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Plasma concentrations of testosterone, dihydrotestosterone, Δ_4-androstenedione, dehydroepiandrosterone and oestradiol-17β in the crab-eating monkey (*Macaca fascicularis*) from birth to adulthood

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Summary. Plasma testosterone, 5α-dihydrotestosterone (DHT), Δ_4-androstenedione, dehydroepiandrosterone (DHA) and oestradiol-17β concentrations of crab-eating macaques after birth were analysed by RIA.

The profiles of plasma testosterone and DHT exhibited four phases: (1) a neonatal phase (0 to 3–4 months of age) with considerable synthetic testicular activity; (2) a phase of 'infancy' (generally up to 29 months of age) during which the values of both androgens were low; (3) a prepubertal phase (generally up to 43 months of age) when circulating values oscillated with wider individual variations, and (4) a pubertal phase when the concentrations increased in parallel and concomittantly with the onset of meiosis and the establishment of spermatogenesis. The testosterone values continued to increase, reaching adult values at about 5–6 years of age, whereas DHT levels tended to stabilize from 4–5 years. Relatively high androstenedione values during the neonatal phase decreased progressively until puberty, then increased again slowly up to the adult stage when they plateaued at about neonatal levels. The DHA levels were high during the first months, decreased at about 1 year, remained stable during infancy and prepuberty and then declined again during puberty. At about 5 years, the values were 28% of those in neonates. There was no evidence of an adrenarche before the first signs of sexual maturity were observed. Oestradiol-17β concentrations were high at birth and until 3 months, then decreased and remained steady from 1 year of age until adulthood, except at the onset of puberty (27–30 months of age) when high values were again noted.

Our results show that, during the neonatal period, the testis exhibited considerable secretory activity.

Introduction

The general pattern of development of steroidogenic activity of the testis from sexual differentiation until puberty is now well documented for many mammals. Nevertheless, several questions remain to be answered about the quantitative differences of the steroids secreted and the temporal relationships between the steroid profiles and testicular activity, especially during the perinatal and prepubertal periods.

In many mammals (rodents, ruminants), puberty begins relatively early, whereas in anthropoids 'infancy' lasts many years. Macaques, occupying an intermediate place, can be used in longitudinal studies in which the steroidogenic and germinal activities of the testis are followed in the same animal from birth to adulthood (Fouquet, Meusy-Dessolle & Dang, 1984). The testis is able to synthesize androgens during the perinatal period. Elevated serum testosterone concentrations are observed at the beginning of postnatal life in several species; these levels are significantly higher in males than in females and are abolished when the males are castrated at birth. The main aim of the present study was to confirm such a synthesis in crab-eating monkey males by extending the work to include four androgens (testosterone, dihydrotestosterone, Δ_4-androstenedione and dehydroepiandrosterone) and one oestrogen (oestradiol-17β) found in the peripheral circulation.

The second objective was to analyse the changing patterns of these 5 steroid hormones at the onset of puberty, at meiosis initiation, during the establishment of spermatogenesis, and up to adulthood. Such a study has not yet been carried out for these monkeys. Furthermore, we wished to ascertain whether there was an 'adrenarche' before or just after puberty, as described for several other mammalian species (Cutler *et al.*, 1978; Castracane, Cutler & Loriaux, 1981) and in man (Dhom, 1973; Collu & Ducharme, 1975) and, if so, whether it plays an important role in pubertal development (Cutler & Loriaux, 1980; Forest, de Peretti, David & Sempé, 1982).

Our research, done at the same time as histological, morphological and ultrastructural studies of the testicular cell populations in crab-eating monkeys (Fouquet *et al.*, 1984; Dang & Meusy-Dessolle, 1985), attempted to establish a chronology of the testicular endocrine functions to give better understanding of the secretory activity of the pituitary–testicular axis.

