Corticosteroid was extracted twice with 1.5 ml of ethylene dichloride from 0.05 ml plasma. The supernatant was transferred to a small test tube, and evaporated to dryness. Bush B5 system was used for separation, if necessary. Corticosteroid-binding globulin (CBG)-isotope solution was made up from 2 ml plasma of Addison's disease administered dexamethasone, 0.2 ml of 3H-corticosterone (10 μCi/ml) in ethanol and distilled water to 100 ml. One ml of CBG-isotope solution was added to each small test tube containing the corticosteroid sample from plasma or standard corticosteroid. Each test tube was then shaken well, warmed 45°C for 5 minutes followed by cooling at 5°C for 10 minutes. Forty mg of Florisil (measured with small spoon) was added to each test tube, which was then shaken for 2 minutes and returned to ice water bath. A half ml of supernatant was pipetted into 10 ml Bray's solution and counted in a liquid scintillation counter. The radioactivity was compared with that obtained by standard cortisol or corticosterone.

Various adsorbent materials including dextran-coated charcoal, Florisil and Fuller's earth were studied for the separation of the protein-bound from the unbound fraction. Florisil gave the most generally useful method of separation. When Florisil was used, 30 to 80 mg was found to be suitable for separating protein-bound and unbound 3H-corticosterone, but very little 3H-cortisol was taken up even by amounts as great as 100 mg Florisil. Since corticosterone and cortisol were bound almost equally and interchangeably by human CBG, no difference in results was found for separating the protein-bound and unbound fractions. The useful range for a particular assay depended on the concentration of CBG present, greater sensitivity being obtained with less CBG. However, the sensitivity was limited principally by the specific activity of the isotope. With greater specific activity it was found possible to dilute the standard plasma. Recoveries of known amounts of unlabeled cortisol added to plasma of a patient with Addison's disease was almost 100%. Comparison of results obtained using this method and fluorometry showed a good correlation. Plasma cortisol levels in healthy individuals ranged from 6.0 to 20.0 μg/dl when determined at 8:30 to 10:30 AM, with a mean of 13.7 μg/dl in 18 subjects. The mean level of plasma corticosterone in eight normal rats was 14.8 μg/dl.

Unlike other methods, the sensitivity was greater in the lower range with a standard deviation of ±1 μg and the method was highly specific for cortisol and corticosterone in human plasma and was affected neither by hemolysis nor by substances which interfered with other methods. The chief advantage of using this method in place of other methods has been the much shorter time required to obtain the results.

**Progestrone Metabolism in Various Diseases**

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Peripheral metabolism of exogenous progestosterone was studied in patients with hepatic diseases, renal diseases and adrenocortical disfunction. Twenty-four hour urine was collected from each patient after the intravenous administration of approximately 2.5 μCi of 14C-progestosterone. An aliquot of the urine was extracted by ethyl acetate and separated into 17-KS and 17-DOHCS by thin layer chromatography. Pd and Pt fractions were further purified by aluminum column.

The radioactivity of the each fraction was counted by liquid scintillation counter. The results obtained suggested that the most part of administered progesterone was degraded in the liver and excreted in urine as pregnanediol. It was also suggested that the small part of the exogenous progesterone was converted to 17-OH-progesterone and excreted in the urine as pregnanetriol and another part
was converted to Δ4-Androstenedione and excreted in urine as Androsterone and Etiocloanelone.

In patient with acute hepatitis, pregnanediol consisted much larger part of excretory products of progesterone than control and conversion to pregnanetriol and 17-KS fractions seemed to be inhibited probably because of decreased activity of 17-hydroxylase. On the other hand, in patients with cirrhosis of liver, conversion to pregnanetriol and X-fraction was increased.

Studies on Radioimmunoassay of Human Growth Hormone
—Labeling and the Damaged 131I-HGH—

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Interestingly, inverse correlation was obtained between Pt/Pd ratio and serum GPT level. In patients with chronic nephritis, we could not find any abnormality in progesterone metabolism, but in nephrotic patients, conversion to pregnanetriol and 17-KS fraction was increased.

Iodine-131 labeled human growth hormone (131I-HGH) with specific activities of 180~650 μCi/μg was prepared by the method of Hunter and Greenwood. This 131I-HGH was further passed through Gephadex G100 column (3.2×35 cm) and the elution pattern, determined by counting 2.5 ml, showed three protein peaks. Standard curve obtained by using 131I-HGH in fraction 3 was the highest in the sensitivity compared with those in other fractions. Immunological activities of 131I-HGH in fraction 1 and 2 to the same antisera were 23 and 39.5 per cent respectively when compared with the standard curve of 131I-HGH in fraction 3.

The damaged 131I-HGH of initial material, fraction 1, 2 and 3 after one week incubation in buffer, separated by chromatoelectrophoresis, was 24, 23, 26 and 16 per cent respectively. The percentage of the damaged 131I-HGH incubated in buffer was 8.4±4.52 when iodine 131 was used within one day of arrival, but 17.4±6.22 when used after more than two days of arrival. The damaged material incubated in serum was 6.7±2.44 per cent greater in buffer and serum increased as incubation time was prolonged, but HGH concentrations of the serum obtained by each standard curve in different days of incubation showed no significant difference when the damaged material was adjusted by the method of Parker et al. The damage increased in proportion to the protein concentration in final incubation medium, but there was no significant difference in the HGH concentrations of the serum obtained by each standard curve in different concentrations of final incubation medium.