Similarity of Glomerular Ultraviolet Absorptions in Diabetes Mellitus and After Cortisone Therapy. (22232)

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In the 20 years since Kimmelstiel and Wilson(1) described intercapillary glomerulosclerosis histologically, it has become a commonly recognized entity. Practically always, nodular glomerulosclerosis is a complication of diabetes mellitus. It is clearly not a subtype of arteriosclerosis or arteriolar nephrosclerosis(2), but little is known of the basis of its development. Experimental glomerular lesions closely simulating intercapillary glomerulosclerosis were described by Mann, Goddard and Adams(3) in rats rendered diabetic with alloxan, after 3 to 6 months, and by Rich and associates(4) in rabbits treated with 7.5 mg of cortisone daily. The pathologic process in rats was described as beginning with glomerular capillary erosions or increased deposits of periodic-acid-Schiff positive material in their walls and progressing to hyalin ball formation with concentric reticulin proliferations. Cortisone produced severe hyperglycemia and lipemia in rabbits. Recent reports by Becker *et al.*(5), Bloodworth and Hamwi(6), Wilens and Stumpf (7) confirm that cortisone therapy will lead to production of intercapillary glomerulosclerosis in normal or alloxan treated rabbits. Other factors such as vit. B_{12} deficiency, increased and altered blood lipids, and fat emboli have been reported as involved. It is uncertain how far the experimental lesions correspond to those of human diabetics, and to what extent cortisone hypersecretion might be present in human cases of glomerulosclerosis. Gordon(8) has reported unusually high levels of glycogenic adrenal steroid excretion and very low levels of 17-ketosteroid excretion in human cases of pathologically confirmed intercapillary glomerulosclerosis.

During an investigation of the histochemical and ultraviolet absorptive characteristics of kidney tissues from hypertensive patients, findings relevant to the hormonal background of diabetic glomerular lesions were unexpectedly encountered, and form the basis of this report.

Materials and methods. Freshly obtained kidney tissues from biopsies or autopsies were prepared by freeze-drying and embedded *in vacuo* in paraffin or polyethylene glycol (carbowax) with apparatus and methods previously reported(9). Microtome sections were cut at 4 μ and mounted on Vicor slides. Glycerin mounting with paraffin ringed Vicor cover slips was employed, with technics originally designed to prevent a loss of soluble substances prior to autoradiography. Ultraviolet microscopy with Polaroid color-translating instrument, was carried out in wavelength range 235-280 mμ as previously described(10). Enzyme treatment *in vitro* employed hyaluronidase in 1% solution, incubated 37°C for 30 minutes, and the solution washed off with distilled water. Treatment with trypsin, lecithinase, streptokinase and fibrinolysin was also employed, but without any significant results in the present problem. Following ultraviolet microscopy, the same sections were stained with Pearse periodic-acid-Schiff method originally introduced for staining pituitary cells(11), and studied for morphologic or histochemical abnormalities.

Results. Among hypertensive patients routinely investigated was a woman 38 years old with Cushing's syndrome, who had kidney biopsies made at the time of bilateral adrenalectomy. Each adrenal gland weighed about 40 g and was mostly composed of multiple cortical adenomas. She was not diabetic or glycosuric, and fasting blood sugar values

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TABLE I. Cortisone Therapy of 5 Cases Investigated by Ultraviolet Microscopy.

Sex	Age	Disease	Dose,† mg/day	Duration, days
♂	16	Osteogenic sarcoma	200, p.o.	30
♂	19	Hodgkin's disease	300, "	9
♀*	38	Postop. Cushing's syndrome	40, i.m.	42
♀	42	Ulcerative colitis	300, "	6
♂	42	Pulmonary fibrosis	100, p.o.	16
			75, "	2
			50, "	2
			25, "	3

* Negative observations after bilateral adrenalectomy; positive findings before operation.
† p.o. = per os; i.m. = intramuscular.

were 82, 115, 120 and 143 mg %. Ultraviolet absorptions of glomeruli and arterioles from this patient were color-translated as orange in the shortest set of wave-lengths, 248, 240 and 235 mμ, previously termed set 3.‡ This abnormality of absorption had been observed before only in diabetes mellitus(9). Three additional cases of Cushing's syndrome available for study had been previously chemically fixed in Zenker's or formalin solutions, and they failed to show any abnormality of ultraviolet absorption comparable to that of frozen-dried tissues.