Materials and Methods

Animals

We used 99 crab-eating monkey males of known age (except for 2 imported adults), ranging in age from 3 days to more than 6 years. They were raised in the laboratory as described by Dang (1977) in individual cages, except for young infants which lived with their mother until weaning. The photoperiod was that natural in Paris and temperature (24–28°C) and humidity were relatively constant; the animals were fed with monkey chow supplemented with fresh fruits and vegetables. Water was available *ad libitum*.

At the beginning of the study, 8 reproductively adult males and 39 postnatal or juvenile subjects were available. During the next 30 months, there were 52 births, and the colony finally contained 12 reproductively active males, 19 postpubertal males, 25 in a pubertal or prepubertal stage (29–59 months of age), 29 juveniles (4–29 months of age) and 14 neonates (0–4 months of age). Three males were castrated at birth and 8 females were used for comparative neonatal investigations.

Experimental design

Without using any anaesthesia, we collected 1150 blood samples from the macaques, capturing them by hand and then gently placing them supine on a table; the oldest or strongest monkeys were restrained in a holding cage with a movable side. The samples were obtained by venepuncture of the cephalic or basilic arm vein, or sometimes the saphenous vein and collected on heparin. After centrifugation, the plasma was separated, divided, and then stored at −20°C until assay. These samples were always taken between 14:00 and 17:00 h, i.e. before the episodic testosterone secretion in the evening described by Steiner & Bremner (1981) for serum samples of crab-eating macaque infants, juveniles, perinates and adults. The animals were bled once every 4 or 2 weeks during critical physiological periods and also before the surgical interventions required to obtain material for histological and cytological analysis of the testis (Fouquet *et al.*, 1984; Dang & Meusy-Dessolle, 1985).

Steroid hormone assays

Radioimmunoassay for Δ_4-androstenedione, dihydrotestosterone (DHT), testosterone and oestradiol-17β. About 6000 d.p.m. of each radioactive tracer for these four steroids were added in a phosphate buffer (pH 7) to a 1-ml plasma sample. To calculate the percentage of recovery, two aliquants of each steroid solution were taken at the same time for counting. After extraction with 7 ml diethyl ether (Gifrer, anaesthesic grade), the solvent was drawn off and dried at 40°C under a filtered air-stream. The dry steroid residues were redissolved in 1 ml iso-octane and transferred onto chromatographic columns.

Pipettes (5 ml) containing about 1 g chromatolithe A (bioMérieux Laboratories, Charbonnières les Bains, France) impregnated with ethyleneglycol–propyleneglycol (50 : 50 v/v) as the stationary phase were used for the chromatographic separation of the 4 steroids above. The column was saturated with 3 ml iso-octane. After the sample was set down, the column was first eluted with pure iso-octane (5·5 ml) to separate the Δ_4-androstenedione and then with iso-octane–ethylacetate mixtures with increasing polarity for the other 3 steroids (7 ml of a 94 : 6 (v/v) mixture for DHT, 7 ml of an 80 : 20 (v/v) mixture for testosterone and 6 ml of a 60 : 40 (v/v) mixture for oestradiol-17β). Each elution was followed by washing with 1 ml of the last elution mixture.

The eluted fractions obtained with this originally adapted separation procedure were dried down at 40°C and the residues redissolved in mono-disodium phosphate buffer, pH 7: 200 µl of each solution were used to calculate recovery and duplicate aliquants of 100 µl were kept for RIA.

Each RIA was carried out by the addition of 100 µl of the appropriate antiserum dissolved in phosphate buffer and about 20 000 d.p.m. of the radioactive tracer ([1,2,6,7-^{3}H]androstenedione, sp. act. 109 Ci/mmol; [1,2,4,5,6,7-^{3}H]DHT, sp. act. 56 Ci/mmol; [1,2,6,7-^{3}H]testosterone, sp. act. 94 Ci/mmol; [6,7-^{3}H]oestradiol-17β, sp. act. 50 Ci/mmol) obtained from the Radiochemical Centre, Amersham, U.K. The standard curves were plotted from solutions containing 100 µg steroid/ml in absolute ethanol. After incubation for 3 h at +4°C, the free steroid fraction was separated from the bound fraction by adding 1 ml of a mixture of 250 mg charcoal and 25 mg dextran into 100 ml buffer. After 15 min at +4°C for equilibration, the samples were centrifuged at 3000 ***g*** for 10 min and the supernatant layers decanted directly into scintillation vials. Radioactivity was measured in the Scintillator 299 Packard System with a Packard liquid scintillation tri-carb spectrometer (Model 3390); tritium efficiency was about 20%.

