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# False Elevation of Plasma 17-Hydroxycorticoids in Diabetic Ketosis

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## SUMMARY

**Plasma corticoids were measured by an acid-fluorescent method and a modification of the Porter-Silber reaction in diabetic patients under good control and in states of poor control including ketoacidosis. In patients with significant ketonemia the plasma ketone bodies may produce a falsely high estimation of adrenal corticoid secretion when measured as Porter-Silber chromogens. Evaporation of the plasma extract, or preferably use of the acid-fluorescent procedure, will obviate such interference.** DIABETES *14*:744-45, November 1965.

An elevation in plasma hydroxycorticoids in diabetic ketoacidosis would be anticipated because of adrenal stimulation by this marked stress. Recently, Kruger and co-workers[1] have shown that in association with elevated ketone bodies there may be a false elevation in the 17,21-dihydroxy-20-ketosteroid (Porter-Silber chromogen) values in obese patients subjected to fasting. The substance producing this increase could be removed by evaporation of the organic extract prior to the color reaction. This aberration was not seen when plasma corticoids were measured by a technic using acid-fluorescence. Since blood ketones, even in controlled diabetic patients, may be somewhat higher than in nondiabetic subjects,[2] we have compared plasma levels of the Porter-Silber chromogens and acid-fluorescent steroids on the same plasma sample in diabetics without glycosuria or ketonuria, in patients with ketosis and in those with frank diabetic ketoacidosis.

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TABLE 1

A comparison of plasma acid-fluorescent corticoids and Porter-Silber chromogens* in diabetes

| Diagnosis | Porter-Silber chromogens μg./100 ml. | Acid-fluorescent corticoids μg./100 ml. | Ketones μg./ml. |
|---|---|---|---|
| Diabetic acidosis | 158.8 | 41.4 | 430 |
| | 139.0 | 91.1 | 311 |
| | 63.0 | 26.1 | 199 |
| Uncontrolled diabetes with ketosis | 89.0 | 20.0 | 256 |
| | 39.5 | 20.0 | 31 |
| | 68.8 | 17.1 | 51 |
| | 73.2 | 25.0 | 274 |
| Controlled diabetes | 16.7 | 16.4 | 2.9 |
| | 20.5 | 21.8 | 12.8 |
| | 15.6 | 21.8 | 2.4 |
| | 9.5 | 12.8 | 8.4 |
| | 18.4 | 23.2 | 6.7 |
| | 6.9 | 11.8 | 3.1 |

*Porter-Silber chromogens measured by the method of Peterson and associates.[4]

## METHODS AND MATERIALS

Plasma ketones were measured according to the method of Lyon and Bloom.[3] Porter-Silber chromogens were measured by a commonly used modification[4] of the Porter-Silber technic[5] which does not employ evaporation. The normal range is 5-25 μg./100 ml. The acid-fluorescent technic for measuring plasma cortisol was modified from Silber and associates[6] by Gantt et al.[7] (normal range 8 to 24 μg./100 ml.).

## RESULTS AND DISCUSSION

Our results are shown in table 1 and reveal that in untreated ketoacidosis there is the expected increased plasma cortisol as measured by acid-fluorescence. This technic measures the unconjugated plasma 11-hydroxycorticosteroids which in normal man is primarly cortisol. The modification of the Porter-Silber reaction that is used in many laboratories, including our own, produced far higher estimates of adrenal response in patients with diabetic ketosis and ketoacidosis. The product of the reaction of acid-phenylhydrazine with 17,21-dihydroxy-20-ketosteroids (Porter-Silber reaction) has an absorption maximum at 410 mμ. Phenylhydrazine will react with other aldehydes and ketones and is therefore nonspecific unless the methods utilized eliminate these interfering compounds prior to the development of the color reaction.

Both acetone added to normal plasma and plasma obtained from fasting ketotic patients produced a similar colored product which resulted in falsely elevated plasma values for the 17,21-dihydroxy-20-ketosteroids. Although the absorption maximum of this product is not at 410 mμ.[1] a marked elevation of the reading at this wave length (410 mμ.) does occur. Evaporation of the methylene chloride extract as suggested by Silber and Busch[8] of either the acetone treated plasma or the ketotic plasma will remove this chromogen.[1] Similarly, in the patients with diabetic ketosis or ketoacidosis, plasma hydroxycorticoids measured by this technic showed a spectrum that was the same as that seen in the plasma of fasting patients and in acetone-treated plasma. This may explain the lack of correspondence noted by Peterson[4] in the plasma of patients with diabetic acidosis when Porter-Silber chromogens were compared by his modification and by the isotope dilution technic. Finding the nonsteroidal chromogen in another state associated with hyperketonemia is additional evidence favoring its identification as acetone.

## CONCLUSION

In patients with marked ketosis or even those with poorly controlled diabetes there may be sufficient ketonemia to elevate the Porter-Silber chromogen values unless the organic extract of the plasma is evaporated. The acid-fluorescent technic for measuring plasma corticoids would therefore seem preferable for the evaluation of adrenal cortical function in diabetic patients unless significant ketosis is excluded. In well controlled diabetic subjects results obtained by both technics are in satisfactory agreement. However, the acid-fluorescent method has additional advantages in requiring less blood and less time for assay.

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