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ANNUAL PROGRESS REPORT

March 1, 1962 to February 28, 1963

J. W. Conn

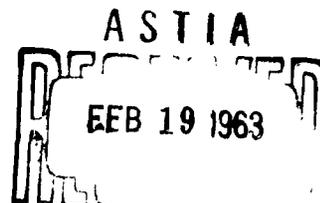
University of Michigan

ISOLATION, IDENTIFICATION AND PHYSIOLOGY  
OF A POSTULATED HORMONE  
CAPABLE OF INDUCING EXCRETION OF SODIUM

Grant DA-MD-49-193-62-G62

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## ABSTRACT

1. University of Michigan, Department of Internal Medicine, Division of Endocrinology and Metabolism and the Metabolism Research Unit
2. Isolation, Identification and Physiology of a Postulated Hormone Capable of Inducing Excretion of Sodium
3. J. W. Conn
4. 5 pages, 1 Table, 1 Figure, February 13, 1963
5. DA-MD-49-193-62-G62
6. Supported by: U. S. Army Medical Research & Development Command  
Department of the Army  
Washington 25, D.C.

Several clinical states are known to be associated with an elevated rate of sodium excretion in response to an acute intravenous saline infusion. These include essential hypertension, Cushing's syndrome, water-loaded people and patients with primary aldosteronism. The physiological mechanism of the phenomenon is unknown. Production of a hormone which induces renal salt loss could be common to all.

Normal young men (before and after administration of aldosterone) and patients with aldosteronomas have been studied using a standardized saline infusion test. Tests were done after standard dietary preparation with both high and low salt diets. Before infusion tests, measurement of plasma volume, extracellular fluid volume, total exchangeable sodium and total exchangeable potassium were done simultaneously by means of a technique developed in this laboratory. Endogenous creatinine clearance, PAH clearance and rates of excretion of sodium, potassium and chloride were measured.

During infusion the mean maximum rate of sodium excretion in normals was 0.72 mEq/min (180 mEq Na diet prep.) and 0.14 mEq/min (8-10 mEq Na diet prep.). Twenty-four hours after aldosterone administration (1.0 mg IM q 8 hr) the rate was 0.36 mEq/min on the high sodium prep. Seven days after starting aldosterone, with no further change in extracellular fluid volume or GFR, "renal escape" had occurred and the rate had increased to 1.53 mEq/min without elevation of blood pressure. This value is similar to that found in patients with aldosteronomas (1.63 mEq/min). These results suggest that "renal escape" from sodium retention is not dependent upon changes in GFR, ECF or blood pressure and that a renal sodium-losing mechanism has been activated secondary to aldosterone administration.

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## PROGRESS REPORT

Work currently being carried forward with support of this research grant falls into four categories:

1. A continuing search for evidence of a salt-losing hormone. Salt loading should be expected to activate this mechanism in normals and to maximize it in the presence of excessive mineralocorticoid activity. For this reason detailed studies (outlined below) are being performed to characterize the responses to acute saline infusions of normal people and of patients with chronic mineralocorticoid excess (primary aldosteronism). In addition, the transitional changes from normal to "abnormal" are being studied by administration of aldosterone to normal subjects for various lengths of time.
2. Development of improved methodology. Work has progressed well in the development of a method for the simultaneous determination (using 3 radioisotopes) of total body exchangeable sodium, total exchangeable potassium and total exchangeable extracellular fluid volume.
3. Establishment on a practical basis of aldosterone secretion rate. Until recently we have had to depend upon urinary excretion of aldosterone (a modification of the method of Neher and Wettstein) as the index of its rate of production. Much effort has been expended during the past year to establish on a working basis the difficult Double Isotope Derivative Assay described by Peterson of Cornell.
4. Related projects supported in part by this grant:
  - a) The absence or diminution of the slowly exchangeable pool of body sodium (bone sodium) in primary aldosteronism.
  - b) Effect of mineralocorticoids upon rat bone uptake of radioactive sodium and potassium.

### Saline Infusion Studies

Several clinical states are associated with an elevated rate of sodium excretion in response to an intravenous saline load. These include Cushing's syndrome, essential hypertension and normal water-loaded people. More recently primary aldosteronism has been added to the list. The physiological mechanism of this phenomenon is unknown. Production of a hormone which induces sodium loss might be common to all of these states. We have chosen to study the mechanism of this phenomenon in the hope that it will lead us to

the elusive salt-losing hormone.

Much speculation, but no definitive information, exists regarding the mechanism of "renal escape" from the sodium-retaining effect of chronically administered mineralocorticoids. During the first few days of aldosterone administration to normal men urinary sodium diminishes greatly, body weight increases about 2 kilograms and the net balance for sodium is strongly positive. Despite continued administration of the hormone, however, urinary sodium now rises until sodium equilibrium is re-established. The initial gain in weight is usually maintained but no further gain ensues.

We chose to superimpose upon this phenomenon the response to acute saline loading. Tests were done before and after "renal escape" while daily administration of aldosterone continued. We have found that after "escape" but not before, the rate of excretion of an administered saline load is abnormally high.