A water blank and a standard plasma from an ovariectomized crab-eating monkey female were assayed with each batch of unknowns. Human plasma pools at three hormonal levels (Steriatrols A, B and C from bioMérieux Laboratories) were also simultaneously treated in the Δ_4-androstenedione, testosterone and oestradiol-17β assays.

Antiserum specificity. Androstenedione was measured by RIA using rabbit antiserum raised against Δ_4-androstenedione 6β-hemisuccinyl-BSA (Steranti, St Albans, U.K.). The cross-reactivity of the antiserum with other compounds was 30·8% with 5α-androstanedione, 6·8% with testosterone, 0·76% with 5α-DHT, 0·02% with DHA and <0·001% with androsterone, oestradiol-17β, Δ_5-androstene-3β,17-diol, oestrone, oestriol, progesterone, cortisol and corticosterone.

The antiserum used for the RIA of the DHT was raised in rabbits immunized against 5α-dihydrotestosterone-3-(CMO)–BSA (Steranti). The cross-reactivity of this antiserum with other compounds was: 50% with testosterone, 5% with 5β-DHT, 4% with 5α-androstane-3β,17β-diol, 1% with Δ_5-androstene-3β,17β-diol, Δ_4-androstenedione, 5α-androstanedione and epitestosterone, 0·5% with DHA, 0·1% with androsterone, <0·05% with oestradiol-17β and -17α, oestrone, oestriol, <0·01% with progesterone and <0·001% with cortisol, corticosterone and desoxycorticosterone.

Anti-testosterone serum was raised against testosterone-3-O-(CMO)–BSA (obtained in our laboratory). Its cross-reactivity with other steroid ligands was: 28·4% with 5α-DHT, 10·8% with 3β,5α-androstanediol, 0·9% with Δ_4-androstenedione, 0·2% with epitestosterone, <0·01% with oestradiol-17β and <0·001% with cortisol and progesterone.

Antiserum to oestradiol-17β was obtained in our laboratory (Dray *et al.*, 1971) by immunization of rabbits against oestradiol-17β-6-O-(CMO)–BSA. It cross-reacted with other compounds as

follows: 12·3% with 6-keto-oestradiol-17β, 5% with 16-epioestriol, 1·3% with 16-keto-oestradiol-17β, 0·9% with oestradiol-17α, 0·7% with oestrone, 0·5% with oestriol and <0·001% with testosterone and progesterone.

The characteristics of the 4 assays are shown in Table 1.

Table 1. Characteristics of the radioimmunoassays for Δ_4-androstenedione (Δ_4), dihydrotestosterone (DHT), testosterone and oestradiol-17β

	Δ_4	DHT	Testosterone	Oestradiol-17β
Recovery (after extraction + chromatography) (%, mean ± s.e.m., $n = 870$)	65·3 ± 0·8	65·0 ± 1·4	72·8 ± 1·3	61·8 ± 1·0
Water blank (ng/ml) (mean ± s.e.m., $n = 6$)	0·009 ± 0·002	0·029 ± 0·007	0·014 ± 0·009	0·007 ± 0·002
Inter-assay precision (% coefficient of variation)	14·0 for 0·57 ng/ml 8·9 for 2·09 ng/ml 8·6 for 5·49 ng/ml	11·8 for 0·22 ng/ml 14·5 for 1·08 ng/ml 8·7 for 1·92 ng/ml	9·9 for 0·42 ng/ml 7·7 for 1·59 ng/ml 6·3 for 6·13 ng/ml	18·5 for 0·21 ng/ml 10·0 for 1·81 ng/ml 6·3 for 4·42 ng/ml
Intra-assay precision (% coefficient of variation)	8·2 for 0·26 ng/ml 5·4 for 2·73 ng/ml	8·7 for 0·41 ng/ml 4·9 for 1·87 ng/ml	12·4 for 0·62 ng/ml 6·5 for 7·6 ng/ml	13·2 for 0·01 ng/ml 10·1 for 0·48 ng/ml
Accuracy (% of mean recovery ± s.e.m.)	103·7 ± 6·1	97·6 ± 4·4	96·3 ± 3·4	101·2 ± 2·5
Sensitivity (pg/tube)	5	5	5	1·6

