is nevertheless considerable. The patient was not previously hypertensive; no evidence for the more common causes for hypertension at this age could be found; and since she stopped taking oral contraceptives her blood-pressure has remained normal, although she has received no hypertensive treatment for the past 14 months. The striking improvement in the electrocardiograph emphasises the reduction in blood-pressure and the apparent reversibility of the hypertensive disease in her case. There is, furthermore, good evidence of persistently normal renal function as illustrated by blood-urea and normal creatinine clearance (130 ml. per minute). It seemed unjustifiable to reintroduce oral contraception to see if the hypertension recurred.

Laragh and his colleagues [9] have attempted to explain the pathogenesis of hypertension associated with oral contraception on the basis of abnormalities in renin-substrate concentration, endogenous renin activity, and in aldosterone secretion, but they concluded that the relevance of these abnormalities to the development of hypertension was not clear because similar effects could be seen in women who were taking oral contraceptives but who had normal blood-pressure. Woods [10] suggested that hypertension in these cases might be intensified because of the metabolic action of œstrogen in causing retention of salt and water. This explanation is unlikely to be relevant to this case, since the patient was not previously hypertensive and salt-and-water retention of a relatively moderate degree (i.e., not associated with œdema), would not alone have been likely to have produced such severe hypertension.

The mechanism for the development or enhancement of hypertension in women on oral contraceptives remains unknown, and it may be that more than one factor is responsible. The occasional development of malignant hypertension in a previously normotensive individual is even more of an enigma, since it is presumably unrelated to dosage or duration of treatment and may apparently develop in the absence of previous renal disease or pre-existing hypertension. Although severe hypertension is probably an infrequent side-effect of oral contraceptives, I suggest that the blood-pressure of women taking these preparations should be checked at regular intervals, say every 3 or 6 months, and that care should be taken in prescribing them for patients with previous hypertension or a strong family history of hypertension.

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Preliminary Communication

INSULIN STIMULATION OF CHOLESTEROL SYNTHESIS BY ARTERIAL TISSUE

R. W. STOUT*
Department of Medicine, Queen's University, Belfast

Summary Rats given sodium [^{14}C]-1-acetate and insulin intravenously were killed 1 hour later. Radioactivity levels in cholesterol in aortic tissue were significantly higher than in rats given labelled acetate alone. Levels in serum-cholesterol were not significantly different, nor were aortic-tissue levels when [^{14}C]-4-cholesterol was given with or without insulin. The result suggests that insulin stimulates cholesterol synthesis in the aortic wall.

INTRODUCTION

IT has been suggested that hyperinsulinism is important in the ætiology of human atherosclerosis,[1] and there is some evidence that insulin influences arterial lipid metabolism.[2 3] Cholesterol is an important constituent of the atheromatous lesion [4] and the factors influencing cholesterol deposition in the arterial wall are of potential significance in the problem of atherogenesis. I have investigated the effect of insulin on cholesterol metabolism in the aorta; the results are discussed below.

METHOD

Male Wistar rats, weighing 90–120 g. fed and unanæsthetised, were investigated by the method previously described.[3] The animals were given an intravenous injection of a solution containing 10 μCi per 100 g. body-weight sodium [^{14}C]-1-acetate (specific activity 40·0 mCi per mmole) and for half the animals, 100,000 μ units insulin per 100 g. body-weight. 1 hour later the animals were killed. After blood had been collected from the neck vessels, the aortas were removed and carefully dissected free of adventitious tissue. The adequacy of removal of the adventitious tissue was confirmed by histological and biochemical tests.[5]

Aortic lipids were extracted in chloroform/methanol (2/1) [6] and the purified solution made up to 2·5 ml. 0·5 ml. samples were pipetted into counting vials, and evaporated to dryness. The lipids were redissolved in the toluene-based scintillation fluid and counted in a Packard 'Tri-Carb' scintillation spectrometer. The defatted aortas were dried in air and weighed. The serum-lipids were extracted in chloroform/methanol by the method of Louis-Ferdinand et al.[7]

The aortic and serum lipids were evaporated to small volume and applied to thin-layer chromatography plates of silica gel G (250 μ thick). Pure standards were run on each plate. The developing solution was hexane/ethyl-ether/acetic-acid (90/15/1).[7] After separation, the lipids were stained with iodine and their position marked. The stain was allowed to fade and the areas of silica gel G containing the cholesterol fraction were scraped into counting vials. The lipids were eluted with the toluene-based scintillation fluid and counted. Experiments with standards showed an 80% counting efficiency, independent of the amount of silica gel G used.

