The prediabetic state of 2 patients was revealed by the development of frankly diabetic glucose-tolerance curves when they were thyrotoxic; the curves reverted to normal with treatment of the thyrotoxicosis. Their insulin release was greatly improved, and although it improved when they were euthyroid it was still subnormal. Conversely, in 2 hypothyroid subjects a diabetic tendency only became manifest on replacement therapy, and these patients also had subnormal insulin release. This situation is analogous to the experimental "diabetes and diabetes" induced in partially pancreatectomised dogs given thyroid extract (Thompson 1944).

Some of the abnormalities of glucose tolerance in thyroid disease could be due to altered absorption within the small bowel. Work is now in progress to measure directly the changes in absorption in thyroid disease and to clarify the abnormalities in insulin release and sensitivity.

We thank Miss J. McAlister for help and facilities in the gastric emptying studies, Mr. D. S. Turner and Miss E. King for plasma and assistance in the insulin immuno-assay, and Prof. E. F. Neyman for his interest and encouragement. The project was supported by grants from the governors of St. Mary's Hospital's and the North-East Metropolitan Regional Hospital Board.

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The association of ischaemic vascular disease and diabetes mellitus has been known for many years (Brunton 1967, Warren et al., 1966), and has been confirmed by the International Atherosclerosis Project (Robertson and Strong 1960). It has recently become apparent that abnormalities of carbohydrate metabolism are present in a large proportion of patients with ischaemic heart-disease, without clinical diabetes mellitus. These abnormalities may be summarised as follows: It therefore seemed important to investigate the action of insulin on vascular metabolism.

**Method**

Male Wistar rats, weighing 90-120 g., fed and unanesthetised, were investigated by a modification of the method of Raffalovich et al. (1965). In any one experiment animals of the same weight were used. The animals were injected intraperitoneally, using the tail vein, with Gey and Cey buffer (2.5 ml. 0.1% w/v, pH 7.4) containing 44C per ml. of the C14-labelled substrate alone or with 4800 units per ml. of insulin. The substrate was sodium-acetate-14C (specific activity 40.0 mC per mmole) in the first series of experiments, and U-glucose-1-14C (specific activity 7 mC per mmole) in the second. In each experiment 50 mg of the animals received buffer and substrate, and the remaining buffer, substrate, and insulin, 1 hour after injection the animals were killed and the radioactivity in the aortic lipids determined. The whole aorta was dissected free of adhering mesentery and adventitia, and its dry weight recorded. The aorta was then homogenised in 0.9% saline and the homogenate centrifuged at 50,000 g for 1 hour. The supernatant was then decanted and the aorta dried at 100°C. The dried aorta was then ashed in a muffle at 500°C for 2 hours and the ashed aorta weighed.

Summary

Intravenous injection of 44C or insulin (5 mC 14C-labelled substrate containing either glucose or acetate led to much greater incorporation of these substances into the aortic lipid than when the substrate alone was injected. Since insulin is known to inhibit tissue-lipase in vitro, excess of circulating insulin could cause accumulation of fat in the arterial wall by increasing its deposition and inhibiting its removal. It is suggested that the insulin thus plays a major role in the pathogenesis of atherosclerosis.

**Introduction**

The association of ischaemic vascular disease and diabetes mellitus has been known for many years (Brunton 1967, Warren et al., 1966), and has been confirmed by the International Atherosclerosis Project (Robertson and Strong 1960). It has recently become apparent that abnormalities of carbohydrate metabolism are present in a large proportion of patients with ischaemic heart-disease, without clinical diabetes mellitus. These abnormalities may be summarised as follows: It therefore seemed important to investigate the action of insulin on vascular metabolism.

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The mean uptake of sodium-acetate-1-14C, in counts per mg. per minute ± s.E.M., was 0.0039 ± 0.0002 (100%) in 16 controls, and 0.0058 ± 0.0068 (124%) in 17 rats which received insulin. The mean uptake of U-14C, in counts per mg. per minute ± s.E.M., was 0.0137 ± 0.0039 (100%) in 16 controls, and 0.0204 ± 0.0032 (157%) in 20 rats which received insulin. The values obtained in each experiment were similar. Application of Student's t test shows that the difference between the control and insulin-treated groups in each site is highly significant (p < 0.01). These results show that insulin greatly enhances the incorporation of both glucose and acetate into the lipids of the rat aorta.

The U-14C labeled lipid in the aortic wall is probably triglyceride synthesized from fatty acid and s-glycerophosphate, both of these having been metabolized from the injected substrates (Chernick et al. 1949). Further work on this is in progress.

Discussion

The results reported here show that insulin stimulates the synthesis of lipid in the arterial wall. The fact that such a striking effect was found in a short time suggests that insulin in sufficient concentration could result in an accumulation of important amounts of lipid. It is well known that maturity-onset diabetes, which has high serum-insulin levels, lays down fat in its adipose tissue. It is suggested, therefore, that the same mechanism can also be utilized by the aortic wall. This process would act for many years, long before the diabetes mellitus becomes clinically apparent. Indeed, a vascular catastrophe may well precede the appearance of clinical diabetes, and appropriate dietary treatment at that time may prevent it ever appearing.

Mahler (1960) has shown that insulin inhibits tissue-lipolysis in the aorta. He suggests that these results in the accumulation of lipids and hence the formation of atherosclerosis. The present work provides evidence that insulin also stimulates lipogenesis in vascular tissue, an action similar to its effect on adipose tissue (Rendel et al. 1965). Excess of circulating insulin would thus cause an accumulation of fat in the vessel-wall by both increasing the incorporation of both glucose and acetate into the lipids of the rat aorta.

The uptake of D-glucose-U-14C, in counts per mg. per minute ± s.E.M., was 0.0365 ± 0.0062 (100%) in 16 controls, and 0.0638 ± 0.0068 (175%) in 17 rats which received insulin. The values obtained in each experiment were similar. Application of Student's t test shows that the difference between the control and insulin-treated groups in each site is highly significant (p < 0.01). During which part of this work was completed; Mr. T. K. Bell, of the Royal Victoria Hospital, Belfast, for a clinical research fellowship during which part of this work was completed; Mr. T. K. Bell, of the Department of Chemistry, Queen's University, Belfast, for advice and helpfulness; and Dr. J. D. Merz for statistical advice and Mr. Ben Finlan for vehicle technical assistance.

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REFERENCES


The published reports contain no consistent evidence that insulin greatly enhances the incorporation of both glucose and acetate into the lipids of the rat aorta.

The uptake of U-14C, in counts per mg. per minute ± s.E.M., was 0.0427 ± 0.0061 (100%) in 16 controls, and 0.0820 ± 0.0061 (194%) in 17 rats which received insulin. The values obtained in each experiment were similar. Application of Student's t test shows that the difference between the control and insulin-treated groups in each site is highly significant (p < 0.01). These results show that insulin greatly enhances the incorporation of both glucose and acetate into the lipids of the rat aorta.

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The results reported here show that insulin stimulates the synthesis of lipid in the arterial wall. The fact that such a striking effect was found in a short time suggests that insulin in sufficient concentration could result in an accumulation of important amounts of lipid. It is well known that maturity-onset diabetes, which has high serum-insulin levels, lays down fat in its adipose tissue. It is suggested, therefore, that the same mechanism can also be utilized by the aortic wall. This process would act for many years, long before the diabetes mellitus becomes clinically apparent. Indeed, a vascular catastrophe may well precede the appearance of clinical diabetes, and appropriate dietary treatment at that time may prevent it ever appearing.

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