Antineoplastic drugs were given as follows: chlornaphazine 300 mg. daily to a total dose of about 78 g. from February to October 1963; cyclophosphamide 100-150 mg. daily from March 1964 to September 1966, from December 1966 to April 1967, and from October 1968 to November 1969; and nitrogen mustard 4 mg./dose, total dosage 24 mg., in November 1969. From October 1968 to October 1969 prednisone 10-7.5 mg. was given daily.

Bladder Symptoms.—In 1968 the patient had cystitis twice, but without haematuria. In September 1969 macroscopic haematuria developed. After this and until her death she had persistent haematuria with periods of massive bleeding, severe dysuria, urgency, and frequency.

Indirect pyelography in September 1969 raised suspicion of a tumour of the right kidney, but this diagnosis could not be confirmed by renal arteriography and ultrasonic scanning. Cystoscopy on three occasions showed no localized tumour. Numerous petechiae were seen as well as a granulated area behind the orifice of the right ureter.

In November 1969 urinary sediment contained numerous tumour cells corresponding to a grade III carcinoma-in-situ. Bladder biopsies in December 1969 showed atypical epithelium, urothelium corresponding to a grade III carcinoma, but without invasive growth.

After bladder biopsies and electrocoagulation massive haemorrhage developed, which was resistant to irrigational therapy, transfusions, and aminocaproic acid. The patient died with rapidly decreasing kidney function.

Necropsy.—The kidneys and the urinary bladder were without macroscopic changes. Both pelves showed acute and chronic inflammation. Both lungs were covered with fibrous visceral pleurae. A tumour measuring 7 by 5 cm. involved most of the upper right lobe, and histological examination showed changes typical of Hodgkin's disease. The spleen had foci of extramedullary haematopoiesis, but no changes compatible with Hodgkin's disease.

Laboratory Tests.—Blood platelet counts 250,000, 20,000, 98,000/cu. mm.; coagulation tests as in Case 1 normal; serum creatinine 0.9-5.4 mg./100 ml.

Comment

Previously described cases of cancer of the urinary bladder induced by chlornaphazine were observed after treatment for 2½ to 10 years and after total dosages of 100 g. or more (Thiede *et al.*, 1964; Videbæk, 1964).

In the present cases the total doses of chlornaphazine were only 78 and 85 g., and five and six years elapsed between discontinuing the drug and the development of bladder cancer. It might therefore be suggested that the bladder malignancies were caused by some other treatment. It is well known that cyclophosphamide can induce a haemorrhagic cystitis with macroscopic changes of the bladder mucosa (Philips, *et al.*, 1961; Gellman *et al.*, 1969), but, to my knowledge, malignant changes have not been described during such treatment, and procarbazine chloride does not produce bladder symptoms. On the other hand, from industrial cancers of the urinary bladder it is known that the time for tumour induction after exposure to the carcinogen is highly variable (Conolly and White, 1969). Consequently, chlornaphazine is thought to be the most likely cause of bladder cancer in the above cases.

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Preliminary Communications

Development of Vascular Lesions in Insulin-treated Animals Fed a Normal Diet

British Medical Journal, 1970, 3, 685-687

Summary: Experiments were undertaken to study the role of hyperinsulinism in the pathogenesis of atheroma. Chickens injected with long-acting insulin for 19 weeks developed lipid containing lesions in their aortas. This provides further evidence in favour of the hypothesis that insulin and atheroma are causally related.

INTRODUCTION

Abnormalities of lipid and carbohydrate metabolism are commonly found in patients with atherosclerotic vascular disease. Hyperglycaemia, hypercholesterolaemia, and hypertriglyceridaemia have all been reported in this condition. More recently abnormalities in insulin secretion have also been described in patients with ischaemic heart disease. These abnormalities consist of raised insulin responses to oral carbohydrate with or without high fasting plasma insulin levels, and are found in atherosclerotic subjects who are neither obese nor hyperglycaemic (Nikkilä *et al.*, 1965; Peters and Hales, 1965; Christiansen *et al.*, 1968; Tzagournis *et al.*, 1968). It has been suggested that hyperinsulinism may be the

common factor linking atherosclerosis with diabetes, obesity, and hyperlipidaemia (Stout and Vallance-Owen, 1969).

As lipid is a major component of the atherosclerotic plaque, and insulin stimulates lipid synthesis elsewhere in the body (Renold *et al.*, 1965), it seemed reasonable to postulate that insulin may have a causal role in atherogenesis. An acute effect of insulin on the lipid metabolism of the arterial wall has been described (Mahler, 1966; Stout, 1968, 1969). It is the accumulation of lipid in the arterial wall over a long period of time, however, that is a feature of atherosclerosis. The effect of long-term administration of insulin on the arteries of experimental animals was therefore investigated.

