Isolation of 17-Hydroxy 11-Dehydro Corticosterone (Kendall's Compound E) from Urine of Normal Males

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In the course of attempting to recover adrenocortical compounds from urine it has been possible to isolate 17-hydroxy 11-dehydro corticosterone from a chloroform extract of pooled normal male urine obtained prior to hydrolysis.

Urine was collected daily and extracted with chloroform without prior adjustment of pH. The extracts were concentrated to a small volume *in vacuo* and stored in the cold. At weekly intervals the pooled extracts were washed with cold 0.1 *N* NaOH and water, taken to dryness *in vacuo*, and stored under nitrogen in the cold. Ultimately the dried residues from 1000 l of urine were combined and part of the caffeine was removed by crystallization from benzene. The noncrystalline portion was divided into 70% alcohol-soluble and petroleum ether-soluble components, and after reduction to dryness *in vacuo* the alcohol-soluble fraction was dissolved in benzene and repeatedly extracted with equal volumes of water. The ketonic parts of both the benzene-soluble and water-soluble fractions were recovered and in turn repeatedly partitioned between benzene and water, using the method outlined by Mason, Myers, and Kendall (*2*). The fraction which passed readily from benzene to water and from water to benzene (designated fraction III by Mason *et al.*) weighed 161 mg and upon being reduced to dryness from chloroform solution crystallized spontaneously.

The first and subsequent crops were recrystallized from absolute ethanol to give 32 mg of colorless rhombohedra, mp 215–218° C. On admixture with an authentic sample of 17-hydroxy 11-dehydro corticosterone (mp 216–218° C) the mp was 215–218° C. Analysis: Calculated for $C_{21}H_{28}O_5$—C = 69.96%, H = 7.83%. Found—C = 69.50, 69.80%, H = 7.61, 7.70%. The addition of concentrated sulfuric acid to a small amount of the crystalline compound gave a yellow solution with a faint green fluorescence. Methanolic solutions of the substance rapidly reduced ammoniacal silver in the cold and formed a bright red precipitate upon the addition of a few drops of a saturated solution of 2,4 dinitrophenylhydrazine in 2 *N* HCl. $[\alpha]_D^{26} = +214° \pm 2°$ (concentration, 0.604 in 95% ethanol). Mason, Myers, and Kendall (*3*) have recorded from 17-hydroxy 11-dehydro corticosterone $[\alpha]_{5461}^{25} = +248° \pm 4°$ (concentration, 0.1 to 0.2), Kuizenga and Cartland (*1*) $[\alpha]_D^{25} = +195°$ (concentration, 1.89), and Wintersteiner and Pfiffner (*4*) $[\alpha]_D^{25} = +209° \pm 1°$ (concentration = 1.2) (all in 95% ethanol).

The compound readily formed an acetate on treatment with a mixture of pyridine and acetic anhydride at room temperature. Recrystallization from absolute ethanol yielded fine needles, mp 236–239° C. On admixture with an authentic preparation of 17-hydroxy 11-dehydro corticosterone acetate (mp 237°–239° C), the mp was 236°–239° C. Analysis of the acetate: Calculated for $C_{23}H_{30}O_6$—C = 68.65%, H = 7.46%. Found—C = 68.41%, H = 7.14%. The compound showed an absorption maximum in the ultraviolet at 237–238 mμ. ε = 13,870 (absolute ethanol). Mason, Myers, and Kendall (*2*) observed an absorption maximum at 237 mμ and have recorded a molecular extinction coefficient of 16,150. The biological activity of the substance is now being determined. A more detailed account of this work will appear at a later date.

References

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Large Scale Paper Chromatography

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In view of the excellent separation of microquantities that can be achieved by paper partition chromatography (*1*), an attempt has been made to apply these same principles on a larger scale.

Two obstacles were encountered initially in large scale chromatography, the first being the difficulty of applying several milliliters of solution to a sheet of paper in a narrow straight band, and the second, obtaining a thick paper which could handle large quantities and still give good separation.

Attempts to apply several milliliters of solution from a capillary by hand were unsatisfactory, since it was virtually impossible to maintain a narrow band. It was found, however, that the application of solutions to paper could be greatly facilitated by the use of an automatically revolving drum to which the paper could be attached. The liquid could then be fed onto the paper, using a narrow capillary tube.

Such an apparatus can be readily set up in the laboratory using a kymograph, modified as shown in Fig. 1. The lower drum (A) of the kymograph is fixed at any convenient height by means of the latch (G). A second drum (B) is then put onto the shaft above (A), but is separated from (A) by means of a one-hole rubber stopper (C) which serves to provide a 1½-in. clearance between the two drums. This is desirable in order to prevent blotting between the paper and the drum when liquid is applied to the paper. A sheet of Whatman No. 1 paper, 17 in. high and 20 in. wide, is wrapped around the drums and fastened by means of two elastic bands. These can be closed most readily and held tightly around the paper if hooks and eyes are sewn on the ends. The paper is fastened in such a way that it overlaps in a direction opposite to that of the rotation of the kymo-

graph. Its lower edge should reach about 1 in. below the gap between drums (A) and (B).

