Production of Hyperlipemia and Early Atherosclerosis in Rabbits by a High Vegetable Fat Diet

By Alfred Steiner, M.D. and Seymour Dayton, M.D.

Feeding of a high vegetable fat diet made up of ground peanuts and Purina chow to rabbits resulted in an increase in the serum cholesterol, as well as other serum lipids, including β-lipoprotein fraction. Microscopic lipid deposits in the intima and underlying media of the ascending and thoracic aorta were present in 10 of 33 rabbits at the end of 5 to 12 months. Small areas of gross atherosclerosis of the aorta were found in two rabbits.

There is considerable controversy as to the importance of dietary fat of vegetable origin in the maintenance of high serum lipid levels in man. Keys, Hildreth, Wilkinson and Page have reported that the ingestion of vegetable fat results in significant increase in the serum cholesterol levels. However, Kinsell and Ahrens have reported reductions in serum cholesterol and serum phospholipids, when plant fats are substituted for animal fats in the diet of man.

In studies on experimental animals it has not been generally possible to produce a significant elevation of serum lipids and resulting atherosclerosis, unless large amounts of cholesterol are added to the diet. The one exception is the production of hyperlipemia and premature atherosclerosis in chickens after the subcutaneous implantation of an estrogenic hormone. Previous attempts to produce hyperlipemia and experimental atherosclerosis by a high fat, low cholesterol diet in rabbits, dogs and chickens have been unsuccessful. The present study was designed to determine the effect of a diet rich in vegetable fat, but free of cholesterol upon the serum lipids and arteries of rabbits.

Method

Fifty-seven chinchilla rabbits, approximately 6 months of age, were used. The animals were about equally divided between the two sexes. The rabbits were kept indoors, in individual cages. Fourteen rabbits were designated as control animals and were kept on the stock diet of Ralston Purina chow. This feed is made from vegetable sources (free of animal sterol) and contains: crude protein 20 per cent, crude fat 2 per cent, crude fibre 18 per cent, nitrogen free extract 44 per cent. The remaining 43 animals were divided into three groups which were given the following diets: 20 animals designated as P 50 were fed a diet containing 50 per cent ground peanuts and 50 per cent Purina rabbit chow. Ten rabbits designated as P 50 M were fed approximately the same diet of 50 per cent ground peanuts and 50 per cent Purina rabbit chow to which 0.5 per cent d.l. methionine were added. The remaining 13 rabbits, P 75 group, were fed a diet of 75 per cent ground peanuts and 25 per cent Purina rabbit chow. The ground peanuts contain 53 per cent fat, by extraction and 27 per cent protein. The rabbits were fed from the ear veins at monthly intervals. Serum cholesterol content was determined by the method of Abell and associates, total lipid by a gravimetric procedure, and serum lipid phosphorus by the method of Fiske and Subbarow. Serum lipid phosphorus was converted to serum phospholipid by multiplying by the factor of 25.

Twenty-eight animals completed the experiment of 12-months duration. Twenty-nine animals died from the second to the eleventh month of the study. Thirty-three of the rabbits were examined at postmortem. Microscopic sections, prepared by paraffin embedding and stained with Hematoxylin and Eosin and frozen sections stained with Oil Red O, from the ascending and thoracic and abdominal portions of
each aorta were studied for deposition of lipid and evidence of atherosclerosis.

**RESULTS**

The serum lipid values for the control animals were fairly constant during the period of observation, averaging 53 mg. per cent for total serum cholesterol and 112 mg per cent for serum phospholipid with a C/P ratio of 0.47. The total serum lipid average was 0.45 Gm./per cent.

The serum lipid values of the animals on the P 50, P 50 M and P 75 diets increased gradually. However, this response was different in each of the three groups. The animals on the P 75 diet exhibited a prompt and sharp increase in the serum lipid values within 4 to 8 weeks, while the animals on the P 50 and P 50 M diets showed a lag of 4 to 6 months before the serum lipid values increased significantly. All of the serum lipid components tended to increase concomitantly as a group. The C/P ratio became greater as the serum lipid values mounted, indicating that the relative increase in serum phospholipids did not keep pace with the increase in serum cholesterol.

At the end of four months on the diet the average serum cholesterol of the P 75 rabbits had increased from 52 to 148 mg., the average serum phospholipids from 102 to 153 mg., the average C/P ratio from 0.51 to 0.97, the average serum neutral fat from 50 to 269 mg., and the average total lipid from 0.44 to 0.84 Gm. At the end of nine months, the average values were as follows: serum cholesterol 207 mg., serum phospholipid 242 mg., C/P 1.10, neutral fat 315 mg., and total lipid 1.16 Gm.

The rabbits on the P 50 M diet showed the least rise in serum lipids; the average serum cholesterol values after 4 months increased from 44 to 73 mg. per cent, serum phospholipid from 98 to 118 mg. per cent, C/P ratio from 0.45 to 0.62, neutral fat from 66 to 112 mg. per cent and total lipid from 0.43 Gm. to 0.54 Gm. At the end of 12 months on the P 50 M diet, the average values were as follows: serum cholesterol 89 mg. per cent, serum phospholipid 125 mg. per cent, C/P ratio 0.71, neutral fat 171 mg. per cent and total lipid 0.63 Gm.

