The Effect of Pancreatectomy on Lipemia, Tissue Lipidosis and Atherogenesis in Chicks

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with the technical assistance of C. Bolene, M.S.

Following complete pancreatectomy, chicks exhibit an excessive hypercholesterolemia when fed a diet containing cholesterol plus cottonseed oil. This inordinate hypercholesterolemia is accompanied by intensified atherogenesis, compared with unoperated controls on the same diet. Dietary neutral fat is essential for these excessive cholesterolemic and atherogenetic responses. The possible significance of these findings for the pathogenesis of atherosclerosis in diabetic man is discussed.

Atherosclerosis is the major unsolved therapeutic problem in diabetes mellitus today. This vascular "complication" is responsible for tremendous morbidity and mortality.1

Despite a mass of clinicopathologic data,1 the causes for this "universality of atherosclerosis in diabetes"2 remain obscure. Considerable evidence is extant implicating alterations in lipid metabolism in the pathogenesis of diabetic atherosclerosis.1 Undoubtedly these data demonstrate an association between lipid disarrangements and atherogenesis in diabetes. However, the precise relationship of this lipid pathophysiology to diabetic atherogenesis remains an unsolved problem.

We undertook to investigate one aspect of this problem experimentally: the interrelationships among the pancreas, lipid metabolism and atherogenesis. Toward this end we studied the effect of pancreatectomy on lipemia, tissue lipidosis and atherogenesis in plain mash-fed, cholesterol-fed and stilbestrol-treated cockerels.

METHODS

Two series1 of chronic experiments were completed involving a total of 125 birds. The experimental regimens are indicated in table 1. Essentially, we assayed the effects of pancreatectomy on plasma and tissue lipid levels, and upon spontaneous and cholesterol-induced atherogenesis, in chicks maintained on one of four different diets: plain chick starter mash (PM—groups 1 and 2); mash supplemented with 2 per cent cholesterol and 5 per cent cottonseed oil (CO—groups 3 and 4); mash supplemented with 2 per cent cholesterol alone (C—groups 5 and 6); mash supplemented with 5 per cent cottonseed oil alone (O—groups 7 and 8). Groups 1, 3, 5 and 7 were pancreatectomized, groups 2, 4, 6 and 8 were unoperated controls. Hy-line cockerels were used throughout. One day old birds were obtained from a certified hatchery and reared in a battery brooder. During the initial five to seven weeks in the laboratory all chicks were maintained on tap water ad libitum and commercial chick starter mash of known composition.1,2 At age 5 to 7 weeks, pancreatectomy was performed under sodium pentobarbital anesthesia. Three to seven days postoperatively experimental and control groups were placed on their particular diets. Chicks were weighed weekly throughout the experiment and a record of feed intake was maintained. The duration of the study varied from 15 to 36 weeks (see tables 3 and 4). Birds were bled periodically from the alar vein. Aliquots of plasma were analyzed for lipid phosphorus, and for free and total cholesterol by the methods of Man and colleagues,4,5 and Schoenheimer and Sperry6 respectively. At the end of the

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During this study C. B. was Deborah V. Dauber Memorial Research Assistant.

* Dr. Raymond Harris, formerly of this department and now in Albany, N. Y., participated in the early phases of the series 1 experiments, and Dr. Ruth Pick in the later phases of the series 2 study.
experiment, surviving chicks were sacrificed by decapitation and exsanguination. At autopsy all the viscera were examined and the gross findings recorded. Completeness of pancreatectomy was confirmed in operated birds. The hearts and great vessels were carefully inspected for evidence of gross atheromatous plaques. Lesions, if any, were recorded graphically on special forms and graded grossly 0 to 4 according to criteria previously described. Specimens from different groups were indiscriminately mixed and graded consecutively as unknowns. Aortas from birds of series 1 and aliquots of liver from chicks of both series were analyzed for the various lipid fractions according to methods previously described. Blocks of tissue were also taken for microscopic examination, fixed in aqueous formalin and stained with either Sudan IV (frozen sections) or hematoxylin-eosin (paraffin sections).

