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Role of the adrenal cortex in diabetic retinitis

James Lorin Karel
University of Nebraska Medical Center

THE ROLE OF THE ADRENAL CORTEX IN DIABETIC RETINITIS

James Lorin Karel, B.Sc. in Med., M.S.

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College of Medicine, University of Nebraska

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Diabetes mellitus is a disease which appears to offer a never-ending challenge to the researcher and clinician in their attempts to understand the physiology of the disorder and the principles of therapy. The era of the exciting discovery of insulin is now past and it would appear that the basic principles of the manufacture of insulin and its use in the clinical management of the diabetic patient are well established. In recent years medical science has begun to face perhaps an even more difficult problem in the management of diabetes mellitus, namely, the prevention and treatment of the complications which cause severe disability and death during the course of the disease process.

It is well known that the complications of diabetes may involve the vascular system, the kidneys, the eyes, and the nervous system. Atherosclerosis and arteriosclerosis, diseases of the vascular system in general, are more common in diabetics than in other members of the general population. Vascular disease appears to be the most important problem in clinical diabetes today; it is estimated that arteriosclerosis is responsible for 47-70 per cent of all deaths among diabetics in comparison with 30 per cent among the general population (1). Investigation into the nature and possible prevention of this form of degenerative vascular disease will be of particular importance to the diabetic patient. In the realm of degenerative vascular disease, there are two conditions

which occur almost exclusively in the diabetic patient, namely, intercapillary glomerulosclerosis (Kimmelstiel-Wilson disease) and diabetic retinitis. The former condition is responsible for progressive renal failure and eventual death; the latter constitutes an important cause of visual disturbance and blindness in the diabetic patient.

In recent years there has been considerable interest in the subject of diabetic retinitis from the standpoint of its etiology and possible prevention. This problem is one of the most frustrating situations in the management of the diabetic patient, for the physician is able to follow the entire course of this disabling condition by direct ophthalmoscopic examination without an available form of therapy or a method of altering its destructive progress. This dissertation will consider some of the recent progress in the study of metabolic alterations in the diabetic patient which may be of importance in the control of diabetic retinitis. It is difficult to discuss a single aspect of such a broad subject as diabetes mellitus without losing the perspective of the disease process as a whole. Nevertheless, the study in endocrine balance to be presented contains far reaching implications which may be important in the control of the entire disease process as well as the singular complication of diabetic retinitis.

CURRENT CONCEPTS OF DIABETIC RETINITIS

The Morphology of Diabetic Retinitis

Several years after the invention of the ophthalmoscope, Jaeger (2) described small, punctate "hemorrhages" in the retinae of diabetic patients. In 1875, Leber (3) reported the pathologic morphology of diabetic retinitis in more detail, noting the tendency of the round, punctate "hemorrhages" to occur chiefly in the perimacular areas. As early as 1877, MacKenzie and Nettleship (4) examined the retina of a diabetic patient at autopsy and found that many of the so-called punctate "hemorrhages" were actually capillary microaneurysms. The exact morphology of retinal capillary microaneurysms was not appreciated until the development of histochemical staining techniques, especially the Hotchkiss-McManus method employing the periodic acid-Schiff stain. In 1944, Ballantyne and Loewenstein (5) showed that most of the punctate "hemorrhages" were capillary microaneurysms occurring almost without exception in the inner nuclear layer of the retina within capillary beds linking deeper and more superficial vascular plexuses. Various studies have demonstrated that the size of the lesions may vary from 20-100 microns (1,5,6). The walls of the aneurysms vary in thickness and are composed of a hyaline substance containing carbohydrate, as evidenced by staining with periodic acid-Schiff reagent. Varying amounts of collagen, sudanophilic lipid, and protein are also found within the hyaline material. The production of the lesion appears to be preceded by changes in the capillary walls; the basement membrane of capillaries free from aneurysms may be thickened with focal

accumulations of the hyaline material (5,6,7). The microaneurysms may persist unchanged for several months or they may undergo absorption and scarring with the production of a small white spot (5). The lesion may be a source of weakness in the capillary wall resulting in local hemorrhage and exudation (5,7). Of great importance is the clinical observation that the capillary microaneurysms may undergo spontaneous remissions and exacerbations (7).

The usual course of progression of diabetic retinitis includes the appearance of the typical "hard", well-defined, waxy exudates some time after microaneurysms have been observed. In later stages, extensive hemorrhages and massive exudates appear; in the final stages preceding blindness, vitreous hemorrhages, retinitis proliferans, and retinal detachment supervene (1,8,9). The retinal veins are frequently uniformly dilated in early stages, becoming tortuous, beaded, and sheathed with gray-yellow infiltrate suggestive of mural thrombi in advanced stages (1,8). The sclerosis and degenerative changes of the arterioles are less striking and not specific in the progress of diabetic retinitis (1).

It is interesting to note that the retinal capillary microaneurysm is not absolutely specific for diabetes mellitus. Lesions similar in gross and microscopic appearance have been described in cases of occlusion of the central retinal vein, glaucoma, severe arteriolosclerosis accompanying nephritis, malignant hypertension, and rarely in apparently normal individuals. In these conditions, however, the microaneurysms are observed in proximity to other

vascular lesions or are located in the periphery of the retina (1,7,10). The diabetic lesions are characteristic in that they are more numerous, of a more saccular form, and are located in uninjured portions of the retina (10).

It is not clearly established whether capillary microaneurysms occur in tissues other than the retina in diabetes mellitus. After careful microscopic examination of all ocular tissues and various body viscera, Ashton (9) maintains that none are found outside the retina. On the other hand, Friedenwald (10) has shown that saccular capillary microaneurysms may be found in the conjunctiva in five per cent of diabetic patients.

The Relationship of Retinal Microaneurysms to the Vascular Lesions of Intercapillary Glomerulosclerosis

There appears to be a definite clinical relationship between the occurrence of retinal microaneurysms and intercapillary glomerulosclerosis. Ashton (9) studied the retinal and renal tissues of diabetic patients at autopsy and observed that the renal lesions are never found when the retinal vessels are normal. This implies that intercapillary glomerulosclerosis is regularly associated with retinal microaneurysms, whether the latter are detectible ophthalmoscopically or not. The converse is not true, however; in Ashton's series of patients with retinal lesions proven at autopsy, the renal lesions were found in only 58 per cent. Other workers have observed the same interesting relationship (6,7).