Tissues prepared by freeze-drying were available from autopsies of 4 other non-diabetic persons who had had cortisone therapy until death. On testing the ultraviolet absorptive properties of their glomeruli, the same abnormal orange color of their glomerular stroma, at short ultraviolet wave-lengths, was observed. Dosages of cortisone and basic clinical information are given in Table I. The original patient with Cushing's syndrome mentioned above was maintained postoperatively on 40 mg of cortisone daily until death 42 days later. At autopsy, kidney tissues obtained and tested as previously described failed to demonstrate the abnormality observed in biopsies. This was thought to indicate that over 50 mg cortisone per day was required to induce the abnormality of glomerular stromal absorptions.

Treatment *in vitro* with hyaluronidase of kidney sections removed the material responsible for abnormal absorption. After enzyme treatment, glomerular stromal absorptions appeared normal for the range 235-280 mμ investigated. It was considered that an abnormal mucopolysaccharide component, but not necessarily hyaluronic acid, had been affected by the hyaluronidase.

Hamster kidneys were also obtained from animals treated with cortisone, approximately 6 mg per week, for use as hosts of human cancer transplants(12). These tissues also showed the abnormal orange color of translated absorptions, and the responsible material was removed by hyaluronidase. It was not possible to determine whether the animals had been diabetic.

The localization of the abnormally absorptive material in the kidneys of patients treated with cortisone differed somewhat from that previously found in diabetics(9). Orange colors of the arteriolar walls in color-translated photomicrographs appeared after cortisone, but not in diabetes mellitus. The glomerular absorptive characteristics were indistinguishable. No similar absorptive abnormality was observed in the kidneys of several persons with essential hypertension or with hypertension secondary to pheochromocytoma, being concurrently investigated.

The renal architecture of kidneys from patients treated with cortisone was not definitely altered as judged by a scrutiny of periodic-acid-Schiff stained slides. The capillary walls stained positively with the PAS technic. Occasionally a dilatation was noted of glomerular capillaries near the glomerular roots. No ectasia of peripheral glomerular capillaries, glomerular capillary aneurysms, or thrombi were observed. No lesions were found simulating intercapillary glomerulosclerosis.

Discussion. Evidence has been presented, although indirect and perhaps tenuous, implicating cortisone as one factor essential for the development of human intercapillary glomerulosclerosis. Under the influence of cortisone administered in moderate dosages the glomerular stromal tissues were altered with the accumulation of an abnormal substance

‡ Color translation by filters of 248 mμ as blue, 240 as green, and 235 as red was employed.

between the capillaries, and in their walls and those of arterioles. From the periodic-acid-Schiff staining of this material and its removal with hyaluronidase *in vitro* it was inferred to be a mucopolysaccharide complex, probably attached to protein, as usually occurs in ground substances and basement membranes.

For the formation of nodular intercapillary glomerulosclerosis in the human kidney other factors are apparently necessary, the chief of which is a part of or accompanies diabetes mellitus. Whether this influence is attributable to hyperglycemia, general changes in the body mucopolysaccharides, hyperlipemia, fat emboli, or various steroid abnormalities in human diabetics is not evident from the present study. Additional information is anticipated from the delicate lipid staining possible with carbowax embedded frozen-dried tissues. Also it is hoped that a pathologic study of the endocrine glands from diabetics with glomerulosclerosis might show some other evidences of the cortisone hypersecretion postulated.

Summary. In the glomerular stroma and arterioles of non-diabetic persons treated with cortisone an abnormal substance was found with ultraviolet absorptive properties indistinguishable from those of the nodules of intercapillary glomerulosclerosis. Histochemical properties of the material suggest that it is a mucopolysaccharide complex. The evidence is considered that implicates cortisone as one factor in the development of human diabetic intercapillary glomerulosclerosis.

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Loss and Repair of Glucose-Disposal Mechanism in Dog Fed Fructose As Sole Dietary Carbohydrate.* (22233)

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Normal rats lose, to a considerable extent, their capacity to utilize glucose when they are fed for several days a diet containing 58% fructose as *sole* carbohydrate. The site of defective glucose utilization in the fructose-fed rats was localized by studying the metabolism of C^{14}-glucose, C^{14}-fructose, and C^{14}-acetate in various tissues of rats previously fed a diet containing either glucose or fructose as sole carbohydrate. The liver was the only tissue in which an altered metabolic pattern was observed after fructose feeding, and this alteration was manifested by a decreased capacity for converting glucose to glycogen, fatty acids, and CO_2. This altered metabolic pattern in the liver was apparently not the result of an insulin-lack, for insulin injections before the fructose-fed rats were sacrificed failed to restore the ability of their livers to utilize glucose(1).

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