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Androstenedione
dehydroepiandrosterone
sexual development
testosterone

Conjugated and Unconjugated Plasma Androgens in Normal Children

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Extract

Methods developed in this laboratory permit measurement of the androgens, testosterone (T), dehydroepiandrosterone (D), and androstenedione (Δ) on a 10-ml sample of plasma. We have determined concentrations of the unconjugated androgens (T, Δ, D) as well as of the sulfates of dehydroepiandrosterone (DS) and androsterone (AS) in the plasma of 85 healthy children of both sexes from birth through the age of 20 years. Our results are shown and summarized, along with those of other investigators.

Sex and age	Mean plasma concentrations				
	ng/100 ml			μg/100 ml	
	T	Δ	D	AS	DS
Male					
Neonates	39	24	30	None detectable	None detectable
1–8 years	25	58	64	2	11
9–20 years	231	138	237	28	74
Female					
Neonates	36	30	83	None detectable	None detectable
1–8 years	11	69	54	5	20
9–20 years	28	84	164	22	44

Testosterone was elevated in both sexes in the newborn as compared with the 1–8-year-old group. In contrast, sulfated androgens, with one exception, were undetectable early in life. In males, there was a marked rise in all androgens, especially T, in the 9–18-year-old group. The increase in plasma androgens occurred before clinical manifestations of increased androgens became evident. In females, there was only a modest increase in plasma androgens in the 9–18-year-old group except in the case of Δ which did not change. The greatest increases were in D, DS and AS. This is the first report of unconjugated androgen concentrations in the adolescent female.

Speculation

Measurement of the plasma androgens will aid in evaluation of children with sexual infantilism or sexual precocity. The prepubertal rise in plasma testosterone in males may be accompanied by a higher level of plasma protein binding than that which is found after puberty becomes evident.

Introduction

Until recently evaluation of androgen secretion has been limited to physical examination and measurement of 24-hr excretion in urine of 17-ketosteroids, the metabolites principally of the weaker androgens. Other procedures not yet in routine general use, which are somewhat more specific, involve the measurement of specific steroids in 24-hr urine specimens. Methods developed in this laboratory [19, 21] permit measurement of the androgens, testosterone (T), dehydroepiandrosterone (D), and androstenedione (Δ) on a 10-ml sample of plasma. We have determined concentrations of the unconjugated androgens (T, Δ, D) as well as of the sulfates of dehydroepiandrosterone (DS) and androsterone (AS) in the plasma of 85 healthy children of both sexes from birth through the age of 20 years since we anticipate that the measurement of these substances will aid in the evaluation of children with sexual infantalism or sexual precocity.

Materials and Methods

Unconjugated Androgen Determination

The double isotope derivative method, using ^{14}C as the reference isotope and ^{82}Br as the measuring isotope first described by Slaunwhite and Neely [21] and its application to androgens as described by Saroff *et al.* [19], was used.

Epitestosterone, if present, is separated by chromatography before labeling with ^{82}Br and would, therefore, not be detected. The specific activity of the brominated derivative varied between 400 cpm/mμg and 2500 cpm/mμg with blank values of about 50 cpm. This procedure would thus allow quantitation down to 1 ng/100 ml plasma inasmuch as recoveries averaged about 20% overall.

Collection of Plasma Samples

Approximately 20 ml whole blood were drawn from each subject into heparinized tubes. The plasma was separated and frozen until analysis. In some cases in which only small amounts of whole blood could be obtained, as in the 1–5-day-old children, plasma samples were pooled prior to analysis. The subjects or their parents, or both, were informed of the purpose of the project and consent was obtained for withdrawing blood samples for analysis.

Conjugated Androgen Determination

A tritium-labeled internal standard of DS (10 nCi) was added to the methylene chloride-extracted aqueous residue of 5 ml plasma which was adjusted to pH 1.0 with 65% sulfuric acid. After dissolving 70% w/v ammonium sulfate in the plasma, the solution was extracted twice with 2 vol tetrahydrofuran (THF), which had been redistilled from potassium hydroxide pellets. After addition of 0.2 ml 70% perchloric acid, the solution was allowed to incubate at 50° for 3 hr. Three milliliters water were then added, and the solution was extracted with 20 ml acid-washed and redistilled heptane. The aqueous layer was discarded, as were the subsequent washes of 8 ml 10% sodium hydroxide and 8 ml water.

