Downman for helpful criticism; to Mr. William Matthews for technical assistance; to Miss F. Ellis, Miss J. Cherry, and Messrs. J. M. Crane and V. Willmott for the photography; and to the Editor of the *Journal of Anatomy* for permission to use Fig. 20.

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METABOLIC AND ENDOCRINE ASPECTS OF DIABETIC NEPHROPATHY

BY

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In the past few years various metabolic and endocrine disturbances have been described in association with the specific vascular lesions of diabetes—namely, retinopathy and nephropathy. It is claimed that many of these disturbances have a pathogenic relationship with the development of the vascular complications.

Becker (1952) gave evidence of excessive adrenal cortical function in diabetics with retinopathy and Kimmelstiel-Wilson (K.W.) lesions. He noted an apparent relationship between the lipoid-laden vacuolated cells in the zona fasciculata of the adrenal cortex and the K.W. lesions in the kidney. A picture similar to early diabetic retinopathy and the K.W. lesion could be produced in alloxan-diabetic rabbits by the injection of corticotrophin or cortisone. Becker, Allen, *et al.* (1954), Becker, Maengwyn-Davies *et al.* (1954), and Maengwyn-Davies (1956) gave further evidence of excessive production of adrenal glucocorticoids in patients with diabetic retinopathy.

Becker *et al.* (1953) also reported a disturbance of vitamin B₁₂ metabolism in patients with diabetic retinopathy. Using the vitamin B₁₂ excretion test, they found that diabetics with retinopathy cannot retain the vitamin, while those with no retinopathy retain most of the given dose. However, Field *et al.* (1957) could not find a definite relationship between the development of degenerative complications of diabetes and the excretion patterns of the B vitamins.

A disturbed carbohydrate metabolism has been found in patients with clinically evident K.W. syndrome. Thus, Berkman *et al.* (1953, 1954), in a study of the serum polysaccharides in diabetics and non-diabetics, reported an increase in the total serum polysaccharides in diabetics with degenerative vascular complications, the highest level being observed in those with clinically evident nephropathy (Adlersberg *et al.*, 1956). It is not clear whether this increase is due to the renal impairment and is thus the result of the diabetic nephropathy, or whether it precedes the nephropathy and provokes the pathological lesions in the kidneys.

Mann *et al.* (1949) have noted hypercholesterolaemia in association with diabetic glomerulosclerosis. Later,

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Barr and Russ (1951), Engelberg *et al.* (1952a, 1952b), Keiding *et al.* (1952), and Collens *et al.* (1954) studied the serum lipids, lipoproteins, and cholesterol levels in diabetics and their relationship to degenerative vascular complications, and found that the serum cholesterol and phospholipid levels were usually raised. The most striking finding, however, was an increase of the S_F 12-20 class of lipoproteins in the serum of patients with diabetic nephropathy out of proportion to the raised level of cholesterol.

The similarity between the pathology of diabetic nephropathy and diabetic retinopathy (Ashton, 1951) has facilitated the study of the various metabolic and endocrine aspects of these two complications. However, Volk (1956) has pointed to a dissimilarity between the retinal micro-aneurysms and the glomerular nodules in diabetes. In his opinion there are probably basic differences in the pathogenesis of the two lesions, so that the results obtained from the study of one lesion may not be applicable to the other.

In a thorough study of diabetic nephropathy, including needle biopsy of the kidney, we have obtained some interesting data about the endocrine and metabolic aspects of this condition. We were also able to investigate human diabetics without having to rely on the ophthalmoscopic changes as an indication of the renal changes.

Material and Methods

Sixty-five diabetic patients were fully studied both clinically and by laboratory methods, and by renal biopsy whenever possible. The following laboratory studies were done for every patient: (1) a complete examination of urine; in cases with proteinuria the amount of protein per 24-hour specimen was estimated, using Esbach's reagent; (2) urine culture to detect infections of the urinary tract; (3) stool examination; (4) total and differential serum protein patterns; (5) Hb, W.B.C., and other haematological investigations when indicated; (6) serum cholesterol; (7) blood urea and urea clearance test; (8) oral 50-g. glucose-tolerance test; (9) serum sodium and potassium, by flame photometry; (10) 17-ketosteroids in the urine, estimated by the Zimmermann reaction in 37 cases; (11) 17-hydroxycorticosteroids in the urine, estimated by the Silber and Porter (1954) method in 28 patients; (12) total non-glucosamine serum polysaccharides were estimated in 39 cases, using the anthrone reagent, according to the method of Graff *et al.* (1951); (13) the vitamin B₁₂ retention test was done in 13 diabetics, 2 normal subjects, and a patient with pernicious anaemia, the vitamin B₁₂ being estimated chemically (Boxer and Rickards, 1951); (14) the Thorn eosinophil test was done on nine patients four hours after the administration of corticotrophin.

