Effect of Insulin in the Induction and Regression of Atherosclerosis in the Chick

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DURING the last 3 decades—the insulin era in the treatment of diabetes mellitus—atherosclerotic vascular complications have become the major causes of morbidity and mortality in diabetic persons, doubtless due, in part, to their increased life span with insulin therapy. Diabetics, as a group, undoubtedly have an increased susceptibility to atherosclerotic disease.[1, 2] The causative and pathogenetic factors responsible for this phenomenon remain obscure.

In view of this problem, experiments were undertaken to explore the effects of exogenous insulin on coronary and aorta atherogenesis in intact chicks.

Methods

Twelve series of chronic experiments, utilizing a total of 461 cockerels, were accomplished over a 5-year period (1954 to 1959). The established control technics of this department for experimentation on atherosclerosis were employed throughout.[1] Over-all, 3 different types of experiments were done to assess the influences of exogenous insulin: (1) regression or "unloading" experiments, in which birds were first fed an atherogenic diet for several weeks (mash + cholesterol + oil), then transferred to plain mash for 2 weeks, with administration of insulin during this regression period (tables 1 and 2); (2) "loading" experiments, in which birds were fed mash + cholesterol + oil, and simultaneously given insulin (table 3); (3) experiments on the effects of insulin on estrogen antiatherogenesis, in which chicks fed an atherogenic diet were simultaneously given estrogen and insulin (table 3).[7, 8]

In accordance with established procedure,[1] a record of feed intake and rate of weight gain was maintained in all experiments. Since groups receiving insulin exhibited decreased feed intake and rate of weight gain, paired-feeding methods were used to assess the possible role of these nonspecific effects in accounting for changes observed with insulin.[9, 10]

Blood glucose concentration was measured as an index of insulin effect.

Results

In control birds, transferred from an atherogenic diet to plain mash for a terminal 2-week period, significant regression of coronary atherosclerosis occurred, in accordance with previous observations, while aortic lesions showed no change during this short period.[8, 11] In contrast, cockerels given insulin during this period exhibited no regression of lesions (table 1). This marked, significant difference between the 2 groups prevailed, although their patterns of cholesterolemia and phospholipemia were very similar, i.e., both exhibited a decline during these terminal 2 weeks from hyperlipemic to virtually normolipemic levels.

The control and insulin groups differed significantly in feed intake, weight and blood glucose level during these 2 weeks. The insulin-treated group had sustained periods of hypoglycemia, ate much less, and lost (rather than gained) weight (table 1).

Since previous work had shown that undernutrition inhibited regression of atherosclerosis,[9, 10] further pair-feeding experiments were undertaken to assess the role of this variable (table 2). The data of this experiment demonstrated that the insulin administration—and not matched underfeeding alone—was associated with complete inhibition of regression of coronary lesions. Thus, this phenomenon in birds receiving insulin cannot be attributed to the accompanying low feed intake, i.e., it is not basically an undernutrition effect.

In experiments on the effects of insulin on cholesterol-oil fed and estrogen-treated birds, no consistent influences of the islet hormone were noted. In 4 of the 9 series of experiments,

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Table 1

Effects of Insulin on Regression of Atherosclerosis Composite Tabulation—Series 33, 38, 48, and 57

Group	No. of birds	Terminal weight Gm.	Δ weight Gm.	Feed intake Gm./bird/day	Blood glucose mg. %‡	Terminal plasma cholesterol mg. %**	Terminal C/P ratio††	Gross thoracic aorta atherogenesis		Microscopic coronary atherogenesis	
								Incidence	Grade	Incidence	% Involvement§§
C-O†	36	1443±47*	+578±24	114± 6	—	907±90	2.00±0.09	75%	1.5±0.2	94%	20.3±2.1
C-O → RM‡	34	1610±62	+181±30§	136±41§	158± 3	137± 6	0.55±0.02	91%	1.1±0.1	56%	11.2±1.6
C-O → RM + Insulin¶¶	38	1268±42	−144±27§	57± 8§	54±10‡‡	168±18	0.74±0.07	79%	1.6±0.2	97%	23.4±2.8

*Standard error of the mean.

