Paperchromatographic Analysis of the Urinary Steroids in Nephrotic Children Treated with Prednisolone

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As early as 1953 Farnsworth suggested that during the treatment of nephrotic patients with ACTH there might be some relationship between the increase in steroid excretion and the occurrence of diuresis [12]. Since then his observations have been confirmed [4]. Thus, Galán et al. [13] found that steroid excretion during ACTH treatment which induced diuresis was twice as high as in the course of ineffective ACTH treatment. In earlier studies carried out by Jobst and the present author [10] it has been established that during prednisolone treatment, too, steroid excretion was increased at the time of diuresis.

Opinion is thus uniform concerning the increase of steroid excretion during diuresis. The question whether the increase in steroid excretion was due to an increased activity of the pituitary-adrenocortical system or to other factors, has, however, remained unanswered.

The aim of the study reported in the present paper has been to approach this question.

Methods

The urines of 5 nephrotic children treated with prednisolone were collected during 24 hour periods, and the steroids which could be released by mild acid hydrolysis and treatment with calf-spleen glucuronidase [7] were extracted from the urine samples with a method described in a previous communication [9]. The crude extracts were purified further by partitioning between benzene and water, according to Talbot [19]. After measuring the extinction of methanolic solutions of these purified extracts in an Unicam SP 500 type spectrophotometer at wavelengths of 220, 230, 240, 250 and 260 mμ, the quantity of unsaturated steroids in the extract was determined by the use of Allen's correction formula [1]. Quantities of about 100 μg were run in Nowaczynski's ethyleneglycol-toluene system [16] on sheets of Schleicher-Schüll paper No 2043b. The steroid spots were localized on the paper according to their ultraviolet light absorption at 240 mμ, using an Uvifot type photometer, on the basis of tetrazolium blue reduction, and were determined quantitatively after elution, with the use of ultraviolet light absorption.

Identification of steroids was done on the basis of the chromatographic mobility of their acetates, prepared by treatment with pyridine-acetic acid anhydride, as well as by comparison of their sulphuric

acid chromogens with those of authentic steroids.*

Results

In the extracts of the urines collected during treatment with prednisolone *(Prednisolon, Chinoin Laboratories, Budapest)* only prednisone (1,4-pregnadiene-17, 21-diol-3, 11,20-trione) and prednisolone (1,4-pregnadiene-11,17,21-triol-3,20-dione) were found in appreciable quantity. The criteria for identification were the correspondence of the R_f value to that of the standard preparation (prednisolone, 0.013; prednisone, 0.058; in comparison to the R_f value of cortisone, 0.16 and 0.71, respectively). The paperchromatographic mobility of the acetylated product coincided with the position of the acetylated standard steroid. In ultraviolet light the absorption spectra of the steroids dissolved in concentrated sulphuric acid is shown in Figs. 1 and 2.

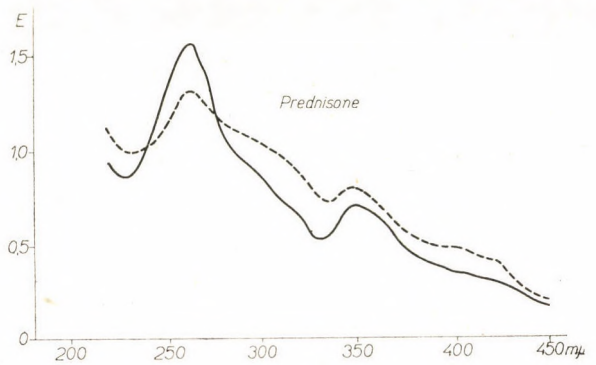

Fig. 1

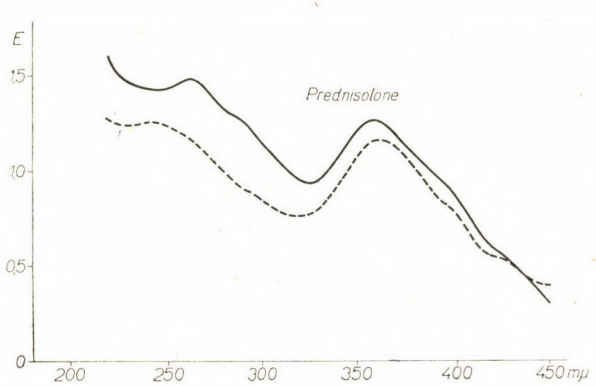

Fig. 2

——— Solid line: authentic steroid ..... dotted line : steroid separated from urine

* Kindly supplied by Organon N. V., Oss, Netherlands.

TABLE

Prednisolone mg/day by mouth	Patient's initials	Oliguric phase		During diuresis	
		Urine ml/day	Steroid* mg/day	Urine ml/day	Steroid* mg/day
80	I. D.	380	4.36	1350	8.16
		350	3.80	1270	7.48
		330	2.72	1500	6.37
				1220	4.69
				1500	11.99
				930	5.36
60	T. V.	440	2.60	1370	3.92
		480	2.57	2050	4.27
	J. P.	360	2.42	1150	3.15
		290	2.25		
40	F. S.	360	2.12	600	4.85
	I. O.	285	1.86	1200	5.93

* Represents the sum of prednisone and prednisolone excretion determined quantitatively after separation by paper chromatography in each case.

