phorylation, or by altering the local availability of ions that are rate-limiting factors. The relevance of this attractive hypothesis to biological systems has not yet been adequately demonstrated.

Summary. Chlorpromazine, injected into the yolk sac of fertile hens' eggs, is lethal to a high percentage of embryos. At dosage levels of 0.9 and 1.8 mg the compound is destructive if it is administered within the first 48 hours of incubation; it shows little effect at 72 hours, and none at 96 hours.

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Urinary Excretion Pattern of Injected H³-Testosterone.* (28690)

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The 17-ketosteroids in urine reflect primarily the production of weak androgenic steroids from the adrenal cortex. It is not surprising that levels of 17-ketosteroids may be quite normal in the presence of various virilizing disorders. Because of this lack of correlation, greater interest has recently been focused on the metabolism of the potent androgen, testosterone. Hollander(1) identified testosterone in the spermatic vein of adult males and Finkelstein *et al*(2) were able to measure free testosterone in the plasma of normal subjects. Camacho and Migeon (3) and Rosner *et al*(4) have found small but measurable amounts of testosterone glucuronide in normal male urine.

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The use of tritiated testosterone of high specific activity, combined with chromatographic and derivative techniques for separation and identification of testosterone and the $C_{19}O_2$ ketosteroids, made possible a study of the urinary metabolites of testosterone.

Methods. 1,2-H^3-testosterone (3 $\mu c/\mu g$) from New England Nuclear Corp. was purified in 2 Bush paper chromatography systems described below. After purification, a trace amount with carrier produced a single radioactive peak in a strip scanner with a mobility identical to the UV absorbing area of standard testosterone.

Testosterone and the $C_{19}O_2$ ketosteroids were separated in 3 chromatographic systems:

(1) Hexane-benzene 1:1, formamide impregnation

(2) Cyclohexane 100, methanol 100, water 10 parts

(3) Cyclohexane 100, methanol 100, dioxane 25, water 10 parts

In these studies 5 μg tritiated testosterone (6×10^6 cpm) was injected into 6 normal male and 4 normal female subjects between 20 and 30 years of age. Urine was collected in 24-hour periods and stored at pH 5 in the cold.

Testosterone glucuronide. One-fifth of a 24-hour urine collection in males (two-fifths in females) was adjusted to pH 4.8, 1/10 vol acetate buffer added and the samples incubated with β-glucuronidase (Ketodase®), 500 U/ml at 40°C for 48 hours. Approximately 1,000 cpm 4-C^{14}-testosterone was added and the hydrolyzed steroid extracted twice into 1 vol ether, which was washed twice with 1 N NaOH and H_2O, 1/10 vol. The extract was dried, dissolved in 70% methanol and partitioned with 1/5 vol n-hexane, then chromatographed successively in systems 1 and 3. The steroid was located by scanning in a Nuclear Chicago strip scanner for C^{14} activity. The extract was oxidized with chromic trioxide 0.5% in 95% acetic acid for 2 hours, extracted with methylene dichloride, washed with water and run in system 2 or 3 for 18 hours. A 1/5 aliquot of the C^{14} area was counted in a Packard Tri-Carb liquid scintillation spectrometer and the remainder quantitated both in UV at 240 mμ and as a 17-ketosteroid against standards (5). The isolated steroid was identified by its mobility in 3 chromatographic systems, by the UV absorbancy at 240 mμ, which agreed within 10% of its quantitation as a 17-ketosteroid in the micro-Zimmermann reaction, and by its sulfuric acid spectrum.

Free testosterone. The free fraction before enzymic or solvolytic treatment was extracted twice with 2 vol ether.

Testosterone sulfate. The urine was brought to a 20% NaCl concentration and the steroid sulfate fraction extracted into ethyl acetate and solvolyzed with 2×10^{-4} M H_2SO_4 at 37°C by the method of Segal and Nes(6).

pH 1 hydrolyzable testosterone. To analyze the pH 1 hydrolyzable fraction, a urine aliquot, after removal of the free fraction by repeated ether extraction, was acidified with 50% H_2SO_4 to pH 1, incubated at room temperature for 24 hours and extracted twice with 1 vol ether.

Either 4-C^{14}-testosterone or 4-C^{14}-dehydroisoandrosterone was added to the sample and parallel-running cold dehydroisoandrosterone, androsterone, and etiocholanolone were used as standards for location and to calculate total recovery. The H^3 and C^{14} were counted simultaneously in the liquid scintillation spectrometer. Efficiency for tritium was 18%.

Results. The excretion of tritium after injection of tritiated testosterone was rapid; 90% or more was recovered in each urine sample within the first 24 hours.

Only a minute amount of *free testosterone* was detected after ether extraction and chromatographic separation, correcting for losses with added 4-C^{14}-testosterone. Recovery in this fraction ranged from undetectable levels (less than 500 cpm/24 hour sample) to 0.2% of the injected dose (12,000 cpm). Since the specific activity of testosterone glucuronide was measured in each subject, assuming an identical specific activity for the free testosterone,§ the excretion of free testosterone was from $<$ 1-8 μg/24 hours in males and $<$ 1 μg in females.

Small but measurable amounts of tritium

§ Personal communication from Dr. Bryan Hudson, Univ. of Melbourne, Australia.

were located in areas having mobility identical with *free androsterone* and *etiocholanolone* in 2 systems. Based on the specific activities of their glucuronides, the mean excretions of free *androsterone* and *etiocholanolone* are 20 and 25 μg/24 hours.

The mean excretion of *testosterone glucuronide* in males was 1% of administered dose or 75 μg (range 50-93); in females it was 4 μg (range 2-8).