The heptane-soluble residue, dissolved in 6 ml 70% methanol, was washed three times with 9-ml aliquots of heptane. After the addition of 6 ml water, the aqueous methanol was extracted twice with 15 ml benzene which had been freshly purified by sulfuric acid extraction and redistillation. The residue of the benzene extract was dissolved in 1.0 ml benzene; 0.1 ml of this solution was counted in a liquid-scintillation spectrometer to determine recovery and 0.5 ml was evaporated for gas chromatography using flame ionization detection.

Pregnenolone (1250 ng) was added as an internal standard. To the residue was added 25 μliters of a solution of *N*,O-bis-(trimethylsilyl)acetamide (BSA) in acetonitrile [20, 21]. After more than 5 min at room temperature, 1 μliter of this solution was injected into a ¼-inch × 6-foot glass column containing 1% OV-1 Chromosorb G (100–120 mesh). At 200° the retention times were as follows: D, 2.2 min; androsterone (AN) and etiocholanolone, 2.8 min; and pregnenolone (P), 4.5 min. The ratio of peak heights (D/P = r or AN/P = r') was calculated, and the mass of D or AN was read from calibration curves (y (ng) = 26.3 r ± 2.1 y (ng) = 29.4 r' + 1.8). The androsterone peak was approximately 1.9 cm/10 ng and the dehydroepiandrosterone peak was 2.4 cm/10 ng. Therefore, assuming a 70% overall recovery and the limit of detection of peak height of 0.5 cm, the lowest plasma concentration accurately measurable using this method would be 4 μg/100 ml for AS and 3 μg/100 ml for DS. Concurrently, blank samples showed no peaks in the areas mentioned nor were any peaks seen in samples reported as ND (none detectable).

The present method for the quantitation of DS and AS in plasma is simpler than existing procedures [9, 15, 17, 18, 20, 23] because of exclusion of one or more chromatographic steps prior to gas chromatography. Adequate resolution from interfering material was obtained in nearly every instance *provided* the solvents

were purified as described. Because OV-1 does not resolve androsterone and etiocholanolone, the AS values will include any etiocholanolone sulfate. The latter is usually negligible in adults, but has not been determined in children. Isoandrosterone ($3\beta,5\alpha$), if present, would also be measured as AS.

Statistical Analysis of Data

The data obtained (Tables I and II) show, in many cases, an extremely skewed distribution with the maximum frequency often falling in the 0–25 ng/100 ml area and the rest of the values strung out over a very wide range. In such circumstances, when there is no gaussian distribution, conventional statistical parameters, such as standard deviation and standard error of the mean, lose their meaning. Even the calculation of "mean" values is misleading, because one value may unduly influence the mean. For example, one determination of T (139 ng/100 ml; see Table I) is responsible for more than 50% of the mean T value (Table III) of the 1–8-year-old male group. We can find no clinical or procedural reason for eliminating such "far-out" values. It is interesting that the females are less prone to such extreme values. Inasmuch as unpublished data on normal adult males obtained in this laboratory indicate that androgen concentrations may vary by a factor of 5 to 10 at different times of the day, but without following a regular circadian rhythm, the collation of data from many individuals will result in a realistic pattern of plasma concentrations.

One useful artifice for obtaining a more representative set of values simply excludes the "tails" of the distribution; it is readily applicable to skewed distributions. Thus, we have reported the median 50% range. We believe that this is more significant than the mean because extreme values, for whatever cause, have been excluded. For example, in 6 of 20 sets of values, the mean is greater than the median 50% range (Table III) and in several others, it lies close to the upper limit of the median 50% range.

Results

Our results are shown in Tables I and II and are summarized, along with those of other investigators, in Table III and Figure 1. Testosterone was elevated in both sexes in the newborn as compared with the 1–8-year-old group. In contrast, sulfated androgens, with one exception, were undetectable early in life.

