DHA sulfate.

H-labelled DHA sulfate with known radioactivities was added for recovery collection to each 15 ml of plasma taken from the subjects examined. Forty-five ml of absolute ethanol was added to the plasma to precipitate plasma proteins. After filtering the mixture, the filtrate was evaporated to near dryness using a rotatory flash evaporator. Secondary butanol was added to the filtrate for a partial separation of steroid conjugates from inorganic salts. The secondary butanol extract was applied to a florisil column for separating free 17-ketosteroids, 17-ketosteroid sulfate and 17-ketosteroid glucuronide. The 17-ketosteroid sulfate fraction was developed on Baulieu's paper chromatography mentioned above. The eluate from the paper part corresponding to the Rf of a pure DHA sulfate was divided into two parts, one for measuring weight of DHA sulfate by Zimmermann reaction, the other for measuring radioactivities by a liquid scintillation spectrometer. Then plasma concentration of DHA sulfate were calculated from recoveries and weights of DHA sulfate.

The obtained values were as follows.

Three normal males: 145μp/100ml, 224μg/100ml, and 260μg/100ml

Three normal females: 211μg/100ml, 258 μg/100ml, and 294μg/100ml

A female pseudohermaphroditism: 224μg/100ml

A female with ihrsutism: 195μg/100ml

Secretion-, production-, interconversion-, and irreversible metabolic rates of dehydroepiandrosterone (DHA) and DHA sulfate in normal controls and patients with various diseases were measured by the method of Vande Wiele et al. (Recent Progr. Hormone Res. 19:275, 1963) using ^{14}C-DHA and ^{3}H-DHA sulfate. No statistically significant difference was found between the mean values in 4 normal males and those in 4 normal females, but significant increases in the rates (except interconversion rates) were found in 3 female patients with adrenogenital syndrome (congenital adrenal hyperplasia) compared with those in 4 normal females. In a case of 4-year-old boy with precocious puberty due to adrenal cancer, secretion rates of DHA and DHA sulfate were remarkedly increased. In two of three patients with Cushing's syndrome due to adrenal adenoma, no increase was observed in secretion rates of DHA and DHA sulfate.

Urinary production rate (UPR) of testosterone (T) was measured by isotope dilution method. From the specific activity of ^{14}C-T isolated from T glucuronide in 48 hr urine after i.v. injection of ^{14}C-T, UPR of T was calculated. Values of UPR of T were 5.18 and 7.91 mg/day in 2 normal males, 3.44 mg/day in a case of Addison's disease and 3.16 mg/day in a patient with adrenal hypofunction after ^{60}Co irradiation for suspected cerebral tumor.

Radioactive urinary androsterone (A)/etiocholanolone (E) ratio, metabolised from ^{14}C-T wa smeasured. Radioactive A/E ratios were 0.81 in a normal male, 0.28 in a case of Cushing's syndrome (adrenal adenoma), 3.25 and 3.30 in 2 cases of hyperthyroidism, 1.10 in a patient with liver cirrhosis and 0.95 in a case of female pseudohermaphroditism.

Measurement of Plasma Transcortin Concentration in Man utilizing Gel Filtration and 4-C^{14}-Cortisol

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Plasma concentrations of transcortin were measured in man by the method of Doe et al. (J. Clin. Endocr. 24:1029, 1964) utilizing gel filtration and 4-C^{14}-cortisol. Values in 8 normal males were 24.2—37.7 (32.25±4.39 mean S.D.) mg/l. Values in 3 normal females were 25.4—33.3 (29.03±5.64) mg/l and showed no significant increase in plasma trans-

cortin by the third lunal month and reached an elevated plateau value by the sixth lunal month (76.7±6.8 mg/l in the eighth lunal month and 76.5±5.68 mg/l in the ninth). Values in 4 patients with Cushing's syndrome showed no significant change. Values in 2 patients with Addison's disease were within normal range. Values in 4 patients with hyperthyroidism were 24.2—37.7 (30.8±5.77) mg/l. Values in 7 patients with nephrotic syndrome were 16.9—35.2 (26.84±6.10) mg/l. Values in 8 patients with liver cirrhosis were 10.4—47.3 (29.9±11.2) mg/l and those in 5 patients with chronic hepatitis were 30.7—52.1 (45.8±6.18) mg/1. Mean values in hyperthyroidism, nephrotic syndrome, liver cirrhosis and chronic hepatitis showed no significant change. One patient with prostate hypertrophy given 250 mg stilbestrol-diphosphate-disodium intravenously daily for 3 weeks showed significant increases in both plasma cortisol (24 μg/100 ml) and plasma transcortin (71.5 mg/l). One patient with metastasis of ovarian granulosa cell tumor and a 35-year-old male with gynecomastia showed normal plasma transcortin concentrations, although urinary total estrogens showed some increase in both cases.

In normal subjects plasma maximum transcortin-bound cortisol (MTBC) was higher than plasma cortisol. In pregnant women plasma cortisol was markedly high and MTBC was higher than plasma cortisol. In Cushing's syndrome MTBC was lower than plasma cortisol, although the latter was markedly increased. These results indicate that an absence of clinical hyperadrenocorticism in pregnancy is due to a marked increase in MTBC and that an absence of increase in MTB in spite of a presence of an increased plasma cortisol in Cushing's syndrome may explain clinical hyperadrenocorticism in this syndrome.

A New and Simple Method for Determination of Blood Corticoids by Using ^{3}H-Prednisolone Resin Uptake

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A new and simple method for the determination of blood corticoids is reported in which the in vitro resin or resin sponge uptake of ^{3}H-prednisolone is used. Principle of this method bases on the competition for blood corticoids between CBG and resin. It is similar to that of Trioscorb Test. ^{3}H-prednisolone is used in place of ^{131}I-T_3, since it has less affinity to CBG than cortisol.

This method can measure blood corticoids directly or indirectly.

Indirect method: 0.5 ml of ^{3}H-prednisolone solution is added to 1.0 ml of serum and well mixed. The radioactivity of one fifteenth of this mixture is measured (A). Then 1.0 ml of the mixture is pipetted to a test tube which contains 200 mg of Amberlite CG 400 Type 1 resin. This tube is incubated at 4 C for 90 min. with shaking at 10 min. intervals. Then one tenth of supernatant is obtained after centrifugation and its radioactivity is measured (B). The samples are prepared for counting by mixing with 0.5 ml of Hyamine solution followed by 10 ml of 30% methanol-toluene scintillator, and counted by the liquid scintillation spectrometer.

^{3}H-prednisolone resin uptake (^{3}H-PRU) is calculated by the formula as follows: $(1-B/A) \times 100(\%)$.

The resin sponge of the Trioscorb Test can be used in place of resin (^{3}H-prednisolone prednisolone solution is added into the 1 ml resin sponge uptake (^{3}H-PRSU)). One ml of of serum with 120 min. incubation period. This method is simpler since no shaking nor centrifugation is required.

Direct method: Four ml of 95% ethanol is added to a 2 ml sample of plasma and mixed. Two ml of supernatant is pipetted into 2nd tube after centrifugation, and is dried under a stream of N_2 gas. The dried material is then redissolved by 1 ml of standard serum.