Results

The diabetic patients were classified into four groups, according to the clinical find-

ings. The 56 patients for whom adequate data were available were grouped as follows:

Group D1, comprising 15 patients who showed not a single complication of diabetes except occasionally diabetic ketosis and the polyneuritis which accompanied it. Laboratory findings were not taken into account until two weeks after control of the ketosis, if present.

Group D2, comprising 20 patients who showed arteriosclerotic complications — palpable tortuous superficial arteries, impending or actual senile gangrene, or coronary artery disease. This group also included cases that showed arteriosclerotic but not diabetic or hypertensive fundus changes, as well as cases of diabetic neuropathy.

Group D3, comprising two patients with diabetic retinopathy, with no clinical evidence of renal involvement (no proteinuria).

Group D4, comprising 19 patients with proteinuria as evidence of diabetic nephropathy. All showed diabetic retinopathy as well, and most of them had arteriosclerotic and neuropathic complications.

Serum Proteins.—The total serum protein levels, the serum albumin levels, and the serum globulin levels in the four groups are shown in Figs. 1, 2, and 3. The cases within group D4 with heavy proteinuria and with the nephrotic syndrome showed the lowest serum albumin levels.

Serum Cholesterol.—The serum cholesterol levels are shown in Fig. 4. The mean value in group D1 is 190.4 mg./100 ml., in group D2 237.7 mg./100 ml., in group D3 243 mg./100 ml., and in group D4 251 mg./100 ml. The highest value was 390 mg./100 ml. in a patient in group D4.

Serum Sodium.—Fig. 5 shows the serum sodium levels in the four groups. Groups D1 and D2 showed a

FIG. 1.—Serum albumin in diabetics.

FIG. 2.—Serum globulin in diabetics.

FIG. 3.—Total serum proteins in diabetics.

FIG. 4.—Serum cholesterol in diabetics.

normal serum sodium; while group D3 and especially group D4 showed a low serum sodium level (mean 131.5 mEq./l.).

Serum Potassium.—Fig. 6 shows the serum potassium levels in the four groups. In Group D1 the level was slightly lower than the average normal, but was still within the normal range. Group D2 showed a slight rise above the average normal value. An especially well-marked rise was seen in group D4, with a mean value of 5.75 mEq./l. Two of them showed values of 7 mEq./l.—the highest value seen among the 56 patients investigated.

Serum Polysaccharides.—The total serum polysaccharides were estimated in 8 normal subjects, and 31 diabetic patients. The normal individuals were taken from the donors to the blood bank of the hospital, all being males. Of 31 diabetic patients 7 were in group D1, 11 in D2, 2 in D3, and 11 in D4. The total serum non-glucosamine polysaccharides as determined by the anthrone reagent had an average normal value of 149.4 mg./100 ml. serum. In group D1 the average value was 146 mg./100 ml.; in group D2, 158; in group D3, 190; and in group D4, 258 mg./100 ml. (Fig. 7).

Urinary 17-Hydroxycorticosteroids.—These were estimated in 28 diabetic patients according to the method of Silber and Porter (1954): 9 were in group D1, 8 in group D2, 1 in group D3, and 10 in group D4. The average value in each group was 7.5, 13.5, 21, and 23.5 mg. respectively (Fig. 8). The following interesting fact was discovered accidentally. When the urinary 17-hydroxycorticosteroids were estimated in the first two cases very high values were obtained—38 and 34 mg. respectively. The result was questioned and the estimation was repeated some two weeks later, the

values then being 8.5 and 11 mg. respectively. It was then realized that the patients had been admitted in diabetic ketosis and that the test was first performed while they were still in this state. After two weeks in hospital this defect was corrected, the diabetic state was stabilized, and consequently the level of the 17-hydroxycorticosteroids was much lower. This finding was later confirmed, for in three other patients admitted in ketosis the levels of 17-hydroxycorticosteroids were found to be 27, 33, and 24 mg.; and two weeks later the figures were 9, 5.1, and 16 mg. respectively. For the purposes of this analysis the results obtained from the patients during ketosis were ignored.

FIG. 9.—17-Ketosteroids in diabetics.