Measurement of plasma DHA. DHA was measured by RIA using [1,2,6,7-^{3}H]DHA (sp. act. 87 Ci/mmol) and rabbit antiserum to DHA-17-(CMO)–BSA (Steranti). The cross-reactivity of this antiserum with other steroids was: 14·6% with Δ_5-androstane-3β,17β-diol; 3·5% with epiandrosterone; 1·1% with Δ_5-pregnenolone; <0·4% with 5α-androstenediol and androsterone; and <0·5% with testosterone, Δ_4-androstenedione, oestradiol-17β, 5α-androstane-3α,17β-diol, progesterone and cortisol.

Plasma samples (0·5 ml) were extracted with diethyl ether, dried under air, redissolved in a cyclohexane–benzene–ethanol mixture (80 : 15 : 5 by vol.) and extracted as described by Cutler *et al.* (1978).

The assay was performed as described for the above RIAs but without chromatography. Recovery (mean ± s.e.m., $n = 477$) was 72·8 ± 0·7%. Water blank was 0·008 ± 0·003 ng/ml (mean ± s.e.m., $n = 6$). Interassay precision (% coefficient of variation, $n = 6$) was 10·6 for 0·41 ng/ml and 6·9 for 2·12 ng/ml. Intra-assay precision (% coefficient of variation, $n = 8$) was 6·2 for 0·27 ng/ml and 4·8 for 3·60 ng/ml. Accuracy (% of mean recovery ± s.e.m.) was 98·7 ± 3·6 and assay sensitivity was 5 pg/tube.

A 9820 A Hewlett Packard calculator was used to analyse the data; a best-fit straight line in logit-log co-ordinates was obtained for every standard curve. In calculating the unknown values from this equation, a correction was made for the mass of radioactive steroid added to the plasma to account for the procedural loss.

Analysis of the data

Each animal was followed from birth or from its age at the start of study, and age-dependent curves for the 5 hormones assayed were established (longitudinal analysis). All the values obtained from these regularly bled macaques and from other subjects of our colony were plotted on two different types of graphs for a cross-sectional study. One concerned all the sample measurements,

and age-dependent hormone variations were revealed by the most significant polynomial curve (orthogonal polynomials regression by Mlp program: G. J. S. Ross, Rothamsted Experimental Station, U.K.). The other graph was plotted by grouping the values into monthly means (±s.e.m.). The successive monthly means were compared by analysis of variance or by Student's *t* test on the paired differences. Bartlett's test (Snedecor & Cochran, 1971) was used to compare the intramonthly variances.