In terms of morphology, there is additional evidence of a relationship between the retinal and renal lesions. The hyaline

material comprising the walls of the retinal aneurysms and the clumped hyaline material in the glomerular tufts show similar staining characteristics for carbohydrate, sudanophilic lipid, collagen, and protein. Some investigators speculate whether the hyaline nodules of the renal lesions represent occluded capillary aneurysms; in addition, they suggest that the overdistended capillary loops occasionally observed in affected glomeruli may be the renal analogues of the retinal microaneurysms (6,7).

The Occurrence of Retinal and Renal Lesions in Relation to the Clinical Course of Diabetes Mellitus

It is well established that the incidence of diabetic retinitis and intercapillary glomerulosclerosis is highest in the young patient with long-standing diabetes and a history of poor control (1,7,10,12,13). The duration of diabetes appears to be one of the most important single factors. In patients of all ages with diabetes of one to ten year's duration, the incidence of retinitis is 9-22 per cent; when the disease has persisted more than fifteen years, retinitis is found in 60-67 per cent, and in patients with diabetes for more than twenty years, retinitis occurs in approximately 73 per cent (7,11,12). Wagener (12) has demonstrated the important effect of duration of the disease in the young patient. In his series, retinitis was found in 76 per cent of patients less than thirty years of age with diabetes for more than ten years; on the other hand, in patients older than thirty years with diabetes of similar duration, only 64 per cent had retinitis.

The degree of control of diabetes is a rather difficult criterion to evaluate in clinical patients; it is usually based upon the number of episodes of coma and the cooperation of the patient in following the diabetic regimen as outlined by the physician. In careful studies among young diabetics, Root and coworkers (13) have shown that retinitis and renal disease are significantly less in patients with excellent or good control. But as Ricketts (1) has pointed out, there is no straight-line relationship between control and the incidence of retinitis, since one-fourth of the patients in this series with good control showed serious retinal damage and one-third of those with poor control had almost no retinitis despite long-standing diabetes. This observation suggests the interplay of other metabolic factors in the determination of the degree of vascular damage.

Factors in the Pathogenesis of Diabetic Retinitis

CAPILLARY FRAGILITY.--Since the formation of an aneurysm and the occurrence of hemorrhage and exudation involve mechanical factors to a certain extent, theories relating capillary fragility to the development of diabetic retinitis have enjoyed varying popularity for many years. Wagener (12) studied capillary fragility in a series of diabetic patients by means of the tourniquet test and observed a significantly greater fragility in those patients with diabetic retinitis. Friedenwald noted that the results of the tourniquet test were difficult to interpret, since the degree

of fragility appears to fluctuate when patients are studied over a period of time (7). However, he did observe a tendency toward correlation of abnormal fragility and the appearance of fresh lesions in the retinae of some diabetic patients. It is especially difficult to evaluate capillary fragility in many patients with diabetes of long standing because of co-existing hypertension which, in itself, is commonly associated with abnormally increased fragility. Barnes, for example, found that hypertension was present in 61 per cent of a group of diabetic patients with retinitis and increased capillary fragility (14).

The special hemodynamics involved in ocular circulation may also play a part in the development of retinal microaneurysms. LeCompte (15) emphasizes that the pressure in the retinal capillaries must be high, if not the highest of all capillary beds in the body, since the intraocular tension represents the highest tissue pressure in the body. In spite of probable participation of mechanical factors in the production of the aneurysmal lesions, the fact that the lesions are almost specific for diabetes mellitus strongly suggests a metabolic defect as a primary factor.

ABNORMAL LIPID METABOLISM AND DIABETIC RETINITIS.--The finding of varying amounts of lipid within the hyalinized walls of retinal microaneurysms has prompted many workers to study the blood lipids as a factor in the production of diabetic retinitis. In general, the results have been inconclusive and have failed to demonstrate a cause and effect relationship.

Early work among clinically controlled diabetic children indicated that values for all serum lipids were normal regardless of the insulin requirements. During insulin deficiency, marked increases in neutral fat levels were observed but cholesterol levels were only minimally increased (16). Among adult diabetics it is difficult to evaluate blood lipid patterns since intercurrent renal disease may produce pathologic alterations. Several workers have demonstrated that serum cholesterol levels bear no relationship to blood glucose levels and are not abnormal even in severe diabetes (17,18). Hirsch and associates (18) have shown, however, that the blood esterified fatty acid levels tend to parallel in time and in intensity the hyperglycemia induced by insulin withdrawal.

Recent studies by Mann (19) indicate that during diabetic acidosis there is an accumulation of S_f 21-100 lipoproteins in serum and that as treatment of the acidosis progresses, the S_f 21-100 levels decrease while those of the S_f 12-20 lipoproteins rise. According to Mann, this implies that diabetes mellitus is associated with a defect in the catabolism of lipid manifested to the greatest extent during acidosis in which there is a block in the normal transition of lipid from low to high density complexes. Studies among large groups of clinically controlled diabetic patients have not shown impressive abnormalities of serum lipoproteins. Barach and Lowy (20) found elevated S_f 12-20 lipoprotein levels in only 33 per cent of males and 43 per cent of females in a group of 618 diabetic patients. Hanig and Lauffer (21) found no significant

elevations of S_f 12-20 lipoproteins in either well controlled or poorly controlled male diabetics.

In regard to retinitis, Keiding and associates (22) report a significant relationship between the presence of diabetic retinitis and elevated S_f 12-20 lipoprotein levels, but no mention is made of the presence or absence of renal disease in their series. Iannaccone and Kornerup (23) found a significant elevation of total plasma lipid in diabetic patients with retinitis, but the levels of cholesterol or phospholipid were not significantly increased.

It is apparent that the investigation of lipid metabolism in relation to diabetic retinitis has produced confusing results, just as it has in the field of arteriosclerosis. After reviewing the recent literature, Ricketts (1) concludes that the hyperlipemia associated with retinitis is probably explained by the presence of intercurrent renal diasease. This appears to be the most reasonable conclusion at this time. It is possible, however, that abnormal lipid metabolism during acidosis may contribute to the metabolic alterations involved in the production of retinitis, since poor control appears to play an important role in this disease process.