Urinary 17-Ketosteroids.—Urinary 17-ketosteroids were estimated in 15 male and 22 female patients, all adults. In the 10 males without diabetic proteinuria the average urinary excretion of 17-ketosteroids was 11.9 mg./24-hour specimen, and in the five males with diabetic proteinuria it was 5.3 mg. In the 14 females with no proteinuria the average daily excretion was 6.3 mg. and in the 8 females with diabetic proteinuria it was 2.5 mg. (Fig. 9). In four patients (two males and two females) the urinary 17-ketosteroids were estimated on two occasions—during ketosis and two weeks after control of diabetes. In the males the figures were 3 and 3.5 mg. during ketosis, and 15 and 14.1 mg. after control. In the females the corresponding figures were 2.7 and 1.0 mg. during ketosis and 5.3 and 4.3 mg. after control.

Vitamin B₁₂ Retention Test.—This was done in 16 cases (13 diabetics, 2 normal subjects, and one patient with pernicious anaemia). The vitamin B₁₂ content of the urine was estimated by the chemical colorimetric method described by Boxer and Rickards (1951). The eight-hour urinary output was ascertained, then 100 µg. of vitamin B₁₂ was given intramuscularly, and the urinary output in the next eight hours measured. The difference was calculated as the percentage excretion from the given dose. The results are shown in Table I, which also records the results of renal biopsy. The excretion in the diabetic patients varied widely, yet the presence or absence of degenerative changes seemed to have some interesting effects on the figures. In Cases 4–9 there were no manifestations of degenerative changes, but all of them showed a marked degree of retention of

FIG. 5.—Serum sodium in diabetics.

FIG. 6.—Serum potassium in diabetics.

FIG. 7.—Total serum polysaccharides in diabetics.

FIG. 8.—Hydrocorticosteroids in diabetics.

TABLE I.—Results of Vitamin B₁₂ Retention Test and of Renal Biopsy

Case No.	Diagnosis	Excretion after 100 µg. vit. B ₁₂	Clinical Degenerative Changes	Renal Biopsy
1	Normal	19%	None	No lesion detected
2	"	17%	"	
3	Pern. anaem.	3.3%	Polyneuritis	
4	Diabetes	9%	None	
5	"	5.2%	"	
6	"	4.2%	"	
7	"	6%	"	
8	"	49.4%	"	
9	"	32.4%	"	
10	"	47.6%	Coronary sclerosis	
11	"	27.2%	Arteriosc. ret. and neur.	
12	"	21.5%	D. retin. and nephropathy	
13	"	69%	"	
14	"	82%	"	
15	"	71%	"	
16	"	58.7%	"	

the given dose, with the exception of the last two cases, which were in a state of ketosis when the test was done. Cases 10 and 11 had degenerative changes but no nephropathy, and in these there was a moderate increase in the excretion of vitamin B₁₂ compared with the normal. In Cases 12–16 diabetic nephropathy was present. The excretion of vitamin B₁₂ was very marked in all of them, ranging from 58.7% to 82%, with the exception of Case 12 in which renal biopsy showed none of the hyaline fibrinoid lesions found in all the other cases. The results are summarized in Table II.

TABLE II.—Summary of Results of the Vitamin B₁₂ Retention Test

Normal	Uncomplicated Diabetes		Diabetes with Degenerative Complications Other than Ret. and Neph.	Diabetic Nephropathy		Per-nicious Anaemia
	Con-trolled	In Ketosis		No H.F.L.	With H.F.L.	
19 µg.	5.2 µg.	32.4 µg.	47.6 µg.	21.5 µg.	69 µg.	3.3 µg.
17 "	4.2 "	49.4 "	27.2 "		82 "	
	9.0 "				58.7 "	
	6.0 "				71 "	

Thorn Test.—The four-hour Thorn eosinophil test was performed in nine patients (four without proteinuria and five with diabetic proteinuria). All nine showed a drop in the eosinophil count of more than 50%, indicating intact glucocorticoid function of the adrenal glands. However, it should be noted that in patients with diabetic proteinuria there was a greater drop (Table III).

TABLE III.—Drop in Eosinophil Count Four Hours After 25 Units of Corticotrophin Intramuscularly in Nine Diabetic Patients

Without proteinuria	57%	50%	60%	58%	
With diabetic proteinuria	86%	75%	83%	100%	100%

Discussion

Serum Proteins

Patients with diabetic nephropathy showed hypoalbuminaemia and hyperglobulinaemia; the total serum protein level was not always diminished, and seemed to depend upon the degree of diminution in the serum albumin or of elevation in the serum globulin.

Hypoalbuminaemia in diabetic nephropathy was recognized long ago, and was described with the nephrotic syndrome accompanying diabetic glomerulosclerosis (Newburger and Peters, 1939). The fall in the serum albumin level was regarded as secondary to the heavy proteinuria and excessive loss of serum albumin

in the urine. This explanation cannot be accepted as the only mechanism of hypoalbuminaemia, since a fall in the serum albumin was noted in diabetic patients with degenerative complications (group D2) and in patients with diabetic retinopathy (group D3) in the absence of proteinuria. However, proteinuria was found in cases with the lowest levels of serum albumin (group D4).

