similar procedure performed in the SERA Study of 1956-57(2). This increase in sensitivity is particularly evidenced by the frequent occurrence of Weakly Reactive (WR) results obtained in the Presumed Normal category of patients (Table I).

Of various factors which have probably played an important part in increased sensitivity of the FTA test, one must certainly consider the introduction and general use of fluorescein isothiocyanate. Other factors may include use of conjugates prepared from higher titered antiserums and incorporation of Tween 80 to enhance antigen-antibody coupling(5). However, regardless of the actual cause of increased sensitivity of the FTA test, it is obvious that this tends to lower the specificity of procedure in terms of syphilis and seriously affects reproducibility of results.

Our quantitative FTA test yielded high titers in secondary and late syphilis. These range from 200 to 25,600 dils. Primary syphilis, on the other hand, demonstrated low titers ranging from 0‡ to 3200 dils. As has been noted, some specimens in this group produced a 1+ reaction as did some serums obtained from nonsyphilitic donors. If one discounts a 1+ (WR) reaction and reports such reading as Nonreactive (N), as is done in the FTA-200 test, false positive reports are avoided without appreciable loss of ability to detect syphilitic patients. Studies to be reported later indicate that the low grade fluorescence obtained when testing presumably nonsyphilitic patients (1:50 and 1:100) is probably due to treponemal antibodies not associated with syphilis. It should be noted also that in no instance was strong fluorescence or a 2+ Reactive (R) result obtained in the Presumed Normal group at the critical 1:200 dilution.

Summary. 1. A modified FTA test technic designated as FTA-200 is described and recommended on the basis of results obtained by examining well-documented serum specimens from various syphilis categories and a Presumed Normal group. 2. The FTA-200 test possesses a greater degree of reproducibility than the FTA test when results were compared on duplicate serums.

‡ 0 dils = less than 200 dils in the FTA-200 test.

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Received January 25, 1960. P.S.E.B.M., 1960, v103.

Plasma Clearance and Glucuronide Conjugation of 11-Desoxycortisol (Substance S) in Man.* (25686)

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The metabolic fate of a number of adrenocortical steroids in the peripheral circulation of man has been described by Peterson(1). Recently Touchstone *et al.*(2) reported that *11-desoxycortisol* was secreted by the human adrenal in significant quantities, amounting to approximately one-tenth the concentration of *cortisol* in adrenal venous blood. Rate of removal of 11-desoxycortisol from plasma and rate of appearance of its glucuronide-conjugated metabolites in "normal" subjects and in patients with liver disease and hypothyroidism are reported here.

* These studies were carried out under grant from N.C.I. (C2-3995).

Materials and methods. Patients. Four

patients with absent or suppressed adrenocortical function were studied. Two adrenalectomized subjects were given 1 mg of 9-alpha fluorohydrocortisone† and 2 mg of dexamethasone‡ daily during the study. Adrenal function in the remaining 2 subjects was suppressed by administration of 2 mg of dexamethasone daily. No attempt was made to limit endogenous corticoid secretion in 2 additional patients studied, one with primary hypothyroidism and another with portal cirrhosis and ascites. Each patient was given an infusion of 100 mg of 11-desoxycortisol§ dissolved in 500 ml of .9% saline intravenously within 30-min. period and heparinized samples of whole blood were collected. The procedure was repeated with each patient on another occasion, substituting cortisol for 11-desoxycortisol. *Measurement of plasma Porter-Silber chromogens (PSC).* Both cortisol and 11-desoxycortisol were estimated as *unconjugated* ("free") PSC in plasma by a modification(3) of the procedure of Peterson *et al.*(4), by which either may be quantitated with equal accuracy. This modification was adapted to the estimation of plasma *glucuronide-conjugated* PSC as follows. Free PSC were extracted from 10 ml aliquots of plasma with dichloromethane in the usual manner(3). The residue was incubated for 18 hr at 45°C with 5000 units of beef liver beta-glucuronidase.‖ 1 ml of 1 M acetic acid-sodium acetate buffer (pH 4.8) and 1 ml of 1% ethylenediamine tetraacetic acid. A reagent blank consisting of 20 ml of distilled water and 2 μg standards of authentic cortisol and 11-desoxycortisol¶ dissolved in 20 ml of distilled water was similarly treated. Following incubation, the hydrolysates were extracted with 80 ml of dichloromethane (freshly redistilled). Emulsions were resolved by centrifugation for 5 min at 3000 rpm. The separated dichloromethane extracts were washed and evaporated to dryness; steroid phenylhydrazones were then prepared and quantitated in the usual manner(3).

