URINARY CORTICOIDS AFTER ADMINISTRATION OF ADRENOCORTICOTROPHIC HORMONE, CORTISONE, ARTISONE OR DEHYDROISOANDROSTERONE

by

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B. S., University of Southern California, 1941

A Thesis submitted to the Faculty of the Graduate School of the University of Colorado in partial fulfillment of the requirements of the Degree Master of Science

Department of Biochemistry

1951

This Thesis for the M.S. degree, by

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Date May 26, 1951

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URINARY CORTICOIDS AFTER ADMINISTRATION OF ADRENOCORTICOTROPHIC HORMONE, CORTISONE, ARTISONE OR DEHYDROISOANDROSTERONE.

Thesis directed by Bernard B. Longwell, Ph. D.

The urinary corticoid excretion of patients suffering from dystrophia myotonica, myotonia congenita, rheumatoid arthritis and disseminated lupus erythematosus was determined before, during and after hormonal therapy. The hormones administered were 17-hydroxy-11-dehydrocorticosterone, pregnenolone, dehydroisoandrosterone and the adrenocorticotrophic hormone. The Corcoran and Page method was used to determine the excretory levels.

In each of the three patients suffering from dystrophia myotonica whose excretory levels were determined during and after treatment with ACTH, a definite adrenal cortical response was noted. In five of the six patients suffering from dystrophia myotonica and myotonia congenita, an improvement in myotonia was realized. It is hoped from this evidence that the adrenocorticotrophic hormone may offer some benefit in the treatment of these diseases.

Two patients suffering from rheumatoid arthritis and one from disseminated lupus erythematosus showed no clinical response to treatment with massive doses of pregnenolone or dehydroisoandrosterone. However, in three of the four series involved, the pattern of corticoid excretion was similar to that noted when the adrenal cortex is under stress. It was postulated that the hormones might be converted into non-active corticoids or that they might produce a stress on the

adrenal cortex.

This abstract of about one hundred and ninety words is approved as to form and content. I recommend its publication.

Signed Bernard B. Longwell
Instructor in charge of dissertation

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URINARY CORTICOIDS AFTER ADMINISTRATION OF ADRENOCORTICOTROPHIC HORMONE, CORTISONE, ARTISONE OR DEHYDROISOANDROSTERONE

Interest was focused on the function of the adrenal cortex in 1855 when Thomas Addison, studying anemias, described a universally fatal type associated with a characteristic set of symptoms and related to hypofunction of the adrenal cortex.

It has been demonstrated repeatedly that removal of both adrenals is fatal and that the resulting symptoms are due to lack of cortical tissue. In 1927, Hartman, MacArthur and Hartman (1) demonstrated that adrenalectomized cats could be maintained by injections of an extract of cortical tissue. The nature of the hormones involved in life maintenance has now been elucidated by extractions and isolation, chemical identification and synthesis of the hormones.

Myers, Mason and Kendall (2), in attempting to identify substances extracted from the adrenal cortex, related the structure of "cortin" and other steroid hormones to cholesterol, thus opening the way to the use of a large number of classification tests known for plant and animal sterols. From their benzene-water fractionation they separated three mixed crystalline substances from which they isolated and identified a number of steroid compounds. Simultaneous investigations by Reichstein, by Wintersteiner and Pfiffner, and by Gartland and Kuizenga contributed to the classification and identification of these compounds. Reichstein (3) has reviewed this work. Sarett (4) confirmed the structural formula of the cortical hormones by a partial synthesis of 17-hydroxy-11-dehydrocorticosterone. He used desoxycholic acid as a starting material and identified

intermediates.

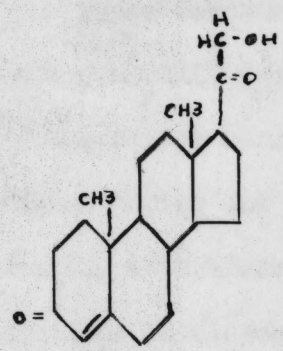
From the combined efforts of these men 43 compounds were isolated and identified from the adrenal cortex. During the past few years, Mason and Sprague (5) succeeded in isolating 17-hydroxy-corticosterone from the urine of a patient with Cushing's Syndrome associated with severe diabetes mellitus. Mason (6) later isolated 17-hydroxy-11-dehydrocorticosterone from two Addisonian patients maintained on that hormone, and he also isolated 17-hydroxycorticosterone and 17-hydroxy-11-dehydrocorticosterone from four patients whose rheumatoid arthritis was treated with these hormones. Small amounts of a compound presumably 17-hydroxy-11-dehydrocorticosterone were isolated from normal urine but positive identification was prohibited by the paucity of the yield. Schneider (7), by chromatographic analysis isolated 55.3γ/liter of 17-hydroxy-11-dehydrocorticosterone from normal male urine.

Of these 3 compounds isolated from the adrenal cortex, six were shown to have physiological activity simulating in part adrenal cortical extracts. These six (listed and structural formulas given in Chart I) were noted to have in common, beside the cyclopentanophenanthrene nucleus common to all forty-three, a 3-keto group and a Δ^4 double bond, a 20-keto group and a 21-hydroxyl group. Evidences that these three characteristics are essential to the physiological activity of these compounds includes the fact that reduction at any one of these positions destroys the physiological activity of the compound.