In addition to these chronic experiments in the chick, the effect of pancreatectomy on the avian plasma lipid response to estrogens was assayed in two further groups of 5 birds each, studied concurrently with series 1. One group was pancreatectomized, and the other unoperated (groups 9 and 10). Both groups subsisted on plain mash and were implanted subcutaneously with 25 mg. pellets of diethylstilbestrol† at intervals of three weeks for a period of 35 weeks. The number of birds surviving to the end of the experiment was inadequate to evaluate the effect of pancreatectomy on estrogen-induced atherogenesis.

### Results

Since the biochemical and morphologic findings in the two series of studies were essentially similar, they have been combined for presentation.

### A. Feed Intake and Weight Change

All groups of depancreatized chicks (table 1) exhibited normal feed consumption and rate of gain in weight, compared with their unoperated paired controls. In contrast to reported findings in the duck, pancreatectomy was not followed by weight loss in cockerels. The depancreatized birds had grossly normal excreta throughout; steatorrhea was not observed.

All birds, pancreatectomized and normal, subsisting on various types of supplemented mash (groups 3 to 8) consistently exhibited normal feed intake and weight change. No gross signs of nutritional deficiency were noted at any time. Thus the experimental diets were apparently without deleterious effects nutritionally.

### B. Plasma Glucose and Lipid Levels

In accord with previous reports, pancreatectomy was without sustained effect on plasma or blood glucose levels in the chick. Intravenous glucose tolerance tests on fed birds revealed essentially similar curves for normal and depancreatized chicks. None of the experimental diets affected the blood glucose level or the glucose tolerance curves in pancreatectomized or normal cockerels, regardless of concomitant induced hyperlipemia. Apparently neither cholesterol, nor cottonseed oil, nor the two in combination in the diet affected carbohydrate metabolism, as assayed by the foregoing tests. Further, the normal and depancreatized cockerels fed plain mash and implanted with

<table>
<thead>
<tr>
<th>Series</th>
<th>Group No.</th>
<th>No. of birds</th>
<th>Type of diet</th>
<th>Procedure</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>10</td>
<td>PM*</td>
<td>Panc.†</td>
<td>%</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>20</td>
<td>PM</td>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>5</td>
<td>CO*</td>
<td>Panc.</td>
<td>93</td>
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<tr>
<td>4</td>
<td>4</td>
<td>5</td>
<td>CO</td>
<td>None</td>
<td>93</td>
</tr>
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<td>5</td>
<td>5</td>
<td>C*</td>
<td>Panc.</td>
<td>98</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>10</td>
<td>C</td>
<td>None</td>
<td>98</td>
</tr>
</tbody>
</table>

† PM = plain mash
CO = mash enriched with cholesterol plus cottonseed oil
C = mash enriched with cholesterol alone
O = mash enriched with cottonseed oil alone

* We appreciate the cooperation of Dr. Samuel D. Loube, formerly of the Department of Metabolic and Endocrine Research, now in Washington, D. C., who participated with us in accomplishing some of these tests. B. Huddleston of the Department of Metabolic and Endocrine Research assisted us by performing some of the plasma glucose determinations for this study. We gratefully acknowledge her cooperation in this project.

† The pellets of diethylstilbestrol were generously supplied by Dr. Kenneth G. Kohlstaedt of Eli Lilly & Co., Indianapolis, Ind.
diethylstilbestrol pellets (groups 9 and 10) also had glucose levels essentially similar to the controls (groups 1 and 2) (cf.3).

Pancreatectomized chicks subsisting on plain mash alone exhibited normal plasma cholesterol and phospholipid levels throughout the experiment (groups 1 and 2, table 2). These determinations yielded no evidence of a chronic disturbance in lipid metabolism in normally fed, depancreatized cockerels.

