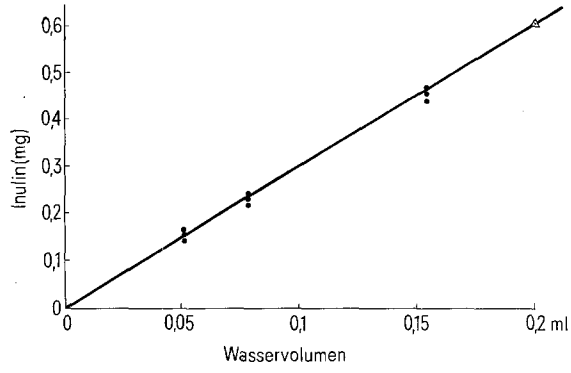
ein unbekannter Anteil des Lichtes durch das emulgierte Fluorocarbon absorbiert wird. Nach der Ausfällung dieses Stoffes mit dem Alkohol wurde zuerst versucht, das Inulin im Überstand zu bestimmen; es gelang jedoch nicht, weil der Alkohol auch das Inulin zur Ausfällung bringt, wenn wässrige Inulinlösungen damit in einem Verhältnis höher als 1:2 behandelt werden[2]. Unter den verschiedenen Verfahren, die zur Vermeidung der Inulinausfällung erprobt wurden, erwies sich als zuverlässigste die Zugabe von verdünnter NaOH[3] oder NaOH mit $ZnSO_4$[4] in den unter Methodik angegebenen Konzentrationen. Die weiteren methodischen Schritte mit dem Überstand stellen eine Modifikation des Verfahrens von HUBBARD und LOOMIS dar[5].



Abszisse: Wasservolumen der Emulsionsproben. Ordinate: Inulinmenge der Emulsionsproben. Die Gerade wird durch Eintragung der Werte aus dem Volumen der wässrigen Phase und der Inulinmenge (Tabelle II) hergestellt. △ Inulinmenge einer Standardprobe ohne Fluorocarbon und ohne Pluronic.

Summary. A method for the quantitative determination of inulin in fluorocarbon emulsions is presented. It consists in the precipitation of the fluorocarbon with ethyl alkohol, and colorimetric determination of the inulin in the supernatant after hydrolisis of the polysaccharide and reaction with resorcin.

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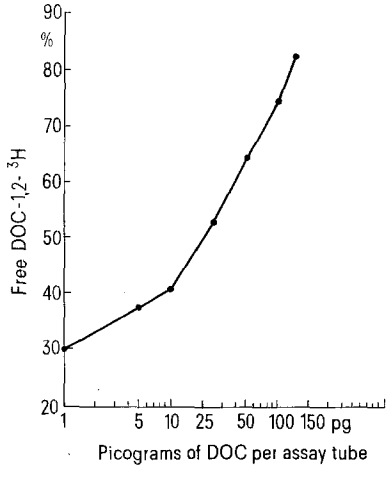
A Radioimmunoassay for Deoxycorticosterone in Human Peripheral Plasma Using Sephadex LH-20 Chromatography

A radioimmunoassay for measurement of deoxycorticosterone (DOC) in human plasma, which is accurate, precise, sensitive and relatively simple, has been developed.

DOC is an active steroid hormone produced by the adrenal cortex which promotes the reabsorption of sodium by the renal tubule and diminishes sodium concentration of sweat, saliva and intestinal secretions. Clinical reports have noted DOC overproduction in some disease processes[1–3]. Excessive DOC is produced in some adrenal disorders caused by elevated ACTH or by neoplasia, and may lead to hypertension and hypokalemia.



A typical standard curve for DOC. The percent free of DOC-1,2-^{3}H is plotted as a function of the amount of unlabeled DOC.

Various methods have been developed based on the double isotope technique for measurement of DOC secretion and excretion in man[4]; however, excessive volumes of plasma (10–30 ml) are required. A sensitive competitive binding assay for DOC was reported[5]; and DOC was detectable in 40% of normal people. A DOC radioimmunoassay involving extraction of 2–3 ml of plasma with dichloromethane and purification by paper chromatography has been published recently. Radioimmunoassay is carried out after elution through silica gel[6,7]. This technique has proven to be tedious. A short, simple radioimmunoassay for plasma DOC with adequate accuracy, sensitivity and specificity to measure levels of DOC in human peripheral plasma is described below. This method involves extraction with dichloromethane and purification by Sephadex LH-20 column chromatography followed by radioimmunoassay.

Preparation of DOC-3-(*O*-carboxymethoxy)-oxime (DOC-3-oxime) conjugate and production of antisera was previously reported by our laboratory[8].

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Plasma extraction and radioimmunoassay. After 1,2-^{3}H-DOC (15,000 cpm) was added to clean 50 ml conical tubes and dried, 2 ml of plasma was put into each tube. Extraction was performed by shaking the plasma with 20 ml of dichloromethane. Tubes were centrifuged and the top plasma layer aspirated. Extracts were evaporated into counting vials using a semiautomatic air blowing device[9]. The dry extract was then redissolved in 0.2 ml of dichloromethane: methanol (98:2). Female plasma samples containing high levels of progesterone (luteal phase or during pregnancy) were pre-extracted with 20 ml of hexane. This step removes 99% of progesterone present and only about 20% of the 1,2-^{3}H-DOC.

