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Isolation and Identification of 17α-Hydroxyprogesterone in Adrenal Venous Plasma of Normal Dogs.* (26637)

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Adrenal venous blood of dogs was analyzed for steroids by Farrell and Lamus(1) and 14 different fractions were isolated of which 10 appeared to be steroids. Three of these fractions were characterized as cortisol, corticosterone, and Reichstein's Substance S. Carstensen *et al.*(2) while reporting the isolation of 5-pregnene-3β.17α-diol-20-one (17α-hydroxypregnenolone) in the canine adrenal vein plasma indicated the presence of 4-pregnene-17α-ol-3,20-dione (17α-hydroxyprogesterone) in this fluid but did not characterize the material. The studies referred to were made on dogs under the 'stress' of an acute surgical operation. The present communication deals with the isolation and identification of 17α-hydroxyprogesterone from adrenal venous blood of 'unstressed' normal dogs.

Experimental. a. *Materials.* The animal preparation used for the experiment was that described by Weaver and Eik-Nes(3). An intravenous injection of 25 I.U. of adrenocorticotropin (ACTH) (Upjohn Co.) was given ½ hour before blood was collected from the adrenal pouch into a heparinized tube. No more than 70 to 80 ml of blood were withdrawn per tapping. The plasma was separated from the red cells by centrifugation for 1 hour at 1800 rpm and stored at −15°C in a deep freeze until processed.

Chemicals used were reagent grade. Solvents were purified according to standard procedures(4) and redistilled before use. All evaporations of organic solvents were done under nitrogen at 40°C.

b. *Extraction and purification of the steroids.* The plasma was extracted 4 times using an equal volume of a mixture of ethyl acetate-ethyl ether (1:1 v/v) each time. The extract was washed once with 0.1 volume of 1 N NaOH and 3 times with 0.1 volume of water, and evaporated to dryness. To the residue 0.01 μg of 17α-hydroxyprogesterone-4-C^{14},‡ equivalent to 800 cpm was added to serve as reference standard for detection on paper chromatograms and for calculation of recoveries.

The dry residue was suspended in 20 ml of 70% aqueous methanol and left overnight (15 hours) at −15°C. The suspension was then centrifuged for ½ hour at 1800 rpm in a refrigerated centrifuge and the supernatant decanted and collected(5). The residue was washed with 5 ml of ice-cold 70% methanol, and the wash after centrifugation was combined with the original supernatant. After evaporating to dryness the dilute methanolic extract, the residue was dissolved in 15 ml of n-hexane and washed thrice with equal volumes of 70% methanol (6). The hexane fraction was discarded and the methanolic solution dried down.

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For separation of 17α-hydroxyprogesterone from other corticosteroids, the solvent system propylene glycol-methyl cyclohexane was used(7). Whatman No. 1 paper was impregnated with propylene glycol-methanol (2:3 v/v), and excess solution removed by blotting between pads of filter paper. The sample was applied using a mixture of chloroform-methanol (1:1 v/v). The chromatogram was developed for 65-70 hours by the descending method with methyl cyclohexane saturated with propylene glycol, then thoroughly dried for 15 hours in a current of dry air.

The paper chromatogram was scanned for radioactivity in a strip counter,§ and the area of the paper strip showing a peak in radioactivity was cut out and eluted with 10 ml of vacuum distilled methanol. After the solvent was evaporated the residue was purified of paper impurities by dissolving in 15 ml ethyl acetate, washing once with 10 ml 0.5% sulfuric acid (by volume) and twice with 10 ml of water. The ethyl acetate was evaporated and the residue analyzed spectrophotometrically for absorption at 240 mμ using a Beckman Spectrophotometer Model DU with micro cells of 0.3 ml capacity.