Operated birds eating a diet of mash supplemented with 2 per cent cholesterol and 5 per cent cottonseed oil exhibited a pattern of plasma lipid levels significantly different quantitatively from their paired controls (groups 3 and 4, table 2). Throughout the experiment in both series, the pancreatectomized birds (group 3) responded to this diet with a far more severe hypercholesterolemia (1310 mg. per 100 cc. mean total cholesterol concentration for the duration of the experiment, group 3; vs. 619, group 4—table 2). Both groups had only a moderate hyperphospholipemia, similar in degree. In accord with previous experience in cholesterol-fed chicks, the increment in plasma total cholesterol markedly exceeded that in lipid phosphorus. Hence the ratio of plasma total cholesterol to lipid phosphorus rose several fold, this alteration being signifi-

Table 2.—Mean Plasma Lipid Levels, Series 1 and 2 Combined

| Group | Determination | Weeks on Diet | Mean§ | Mean C:P ||
|-------|--------------|---------------|-------|----------|
|       |              | 1  | 3  | 5  | 10 | 15 | 20 | 30 |       |
| 1     | T. Chol.*    | 151| 102| 109| 98 | 126| 112| 74 | 110| 14.5 |
|       | Lipid P**    | 7.3| 6.9| 9.1| 7.4| 6.6| 8.4| —  | 7.6|   |
| 2     | T. Chol.     | 155| 110| 84 | 113| 75 | 127| 135| 108| 14.2 |
|       | Lipid P      | 6.9| 8.1| 7.7| 7.0| 8.2| 8.5| —  | 7.6|   |
| 3     | T. Chol.     | 485| 1419|1315|1520|1790| —  | —  | 1310|   |
|       | Lipid P      | 9.8| 16.3|18.7|19.5|19.3| —  | —  | 16.7|   |
| 4     | T. Chol.     | 332| 412 |655 |814 |882 | —  | —  | 619 |44.9 |
|       | Lipid P      | 9.0| 16.8|13.6|14.3|15.4| —  | —  | 13.8|   |
| 6     | T. Chol.     | 210| 426 |533 |700 |666 | —  | —  | 507 |50.7 |
|       | Lipid P      | 7.0| 10.3|11.5|10.4|11.0| —  | —  | 10.0|   |
| 7     | T. Chol.†    | 87 | —   |80 | 88 | 76 | —  | —  | 83  |   |
|       | Lipid P      | —  | —   | —  | —  | —  | —  | —  |     |   |
| 8     | T. Chol.†    | 92 | —   |89 | 83 | 75 | —  | —  | 85  |   |
|       | Lipid P      | —  | —   | —  | —  | —  | —  | —  |     |   |
| 9†    | T. Chol.     | 299| 106 |216 |733 |606 |1125| —  | 514 |9.1  |
|       | Lipid P      | 19.5|11.4|25.1|14.9|73.0|193.0| —  | 56.2|   |
| 10†   | T. Chol.     | 226| 133 |474 |827 |800 |804 | —  | 544 |7.9  |
|       | Lipid P      | 31.3|12.8|47.8|135.0|99.4|86.3| —  | 68.8|   |

* T. Chol. = total cholesterol in mg. per 100 cc.
Lipid P = lipid phosphorus in mg. per 100 cc.
† Series 1 only
§ Mean = average over entire experiment
∥ C:P = ratio of total cholesterol to lipid phosphorus
‡ Series 2 only

In contrast to these findings, pancreatectomized birds fed a mash supplemented with 2 per cent cholesterol (no cottonseed oil) had a plasma lipid pattern essentially similar to that of their unoperated paired controls (groups 5 and 6, table 2). Thus groups 4, 5 and 6 resembled each other closely with respect to degree of hypercholesterolemia and hyperphospholipemia. This was observed in both series. Without oil in the diet, depancreatized chicks apparently exhibited a response to cholesterol feeding quantitatively different from operated birds given cholesterol and oil.
Pancreatectomized and normal cockerels fed a mash supplemented with 5 per cent cottonseed oil exhibited normal plasma cholesterol concentrations throughout (groups 7 and 8, table 2).

None of the foregoing eight experimental groups had any alteration in the ratio of plasma