To date multiple standardized saline infusion tests have been carried out on five patients with aldosteronomas, on four normal subjects, and on two patients with essential hypertension. The normal subjects were tested under four different conditions: 1) After five days of dietary preparation with a normal sodium intake (180 mEq daily), 2) After five days on a diet low in sodium (7-10 mEq daily), 3) 24 hours after intramuscular administration of 1.0 mg d-aldosterone in peanut oil every 8 hours, and 4) 5-7 days after starting aldosterone (when the escape phenomenon was fully established). Each of the seven patients was tested twice; once after five days on a normal sodium intake, and once after five days on a low sodium intake. Four of the five patients with proven aldosteronomas were studied again following removal of their tumors.

Normal saline was infused into all subjects at a rate of 33 ml per minute for one hour (2 liters). Extracellular fluid volume, total exchangeable sodium, total exchangeable potassium and plasma volume were measured before the infusion was begun. Renal blood flow, glomerular filtration rate and the rates of excretion of sodium, potassium and chloride were measured in the pre-infusion and postinfusion periods as well as during the course of the infusion.

The mean maximum rates of sodium excretion for the entire group is listed in Table I.

TABLE I

Maximum Rate (mean) of Sodium Excretion in Response to Acute  
Intravenous Saline Infusion

	Diet (mEq Na/day)	Rate of Na Excretion (mEq/min)
I. Normals	180	0.72
	8 - 10	0.14
II. Normals after 24-48 hr on aldosterone	180	0.36
III. Normals in "escape" while on aldosterone	180	1.53
IV. Primary aldosteronism Pre-op	180	1.63
	8 - 10	0.83
V. Primary aldosteronism Post-op	180	0.6
	8 - 10	0.14
VI. Essential hypertension	180	2.5
	8 - 10	0.62

Note that 24 hours after the beginning of aldosterone administration, the rate of sodium excretion had decreased much below the control value. At this time there was already an increase in plasma volume, extracellular fluid volume and glomerular filtration rate. After "escape" had occurred, there was a marked increase in the rate of excretion of sodium, even though no further increase in the glomerular filtration rate, extracellular fluid volume or plasma volume was measurable. There was no significant increase in blood pressure in the normal subjects receiving aldosterone. Figure 1 shows graphically the results on the first three normal subjects studied and on the five patients with primary aldosteronism.

Thus, changes in GFR, plasma volume, total extracellular fluid volume and blood pressure are not responsible for the sharp increase in the rate of excretion of sodium in the "escape" phase. Renal plasma flow measurements have recently been added to the study and to date they, too, have shown no significant differences in the "pre-escape" and "escape" phases of the study in response to saline loading.

The results indicate that renal tubular rejection of sodium is the mechanism which accounts for the escape phenomenon. Such an effect on tubular function is likely to be hormonal in nature. The ability of adrenalectomized humans to "escape" from the sodium-retaining effects of aldosterone has been reported. This finding makes it unlikely that the salt-losing hormone originates in the adrenal gland. In addition, whatever the salt-losing influence consists of, it does not act as a direct antagonist to aldosterone (See review - Conn, J. W.: Some Clinical and Climatological Aspects of Aldosteronism in Man. (The Gordon Wilson Lecture 1962). Trans. Am. Clin. & Climatolog. A. In press. Also in press in J. A. M. A.)

Publication: Renal 'Escape' from the Sodium-Retaining Effects of Aldosterone. Studies in Primary Aldosteronism and in Normals. D.R. Rovner, J.W. Conn, R.F. Knopf, and M. T-Y. Hsueh. J. Lab. & Clin. Med. 60: 65, (Dec) 1962. (Abstract)

#### Simultaneous Measurement of Fluid and Electrolyte Spaces

It was deemed necessary, in order to evaluate better the various changes taking place upon administration of mineralocorticoids, to develop methods for measuring simultaneously extracellular fluid volume and total body sodium and potassium. Inherent in the simultaneous measurement of these "spaces" are several advantages, technical as well as investigative. In our studies day to day shifts in the equilibrium for sodium, potassium and water may be very large. Measurement of these compartments singly and not simultaneously cannot reflect the true state of affairs. Simultaneous measurements allow one to calculate with greater certainty trans-membrane shifts of water and electrolytes.

A method has been developed which measures plasma volume with Evans Blue Dye (T-1824), total exchangeable sodium with sodium<sup>22</sup>, total exchangeable potassium with potassium<sup>42</sup> and extracellular fluid volume with bromine<sup>82</sup> and sodium<sup>22</sup>. The procedure depends upon the quantitative separation of the bromine<sup>82</sup> from the mixture of sodium<sup>22</sup>, potassium<sup>42</sup> and bromine<sup>82</sup> in urine and blood by ion exchange chromatography. The bromine is then counted directly and the remaining mixture of sodium and potassium is resolved by differential decay counting. The method is simple and accurate. When the normal controls were evaluated with this technique, results agreed well with the published values for normals by other "single" methods. The expected changes in extracellular fluid volume were also measured when changes in dietary sodium intake were made.

