S T U D I E S of the influence of diet on body metabolism have dealt with dietary composition for the most part. Recent evidence has suggested, however, that the daily pattern of ingestion of the diet also may play a significant role in the regulation of overall body metabolism. The manner in which the organism handles ingested foodstuffs is dependent, along with other factors, on whether the diet is eaten as spaced full meals or as frequent small feedings. Force-fed animals or animals trained to eat their food in a limited time period demonstrate increased quantities of body fat, decreased body protein, increased hepatic and fat hexosemonophosphate shunt activity, and decreased thyroid activity, compared with those eating the same diet ad libitum. These differences may be secondary to the rate of influx of the calories, causing alterations of tissue enzymatic activities and differences in the use of alternate enzymatic pathways when multiple pathways are available.

The present report deals with the relationship of eating habits to the pathogenesis of atherosclerosis, and forms one other phase of our studies on the influence of the daily pattern of diet ingestion on body metabolism. The great frequency of clinical atherosclerosis in Western culture may depend in part on the daily pattern of eating. Civilized man has, by habit, custom, and working conditions, become a meal eater. In order to test whether this mode of eating plays a significant role in the development and regression of atherosclerosis, comparable groups of chickens were given various diets conducive to the production or regression of lesions, either at discrete intervals or ad libitum. The results of this study indicate that the manner of eating does affect both the production and regression of experimental atherosclerosis, as well as the cholesterol level of the blood.

Methods

The Hy-Line, series 935, male chickens used in the experiments were received when one day old, were reared in battery brooders, and were fed a chick starter mash until eight to ten weeks of age. The subsequent handling of the birds was as follows:

Experiments on Production (Induction) of Atherosclerosis

Experiment 1

The diet employed contained 20 per cent protein, 5 per cent cottonseed oil, and 0.5 per cent cholesterol. At the start of the experimental period, the chickens were divided into two groups: (a) "nibblers" (10 birds)—allowed continued access to the diet 24 hours per day; and (b) "meal eaters" (10 birds)—given food for one hour in the morning (8 to 9 a.m.) and one hour in the afternoon (4 to 5 p.m.). All birds had access to water at all times. After five weeks of feeding in this fashion, the animals were bled in the morning, the nibblers having eaten all night and the meal eaters having had their last food approximately 18 hours previously. The aortae were examined at necropsy for gross atherosclerotic lesions, graded 0 to 4 +, and the coronaries evaluated for microscopic plaques by previously described tech-
Table 1
Serum Lipid Values of "Nibbling" and "Meal-eating" Chickens Fed a 0.5 Per Cent Cholesterol, 5 Per Cent Cottonseed Oil, 20 Per Cent Protein Diet

<table>
<thead>
<tr>
<th>Manner of feeding</th>
<th>Cholesterol mg. per cent</th>
<th>Phospholipids mg. per cent</th>
<th>Esterified fatty acids mg. per cent</th>
<th>Cholesterol/Phospholipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nibbling (9)</td>
<td>294 ± 19</td>
<td>294 ± 17</td>
<td>397 ± 19</td>
<td>1.02 ± 0.05</td>
</tr>
<tr>
<td>Meal-eating (9)</td>
<td>584 ± 43</td>
<td>396 ± 29</td>
<td>648 ± 44</td>
<td>1.49 ± 0.09</td>
</tr>
</tbody>
</table>

The number in parentheses refers to the number of chicken/group.

The results are the means ± standard errors.

The bloods were drawn in the a.m., after the nibblers had eaten all night, and 18 hours after the meal eaters had consumed their last meal.

The procedure was essentially similar to experiment 1 with the following differences: The experiment was continued for seven weeks, the diet contained 20 per cent protein, 5 per cent cottonseed oil, and 0.3 per cent cholesterol in the first 2 1/2 weeks and 0.4 per cent in the last 4 1/2 weeks. Sixteen nibbling and 15 meal-eating birds were studied.

Experiment 3

Thirty-four chickens (eight weeks old) were made to eat a diet consisting of 20 per cent protein, 5 per cent cottonseed oil, and 1 per cent cholesterol twice a day (meal eating) for five weeks in order to produce aortic and coronary atherosclerosis. At the end of this induction period, 10 birds were bled for serum cholesterol levels and sacrificed; the coronary arteries were examined histologically as a "base-line" for subsequent studies. The surviving chickens were then placed on a regular mash, nonatherogenic diet; 12 were permitted to revert to the nibbling state, while the other 12 were continued as meal eaters. The chickens were kept on the regular mash diet for two weeks. Morning bloods were drawn, before the meal eater's breakfast, on the seventh, eleventh, and fourteenth days. At the end of two weeks, the remaining animals were sacrificed and handled as those described previously. The evaluation of regression was confined to the findings in the coronary arteries, since previous experience has indicated that the regression of aortic lesions is much slower, and so incapable of being evaluated in this two-week period.

