The Effect of a Cholesterol-Free Brain Fraction against Diet-Induced Atherosclerosis


The feeding of a lipid-poor and virtually cholesterol-free residue of mammalian brain mitigates the hypercholesteremia and atherosclerosis ordinarily seen in cholesterol-fed chicks. The probable mechanism depends on the previously demonstrated capacity of oral cerebrosides to convert a large proportion of fecal sterols into unabsorbable coprosterol, thus rendering intestinal cholesterol unavailable to the body economy.

In a preliminary report from this laboratory, it was demonstrated that a crude lipid-poor residue of mammalian brain could mitigate the elevation of serum cholesterol and total lipids ordinarily seen in cholesterol-fed chicks. It seemed important to extend these observations and establish whether this material, used as a dietary supplement, could act over a more extended period to control the elevation of serum cholesterol and thereby the degree of atherosclerosis. The effect of varying dosage was also determined.

Method

Forty-eight white rock cockerels, 10 weeks of age with known dietary histories, were divided equally into four groups. The control group (I) was fed chicken mash mixed (in a Hobart mixer) with 8 per cent cottonseed oil and 1 per cent cholesterol by weight. Groups II, III, and IV obtained supplements of 5, 10, and 15 Gm. of crude lipid-poor brain powder per 100 Gm. of the control diet. These diets were continued for five weeks; each bird was weighed weekly and its growth curve charted. All diets were ad lib, 100 Gm. per bird per day of the control diet being offered, and, though spillage was difficult to estimate, the percentage of food that was eaten must have been approximately equal in each group as judged by their average weight gain.

The lipid-poor extract of brain was extracted repeatedly with acetone and then petroleum ether, as described previously. The final product still contained approximately 15 per cent ether-soluble material, 0.86 per cent cholesterol, and 0.33 per cent lipid phosphorus.

Blood samples for serum cholesterol determination were drawn from the alar vein at the end of the 24-hour feeding period before initiating the diets and again after two, three, and four weeks on the diet. At the end of the fifth week the chicks were exsanguinated, each animal providing enough blood for determination of total serum lipid and total serum cholesterol by the methods of Fiske-Subbarow. After exsanguination each animal was autopsied, special attention being given to the heart, aorta, major arteries, and liver. The aorta, iliac and subclavian arteries were opened and the degree of atherosclerosis grossly visible was estimated, each bird being graded on a scale of 0 to 4 +, as outlined by Horlick and Katz. Frozen sagittal and cross sections of the heart, as well as cross sections of descending thoracic aorta, abdominal aorta just above the bifurcation, and subclavian arteries were stained with sudan III and counterstained with hematoxylin. Coronary artery involvement was estimated by counting the number of coronary arteries visualized under low power, and noting those with lipid infiltration of the media and/or intima. Longitudinal section of vessels were included only if a lumen was flanked by the two more refractile media. All arterial layers were easily recognized in cross section. One sagittal section and one cross section of the heart usually provided a random selection of 15 to 20 coronary arteries of all sizes.

Results

During the third and fourth weeks of the experiment an epidemic of pneumonia caused the loss of several birds. All groups were affected equally, the controls faring no better than those on experimental diets. All the surviving birds gained weight in the last week of the experiment. Growth curves in all groups
were comparable so that it was felt that any effect of the illness on lipid metabolism must have been equally diffused throughout the four groups. Ten birds survived in groups I and III, and nine in groups II and IV. Autopsy yielded no evidence of disease in the birds that survived the experimental period.

Serum cholesterol was reduced below control values in rough proportion to the amount of brain extract fed. Figure 1 compares the effect on average serum cholesterol of the increasing doses of brain extract in the four groups. Individual values showed a greater tendency to overlapping between groups than in the earlier experiment, but this is probably explained by inconsistencies in the extraction of brain powder, or the intercurrent illness, rather than the employment of larger numbers of birds. Terminally, total lipids and lipid phosphorus reflected the differences seen in the cholesterol levels (table 1).