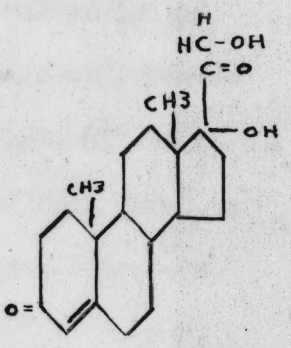
The amorphous material left after the extraction of these compounds from the adrenal cortex has been found to be more active in

CHART I

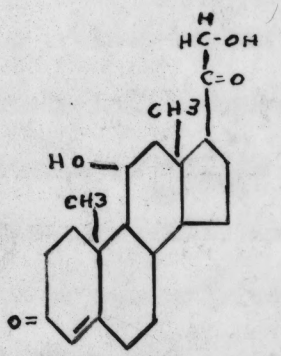
STRUCTURAL FORMULAS OF ADRENAL CORTICAL HORMONES



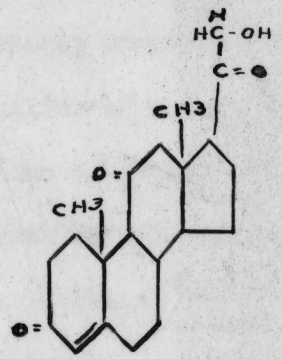
Desoxycorticosterone



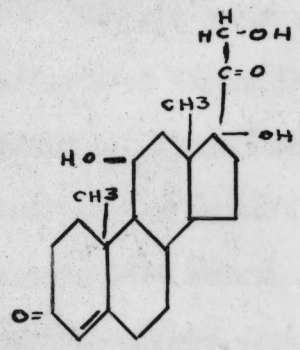
17-hydroxy-11-desoxycorticosterone



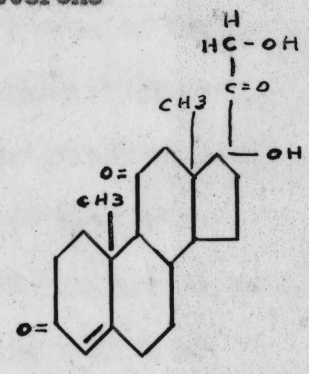
Corticosterone



11-dehydrocorticosterone



17-hydroxycorticosterone
(Kendall's Compound F)



17-hydroxy-11-dehydrocorticosterone
(Kendall's Compound E)

life maintenance than any of them but little is known of its composition.

These compounds which do not exhibit physiological activity have generally been assumed to be precursors or break-down products of the active hormones, or to be the result of the chemical manipulation during the extraction process. In the part of this paper dealing with hormone metabolism the evidence supporting these conclusions is discussed.

In order to evaluate the ability of an adrenal cortical substance to protect the adrenalectomized animal against deficiency symptoms biological assays were developed depending on the ability of a substance to replace in some manner excised tissue. Since each test measures protection against only a portion of the total deficiency it is necessary to conduct several tests in order to establish the potency of a compound.

The six hormones known to be active in combating some of the effects of adrenalectomy divide themselves into two general classifications. Those having an oxygen in the C-11 position affect carbohydrate and protein metabolism and offer protection to the adrenalectomized animal against the deleterious effects of exposure to cold, prolonged muscle stimulation, decrease in the deposition of liver glycogen , and utilization of alanine and pyruvic acid as glycogen formers. They are sometimes referred to as the S-hormones because of their effect on sugar metabolism.

Those which do not contain an oxygen in the C-11 position are active in maintaining the adrenalectomized animal in normal electro-

lyte balance, i.e., they decrease the excretion of sodium, chloride and water and decrease the excretion of potassium. The hormones of this group are able to maintain an adrenalectomized animal in an active condition and normal with respect to appearance, clinical signs, appetite, and blood nitrogen levels. In addition they enable the adrenalectomized animal to react normally to short time muscle stimulation.

Carnes, Ragen, Ferrebee and O'Neill (8) established the fact that desoxycorticosterone may play a prominent role among the hormones of the adrenal cortex by demonstrating that its continual administration to rats resulted in atrophy of the adrenal cortex.

Survival time of adrenalectomized animals is prolonged by the administration of any of these hormones, or more effectively by a combination of several, by adrenal cortical extract, or by the amorphous fraction which remains after extraction. Progesterone in very large doses is also active in increasing survival time.

The significance of the biologically active compounds of the adrenal cortex to human beings has been demonstrated by comparison of the urinary excretion of corticoids, determined both biologically and chemically, of normal persons, of persons suffering from hypofunction of the adrenal gland or anterior pituitary gland, and of persons suffering from hyperfunction of either gland due to hyperplasia or tumors.

Treatment of Addisonian patients with adrenal cortical hormones confirms their biological activity and shows them to have reactions similar to those found in animal experiments. The maintenance of

Addisonian patients in this way is a well established clinical procedure.

Thorn, Garbutt, Hitchcock, and Hartman (9) demonstrated that adrenal cortical extract administered to either normal persons or those suffering from adrenal cortical deficiency resulted in reduction of sodium and chloride excretion, increased elimination of potassium, increased plasma volume and decreased blood non-protein-nitrogen level.

Thorn, Howard and Emerson (10) maintained an Addisonian patient on a salt free diet in order to evaluate the influence of desoxy-corticosterone on both the clinical condition and laboratory findings of the patient. They found it permitted the patient to gain weight and that there was an increase in blood pressure and plasma volume. Blood sodium, chloride and potassium levels returned to normal, and a positive sodium and chloride balance was maintained. There was also an increase in urinary excretion of phosphorus, improved muscle strength, and a feeling of well-being. Withdrawal of the medication resulted in the reestablishment of all these deficiency symptoms, and resumption of treatment produced changes similar to those following the original administration.

One method of evaluating the functional state of the adrenal cortex is by measuring the urinary excretion of cortin. The biological activity of these compounds has permitted the development of bioassay methods for the determination of their excretory levels. Of these methods, the stimulation of liver glycogen deposition in adrenalectomized rats is perhaps the most commonly used (11).

However, chemical testing is largely replacing biological testing where possible, because of the relative ease of manipulation and the

stricter control possible as adequate testing procedures are developed. The term "cortin" has come into use to denote those compounds produced by the adrenal cortex which have a 20-keto and a 21-hydroxyl group, thus excluding the androgenic compounds which are probably produced by the adrenal cortex. The cortin fraction is determined by methods based on the reducing ability of this characteristic group.