Results

Testosterone concentrations

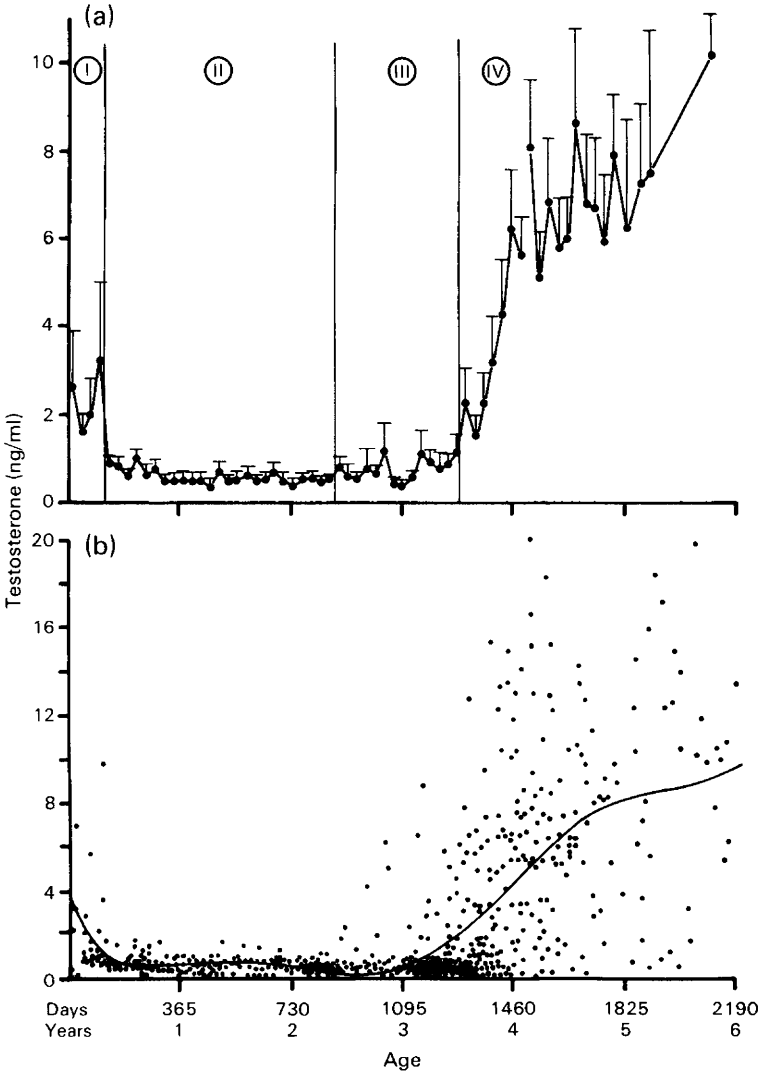
Four phases were distinguished in the changes of testosterone values by analysis of the pattern of the polynomial regression curve and of the variability in individual values (Text-fig. 1): (1) a neonatal phase (from birth to 3–4 months), with mean monthly levels oscillating between 1·6 and 3·2 ng/ml (mean = 2·3 ± 0·6 ng/ml; number of samples (n) = 24; coefficient of variation (CV) = 108%). During the same phase in the castrate, the values were always <0·27 ng/ml; (2) an 'infancy phase' (up to about 29 months) marked by very low levels (0·58 ± 0·03 ng/ml; n = 173; CV = 64%); (3) a prepubertal phase (up to about 43 months) when circulating testosterone oscillated (mean = 1·03 ± 0·11 ng/ml; n = 244) and there were great individual variations (CV = 160%); (4) a pubertal phase (n = 213) when increasing testosterone concentrations accompanied the onset of meiosis and the establishment of spermatogenesis in the testis (Dang & Meusy-Dessolle, 1985). The mean age at the beginning of the testosterone rise was 1315 days, i.e. 43·1 months, but one male showed a progressive and irreversible rise in testosterone level as early as 940 days with signs of the start of spermatogenesis. This macaque was our most precocious subject. One animal exhibited this testosterone rise only at 1510 days (49·5 months). The relatively great variability in the age at which the pubertal phenomena appeared is indicated in Text-fig. 1 by the dispersion of the experimental data. Testosterone concentrations increased to reach high values (mean ~ 7 ng/ml) as the animals achieved puberty (n = 85). The values continued to increase up to adult stage levels; individual values could reach as much as 20 ng/ml, as reported by Dang & Meusy-Dessolle (1981). Moreover, the older the male when the testosterone rise began, the shorter the rise lasted (Text-fig. 2). For example, the testosterone concentrations of Macaques A and B began to rise at about 1100 and 900 days, respectively, but values of 7–8 ng/ml occurred only 300 and 400 days later, while in Macaques C and D the testosterone rise began at about 1350–1400 days but reached adult values (14–19 ng/ml) in less than 2 months.