Although there is a difference in the response of the animals to the P 50 and P 50 M diets, it is not possible to evaluate its significance because of the small number of animals in this study.

Figure 1 shows a comparison of the average values for serum cholesterol for all animals of each group and figure 2 compares the C/P ratio of each group. These charts show that the P 75 diet has the greatest effect upon serum lipids, with the P 50 and P 50 M diets less.

Figure 3 portrays the regression curves of the serum phospholipid plotted against the serum cholesterol in the three groups of rabbits on the P 50, P 50 M and P 75 diets. In addition it shows the regression curve of man and that of rabbits fed cholesterol. It can be seen that the regression curve of the rabbits on the diets of ground peanuts approaches that found in humans, while the regression curve of rabbits fed cholesterol is lower. The serum lipid pattern of rabbits fed ground peanuts assumes the characteristics of that of humans.

The sera of six rabbits, three on the stock diet, and three on the peanut diets have been studied by paper electrophoresis. The results reveal that the β-lipoprotein fraction was quantitatively far greater in the animals on the peanut diet and that rate migration of the β-lipoprotein fraction was also increased, as compared to the rabbits on the stock diet. There was no similar alteration of the α-lipoprotein in these animals.

**AUTOPSY STUDIES**

Twenty-eight of the 57 animals completed the 12 month study, including 12 control.
### Table 1.—Effect of High Vegetable Fat Diet on Serum Lipids of Rabbits

<table>
<thead>
<tr>
<th>Diet</th>
<th>Control</th>
<th>4 Months</th>
<th>6 Months</th>
<th>9 Months</th>
<th>12 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Average</td>
<td>Range</td>
<td>Average</td>
<td>Range</td>
</tr>
<tr>
<td>P50M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser. Phosphol.</td>
<td>52-135</td>
<td>98</td>
<td>50-210</td>
<td>118</td>
<td>42-190</td>
</tr>
<tr>
<td>C/P Ratio</td>
<td>0.27-0.78</td>
<td>0.45</td>
<td>0.45-1.06</td>
<td>0.62</td>
<td>0.45-1.07</td>
</tr>
<tr>
<td>Neutral fat</td>
<td>20-244</td>
<td>66</td>
<td>0-318</td>
<td>112</td>
<td>18-287</td>
</tr>
<tr>
<td>Total lipid</td>
<td>0.21-0.01</td>
<td>0.43</td>
<td>0.29-1.00</td>
<td>0.54</td>
<td>0.23-0.61</td>
</tr>
<tr>
<td>P50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser. Chol.</td>
<td>19-83</td>
<td>50</td>
<td>31-283</td>
<td>96</td>
<td>24-433</td>
</tr>
<tr>
<td>C/P Ratio</td>
<td>0.26-1.12</td>
<td>0.54</td>
<td>0.55-1.12</td>
<td>0.73</td>
<td>0.43-1.09</td>
</tr>
<tr>
<td>Neutral fat</td>
<td>6-225</td>
<td>61</td>
<td>0-380</td>
<td>74</td>
<td>123-340</td>
</tr>
<tr>
<td>Total lipid</td>
<td>0.24-1.10</td>
<td>0.43</td>
<td>0.26-1.34</td>
<td>0.55</td>
<td>0.29-0.94</td>
</tr>
<tr>
<td>P75</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser. Chol.</td>
<td>18-100</td>
<td>52</td>
<td>63-264</td>
<td>148</td>
<td>63-286</td>
</tr>
<tr>
<td>Ser. Phosphol.</td>
<td>55-165</td>
<td>102</td>
<td>75-290</td>
<td>153</td>
<td>55-300</td>
</tr>
<tr>
<td>C/P Ratio</td>
<td>0.32-0.56</td>
<td>0.51</td>
<td>0.72-1.24</td>
<td>0.97</td>
<td>0.94-1.29</td>
</tr>
<tr>
<td>Neutral fat</td>
<td>0-219</td>
<td>50</td>
<td>35-934</td>
<td>269</td>
<td>0-745</td>
</tr>
<tr>
<td>Total lipid</td>
<td>0.31-0.67</td>
<td>0.44</td>
<td>0.44-1.72</td>
<td>0.84</td>
<td>0.36-1.52</td>
</tr>
</tbody>
</table>

Serum cholesterol, serum phospholipid and neutral fat expressed as mg./100 cc.
Total lipid as Gm./100 cc.
P50M Diet containing 50 per cent ground peanuts and 0.5 per cent methionine.
P50 Diet containing 50 per cent ground peanuts.
P75 Diet containing 75 per cent ground peanuts.

