Role of the Adrenal Cortex in Diabetic Retinopathy and Nephropathy

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A number of clinical and experimental observations suggest that the adrenal cortical hormones may be involved in the pathogenesis of diabetic retinopathy and nephropathy.[1-3] The evidence that has been marshalled is rather indirect and circumstantial in nature, with little supporting evidence in the field of clinical diabetes. Attempts have been made previously to evaluate the level of adrenal cortical activity in diabetic patients, based on crude measurements of the urinary 17-ketosteroids and 17-hydroxycorticoids.[4-7] These studies have yielded conflicting results. In view of the variable results reported, and since hypophysectomy and total adrenalectomy[8-10] are already being performed for possible alleviation of the capillary vascular complications of diabetes, we have attempted to reassess with newer technics adrenal cortical function in diabetic patients who have been carefully evaluated with regard to the existence, nature and extent of vascular disease.

The problem, confronting us three years ago when this study was first begun, was how to obtain objective evidence of adrenal cortical activity in a clinical state not characterized by the usual criteria associated with increased or decreased adrenal cortical function.[11] It seemed that probably the most direct approach would be to measure the following parameters of adrenal cortical function:

1. The total identifiable and individual urinary 17-ketosteroids.
2. The total urinary 17-hydroxycorticosteroids. Measurements of the individual urinary corticoids, such as Compounds E, F, Tetra-hydro E, Tetra-hydro F, Cortol and Cortolone, are being studied at present and will be the subject of a future report.
3. The plasma 17-hydroxycorticosteroids.
4. The variations produced in these indices by exogenously administered ACTH.
5. In order to evaluate processes involved in the catabolism of steroids, and renal factors governing excretion of the steroidal metabolites, radioactive hydrocortisone was administered intravenously to several patients, and the appearance of radioactivity in the urine was determined at various time intervals.

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MATERIAL AND METHODS

The present investigation is based on a series of twenty-eight normal patients, ten uncomplicated diabetic patients, fourteen patients with diabetic retinopathy, fourteen patients with the fully developed Kimmelstiel-Wilson syndrome and six nondiabetic patients with various renal diseases. To assure the homogeneity of groups, and to allow comparison of the results obtained, factors such as age and sex were controlled as far as possible by appropriate selection. Renal function was estimated by serial determinations of the blood urea nitrogen, serum creatinine, and endogenous creatinine clearance. Proteinuria was analyzed quantitatively in each twenty-four hour urine sample, and simultaneously analyzed for steroids.

The plasma 17-hydroxycorticosteroids were determined by a modification of the method of Nelson and Samuels.[12, 15] The urinary 17-hydroxycorticosteroids were determined after glucuronidase hydrolysis by the method of Porter and Silber.[13, 14] The individual urinary 17-ketosteroids were analyzed in the following manner:[16, 17, 18] Each urine was hydrolyzed by three methods (glucuronidase, acid *p*H, at room temperature, and acid at 100° C.). The combined neutral extracts from these three hydrolyses were separated by Girard's reagent, yielding a neutral ketonic extract. This ketonic fraction was resolved into its components by gradient elution, chromatography on alumina. The ketosteroids thus isolated were then identified by infrared and

ultraviolet spectroscopy and then estimated by the Zimmermann reaction (figure 1). The C-19-17 ketosteroids can be subdivided into two groups of compounds, those which have an oxygen at C-11 and those which do not, and these will be referred to in the remainder of this report as the C-19-11 oxy and the C-19-11 desoxy compounds, respectively. The C-19-11 desoxy compounds consist of dehydroisoandrosterone, isoandrosterone, androsterone and etiocholanolone, and arise mainly from steroidal precursors which originate either in the adrenals or gonads. The C-19-11 oxy compounds include 11 ketoetiocholanolone, 11 hydroxyandrosterone and 11 hydroxy-etiocholanolone and appear to be derived solely from adrenal precursors (figure 2).