BLOOD AND TISSUE GLYCOPROTEINS IN DIABETIC RETINITIS.--It has already been mentioned that the earliest notable morphologic alteration in diabetic retinitis consists of thickening and hyalinization of the basement membrane and walls of the retinal capillaries. The hyaline material is composed largely of glycoproteins which stain readily with the periodic acid-Schiff reagent. It

would appear reasonable to assume, therefore, that an alteration of glycoprotein metabolism accompanies or possibly precedes the appearance of retinitis. Considerable interest in this problem has resulted in increasing efforts to understand the biochemistry of blood and tissue polysaccharides and glycoproteins.

Jacobs (24) found that the levels of protein-bound serum glucosamine fall within a very wide range in non-diabetic individuals. Diabetic patients, however, seemed to have higher levels at all ages; in addition, higher levels appeared to be related to poor control. In his experiments, the serum glucosamine concentration was found to parallel blood glucose levels very closely. Later work by Berkman and associates (6,25) showed that the concentrations of protein-bound non-glucosamine polysaccharide, serum glucosamine, and polysaccharide of serum mucoprotein were within normal limits in diabetic patients without detectible degenerative vascular disease. When these complications did exist, glucosamine and total protein-bound polysaccharide levels were found to be elevated, but no correlation could be established between blood glucose levels and the amounts of other carbohydrate fractions. Other workers have demonstrated that serum levels of mucoprotein and protein-bound hexosamine are elevated even in diabetic patients without evidence of vascular complications and that a secondary rise in hexosamine concentration occurs with the appearance of minimal retinitis (26). Studies employing filter paper electrophoresis have

confirmed the finding that the concentration of total protein-bound carbohydrate increases as vascular complications of diabetes progress, even before renal damage ensues (27).

It is obvious that the results of the various investigations in this field are not in complete agreement. As Berkman has emphasized (6), the fact that present methods for analyzing serum carbohydrate fractions are not well developed may account for this difficulty, in part at least. The greatest problem in interpreting these findings is deciding whether the alterations in serum carbohydrate and glycoprotein levels are responsible for or merely secondary to the degenerative disease process. It has been known for years that one factor common to all pathological conditions involving tissue destruction or repair is a non-specific elevation of serum glycoprotein and polysaccharide levels (6,25,26). It is still considered possible by several investigators that the elevated serum polysaccharide levels may be related to the progressive deposition of hyaline material in the degenerative vascular lesions (6,27).

Recent Interest in the Adrenal Cortex and Diabetic Retinitis

One of the most important developments in the history of diabetes mellitus is the relatively recent concept of the possibility of multiple endocrine defects in this disease. The diabetogenic effects of the anterior pituitary and the antagonistic actions of the adrenal cortical hormones and insulin are well

established entities. The discovery of the hyperglycemic hormone elaborated by the alpha cells of pancreatic islets promises to further alter theoretical considerations and possibly the clinical management of diabetes mellitus.

Within recent years, certain clinical observations and animal experiments have suggested that excessive secretion of adrenal cortical hormones may be related to the onset and progress of diabetic retinitis. In 1948, Lawrence (28) described two cases of diabetic retinitis which developed rapidly during pregnancy but regressed and apparently completely disappeared within a few years following delivery. Other workers have observed rapid progression of pre-existing retinitis during the last trimester of pregnancy with subsequent regression postpartum (29). It has been shown that the secretion of corticosteroids is enhanced normally in the second and third trimesters of pregnancy, but the diabetic female shows as excessive secretion under these circumstances (13). Poulsen (30) reported the interesting case of a diabetic female who developed Simmond's disease following pregnancy and showed a complete remission of diabetic retinitis within six years.

In 1950, Rich and Barthrong (31) reported the development of renal lesions closely resembling intercapillary glomerulosclerosis in a group of rabbits treated with cortisone. Subsequent studies by Becker and associates (29) demonstrated that alloxan-diabetic rabbits given cortisone showed a much higher incidence of the renal lesions than did normal rabbits with similar treatment. In addition,

retinal microaneurysms were observed in a few cases in the diabetic rabbits receiving cortisone. In no instance, however, did alloxan diabetes alone predispose to the formation of renal or retinal lesions.

Attempts to evaluate the metabolic effects of cortisone in relation to vascular disease in animals have yielded interesting but confusing results. The administration of cortisone to rabbits produces a marked lipemia with elevations of all plasma lipid fractions, the greatest increment occurring in the neutral fat fraction (32,33,34,35). Hyperglycemia and glycosuria tend to parallel the elevations of lipid levels (32,33) and hydropic changes in the beta cells of the pancreatic islets have been described (33). When rabbits are fed atherogenic diets containing large amounts of cholesterol, marked inhibition of atherosclerosis and cholesterol deposition in tissues is noted during cortisone therapy in spite of extreme degrees of hypercholesterolemia (34,35). One group of experimenters suggests that a decrease in tissue permeability induced by cortisone therapy may explain these findings (34). Whether cortisone-induced hyperlipemia is a result of mobilization of depot fat or a phenomenon secondary to deranged carbohydrate metabolism has not been clearly established. The significance of this metabolic effect of cortisone in relation to human vascular disease in diabetes mellitus remains to be determined.

If one assumes that excessive adrenal cortical secretion is a factor in the pathogenesis of diabetic retinitis, it would be

reasonable to expect a high incidence of retinal involvement in diabetic patients with Cushing's disease. However, since hypertension often supervenes in these patients the mixed type of retinal damage is difficult to evaluate. McCullagh and Alavisatos (36) studied 21 patients with both Cushing's disease and diabetes and found two cases showing retinitis after eight and nine years of known diabetes, respectively. Ricketts (1) observed retinitis in two of three patients with acromegaly and diabetes, but in none of seven patients with Cushing's disease and diabetes. The reason for the surprisingly low incidence of retinitis in these patients is not readily apparent; one possible explanation is that perhaps most of the patients with Cushing's disease did not have diabetes long enough to manifest the retinal changes (36).

Several investigators have attempted to suppress or ablate the adrenal cortex in an effort to alter the course of diabetic retinitis. Wortham and Headstream (37) performed bilateral adrenalectomy in seven patients with retinitis and advancing vascular disease. Two of the patients showed minor reversals in retinal damage, restoration of normal blood pressure, and resolution of edema; three showed no further progression of vascular disease after several months of observation. Some workers have questioned whether the decrease in blood pressure may have been responsible for the seemingly beneficial effect on the retinal pathology (1). Testosterone propionate has been used by several investigators in the treatment of diabetic retinitis on a rather empirical basis,

although it is known to suppress the production of ACTH in both man and animals (29,38). Saskin and associates (38) report "unequivocal" improvement in one-third of their cases treated with testosterone, using the number of microaneurysms as the criterion of therapeutic response. Studies such as these will require confirmation and careful follow-up in view of the well known clinical observation that diabetic retinitis shows inherent tendencies toward remissions and exacerbations, especially in the early stages (7,29).