Similarly, a rise in the serum globulin above the maximum normal value of 3 g./100 ml. was noted in all groups of diabetic patients, but especially in those with degenerative complications. These findings point to a disturbed metabolic pattern of the serum proteins in diabetics, which is especially exaggerated in those with degenerative complications, and may be responsible for the development of these complications in some way or other.

It has been suggested that shifts in the serum proteins play a part in the development of the degenerative vascular lesions seen in long-standing diabetics (McManus, 1950b; Friedenwald, 1952; Root, 1952). Schneider *et al.* (1946) described changes in albumin and β -globulin fractions of the serum from 31 diabetic patients with retinitis. The reduction in albumin and the elevation of the β -globulin fractions were more marked in the retinitis cases than in uncontrolled diabetics. Rifkin and Petermann (1952) reported the results of electrophoretic study of the serum proteins in patients with clinically established diabetic glomerulosclerosis. A decrease in the albumin and a significant rise in the α_2 -globulin were found in all of their 10 cases. The β -globulin was normal or only slightly raised. Keiding (1954), using paper electrophoresis, found a definite decrease in albumin and α_1 -globulin, and an increase in α_2 -globulin in all diabetics, but only those with retinitis proliferans and proteinuria showed a diminution in total protein. The β -globulin remained unchanged.

It is concluded that the disturbances in the serum protein pattern, particularly involving albumin and α_2 -globulin, occur at a stage in the development of diabetic vascular lesions when the changes could not be accounted for by loss of protein in urine.

Serum Cholesterol

The mean value of serum cholesterol in diabetic patients with nephropathy was found to be raised in comparison with the other groups of diabetic patients. Because of this hypercholesterolaemia, a metabolic defect affecting lipid metabolism would appear to play a part in the pathogenesis of diabetic vascular disease in general and diabetic retinopathy and nephropathy in particular. The associated severe atherosclerosis and the markedly increased glomerular lipid in hyaline fibrinoid lesions (as seen in the biopsy material) give further support to this view.

Serum Polysaccharides

Normal human serum contains a relatively high concentration of complex polysaccharides bound to protein. The source, composition, and function of these glycoprotein substances are poorly understood. It has been repeatedly demonstrated that their concentration, and in some cases their composition, is altered in a variety of diseases (West and Clarke, 1938; Shetlar *et al.*, 1949). Some published data, notably those of Jacobs (1949), indicate that the concentration of polysaccharides measured as glucosamine is raised in the

serum of diabetic patients. Other figures suggest that diabetic patients have normal serum polysaccharides (West and Clarke, 1938; Shetlar *et al.*, 1949). The data in these studies were presented in such a way that the results could not be correlated with the absence or presence of vascular disease.

In order to explore the possibility of an association between a disturbance of polysaccharide metabolism and the occurrence of vascular disease in diabetic subjects, we have studied the serum polysaccharide levels in several groups of subjects. One group consisted of healthy male volunteers; the others were diabetic patients. All cases in this study were free from other diseases, such as cancer, acute or chronic infections, and myocardial infarction, all of which are known to be associated with raised serum polysaccharide.

Serum polysaccharides were measured as galactosemannose by the method described by Graff *et al.* (1951). From the data already recorded it is evident that the concentration of polysaccharides bound to serum proteins is significantly raised in diabetic patients, but this does not occur in association with the primary metabolic defect, but only in cases with well-established clinically detectable degenerative vascular disease. However, raised values were found in diabetic patients independent of the existence of abnormalities in renal function, as assessed by conventional clinical and laboratory methods. Increased values in the serum polysaccharides, which have been produced experimentally and which occur spontaneously in disease, are currently interpreted as a non-specific response to tissue injury, and in particular to alteration of connective-tissue polysaccharides (Berkman, 1955). Changes in subintimal mucopolysaccharide-ground substance have been implicated in the pathogenesis of arteriosclerosis, a local increase in the subendothelial mucopolysaccharides being an early feature and preceding lipid deposition in the vessel wall (Altshuler and Angevine, 1951; Moon and Rinehart, 1952; Taylor, 1953). It is quite possible that the results obtained simply reflect widespread degenerative vascular disease in the diabetic patients selected for study, the elevated serum polysaccharide levels being the result of concomitant tissue injury. On the other hand, the facts still allow speculation about the possibility of a relation existing between the increased circulating polysaccharide and the eventual development of deposits of hyaline material of a mucopolysaccharide nature around certain capillaries in such patients. Indeed, we believe that the disturbance in the polysaccharide metabolism is related to the development of some of the pathological lesions in the kidney.

