Effects of Dietary Protein and Carbohydrate Level on Cholesterolemia and Atherogenesis in Cockerels on a High-Fat, High-Cholesterol Mash

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Decreased protein intake in the presence of a high-fat, high-cholesterol diet was found to produce marked aggravation of hypercholesterolemia and atherogenesis in cockerels. Sucrose was substituted for protein in this low protein diet. The effect of this diet is not due to sucrose since restoring the protein to a high level, while keeping the sucrose in the ration constant, had no such effect.

Evidence has been presented suggesting that reduction of dietary protein may be associated with enhanced atherogenesis in cockerels on a high-fat, high-cholesterol regimen. The findings on this aspect of the foregoing study were not clearcut, possibly because protein in the ration was only moderately lowered, from 35 to 20 per cent of the diet by weight.

This problem was therefore explored further in the present experiment. An analysis was also made of the effects of incorporation of refined carbohydrate in a high-fat, high-cholesterol mash.

Methods

The methods were those detailed in the previous report. In essence, the design involved combining sucrose (20 and 45 per cent) with commercial chick starter mash containing cholesterol + cottonseed oil (table 1). As a result, the protein content of the ration was lowered from its usual 20 to 22 per cent by weight and by calories to 16 to 10 per cent. In 2 other groups, this protein deficit was corrected by supplementation with soy protein. Vitamins and minerals were added to the ration of the 2 experimental groups receiving soy protein, in order to make up for the deficits resulting from reduction of mash intake. The vitamin and mineral mixtures were those detailed in the previous report. Chicks were on these experimental regimens for 5 weeks, during the age period 9 to 14 weeks.

Results and Discussion

Reduction of protein intake resulted in markedly intensified hypercholesterolemia.
and atherogenesis in both the aorta and coronary vessels (groups 2 and 3, table 2). In contrast, restoration of protein intake by addition of soy protein, with continued inclusion of 45 to 20 per cent sucrose in the mash, prevented this intensified hypercholesterolemia and atherogenesis (groups 4 and 5, table 2).

These findings supplement those of the previous report demonstrating a significant effect of dietary protein level on hypercholesterolemia and atherogenesis in chicks on a high-fat, high-cholesterol ration. They clearly demonstrate that reduction of protein intake to 10 to 16 per cent of total feed intake (by weight and by calories) grossly aggravated the hypercholesterolemic and atherogenic effects of a high-fat, high-cholesterol diet. Thus, these observations lend animal-experimental support to the imbalance hypothesis suggested in the introduction to the previous paper, i.e., that excess of some nutrients (e.g., fat, cholesterol) and inadequacy of others (e.g., protein) may be a particularly pernicious dietary combination for the production of hypercholesterolemia and atherosclerosis.

The data of the present experiment further indicate that sucrose was not deleterious per se. Rather, the effects observed with incorporation of 20 to 45 per cent sucrose in the diet were due to the consequent reduction in protein intake. They did not occur with sucrose when this curtailment of dietary protein was avoided.

These experimental findings pose pointed questions concerning possible relationships among dietary amino acids, proteins, lipids, cholesterolemia and atherogenesis in man. Obviously, these observations in chicks cannot be directly applied to man. They can merely lead to the posing of problems for human research. Only work in man can elucidate whether the findings in chicks have any human parallel. One recent report tends to cast doubt upon the applicability of these findings to man. It was found that varying protein intake (at the expense of carbohydrate) from 8.4 to 17.9 per cent of total calories (63.2 to 138.7 Gm.) had no effect on serum cholesterol levels during 4 weeks in men on an isocaloric diet with 18.9 to 20.5 per cent of calories from fat (65.5 to 68.9 Gm.) and low (374 to 477 mg.) or high (1369 to 1436 mg.) cholesterol intakes. This study clearly involved a relatively low fat intake. Despite this one negative report, certain facts concerning dietary patterns in the United States suggest that this matter merits further exploration: The 1955 Department of Agriculture household surveys indicate that for the average American the foods available in the kitchen for daily consumption yield 3200 calories, 103 Gm. of protein (13 per cent of total calories), 155 Gm. of fat (44 per cent of total calories), 66 Gm. of saturated fat derived from meat, eggs, milk products, lard, shortenings (hydrogenated vegetable oils). Cholesterol is available in amounts ranging from 400 to 1600 mg./person/day, averaging about 800 mg. or more. Of course, these data do not constitute an estimate of foods actually consumed, only foods available for consumption—waste is

### Table 2.—Effects of Experimental Diets

<table>
<thead>
<tr>
<th>Group</th>
<th>Feed intake (Gm./chick/day)</th>
<th>Terminal weight of birds (Gm.)</th>
<th>Serum cholesterol (mg. %)</th>
<th>Thoracic aorta atherogenesis</th>
<th>Coronary atherogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Incidence (%)</td>
<td>Grade</td>
</tr>
<tr>
<td>1</td>
<td>121</td>
<td>1451 ± 18*</td>
<td>663 ± 83</td>
<td>70</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>89</td>
<td>1371 ± 111</td>
<td>1621 ± 118</td>
<td>100</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>123</td>
<td>1463 ± 40</td>
<td>1285 ± 83</td>
<td>88</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>91</td>
<td>1465 ± 68</td>
<td>122 ± 107</td>
<td>45</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>5</td>
<td>116</td>
<td>1411 ± 42</td>
<td>690 ± 90</td>
<td>75</td>
<td>1.5 ± 0.2</td>
</tr>
</tbody>
</table>

* Standard error of the mean.
not evaluated. Nevertheless, together with
the results of numerous other studies yield-
ing similar findings, they give a generally
valid picture of over-all dietary patterns
in the United States at midcentury.