Studies of Adrenocortical Function in Diabetic Patients

Most of the evidence so far presented concerning the function of the adrenal cortex and diabetic vascular disease has been indirect in nature. The ideal situation is, of course, to attempt to evaluate the activity of the adrenal cortex in man in conjunction with the clinical progress of diabetic retinitis. As previously mentioned, the vascular lesions of diabetic retinitis and intercapillary glomerulosclerosis are closely correlated in temporal relationship to the clinical progress of diabetes mellitus. It is noteworthy, therefore, that Becker and associates found that the adrenal glands of patients with intercapillary glomerulosclerosis weigh more, show more lipoid vacuolization in the zona fasciculata, and have a higher incidence of cortical adenomas than the adrenals from diabetic patients without evidence of the renal lesions (29, 39).

In recent years, improvement of laboratory techniques has enabled the investigator to measure the small quantities of adrenal cortical hormones and their derivatives in urine and plasma. Many methods have been devised and applied to the problem of steroid secretion in diabetes mellitus; unfortunately, since the various procedures do not necessarily measure the same chemicals, the results of different studies cannot be readily compared. In general, the results to date have been inconsistent and difficult to interpret.

Practically all studies of urinary 17-ketosteroid excretion in diabetic patients have shown low normal or subnormal values at all age levels (40,41,42,43). Normal values have been reported for urinary phosphomolybdate reducing substances (corticosteroids) (42), glycogenic corticoids as determined by bioassay (41), and reducing corticoids as determined by the tetrazolium blue method (43). It must be emphasized that the determination of urinary 17-ketosteroids is not a good index of adrenal cortical function since the gonadal secretion contributes to this fraction; the excretion of 17-ketosteroids frequently does not parallel that of corticosteroids (41).

Equivocal results have been observed in studies employing eosinophil response tests as indices of adrenal cortical function. Wilson and coworkers (44) observed normal eosinophil responses following stimulation with ACTH, but noted low values for urinary 17-ketosteroids in two of five diabetic patients; they suggested that

adrenal cortical function was low, if anything, in diabetes mellitus. Field and Marble (45) noted that ten of twenty-five diabetic patients showed abnormally low eosinophil responses to the stress of surgery. After finding that the same patients showed sluggish eosinophil responses to ACTH stimulation, they concluded that a decrease in adrenal cortical function existed which, perhaps, was a compensatory change resulting from relative insulin deficiency. In a study of juvenile diabetics, Grinspoon and associates (46) found lower than normal eosinophil responses following epinephrine stimulation. After ACTH stimulation the responses were again less than expected, but there appeared to be no relationship between the magnitude of the responses to epinephrine and ACTH. Furthermore, it was observed that the eosinophil response to hypoglycemia and epinephrine stimulation often showed no relationship in magnitude. According to these investigators, the best explanation for the subnormal responses to ACTH and epinephrine stimulation in the diabetic patient is a state of adrenal "unresponsiveness" or insufficiency which, as they suggest, is the result of the constant stress of rapid changes in blood sugar levels. In recent years there has been considerable doubt cast upon the validity of the eosinophil response tests as indices of adrenal cortical function. The lack of parallel responses to various forms of stress, as noted by Grinspoon and others (46), and the general lack of knowledge of the exact mechanism of this test should emphasize that it must be considered at best only a presumptive index of adrenal cortical

response.

Since the progress of diabetic retinitis and intercapillary glomerulosclerosis appears to be related to the duration of the disease and to poor control, it has been of prime interest to investigate the adrenal cortical function under situations of stress inherent in the disease process. Greenman and associates (47) found an accelerated rate of excretion of urinary 17-ketosteroids and eosinopenia during diabetic acidosis and the subsequent recovery period with gradual return to normal values when recovery was established. Other studies have revealed that the rate of excretion of urinary corticosteroids is two to eight times as rapid during diabetic acidosis as after recovery (48). In extensive metabolic studies using dogs as experimental animals, MacArthur and coworkers (49) found that many of the hematologic changes, the accelerated protein catabolism with loss of body potassium, and the decreased sensitivity to parenteral insulin observed during diabetic acidosis were largely attributable to the increase in adrenocortical activity. It is of interest to note that after withdrawal of insulin from the human diabetic eosinopenia has been observed within 24 hours but the rate of excretion of urinary corticosteroids is not manifest until clinical evidence of mild acidosis appears (48,49). Wallace, Christy, and Jailer (50) recently reported that the plasma levels of 17-hydroxycorticosteroids were distinctly elevated in two patients with diabetic acidosis. Thus, there would seem to be little doubt that diabetic acidosis is a strong stimulus

toward augmented secretion of adrenal cortical hormones.

The relative lack of insulin is not the only mechanism capable of stressing the diabetic patient, for it has been amply demonstrated that insulin-induced hypoglycemia is equally effective. Animal experiments in which plasma corticosteroids and adrenal ascorbic acid were measured have shown that insulin hypoglycemia induced a prompt secretion of corticosteroids, suggesting a participation of the adrenal cortex in counteracting the hypoglycemia (51,52). MacArthur and associates (53) administered large doses of insulin to a diabetic patient in attempting to maintain constant normoglycemia and noted frequent insulin reactions and a measurable increase in the rate of urinary corticosteroid excretion.

The problem still remains to establish the degree of activity of the adrenal cortex in the clinically controlled diabetic patient. With the advantages of newer techniques enabling the investigator to measure the 17-hydroxycorticosteroids in plasma and urine, the project would seem simpler, but uniformity of results has not been achieved. Wallace, Christy, and Jailer (50) report that the plasma levels of 17-hydroxycorticosteroids, as determined by the Porter-Silber method, were within normal limits in seven diabetic patients. Eik-Nes and coworkers (54), using the Nelson-Samuels technique, measured the rise in plasma 17-hydroxycorticosteroid levels following stimulation with intravenous ACTH and found no significant difference between the responses of eight patients with clinically

controlled diabetes of moderate severity and those of normal control subjects. On the other hand, Klein and associates (55) noted that the plasma 17-hydroxycorticosteroids (Nelson-Samuels method) are significantly higher in treated diabetic children than in normal children. The levels were reported to be directly related to the amount of reducing substances in the urine and inversely related to the carbon dioxide content of the serum, suggesting to these investigators that the diabetic child is under some constant stress because of his diabetes or its treatment.