Serum Electrolytes

The serum electrolytes have not been studied before in diabetes (except in ketosis and coma). The present study showed a normal serum sodium level in all groups of diabetics except those with diabetic nephropathy, who showed a low level (mean 131.5 mEq/l.), and especially those with oedema (mean 129 mEq/l.). This finding may appear at first glance contradictory to what is known about the pathogenesis of oedema, in which sodium retention is a prime factor. However, despite the lowered serum sodium level, the total body sodium must be much raised, because an approximate reduction of 7% of the plasma sodium concentration is more than outweighed by an increase of 100–200% in the extracellular fluid volume (plasma and interstitial fluid)

containing this concentration of sodium (Prentice *et al.*, 1951; Warner *et al.*, 1952).

Treatment with low sodium diets and diuretics is instituted so commonly and intensively in nephrotic patients before they are hospitalized and their electrolytes studied, that it is difficult to be certain whether, or to what extent, altered serum electrolyte patterns are due to the nephropathy or are the result of treatment. Lastly, we do not yet know anything about the intracellular electrolyte changes in diabetic nephropathy. It may be quite possible that there is an increase in intracellular water and sodium and a diminution in intracellular potassium, similar to that which occurs in cases of congestive heart failure (Friedberg, 1956).

The serum potassium was lower in the uncomplicated diabetic patients, possibly owing to polyurea, and especially so in patients recovering from ketosis. In the patients with degenerative complications other than nephropathy the serum potassium was normal. In patients with diabetic nephropathy hyperkalaemia was a notable feature. This hyperkalaemia can be explained in part by the renal failure. The highest figures for serum potassium were found in patients with clinically manifest uraemic symptoms, and can be correlated with the levels of blood urea and the results of the urea-clearance test. This effect will become more evident if a shift of potassium from the intracellular to the extracellular compartment (as postulated above) is proved to be true.

Urinary Excretion of Corticoid Substances

Study of the urinary excretion of 17-hydroxycorticosteroids (the end-product of glucocorticoids) has given important results in the diabetic cases studied. Diabetics with retinopathy, and especially those with nephropathy, have shown a marked rise in the excretion of this substance. Increased glucocorticoid activity in those groups of diabetic patients can therefore be assumed. When the figures are seen in relation to the diminished renal function in cases of nephropathy, the substantial rise of the serum glucocorticoids in these cases is still more marked.

The urinary excretion of 17-hydroxycorticosteroids in diabetics has been studied by many authors. Most of them reported a normal excretion of these hormones (Talbot *et al.*, 1947; Venning and Browne, 1947; Shadaksharappa *et al.*, 1951; Perkoff *et al.*, 1954). In these reports there was no attempt to study diabetic patients in separate groups. Maengwyn-Davies *et al.* (1956), however, have studied the urinary excretion of 17-hydroxycorticoid patterns in diabetic patients with and without diabetic retinopathy, and have shown a marked rise in its excretion in the latter group of patients. In our study, also, an important finding was excessive 17-hydroxycorticosteroid urinary excretion in patients with diabetic acidosis. Similar excessive glucocorticoid activity was reported by McArthur *et al.* (1950), Stowers (1951), and Perkoff *et al.* (1954).

Vitamin B₁₂ Retention Test

We have shown that diabetic patients with no complications retain more of a test dose of vitamin B₁₂ given parenterally than normal subjects do. This probably denotes a deficiency of this vitamin—as of other B vitamins—in diabetics, because of the amounts lost through the polyuria. Diabetics in episodes of

acidosis excrete a great part of the test dose of vitamin B₁₂ given. Similarly, diabetics with hyaline fibrinoid lesions excrete the greater part of the test dose.

The interrelationship of vitamin B₁₂, diabetes, and adrenal cortical hormones has been explored (Becker *et al.*, 1953). Animals deficient in vitamin B₁₂ were reported to exhibit hyperglycaemia (Ling and Chow, 1953). Vitamin B₁₂ has been shown to abolish the hyperglycaemia in rats induced by large doses of glucose or by cortisone (Binkley *et al.*, 1951), and in humans it was shown to increase the insulin sensitivity and to antagonize the hyperglycaemic effect of prednisone in normal people and in diabetics (Darwish and Fikry, 1958). Furthermore, the administration of vitamin B₁₂ labelled with radioactive cobalt-60 resulted in a relatively high concentration of radioactivity in the pancreas, kidneys, and adrenals of experimental rats. Cortisone administration increased the turnover of vitamin B₁₂ in all organs studied, and resulted in a greatly increased excretion of vitamin B₁₂ in the urine (Becker *et al.*, 1953). Similarly, in rats, vitamin B₁₂ has been demonstrated to counteract such cortisone effects as thymus atrophy, alopecia, and inhibition of growth. It is therefore evident that vitamin B₁₂ metabolism is intimately related both to the diabetic state and to the function of the adrenal cortex.