<p>| TABLE 3.—Mean Tissue Lipid Levels, Series 1 and 2 Combined |
|----------------------------------|---------|---------|------------------|---------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Weeks on diet</th>
<th>Organ</th>
<th>Lipid P mg. per 100 Gm.</th>
<th>Total cholesterol mg. per 100 Gm.</th>
<th>Free cholesterol mg. per 100 Gm.</th>
<th>Total fatty acids mg. per 100 Gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>15-17</td>
<td>Liver</td>
<td>100</td>
<td>370</td>
<td>294</td>
<td>3180</td>
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<tr>
<td>2*</td>
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<td>Liver</td>
<td>85.6</td>
<td>287</td>
<td>277</td>
<td>5340</td>
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<tr>
<td>3†</td>
<td>36</td>
<td>Liver</td>
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<td>353</td>
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<td>2405</td>
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<tr>
<td>4†</td>
<td>36</td>
<td>Liver</td>
<td>97.3</td>
<td>318</td>
<td>300</td>
<td>2968</td>
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<tr>
<td>3*</td>
<td>15-17</td>
<td>Liver</td>
<td>88.7</td>
<td>1330</td>
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<td>4920</td>
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<tr>
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<td>15-17</td>
<td>Liver</td>
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<td>2860</td>
<td>1260</td>
<td>1600</td>
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<tr>
<td>5†</td>
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<td>Liver</td>
<td>113</td>
<td>2860</td>
<td>1260</td>
<td>1600</td>
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<tr>
<td>6†</td>
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<td>Liver</td>
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<td>3130</td>
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<td>1†</td>
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<td>151</td>
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<tr>
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<tr>
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<td>—</td>
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<tr>
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<td>Aorta</td>
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<td>312</td>
<td>210</td>
<td>—</td>
</tr>
<tr>
<td>6†</td>
<td>15-17</td>
<td>Aorta</td>
<td>36.6</td>
<td>285</td>
<td>211</td>
<td>—</td>
</tr>
</tbody>
</table>

* Series 1 chicks 
† Series 2 chicks

C. Tissue Lipid Levels. Pancreatectomy failed to result in the development of a fatty liver in chicks fed plain mash (groups 1 and 2, table 3). With cholesterol feeding, the degree of hepatic cholesteroisis tended to be less in the operated birds (groups 3 to 6, table 3). No other consistent differences in liver lipids were noted among these groups.

Pancreatectomy had no significant effect on aorta cholesterol concentration in chicks fed either plain mash or cholesterol (table 3). In accord with previous experience, cholesterol feeding (with or without cottonseed oil) induced an increase in aorta cholesterol concentration. This increment was similar in degree in depancreatized and unoperated birds. No significant changes from normal in aorta phospholipid concentration were recorded in these cholesterol-fed cockerels (groups 3 to 6, table 3).

D. Morphologic Findings. In most operated birds, autopsy confirmed the completeness of pancreatic extirpation. A few chicks showed remnants of this organ.

Findings in the aortas and great vessels are summarized in table 4. Despite normal plasma lipid levels in depancreatized chicks fed plain mash, these birds exhibited a slightly higher incidence and a markedly greater severity of atherosclerotic lesions of the spontaneous type after 36 weeks on diet (groups 1 and 2, table 4).* No birds, operated or controls, in either plain mash fed group had gross lesions of the induced type in the thoracic aorta.

In conjunction with the more severe hypercholesterolemia and elevation of the plasma total cholesterol:lipid phosphorus ratio, pancreatectomized cockerels fed mash supplemented with 2 per cent cholesterol and 5 per cent cottonseed oil had significantly more severe cholesterol-induced atherosclerosis.† Greater se-

* This is one of few studies in which the incidence and severity of chick spontaneous atherosclerosis has been influenced in an apparently significant manner by an experimental procedure. Additional investigations are indicated to test this phenomenon further. Our limited knowledge of the pathogenesis of the spontaneous lesion in the chick renders its evaluation difficult.
† These data alone do not permit an evaluation as to the relative roles of hypercholesterolemia per se compared with elevated plasma total cholesterol:lipid phosphorus ratio in contributing to atherosclerosis.
verity of atherosclerosis in chicks of group 3 compared with group 4 could not be correlated with either liver or aorta lipid values of these birds (tables 3 and 4).

In conjunction with their similar degrees of hyperlipemia, cholesterol-fed chicks of groups 5 and 6 had a similar incidence and severity of atherosclerosis. Unlike chicks fed cholesterol and cottonseed oil mash (group 3), birds subsisting on cholesterol mash without oil (group 5) exhibited no effect of pancreatectomy on atherosclerosis.

Feeding mash supplemented with 5 per cent cottonseed oil for 15 weeks had no significant influence on either plasma cholesterol levels or spontaneous atherogenesis in pancreatectomized cockerels (groups 7 and 8, tables 2 and 4). Both groups of oil fed chicks, depancreatized and unoperated, were uniformly free of atherosclerotic plaques of the cholesterol-induced type in the thoracic aorta.

