STUDIES ON A MINERALOCORTICOID FROM HOG ADRENAL EXTRACT

Sir:

During the course of an investigation into the survival-growth-electrolyte activity of hog adrenal extract, a highly active substance with this type of activity was isolated. The substance, which would be classified as a mineralocorticoid, appears identical with the characteristically active component of the classical amorphous fraction[1] since the isolated compound was found to possess the characteristic properties of the amorphous fraction, *viz.*, survival-growth-electrolyte activity, and since the isolated compound was obtained from a similar source, *viz.*, adrenal extract. This mineralocorticoid seems quite similar to one described recently by Tait, *et al.*,[2] and possibly bears similarity to materials being studied currently by others.[3] The isolated mineralocorticoid has been characterized by the use of various bioassay methods, numerous paper chromatographic systems, and a variety of other definitive procedures.

Bioassay data[4] indicate that the mineralocorticoid, qualitatively like desoxycorticosterone, has especially high activity in the growth[5] (modified Grollman[6]) and sodium-retention[7] assays (mineralocorticoid assays) and essentially no activity in the glycogen-deposition[8] and eosinophil[9] assays (glucocorticoid assays). Research preparations which are about 20% pure with respect to the mineralocorticoid are found to possess activity equal to desoxycorticosterone in the growth assay and to be more than 25 times as active as desoxycorticosterone in the sodium-retention assay. Preparations of such a purity usually contain, in addition to the mineralocorticoid, small amounts of cortisone and large amounts of an apparently inert material (a nonultraviolet absorbing, non-reducing substance which presumably originates from the adrenal extract and has a chromatographic polarity similar to the mineralocorticoid). The percentage purity is determined by ultraviolet absorption analysis[10] which involves the elution of differentiated spots from developed papergrams for spectrophotometric quantitation (calculated by use of the k value for cortisone).

The mineralocorticoid has been characterized chromatographically by its mobility in the solvent systems listed below.

TABLE I

CHROMATOGRAPHIC MOBILITY OF THE MINERALOCORTICOID

System	R_E[a]	System	R_E
Burton, *et al.*[11] T/P[b]	1.13	Bush[12] B_2	0.70
B/F[c]	0.72	B_5	0.89
Bush[12] B_1, B_3, B_4, and C	0.77	Bush[13]	0.78

[a] Relative mobility with respect to cortisone. [b] Toluene/propylene glycol. [c] Benzene/formamide.

It is seen that the mineralocorticoid migrates at a rate just slightly faster than cortisone in the toluene/propylene glycol system but somewhat slower than cortisone in all of the other systems.

Various characterization tests have been applied to amorphous, chromatographically homogeneous preparations of the mineralocorticoid (80% pure) in order to obtain information toward the identification of this compound. Ultraviolet absorption maximally at 239 mμ (ethanol), positive reducing tests with both neotetrazolium chloride[14] and modified Tollens reagent,[11] and a negative Porter–Silber test[15] provide evidence that the mineralocorticoid, if assumed to be an orthodox steroid molecule, possesses an α,β-unsaturated ketone, an active α-ketol group, and no 17,21-dihydroxy-20-keto side chain, respectively. Upon acetylation, the mineralocorticoid forms a polyacetate, probably a diacetate, which, although it possesses greatly lessened or no biological activity, can be deacetylated to regenerate the active free compound.

Progressively purer preparations of the mineralocorticoid were obtained in a program of column and paper chromatographic fractionation. Throughout these fractionations, the biological activity was observed to be intimately associated with both characteristic corticoid ultraviolet ab-

TABLE II

COMPARISON OF MINERALOCORTICOIDS

Feature	G.S.T.W.[a]	K.N.H.[b]
Migration in the toluene/propylene glycol system (R_E)	1.18[c]	1.13
Ultraviolet absorption	−	+
Tetrazolium reduction	+	+
Formation of a polyacetate with chromatographic polarity (B/F) intermediate between Reichstein's S and Q acetates	+	+
Activity greatly lessened upon acetylation to polyacetate	+	+
Activity regained upon deacetylation of polyacetate	+	+
Unusually labile toward heat, alkali, and acid	+	+

[a] Grundy, Simpson, Tait and Woodford. [b] Knauff, Nielson and Haines. [c] Calculated by us from their data.