Sephadex LH-20 was soaked overnight with dichloromethane: methanol (98:2) and poured as a slurry into columns of precision bore glass (45 cm length, 0.9 diameter). Samples were introduced in 0.2 ml of solvent. After discarding the first 10 ml, eluates were collected in 1 ml aliquots. Radioactivity of 0.1 ml aliquots was counted to determine the elution pattern. The 2 or 3 peak tubes were pooled, evaporated to dryness in a 40 °C water bath, and the residue dissolved with 3.5 to 5 ml methanol to obtain a radioactivity of about 1500 cpm/ml.

Eluates in the counting vials were mixed, and duplicate 1 ml portions pipetted into 2 ml RIA tubes. Another 1 ml portion was pipetted into a counting vial and dried. This aliquot was employed in the determination of total radioactivity in the RIA tubes and to calculate recovery after extraction and column chromatography. For the standard curve (Figure), 8 standard solutions containing 0, 5, 10, 25, 50, 75, 100, and 150 pg of DOC per 0.1 ml of absolute methanol and 0.1 ml of 1,2-^{3}H-DOC Standard II containing about 1500 cpm were pipetted in triplicate into 2 ml tubes. 1 ml of 1,2-^{3}H-DOC was added to 3 counting vials and dried in a 40 °C vacuum oven for the determination of total radioactivity in the standards. The RIA tubes containing the 1 ml aliquots and the standard tubes were dried down in a 40 °C water bath using a semiautomatic air blowing device[9]. The standard and the sample tubes with anti-DOC rabbit serum (1:12,500) were incubated overnight at 4 °C. Cold saturated ammonium sulfate was used to separate free from bound, centrifuged and counted in a liquid scintillation counter (Nuclear Chicago).

Percent cross reaction of various steroids with anti-DOC rabbit serum

C18 Steroids	Cross reaction (%)
Estriol	<0.01
17β-Estradiol	<0.01
Estrone	<0.01
3,17α-Dihydroxy-1,3,5-estratriene	<0.01
C19 Steroids	
4-Androstene-3,17-dione	<0.1
17β-Hydroxy-5α-androstan-3-one	<0.1
Testosterone	<0.1
3β-Hydroxy-5-androsten-17-one	<0.01
5-Androstene-3β,17β-diol	<0.01
5α-Androstane-3α, 17β-diol	<0.01
17β-Hydroxy-1,4-androstandiene-3-one	<0.1
4-Androstene-3β,17β-diol	<0.01
17β-Hydroxy-5β-androstan-3-one	<0.01
17β-Hydroxy-5-androsten-3-one	<0.1
3β-Hydroxy-5α-androstan-17-one	<0.01
C21 Steroids	
11-Deoxycorticosterone (DOC)	100
11α-Hydroxyprogesterone	<0.1
Corticosterone	0.9
Progesterone	1.8
Aldosterone	<0.01
3β-Hydroxy-5-pregnen-20-one	<0.01
Cortisol	<0.01
5-Pregnene-3β,20β-diol	<0.01
3β,17-Dihydroxy-5-pregnen-20-one	<0.01
5-Pregnene-3β,20α-diol	<0.01
17-Hydroxyprogesterone	<0.01
20α-Hydroxy-5-pregnen-3-one	<0.01
17,21-Dihydroxy-4-pregnene-3,20-dione	0.2
3α,17,21-Trihydroxy-5β-pregnan-20-one	<0.01
4-Pregnene-3,11,20-trione	0.1
17,21-Dihydroxy-4-pregnene-3,11,20-trione	<0.01
5β-Pregnane-3α,17,20α-triol	<0.01
5-Pregnene-3β,17,20α-triol	<0.01

Results and discussion. Pre-extraction of female samples with hexane reduces the overlap effect of DOC and progesterone. Recovery of 1,2-^{3}H DOC varied between 43 and 93% when 35 samples were assayed. Mean recovery was 63.9 ± 13.5 (SD)%. On 12 samples pre-extracted with hexane, mean recovery was 38.2 ± 9.8 (SD)% with values ranging from 33–44%. Intra and inter assay precision was evaluated by multiple measurements of the DOC content of 1 plasma pool in the same assay and in several different assays. Our plasma pool, pre-extracted with hexane, was measured 5 times in 1 assay and the coefficient of variation obtained was 12.4%. On 17 determinations assayed on 2 different days, the coefficient of variation was 27%.

Accuracy of the assay as estimated by recovery of unlabeled DOC was 101.5 ± 39.5 (SD)%. The mean recovery of DOC in 5 determinations was 105.5%, with a range from 79.6% to 117%. The sensitivity of our standard curve allows measurement of 3 ng/100 ml. The mean value for 7 blank samples run in 4 different assays was 1.5 ± 1.8 (SD) ng/100 ml. Specificity of our DOC radioimmunoassay method was tested by direct incubation with 32 different steroids (Table). Percent cross reaction was calculated by measuring displacement of 1,2-^{3}H-DOC at the 50% bound level[10].

Normal DOC levels were determined by assaying 12 normal subjects. The mean obtained was 6.4 ± 4.4 (SD) ng/100 ml. Comparative studies with 3 methods (protein binding, isotope dilution and immunoassay) have shown that in control subjects, similar values are obtained with all 3 techniques, indicating the accuracy and specificity of our method.

Resumen. Se reporta un nuevo método para medir desoxicorticosterona en plasma, usando cromatografía de columna LH-20 y radioinmunoensayo. Las cantidades de plasma necesarios para dicho ensayo son mínimas (2 ml) comparadas con otros métodos en la literatura, es un método practico, sensillo y rápido. Resultados así obtenidos han sido comparados con otros métodos, dichos resultados son más precisos y sensitivos para medir valores de desoxicorticosterona en plasma humano.

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