Since only a few studies of this nature have been completed in diabetic patients, one cannot conclude that the function of the adrenal cortex has been thoroughly evaluated. The direct measurement of plasma and urine 17-hydroxycorticosteroids, which include hydrocortisone and cortisone, will undoubtedly be the technique of choice in this problem. Previous impressions from studies of eosinophil responses and 17-ketosteroid excretion must be re-interpreted as new information accumulates.

A STUDY OF ADRENAL CORTICAL RESPONSIVENESS IN RELATION TO DIABETIC RETINITIS

With the availability of microtechniques for steroid analysis, a study was devised in an attempt to evaluate adrenal cortical function in patients with diabetic retinitis. Since the adrenal cortex is an organ with a definite capacity to respond to stress, it is reasonable to propose that the measurement of hormone secretion in response to a given amount of stress would be a

valid quantitative test of adrenal cortical function. This is the foundation of the intravenous ACTH test which is presently recognized as the definitive diagnostic test in various states of altered adrenal cortical function (56,57). For our purposes the test was performed as follows. A twenty-four hour urine specimen was collected without preservative, refrigerated, and analyzed for 17,21-dihydroxy-20-ketosteroids by the method of Porter and Silber (58). The value obtained represented the baseline excretion of corticosteroids without stimulation. On a subsequent day a similar urine collection was made; however, within one or two hours after beginning the collection, 25 Units of lyophilized ACTH was given intravenously dissolved in a liter of five per cent glucose or physiologic saline and infused over a six hour period. The second twenty-four hour specimen thus contained the baseline excretion of corticosteroids plus the additional steroids excreted under the stimulus of the ACTH. The increment of steroids produced by the ACTH stimulation therefore represents the response of the adrenal cortex to the measured amount of stress and is used as the basis for determining the relative degree of activity of the adrenal cortex.

It was considered advantageous to measure urinary corticoids in preference to plasma corticoids in order to obviate the diurnal variations of plasma levels reported by other workers (50).

Ophthalmoscopic examinations were performed in almost all cases by a member of the department of Ophthalmology. The degree

of diabetic retinitis was classified according to the description of Ballantyne (59):

I. Mild Stage - Changes chiefly in the central area with microaneurysms or small punctate, waxy exudates.

II. Moderate Stage--"Dot and blot" hemorrhages with confluent waxy exudates.

III. Severe Stage - Massive exudates and extensive retinal hemorrhages, vitreous hemorrhages, retinitis proliferans, or retinal detachment.

The degree of control of diabetes was difficult to evaluate and represents only a rough estimate derived from the patient's clinical history. Factors for consideration included willingness to follow a diet, efficiency of testing urine and regulating insulin dosage, and frequency of insulin reactions or episodes of acidosis.

Laboratory Methods

The determination of urinary 17,21-dihydroxy-20-ketosteroids, according to the Porter-Silber method, is performed as follows. One ml. of filtered urine is incubated overnight at room temperature with one ml. of a solution containing 250 Sigma units of glucuronidase. The hydrolysis of glycosides is necessary since the majority of urinary corticosteroids are excreted in the conjugated form. The mixture is brought to a volume of five ml. with phosphate buffer adjusted to pH 6.5 and extracted by shaking with

25 ml. of chloroform for 15 seconds. The aqueous phase is discarded and the chloroform extract is washed by shaking for ten seconds with two ml. of 0.1 N sodium hydroxide. After brief centrifugation the alkali wash is discarded.

Ten ml. aliquots of the chloroform extract are placed in each of two test tubes. One ml. of phenylhydrazine-sulfuric acid reagent with ethanol is added to the first tube; to the other is added one ml. of blank reagent. The tubes are capped, shaken for 15 seconds and centrifuged. The chloroform layer is carefully removed by means of a fine glass tube connected to a special aspirator and discarded.

The samples are allowed to stand overnight at room temperature for color development. 0.5 to 0.8 ml. of the sample is placed in the microcuvette and the optical density is compared at 410 mmu with that of an appropriate blank.

Standard solutions are prepared from a stock solution of hydrocortisone in distilled water and are carried through the same procedure as that for the urine sample.

This procedure is suitable for determining not only the biologically active compounds cortisone and hydrocortisone, but also substances of less activity such as 17-hydroxy-11-desoxycorticosterone and the di- and tetrahydro derivatives of cortisone, hydrocortisone, and 17-hydroxy-11-desoxycorticosterone (58).

Results and Discussion

Twelve patients with a variety of non-endocrine conditions were used as controls. The mean value for steroid excretion in the unstimulated state was 6.7 ± 1.3 mg. per 24 hours. The mean increment in steroid excretion during ACTH stimulation was 15.4 ± 8.2 mg. per 24 hours. Both of these values agree favorably with the findings of other investigators (56,57,58). The values for the control group are presented in Table I, page 26.

The values for steroid excretion in diabetic patients without ACTH stimulation and the comparative degrees of retinitis are shown in Table II, page 27. The mean value for the group is 8.8 ± 3.8 mg. per 24 hours.

Sixteen diabetic patients were given intravenous infusions of ACTH as a test of adrenal cortical responsiveness. About half of the group showed no retinitis; the remainder had diabetic retinitis of varying degrees of severity. The mean increment in steroid excretion elicited by the ACTH in this group was 23.6 ± 13.2 mg. per 24 hours. The values are presented in Table III, page 28.

In Figure 1, page 29, the data from Table II are presented in graphic form to illustrate the relationship between the degree of diabetic retinitis and the excretion of corticosteroids in the unstimulated state. The relationship between the increment in steroid excretion following ACTH stimulation and the degree of retinitis is similarly illustrated in Figure 2, page 29.

