St. is iodide, though monoiodotyrosine and diiodotyrosine are present in small amounts. *In vitro* experiments with homogenates of larval tissue have shown that an enzyme system capable of forming iodide from thyroxine is present only in tissues of larvae fed thyroxine-supplemented diets and not in those of larvae fed control diet alone. Activity of the enzyme is destroyed by boiling for 10-15 minutes.

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Isolation and Identification of Testosterone in Systemic Blood from Normal Human Male Adults.*† (25316)

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It is well recognized that testosterone and Δ^4-androstene-3,17-dione are the predominant steroid hormones of the human testis(1,2). These androgens have also been found in canine spermatic vein blood(3) and in dogs, spermatic vein concentration of testosterone and Δ^4-androstene-3,17-dione increases markedly after human chorionic gonadotropin (HCG) administration(4). Heretofore, neither testosterone nor Δ^4-androstene-3,17-dione have been reported in systemic blood of man. In the present communication, isolation of testosterone from systemic human blood after administration of HCG is described.

Materials and methods. Chemicals, unless otherwise specified, were reagent grade and all solvents used were freshly distilled. Evaporation was done at +40°C under nitrogen. Blood was obtained from 3 normal human male subjects 2 hours after intramuscular injection of 1000 units HCG.‡ Heparin was used as anticoagulant and a plasma pool of 400 ml was processed. The plasma was extracted 4 times with 500 ml ethylacetate/ether (1:1 v/v) each time. The combined extracts were evaporated to dryness and the residue suspended in 25 ml 70% methanol (MeOH) and stored at −15°C for 15 hours. The MeOH solution was then centrifuged 15 minutes at 2000 rpm in a refrigerated centrifuge. The supernatant was carefully removed, the precipitate consisting of lipid material(5) was washed once with 5 ml ice cold 70% MeOH, and the MeOH extracts were combined. Last traces of lipids were removed from the combined 70% MeOH solution by extraction with 30 ml n-hexane. The hexane was discarded and the MeOH solution was evaporated to drynes. The dry residue was transferred to a paper strip with chloroform-MeOH (1:1 v/v) and on another strip 50 γ synthetic

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testosterone were applied in the same fashion (6). Ascending chromatography was then performed with MeOH-benzene (1:9 v/v) to concentrate the material at tip of strip. The tips of these concentration strips were cut off, inserted into appropriate cuts on strips of formamide-MeOH impregnated paper and subjected to descending chromatography in formamide-benzene(7). The chromatograms were removed from the tank and carefully dried when the solvent front reached the lower end of strip. The Rf value of standard testosterone was determined by a Haines UV scanner and the area of the sample strip corresponding to the testosterone spot on the standard strip was eluted with MeOH and evaporated to dryness. The dry residue was rechromatographed in propylene glycol-methylcyclohexane(8) using 50 γ desoxycorticosterone (DOC) as reference compound. Relative mobility of testosterone compared to DOC was 0.68-0.72. This area of the sample strip was eluted and the dried eluate subjected to chromatography on 1.5 g purified silica gel(9), prepared wet with 0.5% ethanol (EtOH) in methylene chloride ($MetCl_2$). Unknown material was applied in 0.5% EtOH in $MetCl_2$. The column was then washed with 25 ml of 1% EtOH in $MetCl_2$ and the fraction 5% EtOH in $MetCl_2$ collected, since authentic testosterone was eluted from silica gel in this fraction. A blank column was processed simultaneously. The final fraction from the column containing the plasma sample was evaporated to dryness and the residue dissolved in 1 ml MeOH. The ultraviolet absorption spectrum of the sample was recorded in a Beckman DK 2 spectrophotometer using 5% EtOH in $MetCl_2$ fraction from the blank column as reference. One-tenth of unknown sample was then evaporated and acetylated with C^{14} labeled acetic anhydride(10), while the rest was submitted to infrared analysis in a Perkin Elmer Model 21 infrared spectrophotometer with ultramicro sampling system (11).§ The acetylated sample was chromatographed in propylene glycol-methylcyclohexane(8) and the radioactivity on the paper was localized by scanning in a strip counter. The area of the chromatogram containing radioactivity was eluted and the eluate divided. To one part was added 25 μg authentic testosterone acetate. The sample was chromatographed in formamide-hexane(12). Localization of radioactivity on the strip was determined in a strip counter and scanning of the strip at 240 mμ was subsequently done by UV scanning devise attached to a Beckman DU spectrophotometer. The second part was dissolved in 15 ml ethyl acetate and washed with 10 ml of a solution consisting of 0.2% anisaldehyde in 0.3 ml absolute EtOH, 0.7 ml conc. sulfuric acid, made up to 100 ml with distilled water. The ethyl acetate was thereafter washed twice with 10 ml water, evaporated to dryness and the residue submitted to a color reaction giving a pink color with peak absorbance at 510 mμ for testosterone, testosterone acetate and Δ^4-androstene-3,17-dione. In this reaction the steroid is dissolved in 0.15 ml of 0.2% anisaldehyde in absolute EtOH, 0.35 ml conc. sulfuric acid is added, the sample is placed in boiling water bath (+92°C) for 10 minutes, cooled under tap water and transferred to a micro cuvette of 0.5 ml capacity. Absorbance is determined in a Beckman DU spectrophotometer at 450, 510 and 570 mμ. The color produced is stable for at least 3 hours at +23°C. If the Allen correction(13), modified by Brown(14) is used to correct maximum absorbance at 510 mμ, 1 γ testosterone dissolved in 1 ml of the described reagent gives a mean absorbance at this wavelength of 0.183 + 0.011‖ (n = 25).