The existence of the carbonyl group in these compounds suggested the possibility of testing procedures based on the reducing action of this characteristic group and early procedures utilized variations of Folin's (12) blood sugar technique.

Reichstein (13) found that formaldehyde was produced by the oxidation of cortin compounds and Lowenstein, Corcoran and Page (14) developed a colorimetric method for determining the amount of formaldehyde released.

A distillation procedure to eliminate certain non-specific chromogenic materials was introduced by Daughaday, Jaffe and Williams (15).

Corcoran and Page (16) modified the Daughaday, Jaffe and Williams procedure and an earlier procedure of their own to include controls that would eliminate error due to chromogenic material in the reagents and non-specific chromogenic material in the urine samples. This latter procedure was utilized in this work.

Venning and Browne (17) utilized their bioassay technique based on glycogen deposition in the liver to determine the level of excretion of cortical hormones of normal persons and those suffering from clinical disorders related to the adrenal cortex. Their unit was the amount of a substance which is equivalent in biological activity to

1 microgram of 17-hydroxy-11 dehydrocorticosterone in increasing liver glycogen deposition in adrenalectomized rat. Chart 12 shows their results.

CHART 2

SEX	CLINICAL CONDITION	AGE	CORTIN LEVEL
Female	Normal	21-48	39 units
Male	Normal	20-51	60 units
Male	Addisonian	28	< 10 units
Female	Addisonian	56	< 10 units
Female	Addisonian	29	< 10 units
Female	Addisonian	39	15 units
Male	Pan hypopituitarism	48	< 10 units
Male	Pan hypopituitarism	32	13 units
Male	Pan hypopituitarism	41	< 10 units
Female	Pan hypopituitarism	45	< 10 units
Male	Pan hypopituitarism	47	< 10 units
Male	Cushing's Syndrome	33	600 units
Female	Cushing's Syndrome	42	316 units
Male	Cushing's Syndrome	11	386 units
Female	Cushing's Syndrome	39	137 units
Male	Cushing's Syndrome	27	42 units

R. D. H. Heard (18) has given a summary of the work of Venning and associates (19, 20, 21), that of Heard, Sobel and Venning (22), and that of Talbot, Saltzman, Wixom and Wolfe (23), all of whom used chemical procedures to determine the levels of corticoid excretion. Their findings are similar in comparing normal persons with those having hypo or hyperfunction of the adrenal cortex. Their results are shown in the Chart 3, which is a part of a table presented by Heard.

CHART 3

Type of Subject	Milligrams excreted per 24 hours		
	Cortin	Reducing Substances	
	Venning *1	Heard	Talbot
Normal female average	0.039	1.3	0.24
Normal male	0.062	1.5	0.24
Hypofunction			
Addison's Disease	0-0.015		0.02-0.26
Panhypopituitarism	0	0.4-0.6	0.10-0.17
Hyperfunction			
Hirsutism	0.050-0.065		0.23-0.32
Cushing's Disease	0.2-0.7	4.8	0.90-12.0
Virilism			0.15-0.57
Stress			
Burns	0.1-0.5	3.0-4.0	0.34-1.70
Postoperative	0.1-0.2		0.34-1.70
Late pregnancy	0.1-0.4	2.8-3.2	

*1 As Kendall's Compound E

As has been noted above, early investigation concerning the function of the adrenal cortex dealt largely with results of cortical deficiency. Some relationship was demonstrated between certain clinical conditions and hyperfunction of the adrenal cortex (Cushing's Syndrome and the adrenogenital syndrome).

In 1937, a different approach to adrenal cortical function was introduced by Hans Selye (24) who described a condition in experimental animals which he called the Adaptation Syndrome. This reaction, which was associated with the development of resistance to many types of stress, was a phenomenon which he attributed to stimulation of the adrenal cortex.

At about the same time and related to this work, Selye and Hall administered large doses of desoxycorticosterone to experimental animals and produced histological changes which simulated those seen in many chronic disease processes.(25).

This work, together with many other observations, led to the belief that the adrenal cortex was involved in some phase of metabolism other than those obvious from the result of studies of adrenal deficiencies.

As the result of his observations that rheumatic disorders improved in clinical states which could be possibly associated with either increased production of corticosteroids or their accumulation for some other reason, Hench (26) demonstrated that the administration of large doses of 17-hydroxy-11-dehydrocorticosterone (Compound E or cortisone) would give relief in rheumatoid arthritis, an observation which has now been extended to many other diseases. This led to a new concept

regarding the function of the adrenal cortex which is at present under intense investigation and has not as yet been fully elucidated.

The dosage of these hormones usually administered is thought to exceed greatly the amount produced by the adrenal cortex. No way of measuring directly the level of secretion of the adrenal cortex is known, however. It might be expected that such massive doses of the adrenocorticotrophic or adrenal hormones would ellicit a response similar to that following the hypersecretion of hormones due to stress or to Cushing's Syndrome. This expectation has been only partially realized.

Sprague, Power, Mason, Albert, Mathieson, Hench, Kendall, Slocum and Polley (27) in a review of patients they have treated, report that in almost every case benefit, at least of a temporary nature was realized. However, the effects they noted to be induced by these hormones in certain of these patients included acne, hirsutism, effects on menstruation, effects on glutathione levels, euphoria, depression, asthenia and a condition simulating diabetes mellitus.

Mason, Power, Rynearson, Ciaramelli, Li and Evans (28) found no significant change in the sodium, potassium, chloride, phosphorus, red blood cells, white blood cells, or electrophoretic pattern of the plasma proteins following administration of adrenocorticotropic hormone to a normal human being. Stimulation of the adrenal cortex was evidenced in this experiment by significant increase in urinary excretion of androsterone, etiochloanone and pregnanediol.