In males, there was a marked rise in all androgens, especially T, in the 9–18-year-old group. The increase in plasma androgens occurred before clinical manifestations of increased androgens became evident. This, however, was not always true, as some of the older teenagers had low values for one or more of the hor-

Table I. Individual plasma androgen concentrations in male children[1]

Age, yr	T, ng/100 ml	Δ, ng/100 ml	D, ng/100 ml	AS, μg/100 ml	DS, μg/100 ml
2 days[2]	ND[3]	22	ND	ND	125
3 days[2]	98	25	28	ND	ND
5 days[2]	19	25	62		
1	ND	2	ND	ND	ND
1.5	62	55	287		
4	139	15	ND	ND	18
5	16	76	21	4	21
6	38	18	ND	8	23
6	ND	339	ND	ND	16
7	15	21	62	5	14
7	ND	20	ND	ND	ND
7	ND	9	246	ND	ND
8	ND	16	61	4	18
8	ND	70	28	ND	ND
9	32	430	1790		
9	178	203	7		
9	29	ND	40		
10		52	10		
10	55		2014		
11	293	40	ND	ND	ND
12	276	83	ND	ND	ND
12	1078	226	166	60	66
12	ND	256	56	ND	29
12	27	176	564	11	80
13	87	98	159	123	228
13	902	ND	113	34	17
13	82	44	61	81	105
13	ND	21	18		
13	245	197	217		
14	16	ND	127	44	78
14	636	78	244	ND	74
14	441	54	31	ND	115
14	202	ND			
15	235	25	318	29	67
16	394	88	268	10	74
16	350	138	524		
16	ND	61	273		
17		44	19	20	58
18	111	ND	199	39	242
19		15	27	30	57
19	8	19	104		
20	102	1250	56		

[1] T: testosterone. Δ: androstenedione. D: dehydroepiandrosterone. AS: androsterone sulfate. DS: dehydroepiandrosterone sulfate.

[2] A pool of several plasmas.

[3] ND: none detectable.

mones. These subjects had normal sexual development.

In females, there was only a modest increase in plasma androgens in the 9–18-year-old group except in the case of Δ which did not change. The greatest increases were in D, DS and AS. The increase in testosterone is reflected in an extension of the upper limit of the 50% median range, whereas the lower limit remained at zero (ND).

Table II. Individual plasma androgen concentrations in female children[1]

Age, yr	T, ng/100 ml	Δ, ng/100 ml	D, ng/100 ml	AS, μg/100 ml	DS, μg/100 ml
1 day[2]		19	70		
1 day[2]	40	48	31	ND[3]	ND
5 days[2]	38	47	91	ND	ND
5 days[2]	29	4	141		
2	55	157	137	ND	ND
3		77	5	ND	ND
4	10	ND	67	ND	ND
4	ND	2	ND	<4	16
5	25	ND	ND	ND	46
6	ND	192	ND	5	6
6	ND	15	ND	ND	27
6	24	46	226		
7	15	43	106	47	102
7	ND	1	ND	ND	ND
7	ND	ND	117	ND	ND
8	ND	348	38	ND	30
8	3	18	2	<4	11
9	ND	275	37	75	ND
9	ND	64	15	15	10
10	8	102	ND	10	26
10	ND	44	88	ND	43
11	ND	ND	228	ND	39
11		47	53	48	74
12	29	7	85	ND	47
12	2	4	104	28	68
12	ND	71	37	ND	7
13	ND	534	146	ND	ND
13	22	36	78	50	106
13		46	77	67	103
13	23	35	130		
13	17	54	56		
14	ND	61	75	ND	11
14	ND	51	34	ND	27
14	11	63	858		
14	43	147	738		
15	39	ND	153	ND	32
15	252	ND	131	ND	ND
15		57	115	61	97
15		45	22	20	25
16	ND	369	97	6	5
16	57	65	487		
17	ND	ND	145	48	95
18	111	ND	271	39	115

[1] T: testosterone. Δ: androstenedione. D: dehydroepiandrosterone. AS: androsterone sulfate. DS: dehydroepiandrosterone sulfate.

[2] A pool of several plasmas.

[3] ND: none detectable.

Discussion

The primary sources of the androgens in the adult are the gonads and the adrenal cortex. In the male, T is principally of testicular origin, Δ is secreted by both the testis and the adrenal, and D and DS are products of the adrenal cortex [2, 11]. The female adrenal secretes the same steroids as the male adrenal, but the ovary secretes mostly Δ and a small amount of T [6]; however, approximately 60% of the T in female plasma is the result of peripheral conversion of Δ [7]. These relations are summarized in Table IV.