METHOD

Of all animals, birds have arteriosclerosis most closely resembling human arteriosclerosis (Dauber, 1944), and the chicken, which like man is omnivorous, has been extensively used for experiments on atherogenesis (Katz and Stamler, 1953). Thirty chickens of the AA hybrid variety, aged 12 weeks, were divided into two groups, each containing 11 females and four males. They were identified by coloured rings, but were otherwise not segregated in any way. They were all housed in one room and fed on commercial chicken feed from one chicken feeder.

One group was injected intramuscularly into the pectoral muscles with insulin zinc suspension. Initially the dose was 10 units daily, but after four weeks it was reduced to 40 units

per week in equally spaced doses. The other group was injected with an equal volume of the vehicle solution, which contained all the constituents of insulin zinc suspension except insulin. There were thus two groups of chickens which differed only in that one received insulin and the other did not.

In the insulin-treated group there was an initial high mortality, which was probably due to hypoglycaemia, as necropsy showed no other cause of death. Four weeks after the start of the experiment the dose of insulin was reduced, and three chickens were transferred from the control to the treated group. Thereafter the mortality was low and occurred in birds in each group.

After 19 weeks the chickens were killed and the aortas examined. There were then 13 chickens in the treated group and eight in the control group. The aortas, fixed in formalin and embedded in gelatin, were sectioned with a freezing microtome, and stained for fat with oil red O, with haematoxylin as counter stain. Three sections of each aorta were examined, taken from the ascending aorta, the arch, and the descending aorta.

The specimens were examined microscopically, the identity of the specimens being unknown at the time. The amount of fat in the wall of the aorta was graded from 0 to 4, the intima and media being graded separately. Where sections from different sites showed different amounts of lipid the maximum grading was taken. As this method was somewhat subjective, a pathologist who was unaware of the identity of the specimens graded them independently. There was a very high degree of correlation ($r=1$) between the results of the two observers.

Biochemical estimations on carbohydrate and lipid metabolism were made during the experiment and will be reported separately. There was, however, no difference in the mean blood sugar levels in the two groups during the course of the experiment.

RESULTS

There were more aortas with significant intimal lipid infiltration (grade 2 and above) in the insulin-treated group than in the control group ($\chi^2=7.15$; $P<0.01$). In the intima the mean lipid grading was 2.08 ± 0.35 in the treated group and 0.37 ± 0.18 in the control group ($t=4.4$; $P<0.0005$). In the media the figures were 1.38 ± 0.55 in the treated group and 0.25 ± 0.25 in the control group ($t=2.6$; $P<0.01$). Medial lipid was seen only in aortas in which there was heavy intimal staining, and there was a high degree of correlation between intimal and medial lipid ($r=0.70$; $P<0.0025$).

Typical sections from the control and treated groups are shown in Figs. 1 and 2. Heavy lipid staining is shown in the intima and media of the aorta from the insulin-treated chicken. The lipid was not uniformly distributed around the circumference of the aortas or in sections from different sites, and thus had an overall patchy distribution.

These results show that treatment with insulin for five months results in the accumulation of lipid in the intima and media of the aortic wall. Spontaneous vascular disease does not occur in chickens under the age of 6 months, but is present in 50% at one year (Dauber, 1944). The birds used in this study were 30 weeks old at the end of the experiment. Thus administration of insulin accelerated the formation of the vascular disease.

DISCUSSION

There are no published reports of experiments designed to test the effect of insulin on arterial lipid accumulation. There is, however, some indirect evidence that insulin has an effect

FIG. 1.—Aorta from control group ($\times 60$) showing no lipid in intima or media.

FIG. 2.—Aorta from insulin-treated group ($\times 70$) showing lipid in intima and adjacent media.

on arterial lipid metabolism. Thus administration of insulin inhibits the regression of arterial lesions induced by a high cholesterol diet, which occurs when the animal is transferred to a normal diet, and abolishes the protective effect of oestrogens on experimental atherosclerosis (Stamler *et al.*, 1960). In pancreatectomized rats fed an atherogenic diet treatment with insulin reduces the serum cholesterol to that of non-diabetic controls but does not decrease the incidence or severity of their vascular lesions, as happens when a comparable reduction of hypercholesterolaemia is effected by dietary methods (Wilson *et al.*, 1969). The nearest parallel to the experiments reported here occurs in the obese hyperglycaemic spiny rat. This animal, which has both hyperglycaemia and hyperinsulinism, develops lipid infiltration of its coronary arteries at a much earlier age than its normal litter mates (Renold *et al.*, 1968).