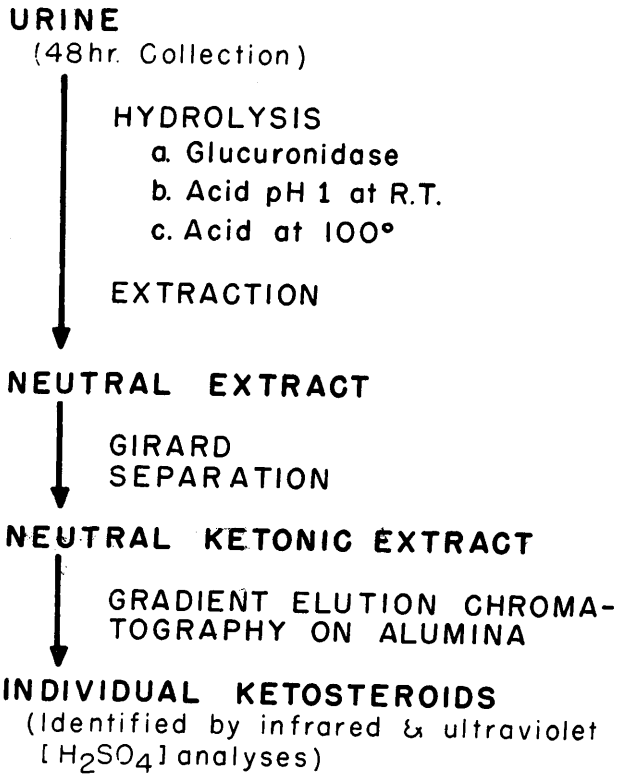

FIG. 1. Method employed for determination of urinary 17-ketosteroids.

RESULTS

The results obtained on the above-mentioned parameters are presented in the accompanying figures.

Figure 3 indicates that the total identifiable ketosteroids, that is, the 11 desoxy- plus the 11 oxysteroids excreted by patients with uncomplicated diabetes are essentially normal, while in patients with diabetic retinopathy and nephropathy, these metabolites vary from relatively normal to low values. In contrast, much

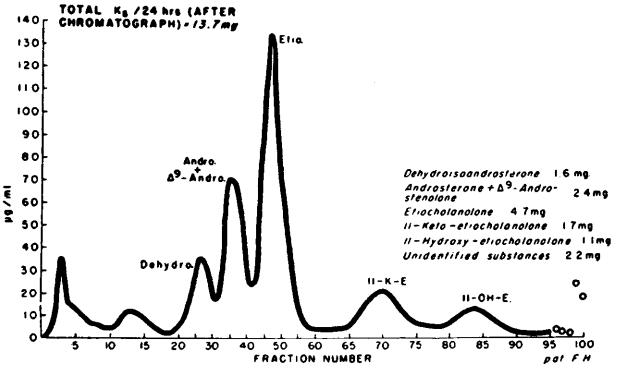

FIG. 2. Chromatogram of patient with diabetic retinopathy.

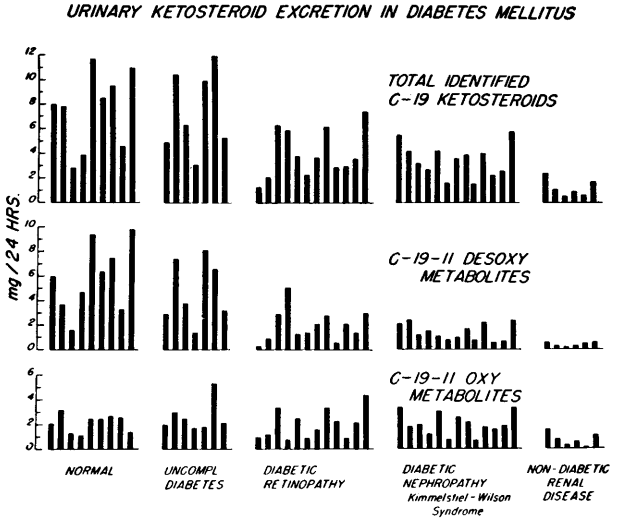

FIGURE 3

smaller quantities of these steroidal metabolites are excreted by nondiabetic patients with renal insufficiency.