From the data thus presented several important observations can be made. First of all, it would appear that the diabetic

TABLE I

ACTH RESPONSE TEST IN NON-DIABETIC PATIENTS

Patient	Age	Sex	17,21-OH-20-ketosteroids mg./24 hr.		
			Unstim.	Test	Increment
C. D.	55	F	7.5	11.8	4.3
M. P.	-	F	8.1	15.8	7.7
E. C.	57	F	5.9	14.4	8.5
N. G.	60	M	5.9	13.5	7.6
R. W.	75	F	8.2	16.8	8.6
M. H.	34	F	4.0	19.4	13.9
M. C.	50	F	6.0	22.5	16.5
G. P.	56	F	7.5	26.2	18.7
E. Es.	60	M	8.6	30.5	21.9
E. E.	74	F	7.1	30.2	23.1
R. B.	20	M	7.4	30.6	23.2
H. B.	28	F	7.6	38.0	30.4
		Mean	6.7±1.3 S.E.=0.39		15.4±8.2 S.E.=2.4

S.E.=Standard Error of Mean

TABLE II

17,21-DIHYDROXY-20-KETOSTEROID EXCRETION IN DIABETIC PATIENTS

Patient	Age	Sex	Years Duration	Control	Retinitis	Corticoids mg./24 h.
V. B.	60	M	38	Good	0	5.5
T. M.	70	F	14	Fair	II	6.0
A. R.	57	F	10	Poor	II	6.0
M. C.	78	F	11	Poor	II	6.5
W. E.	34	M	7	Exc.	0	7.1
P. G.	58	M	1	Good	0	7.0
F. R.	47	F	15	Good	II	7.5
M. B.	36	F	5	Good	0	7.7
E. L.	38	F	11	Poor	III	7.7
C. S.	73	M	2	Good	I	8.6
V. R.	82	M	10	Good	I	9.5
A. G.	79	M	13	Good	0	10.0
H. C.	66	M	3	Fair	I	10.0
V. H.	52	M	17	Good	0	10.0
A. K.	41	F	8	Poor	0	10.1
M. C.	64	F	-	Fair	II	12.0
E. A.	53	F	1	Poor	III	12.8
R. G.	50	F	-	-	0	19.8
A. B.	32	M	28	Poor	II	10.8
L. S.	66	F	5	Fair	0	17.3
J. T.	64	F	4	Good	I	8.5
L. T.	79	F	2	Good	I	8.3
M. R.	18	F	1	Fair	0	2.6
O. H.	58	F	6	Poor	0	2.7
G. M.	30	F	28	Poor	II	5.7
					Mean	8.8 ± 3.8 S.E.=0.76

S.E.=Standard Error of Mean

TABLE III

ACTH RESPONSE TEST IN DIABETIC PATIENTS

Patient	Age	Sex	Years Duration	Control	Retinitis	17,21-OH-20-ketosteroids mg./24 h.		
						Unstim.	Test	Increment
P. G.	58	M	1	Good	0	7.0	27.3	20.3
V. B.	60	M	38	Good	0	5.5	15.8	10.3
W. E.	34	M	7	Exc.	0	7.1	29.7	22.6
V. H.	52	M	17	Good	0	10.0	33.0	23.0
L. S.	66	F	5	Fair	0	17.3	39.5	22.2
M. R.	18	F	1	Fair	0	2.6	12.0	9.4
O. H.	58	F	6	Poor	0	2.7	6.8	4.1
L. T.	79	F	2	Good	I	8.3	14.8	6.5
V. R.	82	M	10	Good	I	9.5	34.5	25.0
H. C.	66	M	3	Fair	I	10.0	41.0	31.0
T. M.	70	F	14	Fair	II	6.0	17.1	9.1
U. C.	64	F	-	Fair	II	12.0	68.3	56.3
M. C.	78	F	11	Poor	II	6.5	28.7	22.2
G. M.	30	F	28	Poor	II	5.7	50.3	44.6
E. A.	53	F	1	Poor	III	12.8	45.5	32.7
E. L.	38	F	11	Poor	III	7.7	47.2	39.5
							Mean	23.6±13.2
S.E.=Standard Error of Mean								S.E.=3.3