Experiment 4

The general plan of this experiment was similar to experiment 3. For a period of five weeks, the birds were divided into three groups: 60 chickens eating their food ad libitum, 95 eating two times/day, and 98 eating three times/day (8 to 9 a.m., 12 to 1 p.m., and 4 to 5 p.m.) were fed the diet described in experiment 3. At the end of this induction period, 20 nibbling, 17 two-meal-eating, and 20 three-meal-eating birds were bled and autopsied to serve as a "base-line." The surviving birds were then placed on the regular mash, nonatherogenic diet, with the nibblers continuing as nibblers and the meal eaters being divided into two groups, one group continuing to meal eat and the other half being allowed to revert to nibbling. Half of each group was autopsied for evaluation of coronary artery atherosclerosis at the end of two weeks, the other half at the end of three weeks. Blood for serum cholesterol values was obtained just before death of the birds.

Results

Effect of Meal Eating Versus Nibbling on Production of Experimental Atherosclerosis

The data in experiments 1 and 2 were similar; therefore, only the results of the former are presented. Table 1 and figures 1 and 2 show that daily pattern of ingestion of the diet plays a role in the induction of experimental atherosclerosis. Figure 1 shows that over the five-week period employed, the birds offered food for one hour twice a day consumed almost one-third less food and gained less in body weight than the birds eating ad libitum. Meal eating, as contrasted to nibbling, was associated with increased serum cholesterol and lipid concentrations (table 1) and with a greater incidence and a greater severity of gross thoracic aorta atherosclerotic lesions (fig. 2). In addition, both the incidence (two times) and involvement (seven times) of microscopic coronary atherosclerosis were significantly greater in the meal-eating birds.
The results reported here indicate that under the experimental conditions meal eating, as contrasted to nibbling, accentuates the development of hypercholesterolemia and experimental atherosclerosis.


discussion

The results reported here indicate that under the experimental conditions, meal eating, as contrasted to nibbling, accentuates the development of and retards the regression of hypercholesterolemia and experimental atherosclerosis.

In the induction experiments, the greater elevation in the serum cholesterol levels and the enhanced atherosclerosis observed in the meal eaters (experiment 4; table 2). However, at the end of the third week, both groups showed evidence of rapid lesion regression.

Discussion

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In the induction experiments, the greater elevation in the serum cholesterol levels and the enhanced atherosclerosis observed in the meal eaters were observed to regress rapidly both at the end of the two- and three-week periods (fig. 4; table 2). By contrast, when the diet was ingested as meals, regression was markedly retarded. In two groups (nibbler—>nibbler, and three-meal eater—>nibbler), the lesions induced by the atherogenic diet were so severe that only moderate regression was observed in the two-week period after reversion to a regular mash diet (experiment 4; table 2). However, at the end of the third week, both groups showed evidence of rapid lesion regression.

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meal-eating chickens occurred despite a diminished intake of food (and cholesterol). Because blood samples for chemical analyses were collected when the meal eaters were in a fasting state, whereas the nibblers could be considered postprandial, serial determinations of serum cholesterol were obtained during the course of a typical day in each group. Little change in cholesterol concentration was noted, regardless of whether the chickens nibbled or consumed their food as meals.

In the regression experiments, the induction period was designed with the birds meal eating for three reasons: (a) in order to adapt the eating habits of the birds to this manner of eating, since it has been our experience that training older birds to consume adequate quantities of diet in the relatively short time period employed is difficult and leads to a poor caloric intake; (b) in order to adapt intermediary enzymatic machinery to this manner of eating; (c) in order to simulate the usual eating habits of civilized man.

During the regression period, the birds allowed to revert back to the nibbling state cleared their coronary arteries of lesions and their sera of the excess cholesterol at a much faster rate than the birds which continued as meal eaters. This finding was true irrespective of whether the birds ate two or three times/day. At the end of two weeks of regression, there was already a marked difference in the rate of clearing of blood cholesterol and coronary atheromas between the nibblers and the birds continuing to consume two meals/day. The difference was still evident after three weeks of eating a regular mash diet. By contrast, there was no difference at the two-week regression period in the rate of clearing in the birds which had had their atherosclerosis produced while eating three meals/day, regardless of whether the birds nibbled or meal ate during the regression period. However, the morphology of the lesions suggested that differences in the rate of regression existed; the nibblers showed a decreased lipid content of their lesions. This finding has happened twice in our experience using this number of meals. It is our impression that during the induction period, the lesions were of such "severity" that they could not be completely cleared in the two-
Atherosclerosis in Chickens

Figure 3
Regression of diet-induced hypercholesterolemia on a regular mash diet under the conditions of meal eating and nibbling.

week regression period. This possibility is supported by our finding that after three weeks of regression, the difference between the three-meal eaters and the nibblers is clearly manifest. Apparently, with the type and severity of the lesion, two weeks were inadequate to reveal the difference by the methods of estimate employed.