Gross autopsy findings showed atherosclerosis in all of the control group and lesions were progressively less severe, on the average, in proportion to the amount of supplemental brain extract fed (fig. 1). Fatty livers were present in all groups, though microscopically there was neither a qualitative nor a quantitative difference in the degree of fatty infiltration. There were no gross abnormalities of kidney, heart or other organs. Three birds in group I and two in group IV were found to be pullets.

In estimating the degree of atheromatous involvement of the aorta and its subdivisions, gross grading of the area involved appears to be more comprehensive and meaningful than microscopic, however subjective. Results of the gross grading (fig. 1) are more significant than those from a random section taken roughly at the same level in each aorta. It is gratifying, however, that microscopic grading was confirmatory of the gross impressions. Patchy subintimal infiltration was graded 1+, diffuse subintimal infiltration 2+, plaque formation 3+, and confluent or extensive plaque formation 4+. The results of this grading can be seen in table 2.

The coronary arteries showed definite intimal

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### Table 1.—Average Values* for Serum Lipid Fractions in Different Dietary Groups at the Termination of the Experiment

<table>
<thead>
<tr>
<th>Diet Fed per Bird per Day</th>
<th>Group</th>
<th>Number of Birds</th>
<th>Total Lipid (Mg./100 cc.)</th>
<th>Cholesterol (mg./100 cc.)</th>
<th>Lipid Phosphorus (mg./100 cc.)</th>
<th>Per cent Phospholipid</th>
<th>Cholesterol Lipid-Phosphorus Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mash 91%</td>
<td>I</td>
<td>10</td>
<td>1750 ± 198</td>
<td>804 ± 131</td>
<td>12.6 ± 1.1</td>
<td>18.5 ± 0.8</td>
<td>66 ± 6</td>
</tr>
<tr>
<td>Cottonseed oil 8%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol 1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% Brain extract per 100 gm. of control diet</td>
<td>II</td>
<td>9</td>
<td>1306 ± 221</td>
<td>655 ± 136</td>
<td>9.4 ± 1.7</td>
<td>19.2 ± 0.7</td>
<td>64 ± 5</td>
</tr>
<tr>
<td>10% Brain extract per 100 gm. of control diet</td>
<td>III</td>
<td>10</td>
<td>1290 ± 194</td>
<td>600 ± 100</td>
<td>10.8 ± 1.6</td>
<td>21.7 ± 0.8</td>
<td>54 ± 3</td>
</tr>
<tr>
<td>15% Brain extract per 100 gm. of control diet</td>
<td>IV</td>
<td>9</td>
<td>926 ± 194</td>
<td>379 ± 46</td>
<td>9.3 ± 0.5</td>
<td>26.0 ± 1.3</td>
<td>40 ± 2</td>
</tr>
</tbody>
</table>

*± Standard Error.

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![Atherosclerosis Gross Grading](image)

**Fig. 1.** The average serum cholesterol for each group during the period of observation. At the right are noted the results of grading the degree of gross atheromatous involvement of aortic and subclavian arteries on a scale of 0 to 4+.4
involvement in all birds. The earliest, and most extensive involvement was in the smaller ramifications of the coronary arteries, including the arterioles. It was sometimes difficult in the smaller arteries to determine whether the fat-staining material might be derived from a surface coating on the intima, a consequence of hyperlipemia, or actual accumulation in the endothelium. However, evaluation by two independent observers led to the same conclusion: In group IV the incidence of coronary involve-

**Table 2.—Microscopic Grading of the Large Arteries—Average Values**

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Aorta</th>
<th>Subclavian</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Thoracic</td>
<td>Abdominal</td>
</tr>
<tr>
<td>I</td>
<td>10</td>
<td>2.2</td>
<td>1.9</td>
</tr>
<tr>
<td>II</td>
<td>9</td>
<td>1.6</td>
<td>1.3</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>1.7</td>
<td>1.0</td>
</tr>
<tr>
<td>IV</td>
<td>9</td>
<td>0.8</td>
<td>0.7</td>
</tr>
</tbody>
</table>

* Each figure represents the average involvement based on grading the microscopic lesions on a 1+ to 4+ scale as outlined in the text.