†C-O is mash + cholesterol + 5% cottonseed oil; the per cent of cholesterol was 2, ½, 2 and 1% in series 33, 38, 48, and 57, respectively.

‡C-O → RM is mash + cholesterol + oil for 5 weeks, then plain mash for the terminal 2 weeks. Birds were 12, 12, 9, and 8 weeks of age at the beginning of the experiment in series 33, 38, 48, and 57, respectively.

§Feed intake and Δ weight during terminal 2 weeks of experiment, on RM.

¶Glucose data collected on series 48 and 57 birds only; Somogyi method.[3]

**Schoenheimer-Sperry method.[4]

††C/P Ratio is the ratio of plasma total cholesterol to total phospholipids.[5]

‡‡Glucose values 3 hours after injecting 10 units Lente insulin; glucose values at 6, 18, and 24 hours were 64±14, 151±63, and 202±18 mg. %, respectively.

§§Per cent involvement is an estimate of severity of coronary atherosclerosis; it is a count of coronary vessels exhibiting atherosclerosis on microscopic examination, in relation to the total number of vessels visualized in standardized sudan IV-hematoxylin stained sections.[6] The figure given represents involvement of vessel in birds with lesions. Negative birds are not included.

¶¶10 units of Lente insulin daily per bird.

data were obtained, suggesting that insulin suppressed estrogen antiatherogenesis in the coronary bed (series 36, 41, 46, 63). However, results were inconsistent, being negative in the other series. Analysis of the over-all data revealed that the 2 groups—estrogen and estrogen + insulin—did not differ significantly (table 3).

Discussion

These experiments are supplementary to previous studies in this department on pancreatic influences on diet-induced atherosclerosis in cockerels.[1] These earlier investigations yielded negative results with respect to the effects of lipotropic factors, such as choline, inositol, lipocaic, lecithin, vitamin B_{12}, and methionine. They also demonstrated that pancreatectomy tended—without inducing diabetes mellitus—to aggravate hypercholesterolemia and atherogenesis in birds fed a diet high in cholesterol and fat. They further showed that diabetes could be induced in this avian species by the administration of hydrocortisone, with or without pancreatectomy or the administration of alloxan, without associated aggravation of diet-induced cholesterolemia and atherogenesis.

It is evident in the present series of experiments, from the findings of the regression data, that insulin in large, hypoglycemia-inducing doses inhibits the regression of coronary atherosclerosis usually supervening in cockerels transferred from a high-cholesterol, high-fat diet to unsupplemented plain mash. To our knowledge, findings indicating an atherogenic property of insulin have been reported on only 1 previous occasion. In alloxan diabetic rabbits fed cholesterol, aorta atherosclerosis is much less severe than in intact animals on the same diet.[12-15] However, when the diabetic rabbits were treated with insulin, severe aorta atherosclerosis supervened.[14] Under these circumstances, therefore, insulin may be regarded as behaving as an intensifier of atherogenesis.

The differences between the foregoing experiments in rabbits and the present ones in chicks should be kept in mind. The rabbits were diabetic, the chicks were not. The insulin effect in the rabbits was observed in a choles-

Table 2

Effect of Underfeeding vs. Insulin on Regression of Atherosclerosis—Series 57

Group*	Weeks of age	Terminal weight Gm.	Δ weight Gm.	Feed intake Gm./bird/day	Blood glucose mg. %¶	Terminal plasma cholesterol mg. %**	Terminal C/P ratio††	Gross thoracic aorta atherogenesis		Microscopic coronary atherogenesis	
								Incidence	Grade	Incidence	% involvement‡‡
1 C-O†	8-13	1420±33	+578±24	108± 7	—	1481±140	2.42±0.08	100%	2.2±0.2	100%	30.8±3.0
1 C-O → RM**											
Ad Lib	8-15	1663±46	+269±26††	169±86††	—	131± 11	0.54±0.03	100%	1.6±0.2	89%	16.8±2.3
1 C-O → RM											
Pair Fed§	8-15	1239±25	- 64±20††	56± 9††	158± 3	289± 30	1.12±0.08	100%	2.0±0.2	94%	17.4±1.9
1 C-O → RM + Insulin†	8-15	1173±36	-186±33††	57± 9††	72± 4¶	243± 51	0.96±0.19	100%	2.2±0.2	100%	32.5±4.4

*Number of birds per group: 10, 9, 18, and 15, respectively.