Considering the C-19-11 desoxy-metabolites alone, these compounds are excreted by diabetic patients with capillary vascular disease as well as by nondiabetic patients with renal insufficiency in considerably smaller amounts than they are by normal individuals or patients with uncomplicated diabetes.

On the other hand, the C-19-11 oxy-metabolites are excreted by all three diabetic groups in normal or somewhat lesser amounts, whereas this class of metabolites is excreted in diminished amounts by nondiabetic patients with renal insufficiency.

Figure 4 shows the excretion of the urinary 17-hydroxycorticosteroids by a smaller but similar group of patients. The values observed in all diabetic groups are essentially normal.

Figure 5 illustrates the plasma 17-hydroxycorticosteroid levels. No significant deviations from the values

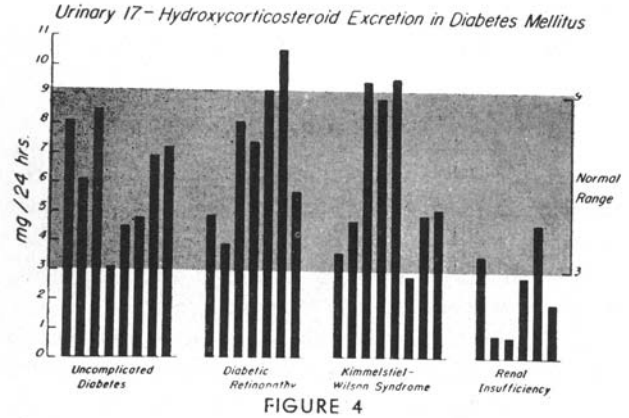

FIGURE 4

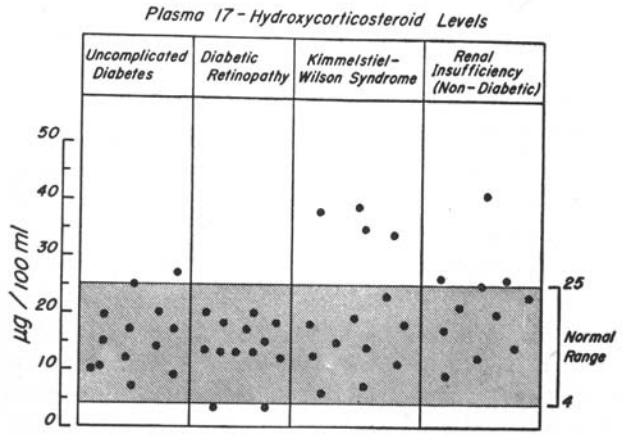

FIGURE 5

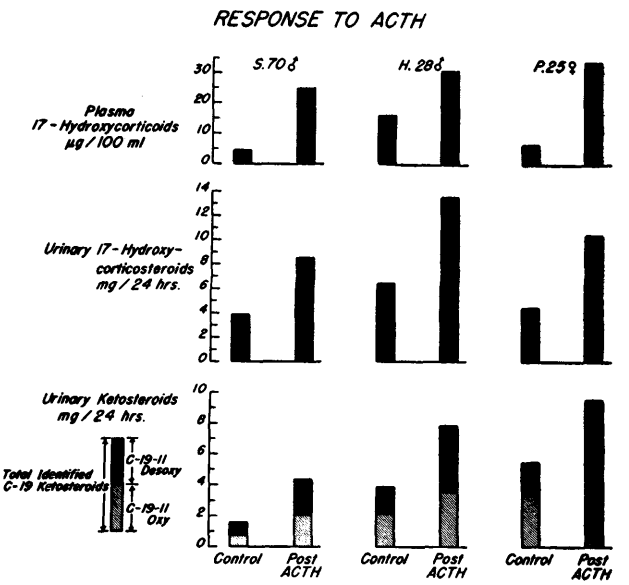

FIGURE 6

obtained in control patients were observed.

