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Salivary Testosterone in Men: Further Evidence of a Direct Correlation with Free Serum Testosterone*

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ABSTRACT. An excellent correlation was found between salivary testosterone (T) and serum T concentrations, as measured by RIA. Using polyacrylamide gel electrophoresis, we have demonstrated that sex steroid-binding globulin could not be identified in the saliva of men with serum sex steroid-binding globulin. After exogenous T administration, saliva and serum T rose abruptly and in parallel. Salivary T concentrations in male patients with thyrotoxicosis were similar to those in normal males, whereas the serum T and sex steroid-binding globulin values were significantly higher in the hyperthyroid patients. This study demonstrates that salivary T levels may be used as an index of free serum T. (*J Clin Endocrinol Metab* **53:** 1021, 1981)

SALIVA has long been used for therapeutic drug monitoring (*e.g.* digoxin, theophylline, and phenytoin) because earlier studies have demonstrated that the concentration of drugs in the saliva is proportional to the concentration in plasma. Furthermore, the salivary concentration also reflects nonprotein-bound drug levels in plasma (1–4). Shannon *et al.* (5) in 1959 demonstrated that the parotid fluid 17-hydroxycorticosteroid concentration closely paralleled that in serum. Subsequent reports confirmed that salivary and parotid fluid corticosteroids reflect the nonprotein-bound (free) corticosteroids in the plasma in physiological as well as pathological states (5–8). However, other reports demonstrated no correlation between salivary and plasma levels of progesterone and estrogen during the menstrual cycle (9), and the concentrations of salivary testosterone (T) far exceeded those reported for free or unbound T (10). Smith *et al.* (11) demonstrated that salivary T showed a close correlation with free T, as estimated by equilibrium dialysis or when calculated from the measurement of sex steroid-binding globulin (SSBG)-binding capacity in normal females and patients with polycystic ovarian disease. Walker *et al.* (12) showed that salivary T paralleled total serum T under physiological conditions in men.

This report describes a direct assay of salivary T and demonstrates that the measurement of salivary T serves as a useful index of free T in men.

Materials and Methods

Twenty-three normal healthy males and eight male patients with thyrotoxicosis provided matched saliva and blood samples. Saliva was collected by the subjects after chewing on dental wax for 10–15 min. Venipuncture was performed during the saliva collection. One patient with hypogonadotropic hypogonadism (after withdrawal of hormonal treatment for 6 weeks) and five normal male volunteers received a single injection of T enanthate (200 mg, im). Simultaneous saliva and blood samples were obtained 2 h before the injection and then hourly for 5 h in three subjects (one patient with hypogonadotropic hypogonadism and two normal volunteers); in the remaining three normal volunteers, the samples were obtained 2 days before and then daily for 4 days after the T injection. Saliva and serum were stored at −20 C until assay. All paired serum and salivary samples from one subject were measured in one assay.

Serum (50–500 μl) and saliva (1–5 ml) were extracted with 5–10 vol ether before RIA. The mean recovery for serum or saliva extraction was over 90%. T was assayed by RIA using Matched Assay Reagents provided by the WHO and the method detailed in the Methods Manual of 1979. The T antibody showed 14% cross-reaction with 5α-dihydrotestosterone (DHT), 6% with 5α-androstenediol, 2.1% with 5-androstenediol, and 0.8% with androstenedione. The intraassay coefficient of variation for the T RIA was 7.5%.

To examine whether SSBG was present in the saliva, paired

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serum and saliva samples were preincubated with [^{3}H]DHT for 16 h at 2-4 C before steady polyacrylamide gel electrophoresis (13). In addition, the paired serum and saliva samples were also mixed with Concanavalin A bound to Sephadex 4D (14). The slurry was allowed to stand for 2 h; then, the supernatant was decanted, and the radioactivity was counted.

SSBG in serum samples was also determined in triplicate by the DEAE-cellulose filter assay described by Mickelson and Petra (15). SSBG in the hyperthyroid men was measured by a charcoal absorption assay, as previously described (16). Free T was calculated from the formula of Pearlman (17) using the association constant for T reported by Moll *et al.* (18).