Publication: A Simple and Precise Method for the Simultaneous Measurement in Man of Extracellular Fluid Volume, Total Exchangeable Potassium and Total Exchangeable Sodium. D.R. Rovner and J. W. Conn, Submitted to J. Lab. & Clin. Med., January, 1963.

#### Measurement of Daily Endogenous Production of Aldosterone

In most of the abnormal states we are studying or inducing, appearance of a salt-losing phenomenon is secondary. The primary abnormality is the sustained, unremitting presence of salt-retaining hormone (aldosterone). It is assumed that the need for homeostasis calls forth the salt-losing hormone as a counter-regulatory device. Assuming normal renal functions the ratio of aldosterone production to sodium excretion could give an indication of the degree of counter-regulation present. This requires the ability to measure

accurately the rate of secretion of aldosterone.

Much effort has gone into this project. A portion of our steroid laboratory is now used exclusively for the measurement of aldosterone secretion rates. Peterson's Double Isotope Dilution Derivative Assay has been set up and tested following a period of observation in his laboratory at Cornell. This is a tedious and difficult procedure which is being mastered.

#### Other Related Projects Supported in Part by Grant

1. We have found in normal subjects a slowly expanding exchangeable body pool of sodium. This exponential increase reaches equilibrium in 10 to 14 days. It is presumed that this represents sodium in bone which is not readily exchangeable with radiosodium during the first 24 hours. Of great interest is the virtual absence of this phenomenon in primary aldosteronism. This means that in the latter condition the tightly bound sodium pool has been depleted or else that aldosterone blocks entry of sodium into bone by a "membrane" effect.

Publication: The Existence of Slowly Exchangeable Pool of Body Sodium in Normal Subjects and its Diminution in Patients with Primary Aldosteronism. D.H.P. Streeten, A. Rapoport, and J.W. Conn. Submitted to J. Clin. Endocrinol. & Metab., Dec. 1962.

2. To answer the question raised above, extensive studies have been carried out to measure in vivo uptake of sodium into bone of rat under a variety of conditions: desoxycorticosterone-treated, aldosterone-treated with and without the addition of aldactone, adrenalectomized, and adrenalectomized treated with mineralocorticoids. The results indicate that mineralocorticoids block entry of sodium ion into bone.

Publication: Content and Uptake of Sodium and Potassium in Bone. Influence of Adrenalectomy, Aldosterone, Desoxycorticosterone and Spironolactone. D.R. Rovner, D.H.P. Streeten, L.H. Louis, C.T. Stevenson, and J.W. Conn. Submitted to J. Clin. Endocrinology and Metab., Dec. 1962.

# RATE OF RENAL EXCRETION OF SODIUM IN RESPONSE TO STANDARD SALINE INFUSION

All Subjects Prepared with Normal Standard Diet for 3 Days

- ▽ 3 Normal Men - Control Infusion
- 3 Normal Men - 24-48 Hrs. of Aldosterone
- × 3 Normal Men - 9-10 Days of Aldosterone
- 5 Patients - Primary Aldosteronism

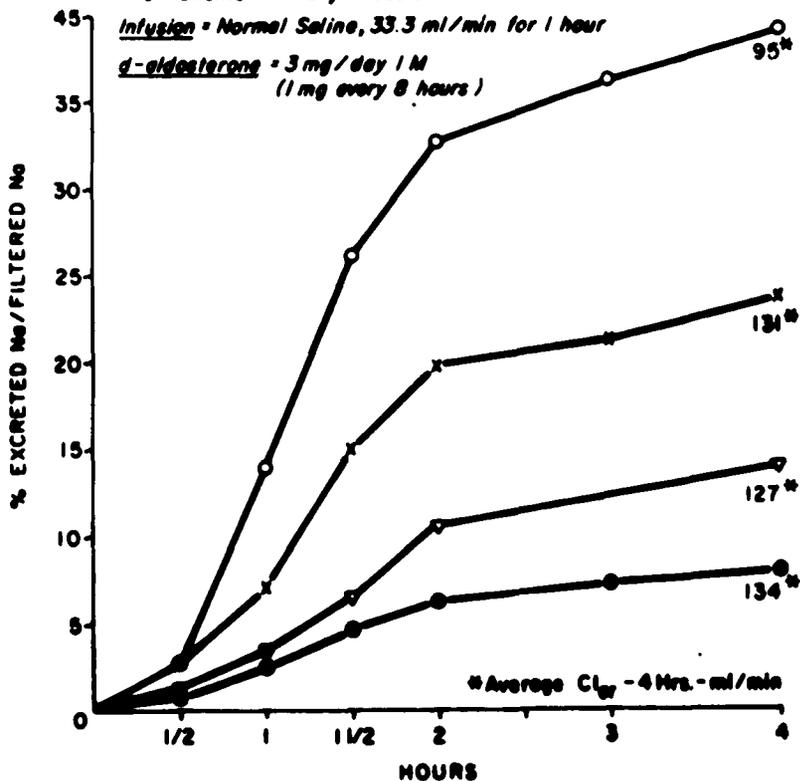


Figure 1