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[1] O. Wintersteiner, H. M. Vars and J. J. Pfiffner, *J. Biol. Chem.*, **105**, c (1934); E. C. Kendall, H. L. Mason, W. J. Hoehn, and B. F. McKenzie, *ibid.*, **119**, lvi (1937); M. H. Kuizenga in "The Chemistry and Physiology of Hormones," American Association for the Advancement of Science, Washington, D. C., 1944, pp. 57–68; D. J. Ingle in "Progress in Clinical Endocrinology," Grune and Stratton, New York, N. Y., 1950, pp. 146–150.

[2] J. F. Tait, S. A. Simpson and H. M. Grundy, *Lancet*, **262**, 122 (1952); H. M. Grundy, S. A. Simpson and J. F. Tait, *Nature*, **169**, 795 (1952); S. A. Simpson, J. F. Tait and I. E. Bush, *Lancet*, **263**, 226 (1952); H. M. Grundy, S. A. Simpson, J. F. Tait and M. Woodford, *Acta Endocrinol.*, **11**, 199 (1952).

[3] G. L. Farrell, *Fed. Proc.*, **12**, 41 (1953); J. A. Luetscher and B. B. Johnson, *J. Clin. Invest.*, **32**, 585 (1953); J. J. Chart, E. G. Shipley and E. S. Gordon, *Proc. Soc. Exp. Biol. Med.*, **78**, 244 (1951); B. Singer and J. Wener, *Am. Heart J.*, **45**, 795 (1953).

[4] Appreciation is expressed to Dr. K. J. Olson and his group for the bioassay data.

[5] R. O. Stafford and K. J. Olson, to be published.

[6] A. Grollman, *Endocrinology*, **29**, 855 (1941).

[7] F. Marcus, L. P. Romanoff and G. Pincus, *ibid.*, **50**, 286 (1952).

[8] M. L. Pabst, R. Sheppard and M. H. Kuizenga, *ibid.*, **41**, 55 (1947).

[9] R. S. Speirs and R. K. Meyer, *ibid.*, **45**, 403 (1949).

[10] E. D. Nielson, R. C. Anderson and C. O. Schwartz, to be published.

[11] R. B. Burton, A. Zaffaroni and E. H. Keutmann, *J. Biol. Chem.*, **188**, 763 (1951).

[12] I. E. Bush, *Biochem. J.*, **50**, 370 (1952).

[13] I. E. Bush, *J. Endocrinology*, **9**, 95 (1953).

[14] R. B. Burton, A. Zaffaroni and E. H. Keutmann, *J. Biol. Chem.*, **188**, 763 (1951). Neotetrazolium instead of triphenyltetrazolium chloride was used.

[15] C. C. Porter and R. H. Silber, *ibid.*, **185**, 201 (1950).

sorption and neotetrazolium reduction. That this mineralocorticoid possesses ultraviolet absorption and the one described by Tait, *et al.*,[2] did not is the only known differentiating feature between these strikingly similar compounds. A comparison of the two mineralocorticoids is shown in Table II.

A detailed description of this work, including the chromatographic characterization of the mineralocorticoid in numerous solvent systems developed in our laboratory and infrared data on the mineralocorticoid and its polyacetate, will be published soon.

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ELECTRODES WITH CONVECTION CONTROLLED LIMITING CURRENTS ("CONVECTION ELECTRODES")

Sir:

Experimental conditions were found under which convection controlled limiting currents at the rotated platinum wire electrode are observed. In addition, a "convection" mercury microelectrode has been developed. In stirred solutions the thickness of the boundary layer at an electrode decreases with increasing rates of stirring and eventually reaches a limiting value of "molecular"dimensions. Under these conditions limiting currents no longer depend on the diffusion coefficient of the electroactive species but on the rate of convective transport to the electrode surface. Assuming that τ microliter of solution is transported per second to a unit area of the electrode, the convection controlled limiting current (corrected for residual currents and expressed in microamperes) is postulated to be:

$$i_{\text{conv.}} = nFA\tau C = knC \qquad (1)$$

where nF represents the number of coulombs per mole involved in the electrode reaction, C is the bulk concentration of the electroactive species in moles per liter and A the area of the electrode expressed in sq. cm. — τ is called the "convection coefficient," expressed in cm. × sec.$^{-1}$, and depends on the prevailing specific hydrodynamic conditions.