c. *Identification of 17α-hydroxyprogesterone.* Material obtained from plasma of several animals showing a running rate in the above mentioned system of chromatography identical with that of authentic 17α-hydroxyprogesterone and exhibiting a distinct absorption at 240 mμ was pooled and further purified for infrared analysis. With corticosterone added as reference compound, the material was chromatographed on paper in a formamide-hexane system for 2 hr over-running, dried for 3 min in air and run in a formamide-chloroform system. As soon as the mobile phase reached almost the bottom of the strip, the chromatogram was removed, solvent front marked and the chromatogram thoroughly dried for 24 hr in a current of air(8). The material behaving chromatographically like 17α-hydroxyprogesterone (R_f 0.88 and R_s 1.33) was eluted, the solvent evaporated and the residue purified as described in step b. The purified extract was rechromatographed on paper in a propylene glycol-toluene system for 4½ hr with testosterone as reference compound. Material exhibiting the running rate of authentic 17α-hydroxyprogesterone (R_s 1.31) was eluted with methanol, solvent evaporated and the product purified by partitioning the material between ethyl acetate and 0.5% sulfuric acid as described earlier.

The material so obtained was further purified by column chromatography on 5 g of Woelm neutral aluminum oxide of activity grade 1. The column was prepared in benzene and developed with benzene and benzene-ether mixtures. Twenty ml eluates were collected as separate fractions. The steroid was eluted with benzene-ether (3:1) mixture. Fifty μg of synthetic 17α-hydroxyprogesterone were also chromatographed on a similar aluminum oxide column. The unknown material and the authentic steroid were subjected to infrared analysis. The spectra were recorded with a Perkin-Elmer Infrared Spectrophotometer, Model 221, employing the potassium bromide technic and a Perkin-Elmer Ultra-micro sampling system(9). The infrared spectrum of the unknown material was identical with that of synthetic 17α-hydroxyprogesterone carried through the last stage of purification (Fig. 1). The peaks in the spectra of the authentic material and the sample, appearing between 4 and 5 μ wave length could be due to some impurity from the aluminum oxide column because this did not appear in the spectrum of the pure, untreated steroid taken in a potassium bromide pellet (Fig. 1).

In these unstressed, ACTH-stimulated dogs an average concentration of 2.2 μg 17α-hydroxyprogesterone/100 ml adrenal venous plasma was found by the measurement of U.V. absorption at 240 mμ and correction for losses on the basis of the ratio of the final radioactivity to that added to the crude extract. Material behaving chromatographically like 17α-hydroxyprogesterone could not be detected in extracts of large pools of systemic blood plasma from the animal preparation used.

§ Nuclear-Chicago, Chicago.

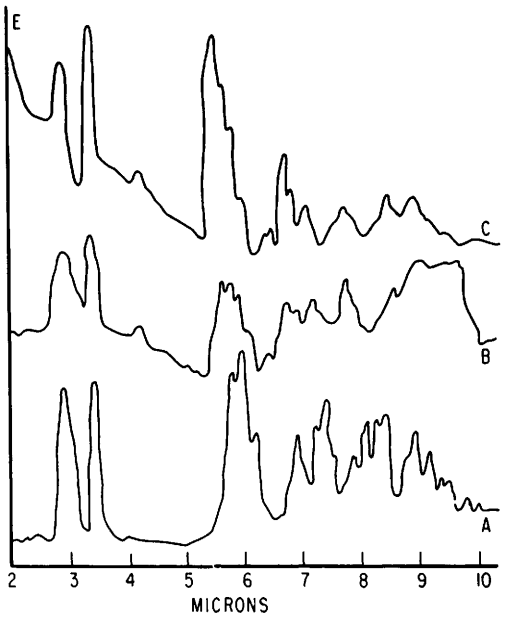

FIG. 1. Infrared spectra of: A, authentic 17α-hydroxyprogesterone; B, authentic 17α-hydroxyprogesterone after chromatography on aluminia oxide; C, compound isolated from adrenal vein plasma.