Mason and Kepler (29) found an increase in androsterone and and etiocholanone which amounted to as much as forty-three per cent of

an administered dose of 100 mg. dehydroisoandrosterone per twenty-four hours to a normal human being. The similarity of the excretory products in these two cases suggests the possibility that dehydroisoandrosterone might enter the metabolic reaction at such a stage that it could produce effects similar to the adrenocorticotrophic hormone (hereinafter ACTH) injections, and thereby bestow the benefits of ACTH while possibly avoiding some of the complications encountered.

The fact that dehydroisoandrosterone excretion is known to be increased to as much as one hundred times the normal amount in some adrenal cortical tumors lends further support to this concept.

Another compound which has been investigated in the hope that it might enter the metabolic chain and which has been reported by Davidson, Koets, Snow and Gabrielson (30) to be of benefit to arthritic patients is 5-pregnenolone (artisone). This compound, an oxidation at the C-17 position yields dehydroisoandrosterone.

The treatment of persons suffering from conditions not known to result from the lack of functioning adrenal cortical tissue is now in its infancy. Basic to the development of such therapy is the work of Collip, Anderson, and Thompson (31) who isolated a substance from the anterior pituitary which could stimulate the growth of the adrenals in hypophysectomized rats. Smith and Engle (32) transplanted the anterior pituitary of rats into immature rats and induced precocious maturity, thus demonstrating the function of the anterior pituitary in regulating the activity of another gland.

Li, Evans and Simpson (33) isolated the adrenocorticotrophic hormone from the pituitaries of sheep, purified it and demonstrated

its ability to stimulate secretion by the adrenal cortex by dogs.

Subsequently, Armour and Company have developed production methods that have enabled them to make large quantities of the adrenocorticotrophic hormone available. This has made possible the extensive research now in progress as well as the treatment of a large number of patients.

It is important to understand the mechanism of the improvement of the patients who have benefited so markedly from treatment with these hormones. In order to understand this improvement it is necessary to determine which of the two danger periods in the "alarm reaction" described by Selye (24) is related to the chronic diseases under consideration. That is: during the stress period when conditions simulating the massive doses occur or during the exhaustive phase when massive doses may be necessary to assist the exhausted cortex.

Tremendous increases in circulating hormones have been shown to occur following such acute stimuli as burns, surgery, trauma, emotional strain and high fevers; conditions which predispose an individual to the chronic diseases benefited by adrenal hormonal therapy. No consistent change in the level of cortin excretion has been demonstrated for persons suffering from chronic diseases. This may be because the patients are tested at varying levels of response, or because urinary excretion of cortin is not a reliable method for quantitating cortical activity.

It is also important to learn of the metabolic pathway of these hormones.

It was hoped that by determining pretreatment and treatment

levels of the persons in this series, some information on these two subjects might be gained.

Eight of the persons in the series were suffering from dystrophia myotonica or myotonia congenita. No adequate treatment is known for these diseases. The etiology of these diseases is unknown, but they are thought to be metabolic in nature. For these reasons, it was hoped they might fall in the category of those diseases which could be helped by adrenal cortical hormonal therapy, and at the same time some information regarding their etiology be gained.

Two persons with rheumatoid arthritis and one with disseminated lupus erythematosus were included in the series. It was hoped they would be benefited by the extremely large doses of the hormoned administered and that the cortical levels following such large doses would help to elucidate the metabolic pathway of the hormones used.

Part of the treatment of these patients was with a representative of the C-19 (androgenic) group of hormones. Although treatment with this compound (dehydroisoandrosterone) and also with pregnenolone has been reported to be of benefit, no attempt has been made to determine whether the benefit might be of the result of their acting as the metabolic precursors of cortin.

Mason, Kepler and Schneider (34) administered 150 mg. dehydroisoandrosterone daily to a woman before and after removal of an adrenal cortical tumor. In either case they noted only a slight increase in 17-ketosteroid excretion. They isolated androsterone, etiocholanone, pregnanediol, androstenediol, androstenediol, and dehydroisoandrosterone from the urine. Consequently, the possibility exists that the

disappearance of dehydroisoandrosterone is due to its acting as a precursor of the adrenal cortical hormones.

In summary the following studies were made:

(1). To determine whether ACTH was of benefit in dystrophia myotonica and myotonia congenita, metabolic diseases in which it had not yet been tried.

(2). To determine whether pregnenolone was beneficial in these conditions and, if so, if its benefit could be attributed to its metabolic transformation into cortin.

(3). To find whether dehydroisoandrosterone, the C-19 steroid could enter into the metabolic pathway and also be of benefit to these patients.

PURPOSE

The purpose of this work was to determine the cortin excretion of patients with rheumatoid arthritis, disseminated lupus erythematosus, dystrophia myotonica and myotonia congenita before, during and after treatment with ACTH, compound E, dehydroisoandrosterone and pregnenolone.

METHODS

Patients: In this study the therapeutic agents ACTH, dehydroisoandrosterone, pregnenolone and Compound E were administered in the amounts and frequencies designated in the protocol for each individual patient. The length of the metabolic periods involved is given for each patient in the individual protocol.

Analytical Methods: Before, during and after the administration of the hormones, twenty-four urine specimens were collected and analyzed for corticoids by the formaldehydogenic method of Daughaday, Jaffe, and Williams (15) as modified by Corcoran and Page (16). This method was chosen because it is adaptible for use with small quantities of urine. In addition, this method seemed to have the following three advantages over other methods: (1) the oxidation and reduction solutions were titrated against one another to insure accurate control of these two important reagents, (2) the distillation step was included to prohibit certain non-specific chromogenic compounds from being present when the colorimetric reaction took place, (3) the use of both an oxidized and an unoxidized urine blank reduced the probability of errors due to chromogenic material in the urine samples which might pass over in the distilling procedure.