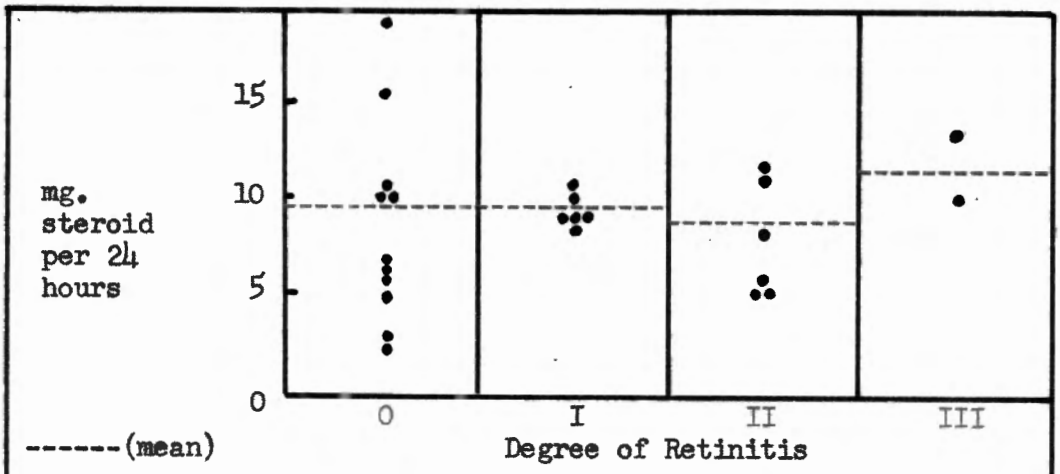

FIGURE 1. RELATIONSHIP OF RETINITIS AND STEROID EXCRETION IN THE UNSTIMULATED STATE

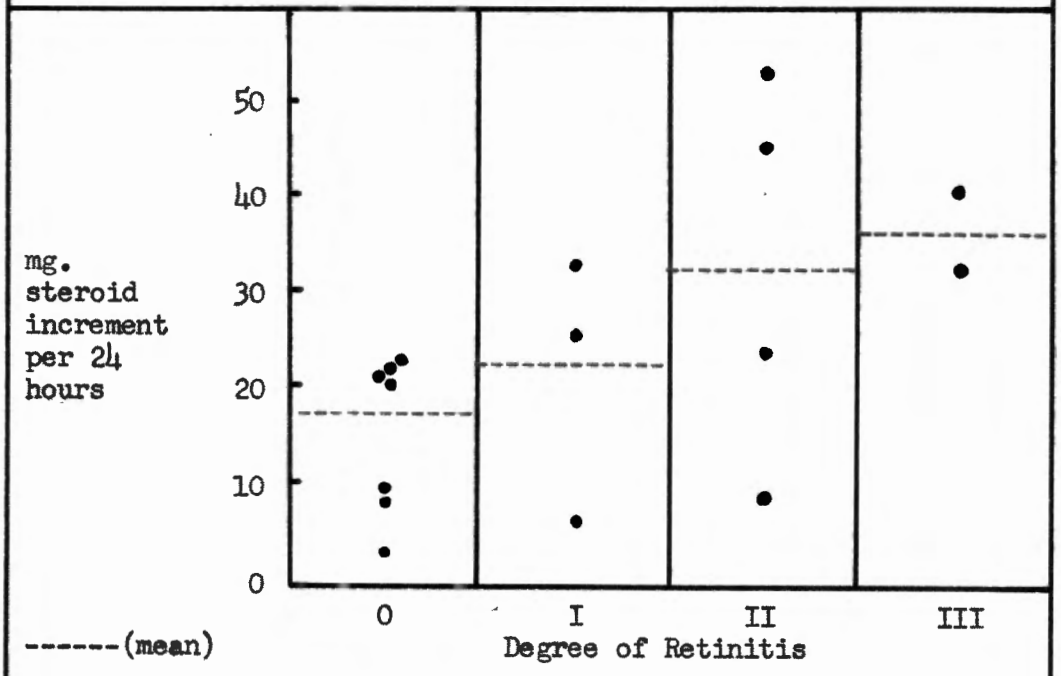

FIGURE 2. RELATIONSHIP OF RETINITIS AND STEROID EXCRETION IN RESPONSE TO ACTH STIMULATION

patients showed a slightly higher excretion of urinary corticosteroids than the control group even without adrenal stimulation. There is, however, considerable overlap of the ranges for each group, and it is evident that retinitis can exist in the presence of normal values for urinary corticoids. Nevertheless, the slightly elevated mean corticoid excretion rate has not been reported by previous investigators, possibly because of a difference in technique.

When intravenous ACTH was used to test the responsiveness of the adrenal cortex, there appeared to be a definite tendency for those patients with retinitis to show abnormally large responses in proportion to the degree of retinal damage. This is best shown by graphic representation in Figure 2. To be sure, some cases of retinitis were associated with normal responses, but there is a distinct rise in the mean response with each succeeding degree of retinitis. The mean response in diabetic patients without retinitis appears to be comparable with the value found in the control group; however, a considerable proportion of this group has decidedly elevated values, as shown in Figure 2. Unfortunately, not enough patients were available to furnish a large series and final conclusions cannot be made until further work is completed. It is interesting to note that there appears to be little if any relationship between the degree of retinitis and the level of corticosteroid excretion in the unstimulated state. If confirmed by future studies, this would emphasize the need for testing the

responsiveness of the adrenal cortex with exogenous ACTH in order to obtain a true evaluation of its functional state.

In this group of patients, there appeared to be a better correlation of lack of control than of duration of diabetes with the degree of retinitis and abnormally large corticosteroid responses. The results of this investigation would tend to discourage the thesis proposed by earlier workers that diabetics with poor control may develop states of adrenal insufficiency (45,46). If the impressions gained from this study are confirmed by further work, the results would seem to justify the clinical impression that cases of advancing retinitis are associated with an endocrine status in which the adrenal cortex is capable of hypersecretion.

The Significance of Adrenal Cortical Hyperactivity as a Factor in the Pathogenesis of Diabetic Retinitis

A detailed discussion of the possible biochemical mechanisms involved in the effect of the adrenal cortical hormones upon the pathogenesis of diabetic retinitis is beyond the scope of this paper.

It is of interest, however, to consider this problem in the light of recent concepts of basic tissue response to inflammation and the adaptation response to stress. Today it is common to speak of the glucocorticoids as anti-inflammatory steroids. Clinically, they are used to suppress inflammatory proliferation of connective tissue and ground substance in certain disease states such as the collagen diseases. Early in the discussion of the pathology of

diabetic retinitis it was stated that the first morphologic alteration was a thickening and hyalinization of the capillary basement membrane and wall; this would presumably indicate a defect in the metabolism of connective tissue and ground substance. Thus, it would seem paradoxical, superficially at least, that this degenerative disease should progress in the presence of a state of augmented adrenal cortical activity. A closer look at the entire picture of the stress reaction may offer some clarification.

According to Selye and associates (60,61), there are three phases of the stress syndrome: (1) the alarm reaction, in which adaptation has not been acquired; (2) the stage of resistance, in which adaptation, including the secretion of glucocorticoids, is optimum; and (3) the stage of exhaustion, in which the acquired adaptation is lost again. It is safe to assume that the diabetic patient probably experiences the general adaptation syndrome to a varying degree with each episode of hypoglycemia or acidosis. There is no method presently available for determining when in the course of the response to stress the metabolic damage to the retinal vessels occurs. In a series of recent experiments, Kramar (62) observed that the capillary resistance of rats and dogs shows a striking pattern of changes in response to stress of several varieties. During the period of adaptation there is an initial rise of capillary resistance; this is followed by a sudden drop and a sustained low resistance during the exhaustion phase, after which the values slowly return to normal. The entire phenomenon lasts

approximatly four weeks. It was found that cortisone was capable of preventing the low capillary resistance during the exhaustion phase and that its effect was antagonized at the peripheral level by somatotrophic hormone. If it can be shown by future investigation that the glucocorticoids play a similar role in regulating capillary resistance in man, it may become reasonable to propose that diabetic retinitis begins and progresses at the time in the stress reaction when the capillary resistance and the circulating levels of glucocorticoids are low. On the basis of this theory, however, one would expect lower levels of adrenal cortical activity with advancing retinitis and it would be difficult to explain the beneficial effects of adrenalectomy and termination of pregnancy on diabetic retinitis. The concept of exacerbation of retinitis during the exhaustion phase following excessive adrenal cortical stimulation is an interesting one and should be evaluated in future studies.

