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 clear-cut, 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 cockerels on a high-fat, high-cholesterol mash.

**Table 1.—Experimental Design**

<table>
<thead>
<tr>
<th>Group</th>
<th>Type group</th>
<th>Mash</th>
<th>Sucrose</th>
<th>Soy protein</th>
<th>Calories from protein as per cent of total calories</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control†</td>
<td>941</td>
<td>—</td>
<td>—</td>
<td>21.6%</td>
</tr>
<tr>
<td>2</td>
<td>Sucrose—45%</td>
<td>49</td>
<td>45</td>
<td>—</td>
<td>10.2%</td>
</tr>
<tr>
<td>3</td>
<td>Sucrose—20%</td>
<td>74</td>
<td>20</td>
<td>—</td>
<td>16.1%</td>
</tr>
<tr>
<td>4</td>
<td>Sucrose—45%</td>
<td>31</td>
<td>45</td>
<td>14</td>
<td>21.0%</td>
</tr>
<tr>
<td>5</td>
<td>Sucrose—20%</td>
<td>65</td>
<td>20</td>
<td>7</td>
<td>21.6%</td>
</tr>
</tbody>
</table>

* All groups received 5 per cent cottonseed oil + 1 per cent cholesterol (1 C–O); groups 4 and 5 received proportional vitamin and salt supplements to replace the nutrients missing from the ration because of incorporation of pure soy protein at the expense of mash.
† 1 C–O control contains approximately 20 per cent protein by weight.
‡ All values are grams per 100 Gm. of mash.

From the Cardiovascular Department, Medical Research Institute, Michael Reese Hospital, Chicago, Ill. This department is supported by the Michael Reese Research Foundation.

Supported by grant no. H-2276 from the National Heart Institute and a grant from the Chicago Heart Association.

Reported initially before the Midwest Section of the American Federation for Clinical Research, October 31, 1957.

Work done during Dr. Stamler's tenure of an Established Investigatorship of the American Heart Association.

Received for publication February 26, 1958.
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>1453 ± 40</td>
<td>1283 ± 83</td>
<td>88</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>91</td>
<td>1465 ± 68</td>
<td>522 ± 107</td>
<td>45</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>5</td>
<td>116</td>
<td>1411 ± 42</td>
<td>680 ± 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 yielding 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 subgroups stratified on a regional, urban-rural and income basis—amply confirm this conclusion. 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 animal fat of high saturation and high cholesterol 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 gms. of protein/person/day available for consumption, 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 contemporary 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 indicate that the need for essential nutrients including 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, particularly 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 revealed increases in protein available to Americans, especially in comparison with the depression 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) available 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 communities. With respect to those low-income persons, the dietary survey data further indicated 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 Americans there is prevalent a unique pattern of dietary imbalance which may be characterized as a tendency to excessive intake of total calories, total fats, saturated fats, cholesterol, and inadequate intake of certain essential nutrients, 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 aggravation of tendencies to hypercholesterolemia and atherogenesis in low-income Americans. 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 understood. It suggests that when intake of calories, fats, saturated fats and cholesterol is high, the level of protein intake may be one factor influencing cholesterolemia and atherogenesis. 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 atherogenesis 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-accleration diet). These findings, therefore, clearly have no meaningful parallel in human experience.

The situation is different with respect to the findings in the present paper. Here incorporation of refined carbohydrate in the ration was used to reduce protein intake from approximately 20 per cent (by weight and by calories) to 10 to 16 per cent in a diet with a high content of fat and cholesterol. Marked intensification of hypercholesterolemia and atherogenesis was observed in the growing chicks. The experimental diet had an overall composition not dissimilar from that of contemporary American diets except for a difference in fat quality (degree of unsaturation) and in cholesterol content.

It would therefore seem appropriate to conclude, particularly in view of these latter animal-experimental findings, that further work in man is in order to test the imbalance hypothesis that relative inadequacy of protein intake may—when the diet is concomitantly high in total calories, total fats, saturated fats, cholesterol—aggravate hypercholesterolemia and atherogenesis.

**Summary**

Reduction of protein intake from approximately 20 to 10 to 16 per cent, by addition of sucrose to a high-fat, high-cholesterol mash, markedly aggravated hypercholesterolemia and atherogenesis in cockerels.

When protein intake was kept at the 20 per cent level by addition of soy protein to the high-sucrose, high-fat, high-cholesterol ration, intensified hypercholesterolemia and atherogenesis did not occur.

**Acknowledgments**

It is a pleasure to acknowledge the contribution of the technical members of the department's atherosclerosis research team, Miss Mildred Michael, Mrs. Eva Miller, Mrs. Charlene Thompson, Mrs. Montez Vankinscott and Mr. Grady Crowley.

**Summario in Interlingua**

Le reduction del ingestio de proteina ab circa 20 pro cento a inter 10 e 16 pro cento per le inclusion de sucrosa in un dieta ric in grassia e in cholesterol resultava in un marcate aggravation de hypercholesterolemia e de atherogenese in gallettos.

Quando le ingestio de proteina eseva mantenite al nivello de 20 pro cento per adder proteina de soja al dieta ric in sucrosa, in grassia, e in cholesterol, le intensification de hypercholesterolemia e de atherogenese non occurreva.
REFERENCES


Effects of Dietary Protein and Carbohydrate Level on Cholesterolemia and Atherogenesis in Cockerels on a High-Fat, High-Cholesterol Mash
JEREMIAH STAME, RUTH PICK and LOUIS N. KATZ

doi: 10.1161/01.RES.6.4.447

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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