REAGENTS

Chloroform: C.P. Chloroform was used. It was redistilled over glass, the head and tail fractions were discarded, 0.75 per cent absolute ethyl alcohol was added to inhibit the formation of phosgene, and it was stored in a dark bottle.

Water: Triple distilled water was used in preparation of reagents and in analyzing the dried residues.

Glacial acetic acid, hydrochloric acid, sodium bicarbonate, and sulfuric acid: C.P. reagents were used.

Periodic acid: C.P. potassium periodate reagent was used and made as specified.

Stannous chloride: C.P. stannous chloride reagent was used. It was made by dissolving 1.4 grams stannous chloride in 50 cc. 2.5 N hydrochloric acid and titrating against the periodic acid solution to a starch endpoint and adjusting so that 10.2 cc. periodic acid oxidized 10 cc. chloride solution.

Chromotropic acid: Eastman Kodak Company 1,8 dihydroxynaphthalene sulfonic acid reagent was used and made as specified. Since no time limit was specified, determinations done within approximately one hour were run on the same chromatropic acid solution. The occasional repeated determinations indicated that this time span was permissible.

Extraction procedure: The extraction procedure varied in some details from the original method. The solvents used and the extraction times were different. Comparisons were made using the method described herein and the author's method for the same urine sample. There were no significant differences observed.

The extractions were carried out as follows: determinations were made from 24 hour samples preserved by toluene and kept cold, and whenever possible, extracted within 48 hours. One hundred cubic centimeter aliquots were acidified with 50 per cent sulfuric acid to approximately pH 1, extracted with 100 cc., 60 cc., 40 cc. chloroform. Emulsions were broken by centrifugation. The chloroform was then washed with 50 cc. saturated sodium bicarbonate solution. This was followed by enough distilled water washes to adjust the pH to between six and seven, (in most cases three washings was sufficient). The chloroform fraction was removed under reduced pressure to about 10 cc. at 40-50° Centigrade and this was transferred with chloroform washings to a small flask. The remainder was evaporated in an atmosphere of nitrogen under reduced pressure. The material extracted in this way was stored for from two to six months. When this method was compared to the extraction procedure originally described, no significant differences in results were encountered.

The dried residue in the flask was dissolved in 1.0 cc. of glacial acetic acid and 17 cc. distilled water were added to it. The contents were then divided into two equal portions, one to be used as an oxidized sample, the other as a urine blank.

The "oxidized" sample was treated with 0.5 cc. periodic acid and oxidation was allowed to proceed at room temperature for thirty minutes. Oxidation was arrested by the addition of 0.5 cc. stannous chloride reagent.

The unoxidized sample was treated with 0.5 cc. stannous chloride reagent followed immediately by 0.5 cc. periodic acid.

Oxidized and unoxidized blanks were prepared using distilled water in place of the solution of urinary extract.

Each sample was then transferred to an all-glass micro distillation apparatus with three one cc. water washings. It was distilled into one cc. of water in a centrifuge tube and the condenser outlet was placed under the surface of the water. About 8 cc. of distillate was collected and the volume was made up to 10 cc. with distilled water.

Three cubic centimeters of distillate were transferred to a tall glass tube. Five ml. of the chromotropic acid reagents were added and mixed immediately. The tube was placed in a boiling water bath for thirty minutes, rapidly cooled to 25° C., made up to 10 C. in a cold water bath.

The color density was determined at 570 millimicrons, reading oxidized sample against unoxidized blank.

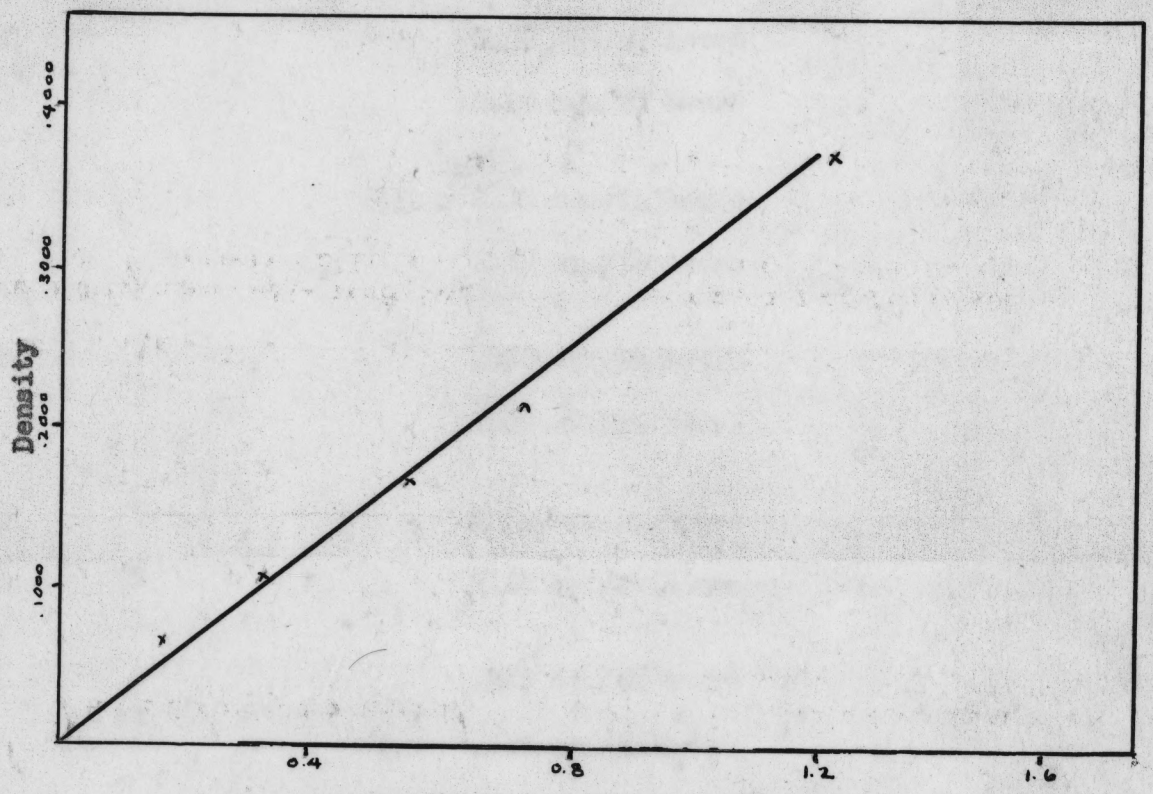
Calculations: The color density of the unoxidized sample was subtracted from that of the oxidized sample. This gave the color density due to formaldehyde liberated from corticosteroid-like substances in the urine and was compared to a predetermined curve for desoxycorticosterone and expressed as milligrams desoxycorticosterone per 24 hours.