Becker *et al.* (1953) concluded that the decreased excretion of a test dose of vitamin B₁₂ by diabetic patients is consistent with a deficiency, or abnormal retention, of this vitamin associated with the diabetic state. The increased excretion of a test dose of vitamin B₁₂ by diabetics with retinopathy is related to the development of this degenerative complication.

We found in our groups of patients that if the diabetics with no degenerative complications were compared with those with degenerative complications, no difference in the result of the vitamin B₁₂ retention test could be arrived at. But, by separating in the first group the cases with ketosis from those without it, and by subdividing the cases in the second group according to the presence or absence of hyaline fibrinoid lesions (ascertained by histological examination of material obtained by renal biopsy) we arrived at the following results: (1) The average normal excretion of vitamin B₁₂ in the eight-hour urine specimen after 100 µg of vitamin B₁₂ intramuscularly was 18 µg. (2) Diabetics without degenerative lesions and without ketosis retained most of the test dose; they excreted an average of 6.1 µg. (3) Diabetics without degenerative complications but with ketosis could not retain as much as did those with no ketosis: they excreted an average of 41 µg. (4) Diabetics with nephropathy and hyaline fibrinoid lesions excreted most of the test dose—an average of 70 µg. (5) Diabetics with nephropathy but with no hyaline fibrinoid lesions showed normal vitamin B₁₂ excretion—21.5 µg.

It seems possible, therefore, that Field *et al.* (1957) could not establish any relationship between the results of the vitamin B₁₂ retention test and the degenerative complications of diabetes because they included cases with ketosis in the group showing no degenerative complications, and because they studied all the degenerative complications together, not distinguishing the group with hyaline fibrinoid lesions from the rest of the diabetics with degenerative complications.

The findings that diabetics with ketosis and hyaline fibrinoid lesions cannot retain vitamin B₁₂ is indirect

evidence of excessive glucocorticoid function in these patients. Experimental chronic alloxan diabetes in rats was noted to induce vitamin B₁₂ deficiency and cause excessive retention of a test dose of this vitamin. Cortisone administered to rats was found to mobilize vitamin B₁₂ from all tissues and increase its excretion in the urine (Becker *et al.*, 1953). This occurred even in the presence of a marked vitamin B₁₂ deficiency, thus aggravating the deficiency state (Wahlstrom and Johnson, 1951). When testosterone was administered to diabetics with retinopathy a marked reduction in the excretion of the test dose of vitamin B₁₂ resulted, approaching the excretion level in those without retinopathy (Becker *et al.*, 1953).

Diabetics with acidosis and with nephropathy show the highest urinary values of 17-hydroxycorticoids. The same group also show the minimum retention of a test dose of vitamin B₁₂. Excess glucocorticoid function is therefore the probable cause of the inability of the diabetic patient to retain vitamin B₁₂, even though an actual state of deficiency exists.

Pathogenic Relationship of Metabolic and Endocrine Disturbances to Development of the Lesions of Diabetic Nephropathy

Serum Mucopolysaccharides

Since McManus (1950b) suggested that the source of the hyaline material of nodular glomerulosclerosis might be an abnormal circulating polysaccharide (because of the mucopolysaccharide nature of this hyaline material) many workers began to study the level of the serum polysaccharides and to correlate it with the development of diabetic glomerulosclerosis and diabetic nephropathy.

We know very little about the basic physiology and metabolism of ground substance, and the interpretation of changes in the levels of serum mucopolysaccharides is fraught with great difficulty. But evidence is accumulating which points to a metabolic disturbance in this substance as a primary defect responsible for the pathogenesis of the hyaline retinal and glomerular lesions in diabetes.

First, the serum mucopolysaccharides are largely bound to the α₂-globulin fraction of the serum proteins in man (Gilliland *et al.*, 1954). A decrease in the serum albumin and α₁-globulin and a significant increase in α₂-globulin were found in diabetic patients long before any proteinuria was present. This can be taken as indirect evidence of the raised serum mucopolysaccharides at this stage in the disease.

Secondly, Berkman *et al.* (1954) have studied the serum polysaccharides in two groups with arteriosclerosis—the first group diabetic and the second non-diabetic patients. In contrast to the pattern in diabetic patients, the values for total polysaccharides and for serum glucosamine were normal or very slightly elevated in the majority of non-diabetics.