Results. Following infusions of 11-desoxycortisol and cortisol, plasma PSC concentrations, expressed logarithmically, decreased as a straight line function of time (Fig. 1). Based on rate of removal of PSC from plasma during initial 150 min. after infusions were begun, the biologic half-life of "free" 11-desoxycortisol was less than one-half that of cortisol in "normal" subjects. Beyond 150 min. the average PSC titer after 11-desoxycortisol (at that time 11.2 μg/100 ml) fell off at a much slower rate, the slope approximating that found for cortisol. In contrast to "normal" subjects the patient with liver disease and another with hypothyroidism showed a marked delay in plasma clearance of both 11-desoxycortisol and cortisol (Fig. 2) with disappearance rates of the 2 steroids remaining proportional, that of 11-desoxycortisol being twice as rapid as that of cortisol.

Rate of appearance in plasma of *glucuronide-conjugated* Porter-Silber chromogens after infusion of each of the 2 steroids differed greatly (Fig. 3). After giving 11-desoxycortisol, a peak concentration was achieved rapidly, the mean amounting to 216 μg/100 ml by the end of 30-min infusion period, with a rapid decline thereafter. At completion of cortisol infusions average level of glucuronide-conjugated PSC, which was only 40.6 μg/100 ml, remained essentially unchanged for 150 min. Once peak levels declined, the conjugates of both steroids were removed from plasma at an identical rate, the half-time for each being 102 min.

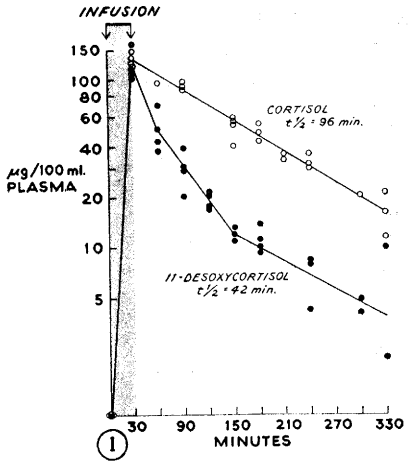
† Kindly provided as Florinef® by S. C. Sutton, E. R. Squibb & Sons.

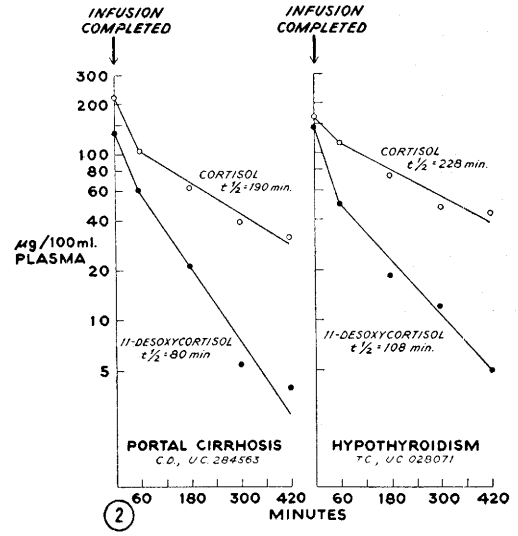
‡ Supplied as Decadron® through courtesy of Dr. Elmer Alpert, Merck, Sharp & Dohme, West Point, Pa.

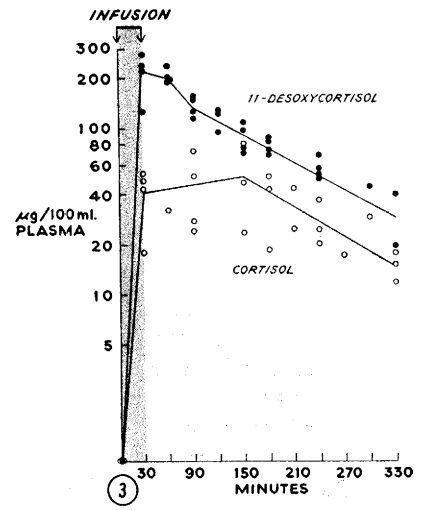
§ 11-desoxycortisol (delta-4-pregnene-17 alpha, 21-diol, 3,20-dione) was supplied by Dr. Harold Upjohn, Jr., Upjohn Co., Kalamazoo, Mich. Cortisol (free alcohol) (delta 4-pregnene-11-beta, 17 alpha, 21 triol, 3,20-dione) was provided by Dr. Elmer Alpert, Merck, Sharp & Dohme, West Point, Pa.

‖ Ketodase®, Warner-Chilcott Labs., Morris Plains, N. J.