Principle: This procedure is dependent upon the ability of the characteristic group of these hormones to be oxidized yielding formaldehyde. The resulting formaldehyde reacts with chromotropic acid to give a lavender color. The density of this color is proportional to the amount of formaldehyde generated. The density was measured by the

use of the Coleman photoelectric colorimeter Model 11 and reported as milligrams desoxycorticosterone equivalents excreted per twenty-four hours.

Standard curve: The standard curve was prepared by using weighed portions of desoxycorticosterone. Its coefficient of extinction determined to be .2404 and the factor relating density of color to milligrams desoxycorticosterone .3125. Figure 4 shows the standard curve obtained.

STANDARD CURVE BASED ON DESOXYCORTICOSTERONE



Micrograms Desoxycorticosterone/cc

$$\text{Factor} = \frac{\text{density}}{\text{micrograms/cc}} = \frac{.125}{.400} = .3125$$

$$\text{Coef. of extinction} = \frac{\dfrac{\text{density}}{\text{micrograms}}}{\text{length (path of light)}} =$$

$$\frac{\dfrac{.125}{.400}}{1.3} = .204$$

NORMALS

N-1	1.33 mg./24 hours
	1.27 mg./24 hours
N-2	1.31 mg./24 hours
	1.17 mg./24 hours
N-3	.952 mg./24 hours
	.908 mg./24 hours
N-4	1.14 mg./24 hours
	1.21 mg./24 hours
N-5	.928 mg./24 hours
	.957 mg./24 hours

PATIENT #1

Patient	J.K.
Age	50 years
Sex	Male
Disease	Dystrophia Myotonica
Duration	25 years
Symptoms	Alopecia, cataracts, testicular atrophy, severe generalized muscular atrophy and myotonia of the hands.
Treatment	ACTH 100 mg./day 5-27-50 to 6-3-50
Response	Moderate improvement of the myotonia

Date	Nature of Specimen	Treatment	Excretion per 24 hours
5-26	Control	none	.382 mg. .370 mg.
6-3	Treatment	100 mg./day ACTH	1.10 mg.
7-24	Control	none	.739 mg. .639 mg.

PATIENT #2

Patient	E. B.
Age	42 years
Sex	Female
Disease	Dystrophia Myotonica
Duration	Two years
Symptoms	Cataracts, atrophy of hands and legs, and moderate myotonia. Her condition at present is mild and her disability only moderate. She is one of three in one family suffering from this disease.
Treatment	She received no hormonal therapy.

Date	Nature of Specimen	Treatment	Excretion per 24 hours
5-15	Control	none	1.80 mg. 2.05 mg.
5-16	Control	none	1.78 mg. 1.68 mg.

PATIENT #3

Patient	G. B.
Age	38 years
Sex	Male
Disease	Dystrophia Myotonica
Duration	15 years
Symptoms	Alopecia, extreme cataracts, testicular atrophy, severe generalized muscular atrophy and moderate myotonia. He is the father of D.B. and brother of E.B.
Treatment	100 mg./24 hrs. ACTH 8-8-50 to 8-11-50 50 mg./24 hrs. ACTH 8-11-50 to 8-20-50 He showed definite improvement of myotonia but no increase in strength.

Date	Nature of Specimen	Treatment	Excretion per 24 hours
5-25	Control	none	.573 mg. .639 mg.
5-27	Control	none	.348 mg. 1.65 mg. *1
7-28	Control	none	.736 mg.
8-20	Treatment	50 mg./day ACTH	2.41 mg. *2

*1 See explanation at end of this section

*2 The volume of this sample is questionable

PATIENT #4

Patient	D.B.
Age	9 years
Sex	Male
Disease	Dystrophia Myotonica
Duration	4 years
Symptoms	Insipient cataracts, atrophy of face, forearms, hands and legs, moderately severe mechanical and voluntary myotonia widely distributed. He is the size of a six-year old and has had almost no increase in size since the onset of the disease.
Treatment	50 mg. ACTH 8-8-50 to 8-18-50
Response	Moderate improvement of myotonia and excellent evidence of adrenal cortical response.

Date	Nature of Specimen	Treatment	Excretion per 24 hours
7-20	Control	none	.158 mg. .186 mg.
8-20	After treatment		2.70 mg. 2.86 mg.

PATIENT #5

Patient	G.S.
Age	29 years
Sex	Male
Disease	Dystrophia myotonica
Duration	5 years
Symptoms	He had no alopecia or cataracts. He had marked general myotonia and atrophy of the face and hands.
Treatment	He underwent no hormonal therapy.