The capillary (F) is clamped on a ring stand by means of a cork, as shown, so that the slightly bent tip of the capillary is pressed against the paper. The tip must be fire-polished to prevent scratching of the paper. The kymograph is then set in rotation and the solution to be

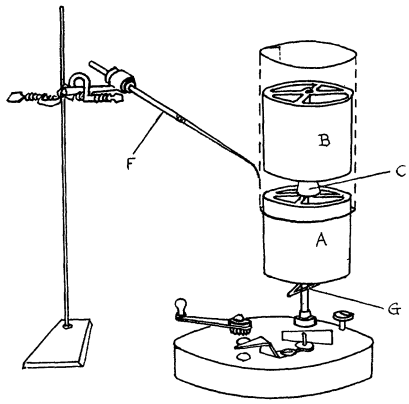

FIG. 1.

applied is added to the capillary tube. Unless this solution is absolutely free from solid particles a small cotton plug should be inserted in the capillary to serve as a filter. A heat source is used to facilitate evaporation of the solvent. A 250-watt infrared lamp has been found useful for this purpose. The band width is determined by the rate of flow of the solution, and may be regulated both by the size of the capillary opening and the speed of rotation of the kymograph. Flow rates can also be readily varied by applying gentle air pressure to the capillary.

By this method it has been possible to apply a 5-ml fraction of a methyl alcohol solution to a sheet of Whatman No. 1 paper in less than 15 min, and at the same time maintain a narrow band of material. The amount of any substance which should be applied in this manner is, of course, ultimately limited by the sharpness of the separation which can be obtained on the paper, and will vary in each case.

After the paper is dry and removed from the drum, any standard method can be used for developing the chromatogram. The authors have found it suitable to cut the sheet into two sections, 13 in. and 7 in. wide, respectively. These can then be stapled into two cylinders, which are simultaneously developed as ascending chromatograms in a glass-covered cylinder, 6 in. in diam and 18 in. high, containing 100 ml of developing solvent. A small section of the developed chromatogram is cut off lengthwise and used in order to detect the position of the desired substance if its R_f value is not already accurately known. Once this has been ascertained, the entire desired section of the paper can be cut out and the material eluted by shaking with successive portions of an appropriate solvent, or by treating the paper with the solvent in a Waring Blendor.

Many different thick filter papers have been tested for suitability in the separation of larger amounts of material. The most satisfactory paper tested so far is Schleicher and Schuell paper No. 470-A[1] (0.025 in. thick). The separation on this paper is not as good as on thinner paper, but has proved very satisfactory for the initial separation of large amounts of material.

Reference

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Relationship in Mice of Intestinal Emptying Time and Natural Resistance to Pig Ascaris Infection

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Rapid intestinal emptying time of young mice is involved in their high degree of natural resistance to the cestode, *Hymenolepis nana* var. *fraterna* (*1*). This emptying-time factor also appears to be operating in the distribution of *Trichinella spiralis* in the intestinal tracts of mice (*3*). It was, therefore, of considerable interest to learn from preliminary experiments that young mice, as compared with guinea pigs, have a remarkable degree of natural resistance to infection with pig ascaris (*Ascaris lumbricoides suum*). The aim of the present study is to determine the relationship of intestinal emptying time and natural resistance to infection for this parasite in mice.

The first three experiments were carried out using female mice, two months old, to establish the minimal infecting *Ascaris* egg dose. The methods of obtaining mature embryonated eggs and infecting the animals have been described earlier (*2*). One half of the mice in each egg-dose group were sacrificed between the fifth and eighth day after attempted infection so as to observe grossly the appearance of the lungs and to search for migrating larvae in that organ. This determination was qualitative only and was made by pressing small pieces of the entire lungs between two glass slides. These slides were examined microscopically with 100 × magnification. The remaining half of the mice were observed daily for 20 days to establish survival rates.

In the first experiment, the mice were divided into five groups of six mice each. Those of group 1 received 600 eggs. This dose for each succeeding group was increased by 600 so that the mice of the last group, group 5, received 3000 eggs. None of the mice showed outward evidence of infection, and of those autopsied the lungs appeared normal and were negative for larvae by the

[1] The author acknowledges with appreciation the technical assistance of Miss Dolores Stough during a portion of the study.

[1] Sample sheets of this paper were kindly furnished by the Carl Schleicher and Schuell Company of New York City.