The variations produced by the intravenous administration of 40 mg. of ACTH over a six-hour period are shown in figure 6. The plasma 17-hydroxycorticosteroids, the urinary 17-hydroxycorticosteroids, and the urinary ketosteroids, both total and the two sub-groups, namely, the 11 oxy and the 11 desoxy components, rose in a manner quite indistinguishable from that expected in normal control subjects.

Thus, independent parameters of adrenal cortical function do not show evidence of hyperactivity. The fact, however, still remains to be explained that normal amounts of urinary ketosteroids and corticoids are excreted by patients with advanced diabetic nephropathy, whereas markedly diminished quantities are excreted by patients with nondiabetic renal disease.

It is possible that other factors related to alterations of renal function may be responsible for this difference. These factors may include varying degrees of diminished glomerular filtration rate, increased tubular secretion of steroids, and possibly, inhibition of tubular reabsorption of these metabolites.[19, 20] Other factors which have recently been considered as influencing urinary steroid excretion are qualitative and quantitative variations in the serum and urinary proteins.[20] In an attempt to evaluate the influence of some of these factors, comparison was made of the excretion of these substances by two groups of patients with diabetic and nondiabetic nephropathy of approximately the same age and sex with similar filtration rates and with similar quantities of proteinuria. Electrophoretic patterns of serum and urinary proteins of both groups were identical. The data indicated that the output of urinary 17-ketosteroids and 17-hydroxycorticosteroids in the patients with diabetic nephropathy still exceeds the amounts found in nondiabetic patients with renal disease.

To test further the role of the kidney, the excretion of radioactive metabolites in the urine following the injection of 4-C^{14} hydrocortisone into five patients was studied. These included three diabetic patients with a fully developed Kimmelstiel-Wilson syndrome, one nondiabetic patient with chronic pyelonephritis and one with chronic glomerulonephritis. These patients were again selected as to be of approximately the same age and sex with similar degrees of azotemia. It can be seen from figure 7 that after twenty-four to seventy-two hours much less of the administered radioactive hydrocortisone appeared in the urines of all these patients than in normal controls.[21, 22] The urinary radioactivity appeared to be closely related to the level of renal function, and no real differences existed between the diabetic and the nondiabetic patient.