Date	Nature of Specimen	Treatment	Excretion per 24 hours
5-6	Control	none	1.95 mg.
5-6	Control	none	1.38 mg.
5-8	Control	none	2.77 mg.
5-8	Control	none	2.60 mg.
5-9	Control	none	.846 mg.
5-9	Control	none	.870 mg.

PATIENT #6

Patient	L. E.
Age	48 years
Sex	Male
Disease	Dystrophia Myotonica
Duration	30 years
Symptoms	Alopecia, testicular atrophy, cataracts, severe general atrophy, especially of the back and lower extremities. Little or no myotonia remains.
Treatment	100 mg./ACTH/24 hrs. 5-17-50 to 5-29-50
Response	He showed no favorable response to treatment.

Date	Nature of Specimen	Treatment	Excretion per 24 hours
8-1	Control	none	.714 mg.
8-1	Control	none	1.12 mg.

PATIENT #7

Patient	H.D.
Age	26 years
Sex	Male
Disease	Dystrophia Myotonica
Duration	9 years
Symptoms	He showed early cataracts and testicular atrophy, generalized moderate muscular atrophy and marked general myotonia.
Treatment	100 mg. ACTH/24 hrs. 4-22-50 to 5-2-50 100 mg. Cortisone/24 hrs. 3-26-50 to 4-2-50
Response	He gained weight, had a feeling of well-being, marked improvement of myotonia, but no change in strength.

Date	Nature of Specimen	Treatment	Excretion per 24 hours
7-24	Control	none	1.32 mg.
7-24	Control	none	1.02 mg.

PATIENT #8

Patient	A.S.
Age	36 years
Sex	Male
Disease	Myotonia Congenita
Duration	22 years
Symptoms	He showed general myotonia, but no atrophy or visceral dystrophies.
Treatment	ACTH 6 days 100 mg./24 hrs. 4 days 200 mg./24 hrs.
Response	Only on 200 mg./24 hrs. was any marked adrenal response noted. Slight improvement of the myotonia resulted.

Date	Nature of Specimen	Treatment	Excretion per 24 hours
7-22	Control	none	2.53 mg.

PATIENT #9

Patient	C.F.
Sex	Female
Age	48 years
Disease	Rheumatoid Arthritis
Duration	15 years
Symptoms	The small joints of the hands and feet, the joints of the upper extremity and of the ankle are chiefly affected. Admission sedimentation rate was 35 mm. by the Wintrobe method. Her condition is only moderate severe.
Treatment	300 mg./24 hrs. IM dehydroisoandrosterone 2-13-50 to 2-19-50 1 gram/24 hours. Oral dehydroisoandrosterone 2-21-50 to 2-23-50.
Response	She showed no clinical response, no significant change in sedimentation rate, no evidence of adrenocortical response nor of androgenic effect.

Date	Nature of Specimen	Treatment	Excretion per 24 hours
2-7	Control	none	1.50 mg. 1.46 mg.
2-13	Treated	300 mg./24 hr. dehydroisoandrosterone	2.41 mg. 2.57 mg.
2-15	Treated	as above	2.66 mg. 2.57 mg.
2-17	Treated	as above	1.92 mg. .866 mg.
2-10	Treated	as above	.866 mg. .863 mg.
2-21	Treated	1 gm./24 hr. dehydroisoandrosterone - oral	.763 mg. .851 mg.

PATIENT #10

Patient	R.D.
Age	46 years
Sex	Male
Disease	Rheumatoid Arthritis
Duration	12 years
Symptoms	His hands, feet, knees and elbows are deformed. He was admitted with acute arthritis of neck, shoulders and wrist and in acute pain. His sedimentation rate by Wintrobe method was 42 mm.
Treatment	300 mg./24 hrs. IM dehydroisoandrosterone 1-24-50 to 1-28-50. 300 mg./24 hrs. Pregnenolone 2-20-50 to 2-28-50
Response	On artisone therapy he experienced no improvement in clinical arthritis, no evidence of androgenic effect or of interference with liver function. There was no significant change in sedimentation rate. On dehydroisoandrosterone therapy there was no evidence of improvement.

Date	Nature of Specimen	Treatment	Excretion per 24 hours
Unknown *1	Control	none	1.31 mg./Liter *2 .996 mg./Liter
2-1	Control	none	.317 mg. .263 mg.
2-2	Control	none	.425 mg. .374 mg.
2-3	Control	none	.768 mg.
2-4	Control	none	.527 mg.

*1 See explanation at end of this section

*2 Volume unknown

PATIENT #10
(Continued)

Date	Nature of Specimen	Treatment	Excretion per 24 hours
2-22	Treatment	300 mg./24 hrs. Artisone	1.35 mg. 1.06 mg.
2-24	Treated	as above	1.10 mg. 1.01 mg.
2-26	Treated	as above	1.48 mg.
2-28	Treated	as above	1.68 mg. 1.30 mg.
3-2	Control	none	1.25 mg. 2.92 mg.
3-4	Control	none	1.82 mg. 1.87 mg.
No date #1	Control	none	.394 mg. 1 L .338 mg. 1 L
1-25	Treated	300 mg./24 hrs. dehydroisoandrosterone	3.97 mg.
1-26	Treated	as above	none demonstrated
1-27	Treated	as above	1.30 mg. .902 mg.
1-28	Treated	as above	.581 mg. .372 mg.
1-29	Control discontinued 1/28	none	.449 mg. 1.10 mg. #1
1-30	Control	none	.726 mg. .467 mg.
1-31	Control	none	.756 mg. .720 mg.

#1 See explanation at the end of this section

#2 Vol. anddate of this sample are not known. See discussion of samples 1-25-50 to 1-29-50 at the end of this section.