In another series of experiments, rats were injected with 2·5 ml. per 100 g. body-weight of a 5% bovine-albumin

* Present address: Hammersmith Hospital, London W.12.

solution counting 2·5 μCi per 100 g. body-weight [^{14}C]-4-cholesterol (specific activity 50 μCi per mmole); half of the animals received 100,000 μ units insulin per 100 g. body-weight, the others acting as controls. Aortic lipid radioactivity 1 hour after injection was estimated as before.

RESULTS

The results are summarised in the table.

The mean radioactivity in aortic cholesterol 1 hour after intravenous injection of labelled sodium acetate was significantly higher in the rats given insulin. Thus treatment with insulin resulted in the appearance of a significant increase in the amount of labelled cholesterol in the aortic wall. Possible mechanisms are that insulin stimulates (*a*) cholesterol synthesis in the aorta, (*b*) cholesterol synthesis elsewhere (resulting in elevated serum-cholesterol levels and infiltration into the aorta), or (*c*) aortic uptake of cholesterol.

Data for serum-cholesterol radioactivity, converted

RADIOACTIVITY AFTER LABELLED ACETATE (EXPERIMENTS 1 AND 2) OR LABELLED CHOLESTEROL (EXPERIMENT 3)

Experiment	Mean ± S.E.M. radioactivity (c.p.m.) in:		*t*	P
	Insulin-treated rats (13)	Controls (13)		
(1) Aortic cholesterol	1·76 ± 0·10	1·34 ± 0·14	2·39	<0·0125
(2) Serum-cholesterol (non-transformed)	136·42 ± 33·01	101·14 ± 12·60	0·99	>0·20
(2) Serum-cholesterol (log)	2·027 ± 0·83	1·955 ± 0·064	0·69	0·5
(3) Aortic cholesterol (after labelled cholesterol) ..	7·68 ± 6·52	11·04 ± 1·20*	0·51	0·3

* 14 animals.

to logarithms since they did not seem to be normally distributed, were not significantly different between the insulin and control groups. Correlation coefficients (z) between aortic and serum-cholesterol radioactivity, calculated using the r to z transformation for small numbers, were −0·0372 (P > 0·9) for the control group and −0·1225 (P > 0·7) for the insulin group, suggesting that the aortic cholesterol radioactivity does not result from an effect of insulin on serum-cholesterol.

The mean aortic-radioactivity levels after administration of labelled cholesterol were not significantly different, showing that insulin does not enhance the uptake of serum-cholesterol by the aorta.

These results suggest that insulin stimulates the synthesis of cholesterol in the aortic wall.

DISCUSSION

The origin of the cholesterol found in the aortic wall has been the subject of conflicting reports. Many workers believe that arterial cholesterol is an infiltrate of plasma cholesterol,[8] but there is experimental evidence that arterial tissue can synthesise cholesterol from acetate.[9 10] The aortic wall contains all the enzymes known to participate in the metabolic pathways of other tissues.[11]

As far as can be ascertained the effect of insulin on cholesterol synthesis has not been previously described in arterial tissue, or, indeed, in any other tissue. The mechanism is not clear, but it may be due to enhanced transfer of acetate into the cells secondary to glucose transport. Work is in progress to determine if this effect is unique to arterial tissue.

The absence of an insulin effect on aortic uptake of labelled cholesterol is similar to that found by Christensen and Jensen,[12] who used subcutaneous insulin in rabbits. On the other hand, insulin inhibits the regression of atheromatous lesions which occurs when cholesterol-fed chickens are transferred to a normal diet [13] and causes increased deposition of cholesterol when infused into the femoral arteries of cholesterol-fed dogs with alloxan diabetes.[14] It has recently been reported that in pancreatectomised rats fed an atherogenic diet, treatment with insulin reduced the serum-cholesterol to that of non-diabetic controls, but did not decrease the incidence or severity of their vascular lesions, as had happened when a comparable reduction of hypercholesterolæmia had been effected by dietary methods.[15] There is thus strong experimental evidence for an effect of insulin on cholesterol deposition in the arterial wall.

There is little direct evidence on the effect of insulin on serum-cholesterol levels. In animals, elevation of serum-insulin levels to over double the normal value by diverting pancreatic venous blood into the vena cava and feeding sucrose results in a twofold increase in serum-cholesterol.[16] In rats, a high-sucrose diet results in elevated cholesterol levels.[17] It is possible that these responses take longer to develop than the 1 hour allowed in my experiments.

These results do not prove that insulin causes accumulation of cholesterol in the arterial wall; further work on this is in progress. However, demonstration that insulin can stimulate cholesterol synthesis in this tissue is relevant to the hypothesis that atherosclerosis and deranged carbohydrate metabolism are causally linked.

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