¶ Chemically pure cortisol and 11-desoxycortisol standards were supplied by Dr. L. H. Sarett, Merck, Sharp & Dohme, West Point, Pa. For most studies, cortisol was the only standard used since its molar absorbance as a Porter-Silber chromogen is essentially identical to that of 11-desoxycortisol(3). Under conditions used for color development in the present study, tetrahydro-cortisol was essentially equal to cortisol in chromogenicity.







Discussion. Unconjugated 11-desoxycortisol disappears from peripheral circulation twice as rapidly as cortisol. Its removal time, while somewhat shorter than that of corticosterone(5), approaches that found for aldosterone and cortisone(1), suggesting that the longer biologic half-life of cortisol, compared to that of any of these other naturally occurring steroids, is a unique property of the cortisol molecule possibly related to hydroxylation of *both* carbons 11 and 17, which may play a critical role in protecting cortisol from metabolic degradation.

A delay in plasma cortisol clearance has been observed in liver disease(6) and hypothyroidism(7). The present findings indicate that catabolism of 11-desoxycortisol is also impaired, and to the same extent, in these disorders.

Rapid appearance of high titers of glucuronide conjugates after administration of 11-desoxycortisol, in contrast to the more prolonged but lower peak levels after infusion of cortisol, suggests that this difference in removal rate of the *free form* of both steroids is related to appearance rate of their glucuronide metabolites. Existence of independent enzymatic mechanisms for reduction of both 11-desoxycortisol and cortisol, which vary in rate as well as specificity, has been demonstrated in preparations of rat liver. A rate-limiting step occurs prior to conjugation of the molecule with glucuronide and involves saturation of ring A by addition of hydrogen to carbon 5, thereby forming the "dihydro-" metabolite(8). Initial *in vitro* studies with incubates of 11-desoxycortisol and rat liver homogenates suggested that its A-ring reduction was catalyzed solely by a 5-alpha reductase, rather than by a 5-beta reductase as in the case of cortisol(9). More recent work, however, has shown that hydrogen may be added to both steroids in either position, al-

FIG. 1. Disappearance of non-conjugated phenylhydrazine chromogen from plasma following infusion of 100 mg of cortisol and 11-desoxycortisol.

FIG. 2. Delayed disappearance of non-conjugated phenylhydrazine chromogens in portal cirrhosis and hypothyroidism.

FIG. 3. Appearance of glucuronide-conjugated phenylhydrazine chromogens in plasma following infusion of 100 mg of cortisol and 11-desoxycortisol.

though the reaction does require hydrogenases which are steroid-specific(10). Moreover, 11-desoxycortisol is metabolized by such *in vitro* preparations at a "considerably faster" rate than cortisol(11). With respect to 5-alpha reductase activity, rat liver microsomes catalyze 11-desoxycortisol approximately twice as rapidly as cortisol(10), a ratio which agrees well with the relative clearance of the 2 steroids from plasma in the present *in vivo* study in man.

These observations suggest that the more rapid removal of 11-desoxycortisol compared to cortisol from the peripheral circulation should be taken into account in estimating total adrenal secretory response to the 11-beta hydroxylase inhibitor SU-4885(12,13) based on periodic plasma levels of free 11-desoxycortisol.

Summary. The biologic half-life of free 11-desoxycortisol in circulating plasma of man, estimated as Porter-Silber chromogens, was 42 min. This may be prolonged to as much as 80 min. in the presence of liver disease or to 108 min in hypothyroidism. Absence of an 11-beta hydroxyl group accelerates clearance of 11-desoxycortisol from plasma, its removal rate being twice as rapid as that of cortisol. 11-Desoxycortisol is rapidly conjugated to a glucuronide. Concentration of its Porter-Silber chromogen metabolites is 5 times greater than that of cortisol at comparable peak levels; the glucuronide conjugates of both steroids disappear from plasma with a half-time of 102 min.

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Received February 1, 1960. P.S.E.B.M., 1960, v103.

Viral Growth and Oncolysis in Krebs 2 Cells as Affected by Substrate.* (25687)

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In roller tube suspensions of Krebs 2 cells (K2) infected with influenza virus and with glutamine and lactate as the only substrates high titers of hemagglutinins were obtained, but when glucose was added in place of lactate HA titers were significantly lower(1). The newly formed hemagglutinins remained in or on the cells, and infectivity titers were very low. The K2 ascites tumor has such high virulence that it is possible to quantitate tumor producing capacity of a cell suspension by inoculating mice with serial 10-fold dilutions. Application of this technic to roller tube suspensions of surviving K2 cells has made possible a quantitative study of the relation between substrate, growth of virus, and reduction of tumor producing capacity. Results with influenza and Newcastle disease virus (NDV) will be compared.

* This work was aided by research grant from Nat. Cancer Inst., U.S.P.H.S., and by funds from Eugene Higgins Trust.