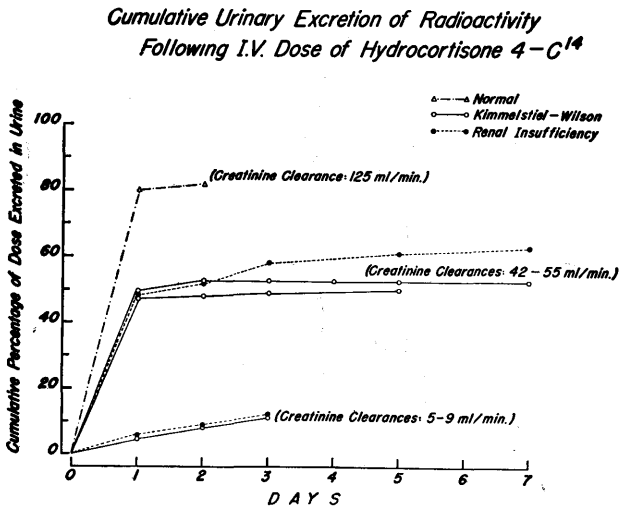

FIGURE 7

DISCUSSION

In spite of these results, which fail to disclose objective evidence of hyperactivity of the adrenal glands of diabetic patients with capillary vascular disease, clinical improvement in the retinopathy and nephropathy following hypophysectomy[9] and adrenalectomy[8] has been reported. This benefit may be totally unrelated to the role of the adrenal and pituitary glands in the pathogenesis of the capillary vascular lesions of diabetes, but may simply reflect nonspecific effects of adrenalectomy and hypophysectomy, which would favorably influence retinal and peripheral edema, hypertension and perhaps proteinuria. It is, of course, conceivable that the methods employed in this study to detect adrenal hyperactivity were inadequate for the task, but this seems rather unlikely. Nevertheless, efforts are now being made by us to estimate the rates of adrenal hormone production by turnover studies utilizing C-14 hydrocortisone, a process which will provide a result independent of the renal and other factors. It is further conceivable that even the normal secretion of adrenal hormonal metabolites found in this study may be damaging to abnormally sensitive end-organs, such as the glomerular and retinal capillaries. On the other hand, it is possible that recurrent stressful situations, such as hypoglycemia and/or acidosis, occurring during the course of the disease, result in periods of adrenal cortical hyperactivity, and it is these intermittent spikes which may trigger sensitive capillary end-organs. Finally, it is suggested that adrenal products, presently unknown, may be involved in the development of diabetic vascular disease, but that these products are not measured by the methods we have employed. It is obvious that these are all speculative considerations which do not lend themselves easily to experimental verification.

SUMMARY AND CONCLUSIONS

1. The plasma 17-hydroxycorticosteroids, the urinary excretion of total 17-hydroxycorticosteroids and of the identifiable and individual urinary 17-ketosteroids, as well as the response of these substances to exogenously administered ACTH have been studied in diabetic patients with and without capillary vascular disease.

2. Results of this investigation indicate that there is no evidence of adrenal cortical hyperfunction in patients with uncomplicated diabetes, diabetic retinopathy and nephropathy.

SUMMARIO IN INTERLINGUA

Le Rolo del Cortice Adrenal in Retinopathia e Nephropathia Diabetic

1. Le 17-hydroxycorticosteroides del plasma, le excretion urinari de 17-hydroxycorticosteroides total e del identificabile 17-cetosteroides urinari individual, como etiam le responsa de iste substantias a administrationes de ACTH exogene esseva studiate in patientes diabetic con e sin morbo de vasos capillar.

2. Le resultatos de iste investigation indica que il ha nulle prova de hyperfunction adreno-cortical in patientes con noncomplicate diabete o retinopathia o nephropathia diabetic.

ACKNOWLEDGMENT

This study was supported by a research grant (P.H.S. No. A-697[2]) from the National Institutes of Health of the United States Public Health Service, Washington, D.C.

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DISCUSSION

GEORGE J. HAMWI, M.D., (*Columbus, Ohio*): I want to take this opportunity to congratulate Dr. Rifkin and Dr. Lieberman on a very beautiful and very thorough paper on the study using technics now available.

Since we were one of the earlier groups involved in performing adrenalectomy in attempting to ameliorate the degenerative vascular complications of diabetes mellitus, and since we had thought for awhile that we were able to produce vascular lesions by the administration of these adrenal steroids, I feel compelled to make a comment. I do agree with Dr. Rifkin in everything that has been presented. I think that the longer we work in this particular area at least with the technics available today, we can find no consistent abnormality in the steroids that we are capable of measuring today. There are deviations with hypoglycemia, or acidosis, and these cannot be evaluated.

The one thing that disturbs all of us is the fact that these degenerative vascular complications are really the result of unknown influences working over a long period of time, and a static determination of any substance at one particular period may be wholly inadequate for interpretation.

Probably the benefits obtained in diabetic retinopathy after adrenalectomy or hypophysectomy are the result of alterations in blood pressure and vascular responsiveness, rather than directly due to the hormonal changes.

JOSEPH L. IZZO, M.D., (*Rochester, New York*): I would like to compliment Drs. Rifkin and Lieberman on their very fine piece of work. However, despite the rather impressive evidence they have presented, I am not yet convinced that the adrenal cortex does not play a role in the vascular lesions of diabetes. We have also been interested in the role of the adrenal cortex in diabetes and as part of our studies have followed the daily excretion of total reducing corticosteroids and 17-ketosteroids in selected cases on the Metabolism Ward under various experimental regimens. One of the things which we have noticed is that while the average or mean values of urinary corticosteroids may be similar to that of normals yet if one observes the pattern of excretion for several days one is impressed by the variability in intra- as well as inter- individual values. In other words, the range of excretion of corticosteroids seems to be somewhat greater than that of normals. This may explain why some investigators have reported values the same as normal while others have reported values less than or greater than normal. We agree with Dr. Rifkin that the average excretion of corticosteroids in diabetics with retinopathy is not different from those without retinopathy.