A review of the theory of diseases of adaptation reveals other possible means of relating retinitis and adrenal cortical hyperactivity. Among the disturbances of the general adaptation syndrome that may cause disease, the following are considered to be important: (1) an absolute excess or deficiency in the amount of adaptive hormones produced during stress; (2) an absolute excess or deficiency in the amount of adaptive hormones fixed by the target organs during stress; (3) a disproportion in the relative secretion (or fixation) during stress of various adaptive hormones; and (4) the production by stress of metabolic derangements which

abnormally alter the peripheral target organ's response to adaptive hormones through the phenomenon of conditioning (60,61). The factor of conditioning of the peripheral tissues in altering normal response to adaptive hormones has received increasing emphasis in recent years. Of great importance is the concept of the "permissive" actions of corticoids, which assumes that the hormones do not affect the target tissues themselves, but only permit stressors to act upon them. The disease diabetes mellitus with its altered carbohydrate and lipid metabolism could conceivably affect retinal vascular tissue in such a way that ordinary responses to stress are not completed in a normal manner. Izzo and Eilers (43) have presented evidence to show that cortisone is converted to 17-ketosteroids at an abnormally accelerated rate in the diabetic patient; thus, there is a possibility that steroid metabolism by peripheral tissues may be abnormal in this disease.

It is obvious that there is no ready definition of the role of corticosteroids in the pathogenesis and progress of diabetic retinitis. It is safe to assume that several factors contribute to this condition, among which the abnormal carbohydrate, lipid, and steroid metabolism appear to be of great importance.

The Clinical Implications of This Study

The results of this study strongly suggest that diabetic retinitis is associated with a hyperactive state of the adrenal cortex. This, in turn, implies that the patient with this

condition experiences frequent episodes of systemic stress, either in the form of diabetic acidosis or insulin hypoglycemia. These findings emphasize the need for close clinical control of the disease, especially in the young diabetic patient who requires large amounts of insulin for control. The evidence to date is overwhelmingly in favor of close clinical control as the only presently available means of preventing or retarding the progress of diabetic retinitis. The medical or surgical procedures for depressing adrenal cortical function may be indicated in select patients in whom the problem of excessive corticoid secretion is outstanding.

SUMMARY

The prevention and control of degenerative vascular disease is one of the greatest problems in the current management of the diabetic patient. Diabetic retinitis is an outstanding form of degenerative vascular disease seen only in diabetic patients and responsible for considerable visual damage often terminating in blindness. Considerable interest has arisen in recent years in an effort to discover the cause and possible prevention and treatment of diabetic retinitis.

The earliest morphologic change in diabetic retinitis is a thickening and hyalinization of capillary walls and basement membranes. Microaneurysms appear which may persist unchanged for months, atrophy and scar, or may be the site of small areas of

hemorrhage and exudation. The early changes of diabetic retinitis are seen mainly in the perimacular areas. In later stages large hemorrhages and patches of hard, waxy exudates appear. Veins become dilated and tortuous and may undergo sheathing and scarring in late stages. In severe form, hemorrhages occur into the vitreous, retinitis proliferans develops, and retinal detachments with subsequent blindness terminate the picture. There is a distinct tendency for retinal arterioles to be spared in this condition, unless they are involved by intercurrent arteriosclerosis or hypertensive changes.

Numerous factors have been postulated in the search for the etiology of this condition. Capillary fragility has been studied and there is some tendency for diabetic patients to show increased capillary fragility. However, capillary microaneurysms do not occur in all conditions manifesting increased capillary fragility. Abnormal lipid metabolism in diabetes mellitus has been a source of conflicting opinions and divergent results. There is no clear-cut evidence that abnormal plasma lipids or lipoproteins predispose to retinitis. Since carbohydrates are an integral part of the hyaline material found in the retinal lesions, many attempts have been made to implicate an abnormal plasma mucoprotein fraction. Although abnormal carbohydrate metabolism in ground substance cannot be disproved by present evidence, most changes in plasma polysaccharide levels are thought to be the result and not the cause of degenerative vascular disease in diabetes.

In recent years, several clinical observations and animal experiments have suggested that excessive secretion of adrenal cortical hormones may be associated with the development of diabetic retinitis. Cases of retinitis have been reported to be worsened by pregnancy and much improved postpartum. A case of complete remission following development of Simmond's disease was recorded. Lesions simulating microaneurysms were produced in alloxan-diabetic rabbits following the administration of cortisone. Some cases of diabetic retinitis have been reported to improve following adrenalectomy.

Studies of adrenal function in diabetic patients have produced inconsistent results. Gross examinations of adrenal glands have shown evidence of hypersecretion in patients with intercapillary glomerulosclerosis and retinitis. Excretion of 17-ketosteroids is apparently normal or below normal in most diabetic patients. Excretion of normal amounts of glucocorticoids has been reported by several workers. Several studies of eosinophil response to stress have shown evidence of diminished adrenal cortical activity in some diabetic patients. Diabetic acidosis has been found to augment secretion of glucocorticoids and excretion of 17-ketosteroids. Insulin hypoglycemia has also been shown to cause adrenal cortical stimulation. Measurement of plasma glucocorticoids in clinically controlled diabetic patients has not yielded uniform results.

A study was devised in an attempt to measure the activity

of the adrenal cortex in relation to the degree of retinitis in a series of diabetic patients. The responsiveness of the adrenal cortex was determined by measuring the increment of urinary corticosteroid excretion in response to 25 mg. of ACTH given intravenously over a six hour period. The corticoids were measured as 17,21-dihydroxy-20-ketosteroids according to the Porter-Silber method. Twelve patients with non-endocrine conditions were used as controls. It was found that the excretion of corticoids was slightly greater in the diabetic patients even in the unstimulated state. There was no good correlation of the degree of retinitis with the unstimulated corticoid excretion level. After ACTH stimulation, excessive responses in corticoid excretion were noted in diabetic patients with retinitis; the highest values were observed in those patients with severe retinitis. There was a better correlation of corticoid response with poor control than with duration of diabetes.

CONCLUSIONS

1. Diabetic retinitis is most common in the young patient with a history of poorly controlled disease for many years.

2. Diabetic patients tend to excrete slightly greater amounts of 17,21-dihydroxy-20-ketosteroids in the urine when compared with patients with non-endocrine disease.

3. The diabetic patient with retinitis tends to excrete excessive amounts of 17,21-dihydroxy-20-ketosteroids in the urine in

response to adrenal stimulation with intravenous ACTH. The excessive responsiveness of the adrenal cortex appears to be proportional to the degree of retinitis. The augmented adrenal activity in these patients is probably related to frequent stress reactions as a result of poor clinical control.

4. The mechanism by which the excessive amounts of adrenal cortical hormones contribute to the pathogenesis of retinitis is not clearly understood.

5. The evidence of frequent stress in patients with retinitis emphasizes the need for close clinical control of the disease as the most important means of controlling or preventing this condition.

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