Dihydrotestosterone concentrations

The profile of plasma DHT concentrations paralleled that of testosterone (Text-fig. 3). High values were detected during the first 4 months (monthly mean: between 1·80 and 0·94 ng/ml); they then levelled off up to 870 days, with values about 0·25 ng/ml with very slight individual variations. More variable values (monthly mean: between 0·19 and 0·69 ng/ml) were then noted up to about 1300 days, which accorded well with the testosterone profile. Plasma DHT concentration further increased until 4·5 years of age, reaching a maximal value of 1·87 ng/ml and then tended to stabilize at around 1·3 ng/ml in adults while testosterone still increased (see Text-fig. 1).

Δ_4-Androstenedione concentrations

Concentrations of this hormone (Text-fig. 4) remained relatively constant, averaging 1 ng/ml between birth and 3 years of age, but showed considerable individual variability; it then decreased slightly but non-significantly ($P > 0·02$) between 3 and 4·5 years of age, again reaching about 1 ng/ml in adults. The androstenedione : testosterone ratio was reversed at about 4 months of age,

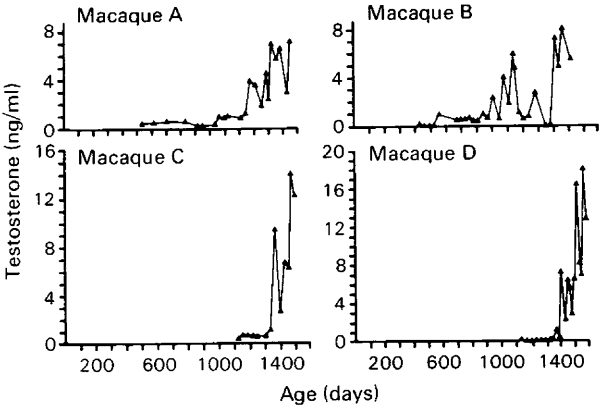


Text-fig. 1. Pattern of postnatal plasma testosterone levels in crab-eating macaques. (a) Monthly means ± s.e.m. values. The number of animals bled for each monthly mean (N) was 5 or 6 for Phase I, 5–10 for Phase II, 7–24 for Phase III, 5–19 for Phase IV. For Phase I *vs* Phase II, $P < 0{\cdot}01$; for Phase II *vs* Phase III, $P < 0{\cdot}01$; for Phase III *vs* Phase IV, $P < 0{\cdot}01$. (b) Individual values. The derivation of the polynomial curve is given by the equation: $y = 1{\cdot}9 - x - 9{\cdot}4x^2 + 12{\cdot}6x^3 - 3{\cdot}4x^4$ with x = age (days)/1000 and y = plasma concentration (ng/ml).