Results. The material in plasma behaving like testosterone in 2 paper chromatographic systems exhibited a distinct UV absorption maximum at 241 mμ. A total of 5.6 γ testosterone was present in 400 ml of plasma. The infrared absorption spectrum strongly indicates that this material is testosterone, though minor additional absorption bands were observed, probably due to admixture of contaminating material (Fig. 1). However, in the fingerprint region adequate infrared resolution was present showing spectral agreement between authentic testosterone and the material isolated from plasma.

§ We are indebted to Dr. Dale A. Clark, Veterans Hosp., McKinney, Texas for the infrared analysis.

‖ Stand. dev.

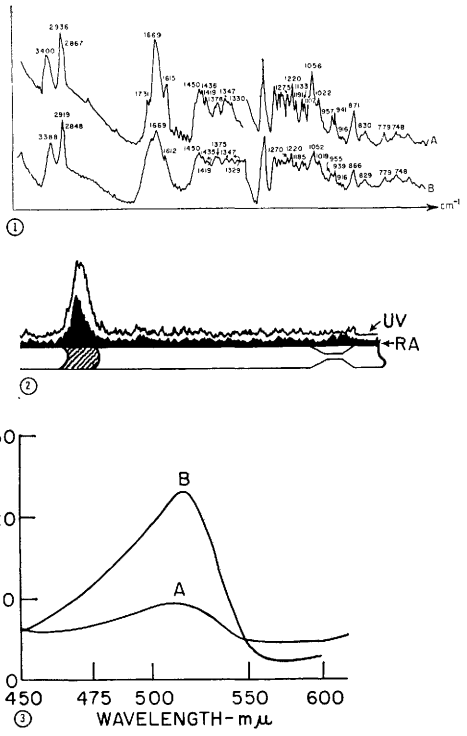

FIG. 1. Infrared spectra of testosterone (A) and "testosterone" from plasma (B).

FIG. 2. Tracings from UV and radioactive scanning: A mixture of testosterone acetate and C^{14} acetate of plasma "testosterone" was used. RA: radioactivity. UV: UV absorbance at 240 mμ. The marked area on chromatogram represents UV absorbance as detected by Haines scanner.

FIG. 3. Spectral curves of testosterone acetate subjected to anisaldehyde-ethanol-sulfuric acid. A: acetylated unknown material in plasma approximately 0.3 γ in 0.7 ml reagent. B: authentic testosterone acetate, 1.5 γ in 0.7 ml reagent.