The production rates of T and Δ in an adult female are 1.5 and 8.1 mg/day, respectively, whereas the corresponding values in a male are 6.2 and 7.4 mg/day, respectively [7]. Production rates of D are 7.4 and 6.6 mg/day for females and males, respectively, whereas the DS rate was 6.9 mg/day for both [8].

The steroidal androgens vary widely in biologic activity, with T being at least 5–10 times more potent than any other [3]. Conjugated androgens have not been tested but, by analogy with estrone sulfate (Premarin), are presumably active since the sulfate group is readily hydrolyzed *in vivo*.

As a group, the androgens are excreted as 17-ketosteroids. Should a specific steroid, such as testosterone, be produced in a female at a rate comparable to a male, the physiologic effect would be readily apparent but the 17-ketosteroid level in the urine would not be markedly changed. Even in a male, a doubling or tripling of the testerone production rate would not alter the 17-ketosteroid output in the urine to a level outside of the normal range. The much smaller body mass and lower levels of all hormones in children would greatly accentuate the effect of abnormal hormone production. Therefore, the determination of plasma androgen concentrations in normal children is a prerequisite to its use in differential diagnosis of children suffering from apparent endocrinopathies.

We realize that the discussion of androgen sources and production rates is based on data obtained only from adult subjects. The dearth of information from younger subjects forces a certain degree of extrapolation. Whether the gonads of prepubertal children are

Table III. Plasma androgen concentrations[1]

Age, yr	No.	T, ng/100 ml	Δ, ng/100 ml	D, ng/100 ml	AS, μg/100 ml	DS, μg/100 ml	Reference
Males							
<1 day	5	53 (27–69)	138 (105–149)	243 (121–345)			Rivarola *et al.* [13]
<1 day	5	43 (34–56)	113 (101–130)				Mizuno *et al.* [10]
1–5 days	7	100 (58–106)	177 (54–236)				Mizuno *et al.* [10]
1–5 days	3	39	24	30	ND[2]	ND	This paper
2–8	10	25 (19–27)				5 (2–11)	Saez and Bertrand [16]
5–8	4			101 (96–104)			Saez and Bertrand [16]
4–8	14, 13	40 (20–59)	63 (29–70)				Frasier *et al.* [4]
2–8	16	16 (5–17)	56 (38–63)	48 (24–79)			Gandy and Peterson [5]
1–8	11	25 (0–16)	58 (16–55)	64 (0–61)	2 (0–4)	11 (0–18)	This paper
9–15	33, 23	194 (52–275)	63 (30–85)				Frasier *et al.* [4]
9–14	4–6	44 (35–70)		177 (145–206)		64 (61–70)	Saez and Bertrand [16]
8–18	18, 21				23 (4–19)	39 (8–64)	Rosenfield and Eberlein [14]
12–18	5	251 (18–234)					Rosenfield and Eberlein [14]
9–15	18–21	199 (48–228)	84 (37–128)	203 (79–229)			Gandy and Peterson [5]
9–20	25	231 (32–293)	138 (21–138)	237 (31–244)	28 (0–39)	74 (57–80)	This paper
Females							
<1 day	4	39 (37–46)	112 (81–145)	153 (145–191)			Rivarola *et al.* [13]
<1 day	5	57 (48–72)	81 (62–100)				Mizuno *et al.* [10]
2–5 days	4	30 (22–36)	8 (0–34)				Mizuno *et al.* [10]
1–5 days	4	36 (38–40)	30 (19–47)	83 (70–91)	ND	ND	This paper
3–9	8	19 (7–30)	30 (26–38)				Frasier *et al.* [4]
4–9	8	8 (3–12)	64 (48–78)	68 (59–69)			Gandy and Peterson [5]
1–8	12	11 (0–15)	69 (2–97)	54 (0–106)	5 (0–2)	20 (0–27)	This paper
9–16	8, 9				34 (7–60)	47 (6–83)	Rosenfield and Eberlein [14]
9–18	26	28 (0–29)	84 (35–65)	164 (56–146)	22 (0–48)	44 (11–74)	This paper

T: testerone. Δ: androstenedione. D: dehydroepiandrosterone. AS: androsterone sulfate.
DS: dehydroepiandrosterone sulfate. The mean of all values and, in parentheses, the median 50% range are shown.
ND: none detectable.

truly quiescent with regard to steroid metabolism is a subject still to be investigated.