PATIENT #11

Patient	W.V.
Age	30 years
Sex	Male
Disease	Disseminated lupus erythematosus
Duration	6 years
Symptoms	He has low grade fever, chronic, indurated, secondarily infected facial rash, marked cervical lymphoadenopathy, and pleuritic chest pain. He showed no evidence of renal damage.
Treatment	300 mg./24 hours pregnenolone 3-14-50 to 3-20-50
Response	He showed no response to therapy.

Date	Nature of Specimen	Treatment	Excretion per 24 hours
3-19	Control	none	1.25 mg. 1.16 mg.
3-14	Treated	300 mg. pregnenolone/24 hrs.	3.03 mg. 3.03 mg.
3-17	Treated	as above	9.14 mg. *1 9.27 mg.
3-19	Treated	as above	.718 mg. .671 mg.
3-22	Control	none	1.32 mg.

*1 See explanation at end of this section

Discussion of difficulties: Seven of the individual samples included in this series gave readings which were so high as to be unbelievable in relation to adjacent samples or in some cases to samples taken from the same urine. No explanation for this variance is evident since the procedure followed in every case was, as much as we could make it so, identical. The obviously erroneous values are indicated in the tables of results.

Samples 1-25-50 to 1-29-50 for the patient, R.D., showed heavy precipitate in the dried extract. There is no likely explanation for this precipitate and no attempt was made to identify it.

CHART 5

Patient	Disease	Therapy	Clinical Response	Mg. Corticoids/24 hours Before Treatment	Mg. Corticoids/24 hours During Treatment
JK	Dystrophia Myotonica	ACTH	++	3.76 to .689	1.10
EB	Dystrophia Myotonica	None		1.73 to 1.92	
GB	Dystrophia Myotonica	ACTH	+++	.348 to .736	2.41
DB	Dystrophia Myotonica	ACTH	++	.172	2.78
GS	Dystrophia Myotonica	None		.853 2.68	
LE	Dystrophia Myotonica	ACTH	OO	.917	
HD	Dystrophia Myotonica	ACTH Compound E	+++	1.17	
AS	Myotonia Congenita	ACTH	+	2.53	
CP	Rheumatoid Arthritis	Dehydro-isoandros-terone	none	1.48	2.62 to .677
RD	Rheumatoid Arthritis	Artisone	none	1.05 to 1.49	.290 to .768
RD	Rheumatoid Arthritis	Dehydro-isoandros-terone	none	.476 to 1.10	
WV	Disseminated lupus erythematosus	Pregnenolone	none	.694 to 3.03	

\+ Slight improvement in myotonia
++ Moderate improvement in myotonia
+++ Marked improvement in myotonia
OO No myotonia remained at time of treatment

Discussion and conclusions: The normal range of cortin excretion as determined by this procedure was .90 to 1.30 mg./24 hours. Five normal determinations were made. A larger number of determinations would undoubtedly have resulted in a wider range.

The control levels of the patients with dystrophia myotonica and myotonia congenita varied from .37 to 2.69 mg./24 hours. Four were below the estimated normal, one within the normal range and three above normal. There was more day to day variation than is the case in the normals presented in the literature.

In three patients, JK, GB, DB, treatment levels of cortin excretion following ACTH therapy were measured. In each, a very marked increase in cortin excretion was noted. This is not surprising in view of the fact that the known function of ACTH is to stimulate the production of the adrenal cortical hormones. It also demonstrated that, whether or not the adrenal cortex is involved in the etiology of dystrophia myotonica and myotonia congenita, these patients have ample adrenal cortical tissue to display a marked response to ACTH.

Five, JK, GB, DB, HD, AS, of the six patients treated with ACTH showed some improvement in myotonia. The disease was too far advanced in the sixth, LE, for any myotonia to remain. Improvement in strength was not noted.

On the basis of these observations, the following conclusions seem warranted:

(1). These data do not warrant any inference that the adrenal cortex is functionally incapacitated in this disease.

(2). In each of the patients whose urine was examined after ACTH

therapy, the adrenal cortex was sufficiently normal to give a marked response to the trophic hormone.

(3). There is considerable reason to hope from this experiment that ACTH may offer some benefit in the treatment of these two diseased states. The one patient who was treated with Compound E showed a clinical response similar to that which he demonstrated on ACTH.

The two patients with rheumatoid arthritis, RA and CF and one with disseminated lupus erythematosus, WV, who were treated with dehydroisoandrosterone and pregnenolone showed no clinical response to these hormones.

However, with both the pregnenolone series and one of the two dehydroisoandrosterone series, the excretion of formaldehydogenic substances increased. These increases followed a characteristic pattern in three of the four series. At first, a marked increase in cortin excretion was noted. Then there was a gradual decline to subnormal levels, even while the administration was continued.

Four methods of explaining these responses present themselves:

First, that the pregnenolone and dehydroisandrosterone acted as a precursor for the adrenal cortical hormones. This explanation seems highly improbable in that none of the three patients showed a clinical improvement.

Secondly, that the variation is due to the variability noted above for control samples of persons suffering from these diseases. This explanation also seems improbable in view of the characteristic pattern of the excretory levels following administration.

Thirdly, that the pregnenolone and dehydroisoandrosterone were

converted into steroids with the characteristic group of the cortin compounds, but that the metabolic pathway traversed did not include any of the active hormones. This theory, in addition to being consistent with the clinical and chemical observations, might explain the tendency noted above for an initial increase to be followed by a gradual decline to subnormal level.

Fourthly, the large doses of hormones administered may have produced a stress on the adrenal cortex. The initial stimulation followed by a decline to subnormal levels is typical of the secretion pattern following other stressful situations.

ACKNOWLEDGMENTS

The author wishes to express her appreciation to Dr. B. B. Longwell for his consideration and guidance in conducting this investigation and in the writing of this paper.

The author also wishes to thank Dr. Dalton Jenkins who supervised the treatment of the patients in this investigation, obtained urine samples and did some of the extractions involved.

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