The compound from plasma formed an acetate with acetic anhydride-C^{14} which behaved chromatographically like testosterone acetate. When the C^{14} labeled acetate of the compound was diluted with non-labeled authentic testosterone acetate and chromatographed in formamide-hexane, the peak of radioactivity coincided with that of the UV absorption maximum at 240 mμ (Fig. 2). Furthermore, the C^{14} labeled acetate of the compound reacted with the anisaldehyde-EtOH-sulfuric acid to give an absorption maximum at 510 mμ like authentic testosterone acetate (Fig. 3).

One hundred and fifty ml pooled plasma from normal male subjects not given HCG were assayed for testosterone by methods described. In this plasma pool, no testosterone was found.

Discussion. The data indicate that testosterone actually is present in the systemic circulation of normal male adults after administration of HCG. A concentration of testosterone 2 hours after injection of human chorionic gonadotropin was approximately 1.4 γ/100 ml plasma. Gallagher *et al.*(15) isolated deuterium-labeled androsterone following administration of deuterium-labeled testosterone in man, and we recently demonstrated that intramuscular administration of HCG increases conjugated plasma androsterone in the normal male(16). This increase is not seen in the normal woman. With the actual identification of testosterone in male plasma at the time when the metabolite is increased in the blood, it can be concluded that human chorionic gonadotropin promotes a prompt secretion of testosterone in normal human male similar to that already demonstrated in the normal male dog(4).

Summary. By criteria of paper chromatography in 2 systems, formation of an acetate derivate, ultraviolet and infrared spectrophotometry, testosterone has been isolated and identified in systemic blood of normal male subjects given HCG intramuscularly. Plasma testosterone could not be found in normal male subjects not stimulated with HCG.

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Hemagglutination of Rabbit Erythrocytes by Pus from Cases of Cat Scratch Fever. (25317)

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The possible viral etiology of cat scratch fever, non-bacterial regional lymphadenitis, has been postulated for some time. The demonstration of positive skin tests and complement-fixing antibodies to lygranum in patients with this disease, as well as observation of cytoplasmic, basophilic inclusion bodies similar to those seen in psittacosis, have indicated specifically a virus related to the psittacosis-lymphogranuloma venereum group. Although Mollaret(1) has been able to transmit the disease in humans and monkeys using filtrates of pus from lymph nodes, numerous efforts to isolate an infectious agent, by use of conventional laboratory media, inoculation of common experimental laboratory animals, and use of tissue culture explants, have failed. This paper is a preliminary report on hemagglutination of rabbit red cells by pus aspirated from lymph nodes of clinical cases of cat scratch fever as possibly a viral type of hemagglutination, as well as attempts to demonstrate specific hemagglutinin inhibiting antibody in serum of patients with this disease, and in rabbits immunized with pus.

Materials and methods for hemagglutination of rabbit red cells consisted of pus from lymph nodes of patients clinically diagnosed as cat scratch fever on the basis of a regional non-bacterial lymphadenitis and a history of one or more cat scratches. The pus, as received, was diluted 1:5 in physiological salt solution and 2 of the samples had been heated at 56°C for one hour on consecutive days for preparation of skin-test antigen. This material, either untreated or heated, was serially diluted in phosphate buffered saline through 1:512. One drop of each dilution was then mixed with one drop of a 2% suspension of washed rabbit red cells. The tubes were incubated in water bath at 37°C for 30 minutes, after which they were centrifuged lightly at 1500 rpm for 30 seconds, and observed for hemagglutination by shaking the tubes gently and noting appearance of clumped red cells. Trypsinized red cells were prepared by treating the suspensions with 0.1% trypsin (Difco) for 30 minutes in water bath at 37°C. The preparation of tanned cells consisted of similar treatment with dilute tannic acid. The human sera employed consisted of samples from 2 infected individuals from whom pus was obtained; serum from a person known to have a positive skin test; and serum from an individual suffering from infection with adenovirus. The rabbit sera were obtained before and after immunization of the animal with his own red cells previously treated with pus. Tests for hemagglutinin inhibiting antibody consisted of incubating equal volumes of various dilutions of serum and a dilution of pus containing 4 hemagglutinating units for 30 minutes in water bath at 37°C and then testing for hemagglutination with red cells as described above.

Results. Rabbit erythrocytes were agglutinated by all 10 samples of pus from known

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