Results

Serum samples showed SSBG peaks in the appropriate gel segments on polyacrylamide gel electrophoresis, whereas no peaks were seen in any of the saliva samples (Fig. 1). In addition, when Concanavalin A/Sepharose 4D (which will remove any glycoprotein moiety) was added to saliva, there was no loss of [^{3}H]DHT, providing further evidence against the existence of any significant amount of SSBG.

Increasing volumes of saliva showed parallel displacement curves with the T standards (Fig. 2). When known amounts of T were added to 1.0 ml female saliva, which contained less than 10 pg T, similar amounts of T were recovered after assay. When saliva samples were assayed after Celite chromatography, an excellent correlation was obtained between the T measured with (y) or without (x) column chromatography (data not shown; y = 13.2 pg ± 0.94x; r = 0.96; n = 19), indicating that column chromatography is not required for saliva T assays.

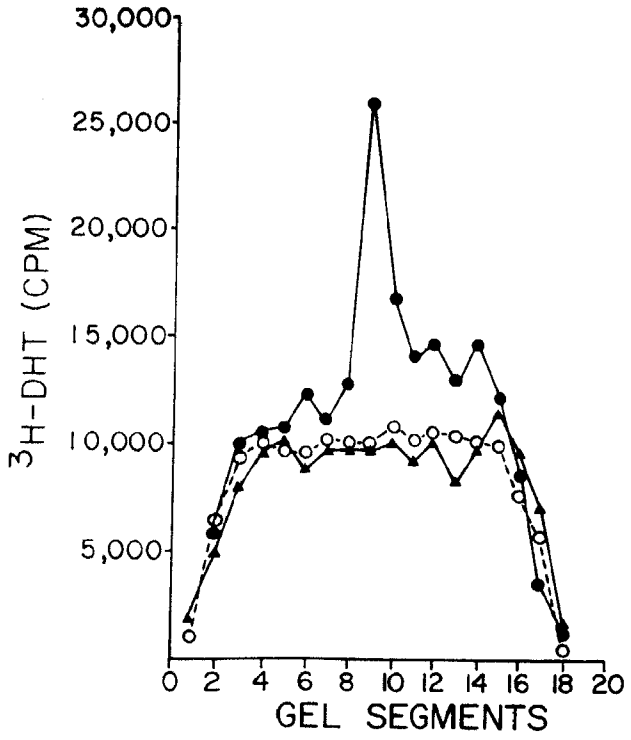

FIG. 1. Steady state polyacrylamide gel electrophoresis of paired serum and saliva from a normal male. ●—●, 1:20 dilution of serum; O- -O, 1:20 dilution of serum with 100 ng unlabeled DHT; ▲—▲, saliva.

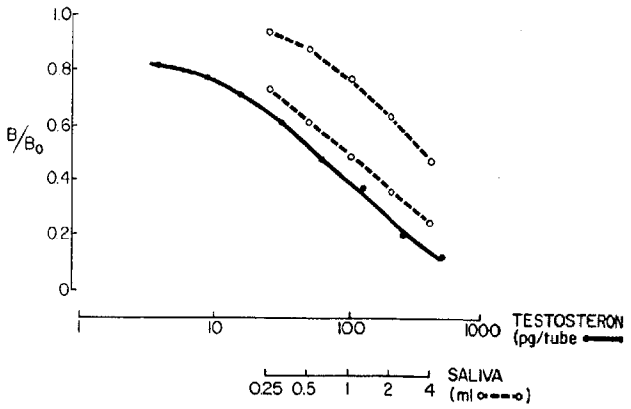

FIG. 2. Increasing volumes of saliva (O- -O) showing parallel displacement with T standards (●—●) in RIA. B, counts per min bound; B$_0$ counts per min bound in the absence of cold T.

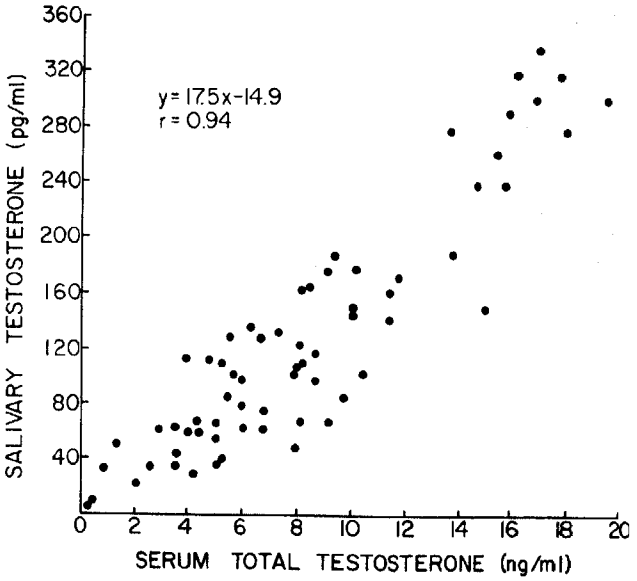

FIG. 3. Correlation between salivary T and serum total T concentrations (n = 65).