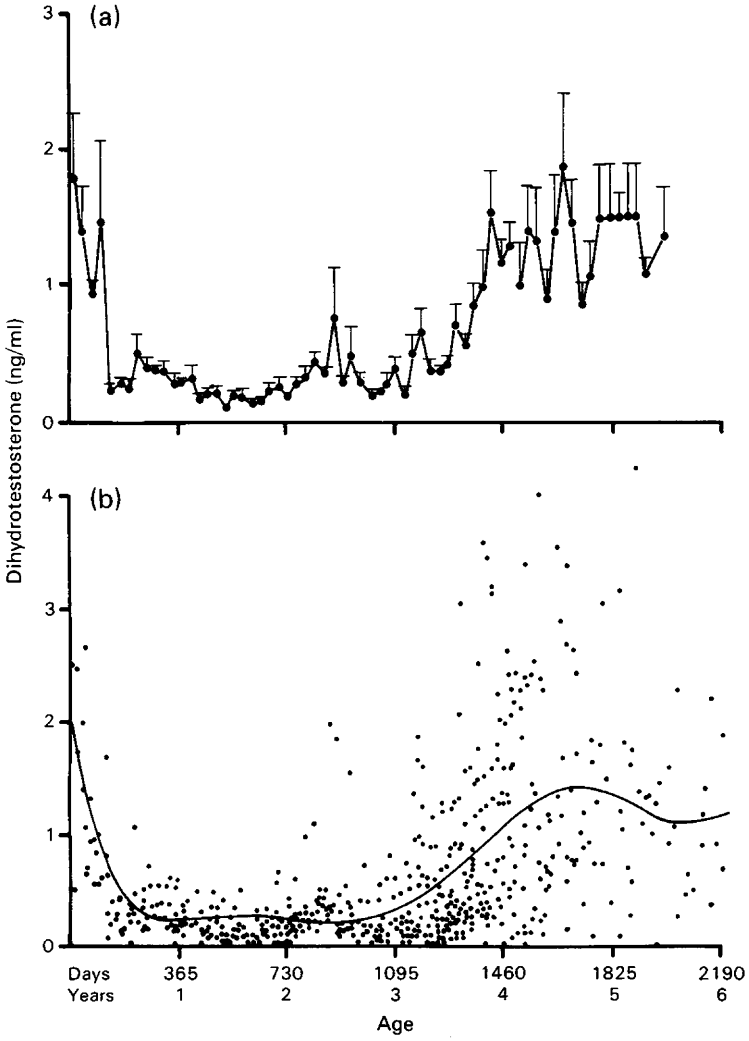
that is at the moment when, after a phase of testicular androgen synthesis, the animal entered relatively silent 'childhood'. The ratio was reversed again at about 1315 days (43 months), the mean age at which the pubertal plasma testosterone rise began.

Dehydroepiandrosterone concentrations

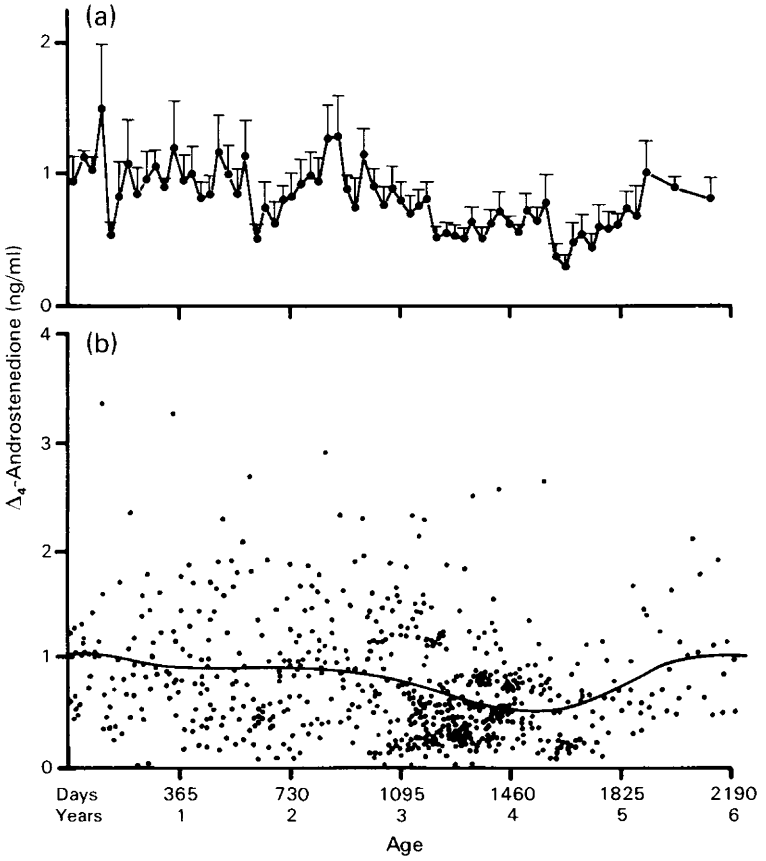
Changes of plasma DHA (Text-fig. 5) showed that after a period from birth to about 3 years of age, during which values remained constant at about 10–15 ng/ml, a clear and progressive decrease occurred during puberty, the adult values ranging between 3 and 4 ng/ml.