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**Fig. 1.** Comparison of average serum cholesterol values of all rabbits on the P 50, P 50 M and P 75 diets. Ordinate, serum cholesterol mg. per cent; abscissa, figure in months.

**Fig. 2.** Comparison of the average C/P ratios of all rabbits on the P 50, P 50 M and P 75 diets. Ordinate, C/P ratios; abscissa, figures in months.

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rabbits, nine on the P 50 diet, seven on the P 50 M diet, and none of the animals on the P 75 diet. Twenty-nine animals failed to survive the entire experiment and either died or were killed by air injection from the third through the eleventh month, or died because of broken backs occurring during the monthly bleeding or from intercurrent infection. All of the 13 animals on the P 75 diet failed to complete the study, and only three survived up to the tenth month. During the latter half of the study the animals on the P 75 diet ate poorly and lost weight.

Control Group: Eight of 14 rabbits in the control group were studied at postmortem examination. In none of the rabbits was gross or microscopic evidence of atherosclerosis present. The spontaneous medial calcification of rabbits was observed in three animals.
Relationship between serum phospholipid and cholesterol in cholesterol and high vegetable fat fed rabbits and in man. Ordinate, serum phospholipid mg. per cent; abscissa, serum cholesterol mg. per cent.

**P 50 M Group:** Nine of 10 animals of this group were examined at post mortem. On microscopic section of the ascending and thoracic aorta in one rabbit, lipid infiltration in the intima and in the underlying media were present. The sudanophilic material was deposited in the form of streaks. No proliferation of cellular elements was present. These lesions were present in rabbit 22 whose serum lipids demonstrated the greatest increase of the group.

**P 50 Group:** Sixteen of 20 animals in this group were examined at post mortem. In six of the 16 animals studied by microscopic section, lipid infiltration of the intima and underlying media was present. In addition to the lipid infiltration, proliferation changes were present in the intima in two animals. In one of these animals, a gross atherosclerotic plaque was visible in the ascending aorta. On microscopic examination, this was a typical atheromatous plaque with cellular proliferation. It contained an abundant amount of lipid and was sharply delineated by the internal elastica. Lipid also appeared in the underlying media. The lesions could be correlated with the elevation of the serum lipids. Five animals that were free of lesions failed to survive six months of the study.

**P 75 Group:** Eight of 13 animals of the P 75 group were autopsied. In three of the 8 animals, definite lipid infiltration of the ascending and thoracic aorta was present on microscopic examination. In one of the animals, lesions were present after eight months on the diet and in another, infiltration of the intima in the region of the sinus of Valsalva after only five months on the diet. In one animal that survived 10 months, a gross atherosclerotic plaque was found in the arch of the aorta.

**Discussion**

The above results reveal that the serum lipid levels of rabbits can be significantly elevated, following the ingestion of diets of ground peanuts and rabbit chow. These diets are rich in vegetable fat, containing 20 or 40 per cent, and free of animal sterol. A certain proportion of the ingested fat is probably metabolized to cholesterol by the rabbits. However, the conversion to phospholipid does not keep pace with the cholesterol resulting in an increase in the C/P ratio. The C/P ratio of the rabbits on a diet of ground peanuts does not increase as much as the C/P ratio of the rabbits fed cholesterol. Likewise, the degree of lipid deposition in the arteries is less in the rabbits fed ground peanuts than in rabbits on a high cholesterol diet.

A lag of 4 to 6 months may occur before appreciable increase in serum lipids is found in animals on the P 50 or the P 50 M diets. The arterial lesions produced by the diet of ground peanuts can not be distinguished from the early lesions of rabbits fed diets rich in cholesterol. These studies again raise the question whether fat of vegetable origin may possibly play a role in the genesis of atherosclerosis in human beings.

**Summary**

The results of feeding high vegetable fat diets composed of ground peanuts, containing 53 per cent fat by extraction and purina chow (prepared from vegetable sources) to rabbits were:

- An increase in serum cholesterol, serum phospholipid, total serum lipid and C/P ratio.
- An increase in the β-lipoprotein fraction, as determined by paper electrophoresis.
- Lipid deposits in the intima and underlying media of the ascending and thoracic aorta in 10 of 33 rabbits at the end of 5 to 12 months.
Small areas of gross aortic atherosclerosis developed in the aorta in two rabbits.

ACKNOWLEDGMENT

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SUMMARY IN INTERLINGUA

Le sequente resultatos esseva obtenite per mantener conilios super un dieta ric in grassia vegetal, consistente de arachides molite (con un contento extrahibile de 53 pro cento de oleo) e purina (preparate ab materiales vegetal):

Un augmento del cholesterol serai, del phos- phospholipide serai, del lipide serai total, e del proportion C/P;

Un augmento del fraction lipoproteinic beta, determinate per electrophorese a papiro;

Depositos de lipide in le intima e le sub- jacente media del aorta ascendente e thoracic in 10 inter 33 conilios al fin de periodos de inter 5 e 12 menses.

Parve areas de grossier atherosclerosis aortic in le aorta de duo conilios.

REFERENCES

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