cortin by the third lunar month and reached an elevated plateau value by the sixth lunar month (76.7±6.8 mg/l in the eighth lunar 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 7 patients with nephrotic syndrome were 16.9–35.2 (26.84±6.10) mg/l. Values in 8 patients with chronic hepatitis were 30.7–52.1 (45.8±6.18) mg/l. 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 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 ³H-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 ³H-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. ³H-prednisolone is used in place of ¹³I-T₂, since it has less affinity to CBG than cortisol.

This method can measure blood corticoids directly or indirectly.

**Indirect method:** 0.5 ml of ³H-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 90% methanol-toluene scintillator, and counted by the liquid scintillation spectrometer.

³H-prednisolone resin uptake (³H-PRU) is calculated by the formula as follows: (1-B/A) × 100(%).

The resin sponge of the Trioscorb Test can be used in place of resin (³H-prednisolone prednisolone solution is added into the 1 ml resin sponge uptake (³H-PRSU)). One ml 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 N₂ gas. The dried material is then redissolved by 1 ml of standard serum.
$^3$H-PRU or $^3$H-PRSU of this solution is measured and then calibrated in amount of corticoids in 2 ml supernatant ($\mu$g) using the standard curve of cortisol. Recovery of corticoids in 2 ml supernatant is determined by adding $^{14}$C-cortisol to the plasma.

This method is based on the same principle as the method to measure blood thyroxine reported by Nakajima et al (1966).

Results: (1) Specificities, reproducibilities and sensitivities of these methods are examined with satisfactory results. (2) No significant effect with cortisone, 11-deoxycorticisol, THF, THE, dexamethasone, paramethasone and triamcinolone on the $^3$H-PRU is observed except with cortisol, hydrocortisone acetate and prednisolone. (3) Significant increase after the ACTH administration and circadian change in $^3$H-PRU are observed. (4) Good correlation between the plasma corticoids determined by our direct method and the plasma 11-OHCS level determined by DeMoor's method is observed.

Simultaneous determination of $^3$H-PRSU and $^{131}$I-$T_3$RSU by our indirect method is studied with satisfactory results. No interference between $^3$H-PRSU and $^{131}$I-$T_3$RSU is observed. Both blood corticoids and thyroxine level are simultaneously determined by the same method as our direct method.

The advantage of these methods over the conventional method is found in their simplicity and their requirement of only a small specimen, and their capability of simultaneous determination of $^3$H-PRSU and $^{131}$I-$T_3$RSU.

Clinical Studies on Cortisol Secretion and its Metabolism Using Radioactive Cortisol

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Cortisol-4-C$^{14}$ was administered intravenously to six normal subjects, fifteen patients with thyroid dysfunction and to nine patients with liver disease. Twenty-four urine sample after the injection was collected. Cortisol secretion rate was estimated by isotope dilution method and daily excretion of major urinary metabolites of cortisol, i.e. cortols, cortolones, tetrahydrocortisol (THF), allotetrahydrocortisol (ATHF) and tetrahydrocortisone (THE) were calculated by multiplying the secretion rate by per cent dose injected of each radioactive metabolite on paper chromatogram.

In ten patients with hyperthyroidism, cortisol secretion rate and urinary excretion of total glucuronide, THE, ATHF and of cortolones were remarkably increased (26.4±5.7**, 17.8±1.2***mg/day, 9.2±2.2***mg/day, 1.67±0.63***mg/day, and 5.40±1.69mg/day, respectively) as compared with those in normal subjects (16.8±1.5mg/day, 7.0±1.2mg/day, 3.2±0.4mg/day, 0.93±0.33mg/day, and 1.30±0.45mg/day, respectively). And THE/THF ratio, ATHF/THF ratio and cortolones/cortols ratio were also increased (9.8±4.0**, 1.6±0.3*, and 16.3±6.5**, and 5.3±1.3, respectively), suggesting acceleration of both 11 β-dehydrogenation and 5 α-hydrogenation in cortisol metabolism. These changes were statistically significant (*: $p<0.001$, **: $p<0.01$, ***: $p<0.05$).

In five patients with hypothyroidism, the reverse was observed (secretion rate 8.3±2.3* mg/day, total glucuronide 3.9±1.6***mg/day, THE 1.12±0.40**mg/day, ATHF 0.05±0.02**mg/day, THE/THF 0.81±0.04*, ATHF/THF 0.05±0.07*, cortolones/cortols 2.28±0.43**).

In all patients with liver disease (liver cirrhosis 3, chronic hepatitis 5, acute hepatitis 1), cortisol secretion rate was decreased (11.7±4.1***mg/day, 10.8±1.3***mg/day, and 8.6 mg/day, respectively). And was observed decreased tetrahydro (THF+ATHF+THE)/hexahydro (cortols+cortolones) ratio (1.74±1.0*, 2.10±0.21*, and 0.39, respectively) as compared with normal value (3.85±0.94), suggesting that hexahydro metabolites of cortisol might be produced by both liver and extrahepatic tissues, whereas tetrahydro metabolites were mainly produced by liver.