The mean serum total T in normal adult men was 6.09 ± 1.8 ng/ml (mean ± SD; n = 23), and the salivary T level was 84 ± 35 pg/ml. Thus, the free T in saliva represented 1.38 ± 0.44% of the total serum T. The correlation between salivary T and serum T titers in normal males before and after T treatment is depicted in Fig. 3. Salivary T levels showed excellent correlation with the total serum T values (r = 0.94; n = 65).

The effects of the administration of T enanthate (200 mg, im) on serum and salivary T levels are shown in Figs.

4 and 5. Abrupt and parallel rises in both serum and salivary T were observed 1 h after exogenous T administration in the patient with hypogonadotrophic hypogonadism (Fig. 4A) and the two normal males (Fig. 4, B and C). In contrast, the SSBG levels in these three men remained stable (Fig. 4). In three additional men, daily blood samples showed a parallel rise and fall in salivary and serum T levels after T enanthate administration (Fig. 5). SSBG levels were measured in only one of these men. They remained unchanged (range, 0.34–0.46 μg DHT bound/100 ml serum).

Plasma and salivary T levels in eight male patients (aged 20–45 yr) with untreated thyrotoxicosis are shown in Table 1. The mean total serum T titer in the patients with thyrotoxicosis was 13.48 ± 3.74 ng/ml, which was significantly higher than that in our normal men (6.09 ± 1.8 ng/ml; $P < 0.001$), whereas the mean salivary T titers for the two groups were similar. The SSBG levels in the hyperthyroid men were markedly elevated compared to those in normal male controls. Similar calculated free T levels and salivary T concentrations were found in the hyperthyroid men and normal male controls (Table 1), but when the salivary T concentration was expressed as a percentage of the total serum T titer in the patients with thyrotoxicosis, it was lower than that in normal men (0.64 ± 0.16% *vs.* 1.38 ± 0.44%; $P < 0.001$). This was expected and is due to the increase in SSBG.

Discussion

We have shown in this study that SSBG was not identified in the saliva of men with normal serum SSBG levels. Furthermore, the salivary T concentration showed a good correlation with total serum T. After the exogenous administration of T, salivary T rose in parallel with total serum T without significant changes in the SSBG-binding capacity in the serum. The results strongly suggest that salivary T reflects the nonprotein-bound or free T in the serum. Furthermore, the salivary T levels, expressed as a percentage of the total serum T level reported in this study for normal men (1.38 ± 0.44%), agreed closely with the percent free T (2%) reported by other studies (19, 20). Our salivary T values are also in good agreement with those reported by Walker *et al.* (12). This is further strengthened by the estimations of salivary and serum T in patients with thyrotoxicosis. These patients show an increased SSBG-binding capacity and usually exhibit elevated total T levels but normal serum free T titers (19, 20). We have shown in this study that our patients with thyrotoxicosis had significantly higher total T levels, but their salivary T concentrations and calculated free T levels were similar to the normal male control values. This suggests that salivary T reflects free T. The salivary T to total T ratio (as a percentage) in thyrotoxicosis reported in this study is also similar to the percent free T reported in other studies in hyperthyroid men.