Because of the difficulty in obtaining sizable blood samples from newborn infants, few values have been reported. Mizuno *et al.* [10] studied 11 babies and we have studied 7, all pools from many persons. The former study was not performed on normal babies, but on those about to undergo exchange transfusion for blood type incompatibility. More studies have been performed on umbilical cord blood (Rivarola *et al.* [13], Mizuno *et al.* [10]). All three androgens appear to be elevated in both sexes (relative to the 1–8-year-old group) when cord blood is examined.

Resko *et al.* [12] have shown that in rats there is a parallel to the phenomenon of high androgen concentrations in babies. They showed that the concentration of T in 1-day-old male rats was 10 ng/100 ml which dropped to 3 ng/100 ml at 10 days and then increased gradually to the adult level (a maximum of 53 ng/100 ml in their series). No Δ, however, was detectable until 30 days of age.

In the 1–8-year-old range, our values for androgens are in excellent agreement with those of Gandy and Peterson [5], but lower for T than those of Saez and Bertrand [16] and of Frasier *et al.* [4]. Our DS values in males, however, are in agreement with those reported by Saez and Bertrand [16]. All three unconjugated androgens are apparently lower in the 1–8-year-old age group than in either the neonatal or older children, whereas the conjugated androgens, which were undetectable in the neonatal group, begin to appear sporadically.

As puberty approaches, androgen concentrations in all categories increase dramatically, but not necessarily evenly, in male plasma about 2 years before clinical manifestations become evident. In the case of the females, the principal increase occurs in the conjugated androgens with only a small (and perhaps questionable) increase in T and D. From Table III it can be seen that there is general agreement on the values for the 9–18-year-old males [4, 5, 14, 16] except for the T values of Saez and Bertrand [16]. Apparently ours is the first report of unconjugated androgen concentrations in the adolescent female.

The 9–18-year-old males exhibited quite wide ranges of T and D (Fig. 1, Table III). We have observed the same phenomenon with adult males. For example, in 37 determinations of T on 10 men, a range of 20–795

