methanol, crystallization started promptly. After 20 hours at 5° the precipitate was collected, washed with a little cold methanol, and dried *in vacuo,* giving 3.3 g mp 161–3°. After one more crystallization from 30 ml of 90-percent methanol, 2.8 g of pure L-arterenol L-bitartrate, mp 163–4°, was obtained. An additional recrystallization of a portion did not change the melting point. By reworking the liquors, 1.2 g of L-bitartrate, mp 163–4°, was recovered, making a total equivalent to 2.5 percent of the epinephrine sample.

Portions of this pure L-bitartrate were converted to the D-bitartrate monohydrate and to the hydrochloride, as described previously for synthetic L-arterenol (*4*). The identity of these salts with the corresponding synthetic L-arterenol salts is shown in the table.

In summary, natural epinephrine contains appreciable quantities of levo-arterenol, as shown by isolation of the latter compound. The adrenal gland therefore contains arterenol as well as epinephrine. The present study is believed to be the first isolation of levo-arterenol in chemically pure form from biological material and is thus the final step of proof in establishing the hormonal nature of this substance.

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The Determination of Arterenol in Epinephrine

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USING A PAPER CHROMATOGRAPHIC TECHNIQUE, Goldenberg, Faber, Alston and Chargaff (*1*) were recently able to demonstrate the presence of arterenol (nor-epinephrine, nor-adrenaline) in supposedly pure crystals (U.S.P. grade) of epinephrine derived from adrenal glands. This most interesting finding prompted investigations which led to the actual isolation of arterenol (*2*) from natural epinephrine, and to the development of a chemical method somewhat more convenient for the assay of arterenol in epinephrine than that offered by paper chromatography. It is the purpose of this note to describe the method, and to report the values found for several lots of natural epinephrine.

Reagents. (1) Buffer, pH 9.6 (Clark and Lubs) 50 cc of a solution which is M/5 in both H_3BO_3 and KCl, plus 36.85 cc M/5 NaOH, diluted to 200 cc. (2) Five-tenths percent sodium β-naphthoquinone-4-sulfonate (Eastman Kodak) in water. This reagent must be used before it is one hour old. (3) One percent aqueous solution of alkyl dimethylbenzylammonium chlorides (Roccal or Zephiran) (benzalkonium chloride). (4) Mixed solvent. Eighty-five volumes C.P. toluene plus 15 volumes redistilled ethylene dichloride. Mix only enough for one day's use. Wash the mixture with a little of the borate buffer, then filter through dry paper to remove droplets of water. (5) Epinephrine control solution. One hundred mg pure synthetic epinephrine base dissolved in 2.5 cc of 5 percent aqueous solution of sodium borate, and diluted to 100 cc with water. (6) Arterenol standard solution. Forty mg of pure arterenol base (or its equivalent of the bitartrate) dissolved in 5 cc of 5 percent aqueous solution of sodium borate, and diluted to exactly 200 cc with water. Store in a cold dark place and discard after 24 hours.

Procedure (setting up the standard curve). To each of five 50-cc glass-stoppered graduated cylinders, add 1 cc of the control epinephrine solution, and to four of these add respectively 0.25, 0.50, 0.75, and 1.00 cc of the standard arterenol solution. To each cylinder add 1 cc. buffer solution, swirl, add 0.5 cc naphthoquinone reagent, swirl; let stand at room temperature for 45 minutes. Add 0.15 cc of the benzalkonium chloride (use of a calibrated dropping bottle is convenient), then add exactly 10 cc of the mixed solvent and shake thoroughly. Let stand for 45 minutes, shaking the mixtures gently at least five times more at regular intervals during this time. After the final shake, the solvent layer (purplish red in the presence of arterenol) should separate clearly. If it is turbid, transfer to a small centrifuge tube, stopper, and centrifuge for a few minutes to clarify. Transfer the extracts to appropriate colorimeter tubes and read the percentage transmittancy in a suitable photo-

electric colorimeter, using a green glass filter (540 mμ). Set the instrument to 100 percent transmittancy with the extract from the cylinder containing only epinephrine. When plotted on semilog paper, the values obtained will result in a straight line.

Assay of Epinephrine Crystals. Dissolve a weighed amount of sample in a little 5-percent sodium borate solution and dilute with water, so that the final dilution will be approximately 0.1 percent in borax and will contain about 2 mg epinephrine per cc. Run 0.25-cc, 0.5-cc, 0.75-cc and 1.0-cc portions in exactly the manner already specified, and interpret the final transmittancies by reference to the standard curve. If the content of arterenol is known approximately, there is no need to run a whole series. One sample, estimated to contain 0.1 mg to 0.2 mg arterenol, is sufficient.

Discussion. The absorption spectrum of the colored extract shows a very broad maximum in the area 530 μ–560 mμ.

The borate medium functions not only to maintain a desirable pH level, but also to form a complex with the catechol group and thus diminish chromogenic side reactions. The formation of fatty salts of acidic dyes with long-chained quaternary ammonium compounds has been used very generally in these laboratories as an aid in analytical colorimetry. Some of these salts show a truly impressive hyperchromic and bathochromic effect on being extracted from an aqueous into a nonpolar solvent. Further, by extracting the colored derivative from the original reaction medium, an important degree of selectivity is often achieved. In the present case, the extraction eliminates interference by dihydroxyphenylalanine (dopa) and dihydroxyphenylethylamine (3-hydroxytyramine).

TABLE 1

THE ARTERENOL CONTENT OF EPINEPHRINE FROM NATURAL SOURCES

	Percent arterenol
U.S.P. epinephrine reference standard 1947-1G	17.5
U.S.P. epinephrine reference standard 1949-1H	16.3
U.S.P. epinephrine crystals, Lot No. 73016 ...	18.5
U.S.P. epinephrine crystals, Lot No. 73491 ...	17.0
U.S.P. epinephrine crystals, Lot No. 72354 ...	10.5
U.S.P. epinephrine crystals, Lot No. T-22849 .	10.5

Many of the natural amino acids also yield colored extractives with the proposed assay method. The authors hope to publish elsewhere further details, in connection with experiments now under way on the assay of fresh adrenal glands.

Results. Using the method described, we have examined samples of U.S.P. grade natural epinephrine from several sources. In each case, as shown in Table 1, a considerable amount of arterenol was found.

The authors are indebted to several individuals for help in obtaining representative samples of U.S.P. epinephrine crystals.

References

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