Thirdly, the observation of McManus (1950a) that the hyaline material of glomerulosclerosis is not deposited in ischaemic glomeruli indicates that it probably originates from circulating serum polysaccharides.

Lastly, the fact that all observers, including ourselves, have reported very high values for the serum polysaccharides in diabetic retinopathy and diabetic nephropathy—values much higher than in any other generalized degenerative vascular disease—may be seen to be related to the finding that these specific diabetic

capillary lesions contain the largest known deposits of this hyaline material.

Serum Lipoproteins

Another aspect of disordered metabolism of the diabetic has been illuminated by the observations of Engelberg *et al.* (1952b) and by Keiding *et al.* (1952) on serum lipoproteins. These workers noted a marked increase in the S_f 12-20 class of circulating lipoproteins in diabetic patients with retinopathy and nephropathy. Simon (1940) first stressed the lipohyaline nature of the deposits in glomerulosclerosis. Wilens *et al.* (1951), in an extensive study of glomerular fat, demonstrated the inordinately high lipid content of the glomerular nodules and drew attention to the possibility that a fatty deposit was one of the initial occurrences in the development of these pathological defects. Now, by the histochemical examination of renal biopsy material, we have found that the nodular lesions are devoid of fat, but that the hyaline fibrinoid lesions are probably a lipoprotein (Sabour *et al.*, 1959). Thus an equally attractive argument can be postulated for a disturbed metabolism of lipoproteins as for mucoids, the latter being responsible for the development of diffuse and nodular glomerulosclerosis and the former for the development of the hyaline fibrinoid renal lesions.

However, it should be noted that other diseases with raised serum lipoproteins, such as myxoedema, nephrosis, and atherosclerosis, do not show the specific fibrinoid lesions of diabetes. This can be accounted for by the fact that these hyaline fibrinoid lesions were found only in glomeruli which were already the seat of glomerulosclerosis, nodular or diffuse. This probably shows that a local mechanical factor is necessary to provoke the onset of these lesions in the presence of a high lipoprotein content of the plasma.

Adrenocortical Function

We have found a significantly increased excretion of glucocorticoids in patients with diabetic nephropathy. Similar results had been previously obtained in cases with diabetic retinopathy. The Thorn test showed that patients with diabetic nephropathy develop a more marked eosinopenia four hours after 25 units of corticotrophin than diabetics without nephropathy. These findings point to a possible role of increased glucocorticoid activity in the pathogenesis of diabetic nephropathy. Moreover, the following observations provide additional evidence of the importance of the adrenal cortex in the pathogenesis of the retinopathy-nephropathy syndrome in diabetics.

A: Suggestive Clinical Observations.—(1) Diabetic retinopathy has appeared for the first time in non-hypertensive diabetics during pregnancy, and cleared completely after delivery (Lawrence, 1948; Becker, 1952). (2) Many diabetics with retinopathy have been observed whose ocular disease showed startling progression during the last trimester of pregnancy and subsequent post-partum regression (Becker, Maengwyn-Davies *et al.*, 1954). (3) Berkman *et al.* (1953) have noted an increase of the urinary signs of diabetic nephropathy after the administration of corticotrophin. (4) Retinal capillary aneurysms have been observed clinically to appear in non-diabetic patients during intravenous corticotrophin therapy and to disappear when treatment ceased (Becker, Maengwyn-Davies *et al.*, 1954).

B: Therapeutic Reports.—Mechanisms that decrease the activity of the adrenal cortex have occasionally been seen to improve diabetic retinopathy (and nephropathy). Thus hypophysectomy (Kinsell *et al.*, 1954), bilateral

adrenalectomy (Green *et al.*, 1950; Wortham and Headstream, 1954), and administration of D.D.D. (Törnblom, 1956) were all reported to have this effect and to correct the disturbed chemistry in patients with diabetic retinopathy and nephropathy.

C: Histopathological Data.—Becker (1952) stated that "diabetics with retinopathy and K.W. disease, in contrast to uncomplicated diabetes, had heavier adrenals with excessive lipid vacuolization of the zona fasciculata, and an abnormally high incidence of adrenal cortical adenomata."

D: Experimental Evidence.—Glomerular lesions identical with the hyaline fibrinoid lesions can be produced in rabbits by the administration of cortisone (Rich *et al.*, 1950). When cortisone or corticotrophin was administered to alloxan-diabetic rabbits, the incidence of the renal lesions and the retinal capillary aneurysms increased (Becker, 1952).