Finally, it should be pointed out that the present authors have examined mainly the 17-ketosteroid metabolites. As you know, this comprises only one fraction of the steroid metabolites. I should like to ask Dr. Rifkin if he has any information on the pattern of

excretion of the alpha-ketolic or the glycol metabolites in the urines of diabetics.

DR. RIFKIN: I should like to thank both Dr. Hamwi and Dr. Izzo for their kind comments. We have had little personal experience in the use of hypophysectomy and bilateral adrenalectomy for the alleviation of diabetic capillary vascular disease. Two of the patients included in this afternoon's presentation have been under the care of Dr. Irving Graef and were submitted to bilateral adrenalectomy. He informs me that one patient has had marked improvement in retinopathy while the other has shown no change since operation. We have, however, subjected three of our patients to intensive cobalt irradiation directed to the pituitary gland, but thus far have been unable to produce any clinical or metabolic changes.

I suspect that the variability of excretion patterns that Dr. Izzo has noted might be expected in diabetic patients when subjected to various stress reactions. We are aware that there are other steroidal metabolites which we have not yet measured, but we hope to have an opportunity to do this in the near future.

The Purpose of Research

Bacon asserted that it had not yet, in his time, been recognized that the true aim of all science is "to endow the condition and life of man with new powers or works" or "to extend more widely the limits of the power and greatness of man." Bacon, however, does not take the position that these are the sole purposes of research. Truth for the sake of truth is by no means excluded. Indeed, by pursuing truth for the sake of the power with which it endows mankind, one also arrives at true knowledge, since without knowledge there is no power. Whether one seeks truth first and utility second, or vice versa, is in fact, the same, since "works themselves are of greater value as pledges of truth than as contributing to the comforts of life." Bacon must be credited with emphasizing the fact that knowledge should proceed in the consciousness that the growth of knowledge is the way to increased power over man's environment.

The prescience of one great intellect perceived, as long as three centuries ago, that the role of science and invention and of technologic education must be not only to search for truth but also to direct the fruits of science in the interests of the community and mankind.

By M. H. Trytten
From *Science,* Vol. 126, No. 3262, July 5, 1957.

Fatal Acute Myocardial Infarction

The data obtained from autopsy series by the Washington University group confirm some widely held views regarding acute myocardial infarction but do not confirm others. The facts regarding the incidence of acute myocardial infarction among 17,000 autopsies that are the most interesting are: (1) the discovery that a change has occurred in the relative incidence of the disease in white individuals of the two sexes from 2:1 (males: females) before 1940 to 1:1 since 1940; (2) the discovery that the incidence in older white women has increased more since 1940 than in other age-sex-groups; (3) the discovery that the difference between the incidence in Negroes and whites is increasing rather than decreasing; (4) the discovery that diabetes mellitus did not have a significant effect on the incidence in Negroes although diabetes mellitus unquestionably has a profound effect on the incidence of acute myocardial infarction among white individuals.

Many investigators have noted a close association between diabetes mellitus and coronary heart disease. The Washington University group found that acute myocardial infarction was four times more common among autopsied white diabetic patients than among autopsied white nondiabetic patients. However, among Negro diabetic patients, acute myocardial infarction was not significantly more common than among nondiabetic patients. Thus a Negro diabetic patient is even less likely to die of myocardial infarction than a nondiabetic white patient. It is interesting to note that diabetic patients with acute myocardial infarction did not die at an earlier age than nondiabetic patients.

From "Fatal Acute Myocardial Infarction: Sex, Race, Diabetes and Other Factors," by Wilbur A. Thomas, M.D., in *Nutrition Reviews*: Vol. 15, No. 4, April 1957, pp. 97-101.