†10 units Lente insulin daily.

‡Chick starter mash + 1% cholesterol + 5% cottonseed oil + 20% sucrose (to reduce dietary protein to 15% by weight).

§Pair fed to insulin group.

¶Three hours after insulin.

**1 C-O → RM is a diet of 1 C-O for 5 weeks, followed by a diet of chick starter mash (RM) for 2 weeks (the regression period).

††For weeks 5-7.

‡‡Per cent involvement is an estimate of severity of coronary atherosclerosis; it is a count of coronary vessels exhibiting atherosclerosis on microscopic examination, in relation to the total number of vessels visualized in standardized sudan IV-hematoxylin stained sections.[6] The figure given represents involvement of vessel in birds with lesions. Negative birds are not included.

terol-oil feeding experiment, whereas in the chick studies, no atherogenesis-intensifying effects of insulin were noted in the cholesterol-oil feeding experiments.

No additional data are available to clarify the reasons for these apparent differences in the 2 species. Nor is it clear why in cockerels, insulin had an effect on regression of lesions, but apparently none in the cholesterol-oil feeding experiments, with or without estrogen added.

With respect to possible mechanisms of the insulin effect on regression of coronary lesions, several hypothetical possibilities present themselves, e.g.: a direct effect of insulin on the metabolism of vascular tissue; an indirect effect, possibly related to cyclical hypoglycemia and consequent changes in adrenal medullary and cortical hormonal secretion.[1, 8, 16-18] It is clear from present data that the inhibitory effects of insulin on regression of coronary lesions cannot be related to plasma cholesterol-phospholipid levels, or to patterns of feed intake.

Summary

Insulin administration to intact cockerels made atherosclerotic by the feeding of a cholesterol-oil-containing diet prevented regression of coronary atherosclerosis. Insulin administration during the induction of atherosclerosis had no effect on the development of lesions. In some experiments, insulin slightly inhibited estrogen-induced protection of the coronary arteries when the birds were on a high cholesterol-oil diet.

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Table 3

Effects of Insulin on Cholesterol-lipid, Atherogenesis, and on Estrogen Antiatherogenesis
Composite Tabulation—Series 36, 38, 41, 43, 46, 47, 52, 53, and 58

Group	No. of birds	Terminal weight Gm.	Δ weight Gm.	Feed intake Gm./bird/day	Blood glucose mg. %	Terminal plasma cholesterol mg. %	Terminal C/P ratio	Gross thoracic aorta atherogenesis		Microscopic coronary atherogenesis	
								Incidence	Grade	Incidence	% involvement
C-O Control	82	1699±43	+496±30	108± 5	166± 9	770± 54	1.83±0.07	72%	1.5±0.1	86%	20.6±1.6
C-O Insulin	65	1476±28	+441±40	87± 4	94± 8	540± 43	1.56±0.07	62%	1.4±0.1	77%	20.4±1.9
C-O Estrogen†	78	1701±82	+569±46	104± 5	185±14	982± 43	0.75±0.01	87%	1.5±0.1	15%	14.0±3.6
C-O Insulin + Estrogen†	76	1499±40	+497±44	83± 4	94± 8	982± 53	0.83±0.04	83%	1.3±0.1	39%	9.4±1.2

*Three hours after injection of Lente insulin, 10 units. Hypoglycemia was also noted in both insulin-treated groups at 1½, 4, and 6 hours. Blood glucose values were at normal levels, 24 hours after insulin injection.

†Estrogen was 25 mg. per bird, per day, of conjugated equine estrogens (Premarin) in the drinking water.

For further explanation, see table 1.

Summario in Interlingua

Le administration de insulina a intacte gallettos que habeva essite rendite atherosclerotic per un dieta a contento de cholesterol e oleo preveniva le regression de atherosclerosis coronari. Le administration de insulina durante le induction de atherosclerosis habeva nulle effecto super le disveloppamento del lesiones. In plure experimentos, insulina inhibiva levemente le protection estrogenogenic del arterias coronari durante que le aves recipeva un dieta ric in cholesterol e oleo.

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