A platinum wire electrode, 0.5 mm. in diameter and 5 mm. in length, rotated at 600 to 900 r.p.m., yielded convection controlled limiting currents when the solution was stirred with the aid of a rectangular propeller with four blades (13 × 5 × 1 mm. each), mounted concentrically 2 cm. above the electrode and rotated at the same rate as the latter. Identical convection controlled limiting currents were obtained in equinormal solutions of iodide, triiodide and an ethylenediamine tetraacetate complex of thallium(III), the diffusion coefficient of the latter being about one-twentieth as large as that of iodide.

Well-defined limiting currents were obtained with a stationary mercury microelectrode which is being further studied and which consisted of 0.005 ml. of mercury in a 2-mm. bore capillary cuvette attached to the bottom of a 250-ml. electrolysis cell, the solution being stirred at 900–1200 r.p.m. In 0.1 M potassium chloride as supporting electrolyte the convection current of thallium(I) was proportional to concentration in the range between 5×10^{-6} and 5×10^{-3} M and about twenty times as large as the corresponding diffusion current at the dropping mercury electrode. The half wave potential was independent of concentration at a given rate of change of applied voltage.

The value of the convection coefficient, τ, was estimated from convection currents at 900 r.p.m. for the rotated platinum wire electrode and the stationary mercury microelectrode as 19 and 18 cm. × sec.$^{-1}$, respectively.

Most prominent advantages of convection electrodes are: (1) stationary electrodes can be used. (2) The limiting current is independent of the diffusion coefficient of the electroactive species. (3) The use of a synchronous motor is not necessary.

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PROPERTIES OF A SODIUM-RETAINING PRINCIPLE FROM BEEF ADRENAL EXTRACT

Sir:

Current interest in the amorphous fraction of adrenal extracts[1] makes it desirable to present results of a study of this fraction since further work must await processing of more material.

The extract (furnished by the Upjohn Co.) of 1200 lb. of beef glands was chromatographed on a column of silica gel with the propylene glycol–toluene system. The active fractions, assayed in rats using a method similar to that of Simpson and Tait[2] were combined and rechromatographed. The active fraction was acetylated and chromatographed successively in the formamide–benzene and formamide–benzene–cyclohexane systems. It was then chromatographed three times in the latter system on paper.

The acetate isolated weighed 13.2 mg. and assay for α-ketol with blue tetrazolium indicated 13.2 mg. in terms of cortisone. It could not be crystallized. Its biological activity in dog and rat assays was similar in type and approximately equal to that of desoxycorticosterone acetate. Absorption maxima at 239 mμ ($E^{1\%}_{1\,\text{cm.}} = 370$, methanol) and at 1618 and 1671 cm.$^{-1}$ (chloroform) indicated the presence of a Δ^4-3-keto group assuming the substance is a steroid. An acetylated α-ketol side chain was evidenced by absorption maxima at 1736 and 1750 cm.$^{-1}$ (chloroform). The absence of absorption maxima near 1710 (CS_2) and 3600 cm.$^{-1}$ denoted absence of an 11-keto and of unacetylated hydroxyl groups. A negative Porter–Silber[3] reaction indicated absence of a 17-hydroxyl group, of a 16–17 double bond, and probably of a 16-acetoxy group.

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[1] H. M. Grundy, S. A. Simpson, J. F. Tait and M. Woodford, *Acta Endocrinologia*, **11**, 199 (1952).

[2] S. A. Simpson and J. F. Tait, *Endocrinology*, **50**, 150 (1952).

[3] C. C. Porter and R. H. Silber, *J. Biol. Chem.*, **185**, 201 (1950).