They indicate that high-calorie, high-fat,
high-saturated fat, high-cholesterol diets are
commonplace. The detailed data of the 1955
surveys—including the dietary data on sub-
groups stratified on a regional, urban-rural
and income basis—ample confirm this con-
clusion. Thus, in all subgroups, only a small
percentage of the population was found to
have available a diet yielding less than 36
per cent of total calories from fat. The vast
majority had foodstuffs yielding over 40 per
cent of calories from fat, predominantly ani-
mal fat of high saturation and high choles-
terol content.

These American diets with their generally
high levels of meat, poultry, fish, eggs, milk
products also tend to be generally high in
protein. Thus, the average figure of 103 6m.
of protein/person/day available for consump-
tion, with animal protein constituting a large
percentage of the total, is above that for the
people of most other countries. Nevertheless,
because of the concomitant high intake of
fats and carbohydrates, protein supplies only
about 13 per cent of total calories in contem-
porary American diets. This value is not
higher than that for most other countries,
and is in fact lower than that for several.

Research findings from many sources indi-
cate that the need for essential nutrients in-
cluding essential amino acids, is, among other
things, a function of the intake of calories,
fats and carbohydrates. Based on this fact
and the foregoing data, it would seem valid
to pose the question: Are present-day levels
of protein in American diets optimal, particu-
larly in relationship to the high intakes of
calories, fats, saturated fats, carbohydrates,
and refined carbohydrates? In view of the
findings of the chick experiments reported,
this problem cannot be considered settled, but
merits continued exploration.

Further, although the 1955 surveys re-
vealed increases in protein available to Amer-
icans, especially in comparison with the de-
pression year 1936, and even compared to
1948, it was still observed in 1955 that a
significant percentage of the population had
an inadequate amount of protein (based on
National Research Council standards) avail-
able for consumption. Thus, the 1955 surveys
revealed that 8 per cent of the population
had less than the N.R.C. recommended protein
allowance. The figure was considerably
higher—14 to 23 per cent—for low-income
persons in urban and rural nonfarm com-
munities. With respect to those low-income
persons, the dietary survey data further indi-
cated that total calories, total fats and
saturated fats were available in large
amounts, approaching those for higher-income
individuals.

It may be suggested, therefore, that at
least among a segment of low-income Ameri-
cans there is prevalent a unique pattern of
dietary imbalance which may be character-
ized as a tendency to excessive intake of total
calories, total fats, saturated fats, cholesterol,
and inadequate intake of certain essential nu-
trients, including protein.

Based on presently available data, it is not
possible to assess whether such patterns of
dietary imbalance do in fact result in an ag-
gravation of tendencies to hypercholesterol-
emia and atherogenesis in low-income Ameri-
cans. Further work is needed on this matter.
However, considerable data are available
indicating that for the middle-aged white
male population of the urban North, mean
serum cholesterol levels and incidence rates
of coronary heart disease tend to be high in
all subgroups, including low-income manual
workers.

It is essential that the precise nature of
this imbalance hypothesis be clearly under-
stood. It suggests that when intake of cal-
ories, fats, saturated fats and cholesterol is
high, the level of protein intake may be one
factor influencing cholesterolemia and athe-
rogenesis. The significance of this may be
clarified by reference to the oft-cited fact
that among peoples with low protein intake,
like the South African Bantu, levels of cho-
Lesterolemia and occurrence rates of atherosclerotic disease are significantly lower than among Americans. This fact is frequently mentioned to buttress the conclusion that low protein intake cannot be a factor contributing to hypercholesterolemia and atherosclerosis and that the opposite may rather be the case, i.e., that low protein is protective. It should be emphasized, however, that the diets of these economically less developed peoples, including the South African Bantu, are not only low in protein, they are also low in total fats, saturated fats and cholesterol. Therefore, findings in these peoples do not constitute a test of the imbalance hypothesis that a high-calorie, high-fat, high-saturated fat, high-cholesterol intake combined with relatively low protein may be particularly pernicious in inducing hypercholesterolemia and atherosclerosis. This nutritional pattern of imbalance almost certainly is never present among the broad strata of the populations of the economically less developed countries. It is confined to strata of the economically more developed countries, especially the United States.

It should again be emphasized that this concept is being advanced as an hypothesis, to be tested in further work.

Finally, one additional comment is appropriate concerning the findings of the chick experiments: In the studies with high-protein, high-vitamin supplementation, a level of protein intake was given that far exceeded any consumed by present-day man (with the possible exception of the Eskimo subsisting on his pre-acclu-
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