As seen in Figs. 1 and 2, the characteristic absorption maxima and minima appear at the same wavelength as in the case of the standard steroids and are in good agreement with the data of SLAUNWHITE and SANDBERG [13]. Quantitative determination following chromatographic isolation showed that in the fraction hydrolysed by mild acid, 20 to 30 per cent of the estimated steroid quantity was prednisone, and 70 to 80 per cent prednisolone. In the glucuronoside fraction, which constituted about 20 per cent of the excreted unsaturated steroids, about 10 per cent prednisone and 90 per cent prednisolone could be found.

The results of the examinations are listed in Table I.

The data in Table I show that in the oliguric phase (before the onset of diuresis) only 4 to 6 per cent of the daily prednisolone dose could be recovered in an unchanged form from the urine. This is about 1/3 of the value reported by SANDBERG and SLAUNWHITE [17] for normal persons without renal abnormality.

In the urines collected during diuresis prednisone and prednisolone could only be demonstrated. The whole amount of these steroids, however, as compared with the quantity of the prednisolone administered, was about 8 to 15 per cent, consider-

ably higher than during the oliguric phase. This value practically agreed with the 10 to 15 per cent mentioned by SANDBERG and SLAUNWHITE. The amount of steroids (prednisone and prednisolone) bound to glucuronic acid did not change during diuresis, it corresponded to about 1 per cent of the quantity of the steroid administered.

DISCUSSION

The increase in the excretion of steroids during diuresis may be brought about in several ways.

(i) As a result of activation of the pituitary-adrenocortical system the quantity of steroids released into the circulation by the adrenals may increase. A stress reaction may thus be assumed to be at play.

(ii) Another possibility may be that the steroids dissolved in the oedema fluid leave the organism when that fluid is excreted.

(iii) The increased excretion of steroids might be due to a change in renal function, and may reflect the changes of glomerular filtration or tubular reabsorption during diuresis.

In deciding the question which of the above assumptions is correct, examination of the steroid excretion of nephrotic children treated with prednisolone seemed to be the most suitable way. First, steroids with a double unsaturated ring A are not formed in the organism. As a result of the particular structure of ring A, these steroids are not reduced to tetrahydro compounds in the liver [20], which otherwise is a characteristic step in the breakdown of natural steroids [5]. Consequently, a considerable proportion of the administered steroids may be recovered from the urine in an unchanged form, in contrast to the quantity of about 1 per cent observed with natural steroids [5]. At the same time this facilitates the identification of the excreted steroids. A further point is that in our patients the daily dose amounted to 40 to 80 mg, exceeding by far the 8 to 9 mg dose of prednisolone sufficient for substituting the normal adrenal hormone production. A natural consequence of this was that the adrenals discontinued to synthesize their own hormone and this explains why only prednisolone derivatives were present in the urine during treatment. Similarly, only prednisolone and prednisone were found in the urine during diuresis; although the excretion of steroids increased, it was invariably the exogenous derivatives that were present. All this argues against an increased activity of the pituitary-adrenocortical system having been the cause of the increased steroid excretion. Had this been the case, natural steroids or their derivatives should have appeared in the urine. In their absence the role of endogenous steroid mobilisation in the observed phenomenon can be excluded.

On the basis of the present investigations it cannot be decided which of the further possibilities plays a decisive role in the increase of steroid excretion. Computed from the weight

loss during diuresis, the oedema fluid may amount to from 4000 to 7000 ml. The steroid content of the ascites fluid of patients treated with prednisolone is low, 1—2 μg/100 ml, perhaps because of the low protein content [8]. Thus, only 10—40 μg of steroid are excreted with 100 to 2000 ml of oedema fluid daily. This quantity is by two orders of magnitude less than the increase in steroid excretion as estimated by chromatographic analysis, and throws doubt upon the importance of the steroids dissolved in the oedema fluid for increased steroid excretion.

The diuresis induced by steroid treatment is the consequence of changed renal function. In some of the cases a certain increase in glomerular filtration rate occurs [2], then invariably a marked decrease in tubular salt and water reabsorption sets in, followed by the phenomenon of diuresis [14].

Part of the steroids is well-known to be excreted as a compound easily hydrolysable by mild mineral acids. The rest is bound to glucuronic acid and can be hydrolysed by glucuronidase only. The steroids bound to glucuronic acid find their way into the urine by filtration, and the so-called free steroids, which are linked to serum, proteins, *via* the tubular mechanism [5, 11].

As has been pointed out, in our cases the quantity of the glucuronidase fraction during diuresis did not change much; this indicates that no marked change in glomerular filtration had taken place in that stage. On the other hand, the quantity of the steroids which are excreted in loose chemical linkage and which are hydrolysable with diluted mineral acids, showed a considerable increase during the decrease of tubular reabsorption. This indicates that a changed, or rather a normalized tubular function considerably influences the quantity of excreted steroids.

Thus, the increase in the excretion of free steroids during diuresis is not caused by increased steroid production in the adrenals but is probably a result of changed renal function, namely, of altered tubular reabsorption.

Summary

Steroid excretion in nephrotic children treated with prednisolone has been examined paperchromatographically before and during diuresis.

The increase in steroid excretion during diuresis was not due to an increased pituitary-adrenocortical activity but to derivatives of the administered steroid. The increase in steroid excretion during diuresis is most probably a consequence of the reduction of tubular reabsorption.

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