**Table 3.—Microscopic Estimation of the Incidence of Coronary Artery Lipid Infiltration**

<table>
<thead>
<tr>
<th>Group</th>
<th>Sagittal Section</th>
<th>Cross Section</th>
<th>Total</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Arteries Involved</td>
<td>Number of Arteries Involved</td>
<td>Number of Arteries Seen</td>
<td>Number of Arteries Involved</td>
</tr>
<tr>
<td>I</td>
<td>109</td>
<td>91</td>
<td>102</td>
<td>143</td>
</tr>
<tr>
<td>II</td>
<td>88</td>
<td>47</td>
<td>109</td>
<td>89</td>
</tr>
<tr>
<td>III</td>
<td>111</td>
<td>53</td>
<td>118</td>
<td>58</td>
</tr>
<tr>
<td>IV</td>
<td>66</td>
<td>28</td>
<td>101</td>
<td>44</td>
</tr>
</tbody>
</table>

The original observations that led us to employ brain substance, in an effort to control the serum cholesterol, were those of Rosenheim and Webster. They demonstrated in rats that brain matter, especially the "white matter" of Thudichum, and particularly the cerebrosides, enhanced the intracolonic conversion of cholesterol to coprosterol. That this may be the mechanism responsible for the mitigating effect on serum cholesterol is suggested by unpublished evidence that feeding of brain powder or extracted cerebrosides lowers serum cholesterol and increases the percentage of saturated sterols in the feces; large doses of cerebrosides, for example, 5 per cent of the diet, show approximately the same activity as 10 per cent whole brain powder. However, the fact that brain powder extracted four times by hot ethanol is still active in lowering the dietary-elevated cholesterol level in individual chicks leads us to suspect that either there is another assisting mechanism, or a large amount of cerebroside is resistant to extraction by this means.

Insofar as the lipid-poor residue under study is predominantly protein (8.6 Gm. nitrogen per 100 Gm.), the effect may be due in part to the protein content. According to Wissler (personal communication) protein-deficient diets cause elevation of serum cholesterol levels in rats. Mellinkoff and co-workers demonstrated a striking fall in serum cholesterol levels of patients receiving a protein hydrolysate and maltose solution as the only diet. We have found that substitution of 10 per cent casein, instead of brain extract, in a 1 per cent cholesterol high-fat diet also significantly lowers serum cholesterol in chicks.

The possibility that our extraction of phrenosin from the dry brain powder was incomplete has not been excluded. The analysis for phrenosin depends upon its solubility in hot, but not cold, ethanol and quantitative acid hydrolysis. While the purity of the isolated material could be tested, the original crude brain powder could not be so analyzed with any confidence. Other compounds, such as strandin and gangliosides, are present in crude brain and may conceivably work in the same fashion as phrenosin to promote coprosterol formation. Thus the hypocholesteremic activity of crude brain powder cannot be explained by the extractable cerebrosides, yet in large enough quantities an impure phrenosin can be as active as the crude preparation. This means that either the phren-
osin is not released quantitatively by boiling ethanol, some other substance with similar properties is yet to be isolated, or the hypcholesteremic effect may be partly due to a different property, perhaps residing in the more bulky protein.

**Summary**

1. The mitigation of dietary-induced hypercholesteremia in cockerels by a lipid-poor residue of brain is confirmed.
2. The effect can be sustained for at least five weeks and will reduce atherogenesis in that period.
3. The degree of reduction in hypercholesteremia is roughly proportional to the dose of brain extract.
4. The active agent in brain powder is apparently a cerebroside, though an additional protein effect has not been excluded.

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**References**

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