Previous data in the literature can be found which support our findings and interpretation on the influence of the daily pattern of eating on the induction and regression of atherosclerosis. Thus, Cox et al.15 found that the production of atherosclerosis in the monkey was facilitated by feeding the animals once daily. Their findings may be attributed to either a greater food (and cholesterol) intake under these conditions or to the periodicity of their feeding regimen. McAllister and co-workers,10 in an experiment designed to study the influence of exercise on experimental atherosclerosis, fed two groups of dogs identical amounts of food containing cholesterol and thioacetil. Over a 15-month period, five dogs were exercised and five animals confined to their cages. At the end of the experimental period, it was found that the exercised dogs had higher serum cholesterol levels and a greater amount of atherosclerosis than the nonexercised animals. McAllister et al. pointed out in their discussion that the nonexercised animals required 24 hours to consume their food while the exercised dogs, the appetites of which had been stimulated by running, bolted the food quickly as soon as it was offered to them. It therefore appears to us that the results of McAllister et al. might be subject to an alternative explanation, i.e., that the increased atherosclerosis observed in the exercised dogs might have been caused by their being meal eaters, whereas the nonexercised animals could be considered to be nibblers. This is a possibility that should be considered in other experiments dealing with the effect of exercise. Finally, Okey et al.17 have presented independent data showing that meal-eating female rats consuming a cholesterol-rich diet had higher serum cholesterol levels than nibblers eating the same diet.

A number of possibilities suggest themselves to explain our findings. Thus, the atherogenic potentialities of meal eating could be attributed to all or one of the following:

1. Deficient protein intake in the meal eaters, because of the smaller dietary intake. Two facts argue against this being the cause: (a) although the meal eaters consumed one-third less diet, their food intake still exceeded the minimum daily allowances of protein,
calories, and vitamins suggested by the National Research Council; (b) while the induction of atherosclerosis is enhanced by a deficient protein intake, regression is favored under these circumstances. One cannot invoke a protein deficiency as a factor under both conditions, since they apparently mutually exclude each other.

2. Cholesterologenesis might be enhanced with the changes in overall body metabolism that accompany meal eating. We attribute these latter changes to adaptive enzymatic alterations and to the use of alternate metabolic pathways accompanying the different rate at which the calories are ingested. Data have previously been presented demonstrating that meal eating is accompanied by increased tissue hexosemonophosphate shunt activity. This pathway generates NADPH, considered necessary in the present scheme of lipogenesis and cholesterologenesis.

3. Meal eating may alter lipoprotein lipase activity, resulting in a slower rate of clearing of endogenous cholesterol. If this is the explanation for the higher levels of serum lipids seen in the meal-eating chicken, the arteries of the birds eating in this manner would be subject to the atherogenicity of greater concentrations of serum cholesterol levels.

As indicated previously, the implications of our findings with respect to man appear to deserve careful study. If man reacts physiologically to meal eating and nibbling as do other species, both herbivores and carnivores, one factor in the pathogenesis of human atherosclerosis might well be his eating habits.

Bearing on this point are the recently reported studies of Hashim, Arteaga, and Van Itallie. These workers presented data demonstrating that when humans were transferred from a meal eating to a formula diet fed six times/day, regardless of the fat in the diet and the formula, the increased number of feedings was accompanied by a decrease in serum lipids. We, ourselves, have observed one individual with idiopathic hyperlipemia and hypercholesterolemia whose serum lipids fell from an average value of over 2,000 mg. per cent to 700 mg. per cent and whose serum cholesterol declined from a level of 425 mg. per cent to 225 mg. per cent when changed from a meal-eating to a nibbling pattern. These changes have persisted for over a year of nibbling. The elevated values had been observed for over a 12-year period. One case does not prove the point, but it suggests that this line of study must be pursued in man. This we are undertaking.

Summary
Both the production and regression of experimental atherosclerosis, as influenced by eating habits, were studied in the chicken. Birds were either allowed free access to food 24 hours/day ("nibblers") or only for two one-hour periods per day ("meal eaters"). In the induction experiments, it was found that the meal eaters exhibited double the serum cholesterol levels and seven times the severity of coronary atherosclerosis as the nibblers. In studies designed to explore the effects of these eating habits on the regression of established atherosclerosis, lesions were induced in the usual way by feeding an atherogenic diet under the conditions of meal eating. Then the birds were put on regular mash and divided into two groups: meal eaters and nibblers. Meal eating a regular mash diet was associated with (a) slower rate of fall of serum cholesterol levels to normal, and (b) a marked decrease in the rate of healing of the coronary lesions as compared to nibbling. It is concluded that the manner of ingestion of the diet plays a significant role in accentuating the production and in inhibiting the regression of experimental atherosclerosis.

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References
ATHEROSCLEROSIS IN CHICKENS


Effect of Meal Eating Compared to Nibbling upon Atherosclerosis in Chickens

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