplement	Body wt (g)*	Plasma cholesterol (mg %)	Liver cholesterol (% wet wt)	Liver lipid (% wet wt)	Fecal lipid (% dry wt)	Fecal sterol† (% dry wt)
Corn starch, 43%	317 ± 12‡	283 ± 20	1.7 ± .2	6.0 ± .4	7.2 ±1.0	1.2 ± .1
Ground oats, 43%	297 ± 12	184 ± 10	1.0 ± .1	5.3 ± .5	4.6 ± .1	.8 ± .2
Oat starch, 43%	309 ± 7	282 ± 29	1.9 ± .3	6.8 ± .5	5.9 ± .4	1.0 ± .0
Oat hulls, 15% + corn starch, 28%	292 ± 10	199 ± 12	.9 ± .1	4.9 ± .2	4.9 ± .1	.8 ± .0
Dehulled oat flour, 28% + corn starch, 15%	283 ± 10	210 ± 14	1.4 ± .2	5.9 ± .2	9.2 ± .4	1.2 ± .1
Oat oil, 2% + corn starch, 41%	321 ± 9	248 ± 20	2.0 ± .3	7.1 ± .4	8.6 ± .8	1.4 ± .1

* At 3 weeks; chicks started at one week of age on experimental diets; 12 chicks (duplicate lots of 6) per group.
† Lieberman-Burchard-positive.
‡ Mean ± S.E.

hulls showing very significant lowering effects in comparison with the corn starch controls, and the dehulled oat flour an intermediate lowering effect. The oat oil-fed birds had higher liver cholesterol and total liver lipid values than any other group. The results for the liver lipids given as % of wet weight (Table IV) show essentially the same pattern when calculated on an absolute basis, since body weights and liver weights were similar for the birds in the various groups. As in the earlier fecal analyses, the whole oats and the oat hulls produced lower lipid and Lieberman-Burchard-positive sterols than the corn starch controls, whereas the dehulled oat flour as well as the oat-oil-fed groups gave rise to values as high or higher than those observed with the control birds.

Discussion. These studies indicate a marked cholesterol-lowering effect from certain common cereals and suggest that in the case of oats much of the effective material may be in the form of complex carbohydrates other than starch. That the effect is not due to cellulose has been previously confirmed in our pectin studies, where cellulose was used as the control material(1). Results of an experiment not given here also showed that the protein content of oats did not contribute to the cholesterol-lowering action. Differences in blood cholesterol levels among experiments for similar treatment may be explained on the basis of differences in age at time of bleeding, as well as duration of experimental feeding.

In contrast with the results of De Groot *et al*(4), who studied what we have herein called a dehulled oat flour (rolled oats), we could not ascribe any cholesterol-lowering effect to the lipid component of the oats. At the same time the 15% oat hulls were more potent than double this amount of dehulled oats.

The cholesterol-lowering effect of oats did not result in increased lipid or sterol excretion, in contrast with the effects observed from the ingestion of pectin or scleroglucan(3). Furthermore, the plasma cholesterol-lowering effect did not result in accumulation of cholesterol or lipid in the liver, since the oat-fed birds also had significantly lower liver lipid and cholesterol levels (Table IV). The mechanism of the oat effect, therefore, appears to be different from that of pectin, which can, at least in part, be explained on the basis of interference with lipid and cholesterol absorption(1). It remains to be determined whether the active principle in oats might also be effective in lowering blood cholesterol levels of birds fed cholesterol-free diets. This aspect as well as consideration of possible mechanisms are currently under investagation.

Summary. The plasma cholesterol-lowering activity of whole ground oats and its components was studied in chicks fed hypercholesterolemic diets. Oat hulls were most effective whereas oat starch and oat oil had no cholesterol-lowering activity. Liver lipids and cholesterol were also significantly reduced by whole oats and oat hulls, and, to a lesser extent, by dehulled oats. In contrast to observations made with pectin or scleroglucan, fecal lipids and sterols were not increased by the feeding of whole oats or oat hulls.

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