The glucuronides and sulfates of androsterone, etiocholanolone, and isoandrosterone-dehydroisoandrosterone were separated by chromatography. The specific activities of the glucuronide and sulfate conjugates of androsterone and etiocholanolone were identical. The total recovery of tritium in this $C_{19}O_2$ ketosteroid fraction was 40 ± 5% of the total injected dose.

0.1% of the administered dose or 3-15 μg of testosterone was present as a *pH 1 hydrolyzable fraction.* In 4 normal male subjects 0.03% of the dose was isolated in a solvolyzable fraction having mobility identical with testosterone in 2 systems. It is not possible to state whether this very small amount of radioactivity actually arises from a sulfate conjugate of testosterone or is the result of acid hydrolysis of testosterone glucuronide in the sample.

Discussion. These studies support the work of Schubert and Wehrberger(7) and Camacho and Migeon(3) who observed that a significant amount of testosterone glucuronide is excreted by normal subjects. Sandberg and Slaunwhite(8) performed similar studies in 1956; however, the specific activity of the testosterone they injected was low, necessitating administration of larger quantities. They noted 50% excretion of the dose within 4 hours and trace amounts in the stools. In our studies, 90% or more of the tritium injected was excreted in the urine within 24 hours.

Most of the radioactivity from injected testosterone recovered in urine is in the form of the ketosteroid glucuronides and sulfates. We found about 0.1% of the injected dose in free form, 1% as the glucuronide of testosterone, and 0.03% in a solvolyzable fraction, presumably as a sulfate conjugate.

Forty per cent of a tracer dose of testosterone is excreted in the urine as a $C_{19}O_2$ ketosteroid. Using the normal production rate calculation of Hudson *et al*(9), about 2-3 mg of the ketosteroid is derived from testosterone.

The relatively small amount of ketosteroid derived from testosterone in comparison to the large contribution of ketosteroids from the adrenal cortex explains why a significant increase in testosterone secretion resulting in various androgenic syndromes will not usually lead directly to elevation of the total 17-ketosteroids above the normal range.

Since testosterone is excreted as both pH 1 hydrolyzable and glucuronide conjugates, it will be of interest to determine which conjugate most accurately reflects secretion rate and blood levels of testosterone(10).

Summary. A tracer dose of tritiated testosterone was injected into 6 normal male and 4 normal female subjects and the excretion pattern studied. Ninety per cent or more of the radioactivity appeared in the first 24 hours. Minute but detectable amounts of free testosterone, androsterone and etiocholanolone were found. Testosterone glucuronide averaged 75 μg and 4 μg respectively in this group of male and female subjects, about 1% of the injected dose, and 0.1% of the dose was present as a pH 1 hydrolyzable fraction. Most of the radioactivity recovered was found in the $C_{19}O_2$ glucuronide and sulfate fractions. Comparison of specific activities suggested that a third of the androsterone and etiocholanolone (totaling about 2.5 mg) was derived from testosterone.

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Secretion of 17-Hydroxycorticosteroids in Conscious and Anesthetized Dogs Exposed to Simulated Altitude.* (28691)

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Armstrong and Heim were the first to observe adrenal hypertrophy in rabbits exposed daily to a pressure equivalent to 18,000 feet (1). Metabolic and histologic extensions of these studies(2,3) revealed that maximal activation of the adrenal cortex occurred in rats, rabbits and dogs within 2-3 days. Others have demonstrated that, although urinary 17-ketosteroids (17-KS) and 17-hydroxycorticosteroids (17-OHCS) of men living at altitudes of 15,000-21,000 feet for over 2 weeks were not significantly different from those observed in subjects living at sea level(4,5), urinary and peripheral plasma 17-OHCS markedly increase during the first 3 days at high altitude before returning to sea level values(6). Hale *et al*(7), however, reported that plasma 17-OHCS were not significantly elevated in humans breathing ambient air for 45 minutes at 14,000 feet simulated altitude.

Increased urinary excretion of 17-KS in humans(8-10) and accelerated adrenocortical secretion of 17-OHCS in anesthetized dogs (11) have been observed during hypoxia induced at ground level by breathing gas mixtures low in oxygen. Under similar conditions Biddulph *et al* reported that peripheral plasma 17-OHCS and corticosterone-like steroids of dogs remained essentially unaltered (12). Since concentrations of peripheral plasma and urinary adrenocortical steroids are dependent on their secretion by the adrenal gland, metabolism and excretion, the present study was undertaken to determine the effect of hypoxia induced by simulated altitude on the secretion of 17-OHCS of conscious and anesthetized dogs.

Materials and methods. Eight male dogs, weighing 11 to 16 kg, were prepared 1 to 2 months prior to collection of adrenal venous blood in anesthetized and conscious dogs by a modification(13) of the method of Satake *et al*(14). Essentially the procedure consisted of bilaterally sectioning the dorsal spinal roots (T_{11}-L_3) through which the sensory nerve fibers from the lumbar region run. Approximately 24 hours prior to collection of blood samples, the accessory branches of the lumbo-adrenal vein were doubly ligated and a glass cannula fitted with a small length of rubber tubing was inserted into the vessel lateral to the adrenal gland. The cannula and tubing were filled with a heparin-saline solution and then clamped. A silk ligature was placed loosely around the central vein which drains adrenal secretions into the inferior vena cava. The skin was sutured and the animal allowed to recover.

Eighteen experiments (both right and left lumbo-adrenal veins were used in 5 dogs) were conducted in a continuously ventilated decompression chamber at 23°C for 2 hours. The dogs respired ambient air which resulted in oxygen tensions of 150 mm Hg in 10 ground level experiments (4 conscious; 6 anesthetized; sodium pentobarbital, 30 mg/kg), and 78 mm Hg in 8 experiments (4 conscious; 4 anesthetized) at 17,000 feet simulated altitude (395 mm Hg). Ascents and

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