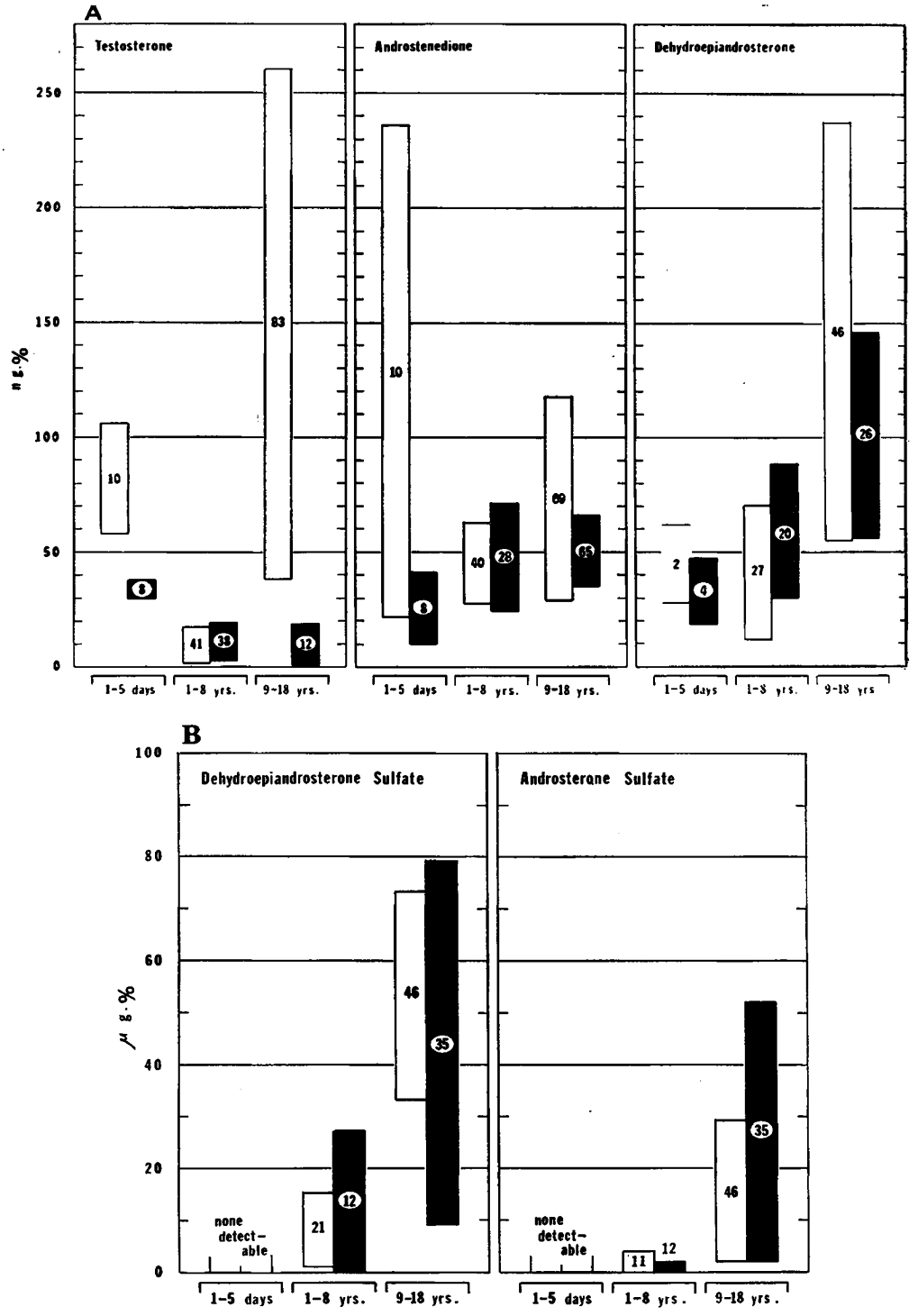

Fig. 1. A and *B:* best estimates of median 50% range of plasma androgen concentrations in children. The values were obtained by averaging most of the values shown in Table III, omitting only those in which the results of one investigator were obviously different from those of two or more investigators. Where only two investigators disagreed, both results are included; this leads to an unrealistically large range in the case of androstenedione for 1–5-day-old boys. The numbers within the bars are the number of determinations falling in that range. The open bars represent male values, the solid bars female values.

Table IV. Summary of relative direct contribution of steroid-producing organs to plasma concentration of hormone

Hormone	Organ		
	Adrenal Cortex	Ovary	Testes
Testosterone	?	+	+++
Androstenedione	+	++	+
Dehydroepiandrosterone	+++		
Dehydroepiandrosterone sulfate	+++		+

ng/100 ml, with a mean of 175 ng/100 ml and a 50% median range of 70–195 ng/100 ml, was found [1]. Similarly, Steeno *et al.* [22] have reported wide individual variations in urinary D in 11–15-year-old boys.

Upon analysis of duplicate aliquots of a plasma sample, the range on the same day is within 5% of the mean value. When additional duplicates were run on subsequent weeks, the range was within 20% of the mean. As with any sophisticated microanalytic procedure, occasionally a grossly erroneous value was found and, when this was obvious, the numbers thus obtained were disregarded. Some obvious signs of error would be extremely high blank values, low recoveries of internal standard, inappropriate specific activity values for the standards, or, as once occurred, very high bromine count rates which were traced to contamination by the plasticizer from some plastic tubing which had been used in that particular assay.

Yen *et al.* [24] correlated testosterone and testosterone binding affinity (TBA) levels in the plasma of boys from the ages of 8 through 14 years and showed that, although the testosterone concentrations increased, the TBA decreased; thus, perhaps the lag time between initial testosterone increase and obvious puberty could be related to the amount of unbound testosterone.

In summary, both male and female neonatal humans exhibit substantial concentrations of androgens which decline with age. Sexual maturation in the male is preceded by a dramatic rise in the testosterone concentration and real, but less dramatic, increases in the other steroids. In the female, it is primarily the conjugated androgens which increase concomitantly with sexual maturation. Wide ranges of individual values preclude using a single androgen concentration value in making diagnosis; as an adjuvant to other data, however, such information can be of value.

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