**Discussion**

Our findings in this study demonstrated the existence of subtle defects in lipid metabolism in depancreatized cockerels. These defects were brought out particularly by a diet of mash enriched with cholesterol plus cottonseed oil. On this regimen pancreatectomized chicks exhibited an inordinate hypercholesterolemic response. Increased incidence and severity of atherosclerotic lesions accompanied this excessive hypercholesterolemia. Thus the lipid metabolic defects in depancreatized cockerels were associated with, and apparently related to, intensified atherogenesis. Hence in the chick—as in man—the pancreas apparently influences the metabolism of lipids related to atherogenesis. This biologic similarity emerges despite initial data indicating that pancreatic deficiency in chick and man has fundamentally different effects on lipid metabolism.

Concurrent studies in our laboratory by Stamler and Pick demonstrated that similar subtle defects in carbohydrate metabolism were present in masked form in pancreatectomized and alloxanized cockerels. These birds, with normal fasting blood glucose levels, exhibited inordinate hyperglycemic responses to adrenal cortical extract (ACE).* Gluconeogenesis induced by adrenal cortical extract apparently overwhelmed limited glucose regulatory mechanisms in these chicks. Corresponding to their lack of pancreatic β cells, these depancreatized or alloxanized cockerels apparently had a relative insulin lack (latent diabetic or subdiabetic tendency).21-23

Together with the lipid data of the present experiment, these findings indicate that chicks are not so fundamentally dissimilar from mammals (dog, rabbit, rat, man)† in carbohydrate and lipid metabolism as one might conclude from initial study of a pancreatectomized preparation. The apparent differences between man and chick may not necessarily be qualitative ones, but rather at least in part quantitative.

The data of the present study further demonstrated significant effects of dietary neutral fat ingestion on cholesterolemia and atherogenesis in cholesterolized, depancreatized chicks. On a 2 per cent cholesterol mash without cottonseed oil, pancreatectomized and unoperated chicks exhibited similar cholesterolemic and atherogenic responses. With the incorporation of 5 per cent cottonseed oil in the 2 per cent cholesterol mash, operated and control birds manifested significantly different responses.‡ This finding is generally in agreement with work indicating that neutral fat ingestion may in some circumstances influence cholesterolemia and atherogenesis in man, whereas in other instances it may be without effect.1,24-32 The factors responsible for these phenomena are as yet obscure.

The present experiment could not yield data indicating the mechanisms of the altered cholesterolemic and atherogenic responses of de-

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* This production of marked hyperglycemia with adrenal cortical extract in pancreatectomized or alloxanized chicks makes available a preparation more closely similar to diabetic man. Studies on lipid metabolism and atherogenesis in this preparation are being pursued in this laboratory.

† Elsewhere we have reviewed the findings in these mammalian species on the interrelationships among the pancreas, lipid metabolism and atherogenesis.1

‡ In contrast, addition of 5 per cent cottonseed oil to plain mash, devoid of a cholesterol supplement, had no effect on lipemia or atherogenesis in normal or depancreatized cockerels.
pancreatized cockerels to cholesterol-cottonseed oil mash. Further studies on this problem are currently in progress in our laboratory.1

SUMMARY

Pancreatectomized chicks exhibit an excessive hypercholesterolemia when fed a diet containing cholesterol plus cottonseed oil. This inordinate hypercholesterolemia is accompanied by intensified atherogenesis, compared with unoperated controls on the same diet.

Neutral fat is essential for these excessive cholesterolemic and atherogenic responses, since they are absent in depancreatized cockerels fed mash supplemented only with cholesterol (i.e. without oil).

Plain mash-fed pancreatectomized cockerels exhibit intensified spontaneous atherogenesis, although they have normal plasma lipid and glucose levels.

ACKNOWLEDGMENTS

We wish to express our appreciation to the other members of the department's atherosclerosis project research team, whose intelligent cooperation made it possible to consummate this study: Miss Marilyn Dudley and Mrs. Eva Levinson, research chemists; Messrs. P. Johnson and G. Crowley, research technicians.

REFERENCES


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