From the above data it is apparent that increased adrenal cortical activity is involved in the pathogenesis of diabetic retinopathy and diabetic nephropathy. It is of interest to note that cortisone administration to rabbits results in marked lipaemia (Rich *et al.*, 1951; Cook *et al.*, 1952), with elevation of the lipoprotein component in the S_f 10-30 class. Alloxan-diabetic animals are more susceptible to lipaemia, and alloxan diabetes blocks the conversion of lipoproteins of the S_f 80-100 class into lipoproteins of a lower S_f class. Thus, alloxan diabetes and cortisone, which act together to produce renal lesions in the rabbit (Wilens and Stumpf, 1955; Bloodworth and Hamwi, 1956), also behave similarly in interference with lipoprotein metabolism. The experimental lesion produced by cortisone administration to alloxan-diabetic rabbits is not identical with K.W. hyaline nodules, but with the hyaline fibrinoid lesions (Le Compte, 1957). Thus since an alteration in lipoprotein metabolism is involved in the pathogenesis of the hyaline fibrinoid lesions, and since changes in adrenal cortical activity are associated with alterations in the serum lipoproteins, the abnormal levels of serum lipoproteins reported in the nephropathy group of diabetic patients are entirely consistent with the hypothesis that the adrenal cortex plays a part in the development of these lesions.

Vitamin B₁₂

Excess glucocorticoids have been shown to cause excessive excretion of vitamin B₁₂ in animals (Wahlstrom and Johnson, 1951), in humans treated by cortisone (Becker, Allen, *et al.*, 1954), and in diabetics with acidosis or with hyaline fibrinoid lesions. Diabetics are themselves deficient in vitamin B₁₂, as they are in other B vitamins, and hence retain most of the test dose given. Hyperfunction of the suprarenal gland, when it occurs, will prevent the diabetics from retaining the vitamin B₁₂ and thus will exaggerate the deficiency state.

By performing the vitamin B₁₂ excretion test and examining renal biopsy specimens, we have shown that excessive excretion of vitamin B₁₂ does not occur in all cases of diabetic nephropathy. It did occur in cases in which there were hyaline fibrinoid renal lesions, but not in cases with diffuse or nodular glomerulosclerosis, no matter how advanced such lesions were. Therefore this metabolic defect is shown to be related to the development of the hyaline fibrinoid lesions and not to the other renal lesions.

Summary

Sixty-five diabetic patients were investigated to elucidate the endocrine and metabolic defects associated

with diabetic nephropathy and to demonstrate by needle biopsy studies the pathogenic relationship of these defects to the development of the renal lesions.

Cases of diabetic nephropathy showed hypoalbuminaemia, hyperglobulinaemia, hypercholesterolaemia in many instances, a rise in the protein-bound serum polysaccharides, hyponatraemia, and hyperkalaemia. The urine showed excessive amounts of 17-hydroxycorticosteroids and low values of 17-ketosteroids. Most patients with diabetic nephropathy could not retain the greater part of a test-dose of vitamin B₁₂ administered parenterally.

The following endocrine and metabolic disturbances are believed to be probably responsible for the development of the renal lesions: a disturbance in protein metabolism resulting in a rise in α_2 and β -globulins; a disturbance in lipid metabolism causing lipaemia and especially a rise in the smaller aggregates of the serum lipoproteins; a disturbance in carbohydrate metabolism leading to a rise in the serum polysaccharide; excessive glucocorticoid activity; and inability to retain vitamin B₁₂.

The high serum mucopolysaccharides are probably related to the development of the lesions of diffuse and nodular glomerulosclerosis. The disturbed lipoprotein metabolism, vitamin B₁₂ metabolism, and the excessive glucocorticoid activity are probably related to the development of the hyaline fibrinoid lesions.

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MITRAL VALVOTOMY AND RESTENOSIS

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The occurrence of restenosis after an adequate mitral valvotomy has been questioned, but reports in the literature of well-documented cases imply that it does undoubtedly occur (Jordan and Hellemis, 1952; Keyes and Lam, 1954; Donzelot; *et al.*, 1953; Santy *et al.*, 1954; McKusick, 1955; Glenn and Dineen, 1956). However, the true incidence of restenosis is not known.

In many patients apparent restenosis has been attributed to inadequate initial valvotomy. Thus Harken and Black (1955) have declared that restenosis is most uncommon. Glover *et al.* (1955) have seen no case of restenosis following valvotomy in their series. From their experience in a review of 250 cases subjected to mitral valvotomy followed from one to six years, Turner and Fraser (1956) felt that refusion of the commissures must be very rare. Of their cases five had second valvotomies and three more were under consideration at the time of writing. It was the opinion of the surgeon that in no case had refusion occurred, but that the original split had been inadequate.

On the other hand, Belcher (1958) described 12 cases in which revalvotomies were undertaken, and drew a