Our findings in this study substantiate and extend the studies of Smith *et al.* (11) and Walker *et al.* (8, 12), who demonstrated that salivary steroids (T as well as cortisol) represents the free or nonprotein-bound fraction of the respective steroid. The use of a direct salivary T assay

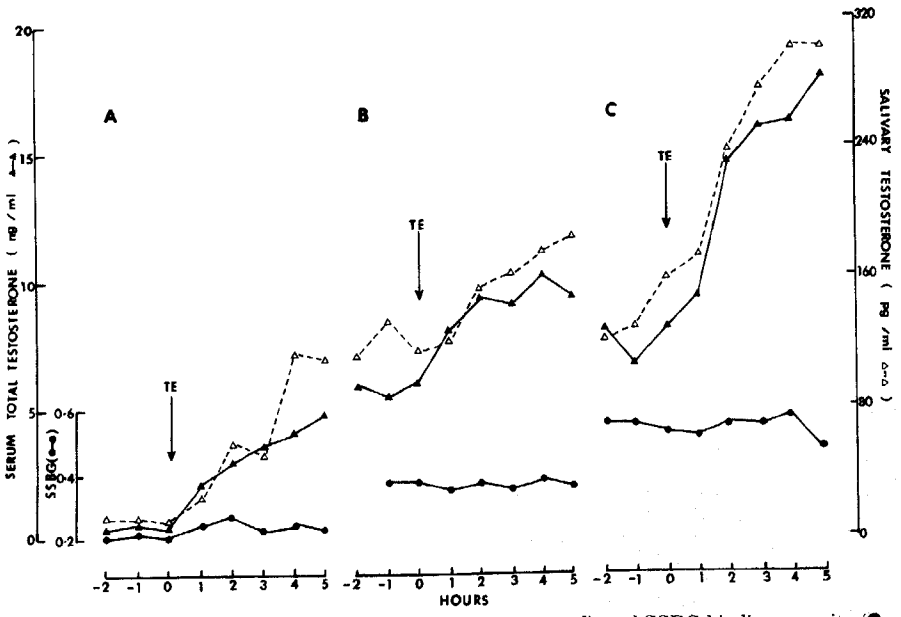

FIG. 4. Hourly serum T (▲—▲; nanograms per ml), salivary T (△—△; picograms per ml), and SSBG-binding capacity (●—●; micrograms of DHT per 100 ml serum) before and after the administration of T enanthate (200 mg, im; TE) to subjects A (patient with hypogonadotropic hypogonadism), B (normal male), and C (normal male).

TABLE 1. Serum and salivary T concentrations in patients with thyrotoxicosis

Patient	Sex/age (yr)	Serum T (ng/ml)	Salivary T (pg/ml)	SSBG (μg DHT bound/100 ml)	Salivary T: serum T (%)	Calculated Free T (pg/ml)
C.Y.W.	M/31	14.14	123.8	5.2	0.80	95
C.K.C.	M/33	13.13	67.1	10.3	0.51	40
L.K.C.	M/31	15.31	120.9	8.3	0.79	61
L.K.S.	M/20	19.46	96.8	5.9	0.50	125
L.W.T.	M/45	10.01	66.2	7.7	0.66	40
L.H.C.	M/31	17.04	62.8	7.3	0.37	81
M.Y.S.	M/30	9.43	63.4	4.6	0.67	65
T.H.	M/40	9.33	74.6	3.3	0.80	92
Mean ± SD		13.48 ± 3.74[a]	84.4 ± 26	6.5 ± 2.2[a]	0.64 ± 0.16[a]	74 ± 29
Normal male controls (n = 23)		6.09 ± 1.8	84 ± 35	0.8 ± 0.3	1.38 ± 0.44	119 ± 47

[a] $P < 0.001$ compared with controls.

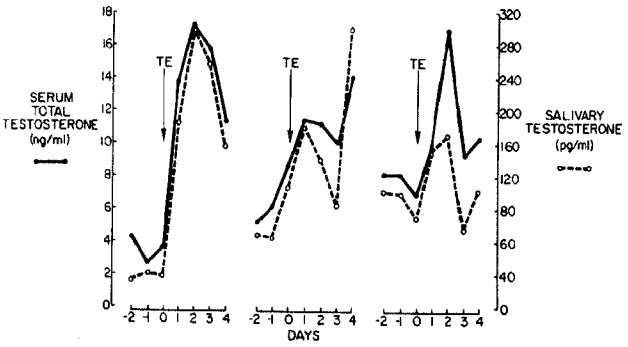

FIG. 5. Daily serum T (●—●; nanograms per ml) and salivary T (○- -○; nanograms per ml) before and after the administration of T enanthate (200 mg, im; TE) to three normal male subjects.

after simple extraction circumvents the necessity of using the tedious and indirect methods described previously for the measurement of free T in a variety of physiological states when the SSBG-binding capacity may vary (19, 20).

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