The earliest evidence of human atherosclerosis is the fatty streak. This lesion is characterized by lipid accumulation in the intima and the adjacent media (Geer and McGill, 1967). It bears a strong resemblance to the lesion produced by hyperinsulinism in chickens. The fact that only aortas with heavy intimal lipid deposits showed medial lipid suggests that lipid is first deposited in the intima and later overflows into the media.

Thus the present experiments show that treatment with long-acting insulin results in vascular lesions similar to the earliest lesions in human atherosclerosis. This is the first report of the induction by insulin of lipid accumulation in the

arteries of experimental animals without dietary manipulation. It provides further evidence in favour of the hypothesis that atherosclerosis and deranged carbohydrate metabolism are causally linked.

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Medical Memoranda

Thrombectomy for Renal Venous Thrombosis in Infant of Diabetic Mother

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Renal venous thrombosis can occur in newborn infants of diabetic and "prediabetic" mothers (Avery *et al.*, 1957; Takeuchi and Benirschke, 1961; Stark, 1964; Aurelius, 1969). In this paper we describe successful treatment by thrombectomy in an infant with thrombosis of the renal veins and inferior vena cava.

CASE REPORT

A male infant was born on 1 June 1968 to a 33-year-old woman in whom diabetes mellitus had been diagnosed during this, her third, pregnancy. Treatment consisted of metformin and dietary carbohydrate restriction. Surgical induction was performed at 35 weeks' gestation because of pre-eclamptic toxæmia and hydramnios. The baby was delivered normally but was mildly asphyxiated at birth, with an Apgar score of 6 at one minute.

The infant weighed 3.8 kg. and showed the physical features of infants of diabetic mothers. Hypoglycaemia (blood sugar 8 mg./100 ml.) and respiratory distress were early problems and responded to treatment. Pooled human milk was fed by nasogastric tube from the age of 5 hours. Though respiration improved, cyanosis occurred whenever the ambient oxygen concentration was reduced to less than 30%. On the fifth day a rounded left renal mass 8 by 6 cm. in size and later a smaller right-sided renal mass, were felt. Dilated veins appeared on the abdominal wall. The blood urea level was 142 mg./100 ml., the platelet count was 95,000/cu.mm., and the urine contained numerous red blood cells.

Contrast radiography of the inferior vena cava showed narrowing of the lumen, and an extensive collateral circulation was present. There was no retrograde filling of either renal vein. Faint nephrograms confirmed the bilateral renal enlargement.

Open disobliteration of the inferior vena cava and renal veins was carried out on the sixth day. Clots and pultaceous material were removed. Suction catheters were passed up and down the inferior vena cava and into both renal veins. Free flow of blood was obtained from both ends of the inferior vena cava and the left renal vein, but not from the right renal vein in spite of an apparently satisfactory disobliteration. The gross enlargement of the kidneys was confirmed, but in order to preserve the collateral circulation the overlying peritoneum was not disturbed. Histological examination of the clots showed recent thrombus with no evidence of organization.

Postoperative progress was satisfactory. Within three days the kidneys were of normal size, the blood urea was 38 mg./100 ml., and the urine was normal. The creatinine clearance on the tenth

postoperative day was 39 ml./min./1.73 sq.m. and the platelet count 300,000/cu.mm. We attributed the baby's earlier thrombocytopenia to the consumption of platelets during the intravascular coagulation. Neither cyanosis nor respiratory difficulty was present after the operation.

The baby's renal function improved, the creatinine clearance of 71 ml./min./1.73 sq.m. being within the normal range for his age at 8 months. Intravenous pyelography at the age of 4 months showed normal kidneys with good excretion of dye; slight perirenal calcification present on the left side later disappeared. The baby's blood pressure, blood urea levels, and urine remained normal.

COMMENT

The aetiology of renal venous thrombosis in the infants of diabetic and prediabetic mothers was discussed by Avery *et al.* (1957), who suggested that abnormalities of the renal capillary vasculature might be present, while Takeuchi and Benirschke (1961) thought that intermittent fetal glycosuria led to haemoconcentration and thrombosis within the kidney. Various mechanisms exist whereby these infants may be at risk of intravascular coagulation. Placental damage may allow placental thromboplastin or other coagulation initiators to pass into the fetal blood, while in poorly controlled diabetes certain free fatty acids and phospholipids may cross the placenta to influence the infant's platelet function.

Nephrectomy, conservative management (Stark, 1964; McFarland, 1965; *Lancet*, 1966) and heparin (Stark, 1964) have been used successfully in treatment.

We believe that thrombectomy is indicated in selected cases—for example, where the inferior vena cava is involved. Rapid resolution of the renal enlargement in the present case and the baby's subsequent progress appear to support our view.

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