Discussion. 17α-hydroxyprogesterone was first isolated from adrenal cortical extracts as an inactive corticoid(10,11). Hechter *et al.*(12) and Kushinsky(13) isolated this steroid after perfusion of the bovine adrenal gland with non-labelled and with radioactive progesterone. Plager and Samuels(14) and Levy *et al.*(15) have established the conversion of progesterone-4-C^{14} to 17α-hydroxyprogesterone-4-C^{14} by incubation of adrenal tissue homogenates. In the many studies of steroids in the adrenal vein blood of the dog (1,16,17,18,19) this steroid was neither detected nor characterized. Carstensen *et al.* (2) indicated the presence of this steroid in the adrenal venous effluent of dogs from its partition coefficients in 2 systems of counter-current distribution and its maximal ultraviolet absorption at 240 mμ but did not confirm the indication by more definitive methods.

The fact that this steroid was found in the adrenal venous blood of the dog but not in the circulating blood under similar conditions supports the assumption that it represents a secretory product of the adrenal gland(17, 20). Apparently this steroid undergoes rapid metabolism.

17α-hydroxyprogesterone is an important intermediate in biosynthesis of corticoids. Generally, this steroid has been thought to be metabolized so rapidly in the adrenal gland that it does not leave this organ and appear in significant amounts in the venous blood. By experiment reported here, however, 17α-hydroxyprogesterone has been conclusively proved to be a secretory product of the normal canine adrenal cortex.

Summary. By criteria of paper chromatography in several systems, ultraviolet and infrared spectrophotometry, 4 pregnene-17α-ol-3,20,dione has been isolated and identified in adrenal vein blood of normal dogs given adrenocorticotropin intravenously. This steroid could not be detected in blood from the systemic circulation of dogs given adrenocorticotropin.

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Triiodothyronine Uptake by Erythrocytes in Mongolism. (26638)

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In view of the recent discovery that mongolism is associated with a chromosomal anomaly, there is good reason to believe(1) that certain enzyme anomalies also exist. During a series of experiments on enzyme systems in mongolism we have observed that red blood cells of mongoloid subjects tend to have a higher oxygen uptake and lower rate of phosphorylation than control subjects(2). In an attempt to explain this phenomenon, we decided to follow up the suggestion of Kurland *et al.*(3) who noted in a series of 9 cases that mongoloid rbc had a "tendency" to a higher triiodothyronine uptake *in vitro*. Our present studies show that this high triiodothyronine uptake is highly characteristic of mongolism.

The method used was the standard radioiodinated triiodothyronine *in vitro* rbc uptake test of Hamolsky, Golodetz and Freedberg(4). We used 23 euthyroid individuals, both male and female, as controls. Their normality was established by one or more of the following criteria: 1) lack of thyroid disorder symptoms, 2) normal protein-bound iodine, and 3) normal neck $iodine^{131}$ uptake. Since some of these controls were individuals being tested for thyroid malfunction, the number of borderline cases is probably somewhat higher than might be found in a random normal population. The mongoloid group consisted of 22 individuals, 12 of which were out-patient mongoloid children ranging in age from 5 to 11 years, 5 mongoloid infants under one year of age, and 5 institutionalized mongoloid adults.* A small group of non-mongoloid retarded children and another group of institutionalized adult psychiatric cases, mostly schizophrenics, were studied for comparative purposes. Abnormal thyroid activity due to medication was ruled out in all cases.

It was found that the triiodothyronine uptake increase was almost invariably present in the rbc of the mongoloid subjects. The mean for the control group, consisting of 23 samples, was 15.4, with a range between 11.8 and 19.3. The mongoloid group, consisting of 22 samples, had a mean value of 22.9, with a range between 13.9 and 29.8. Only 2 mongoloid values fell into the normal range. One of these was a borderline value, while the other was that of an obese borderline hypothyroid mongoloid with a PBI of 3.2. Values in female subjects tended to be slightly lower. When the 2 sexes were considered separately, the borderline hypothyroid mongoloid mentioned above was the only mongoloid subject falling into the normal range (Fig. 1). When the adult psychiatric cases were used as controls a slight

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