Text-fig. 2. Patterns of plasma testosterone increase in 4 macaques during the pubertal period. Macaques A and B showed a precocious testosterone rise, Macaques C and D showed a later rise.



Text-fig. 3. Profile of changes in postnatal plasma DHT concentrations: (a) monthly means ± s.e.m.; (b) individual values. The derivation of the polynomial curve is given by the equation: $y = 1{\cdot}3 - 4x + 3{\cdot}9x^2 - 0{\cdot}8x^3$ with x = age (days)/1000 and y = plasma concentration (ng/ml).



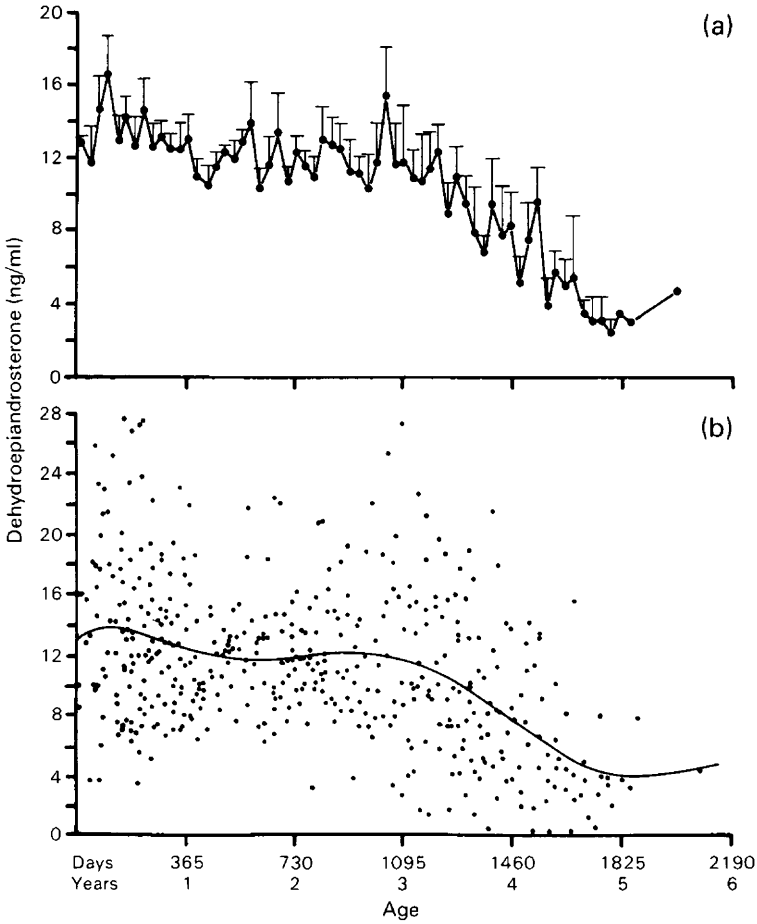
Text-fig. 4. Androstenedione profiles during postnatal life: (a) monthly means ± s.e.m.; (b) individual values. The derivation of the polynomial curve is given by the equation: $y = 1{\cdot}1 - 1{\cdot}4x + 3{\cdot}3x^2 - 3{\cdot}1x^3 + 0{\cdot}9x^4$ with x = age (days)/1000 and y = plasma concentration (ng/ml).

Oestradiol-17β concentrations

The pattern of circulating oestradiol concentrations (Text-fig. 6) showed high values from the beginning of postnatal life until about 6 months of age, when they declined. Except during the first part of prepuberty at about 27–30 months of age, when values could be high, these levels then remained constant from 1 year until adulthood, with mean monthly levels oscillating between 40 and 100 pg/ml.

Discussion

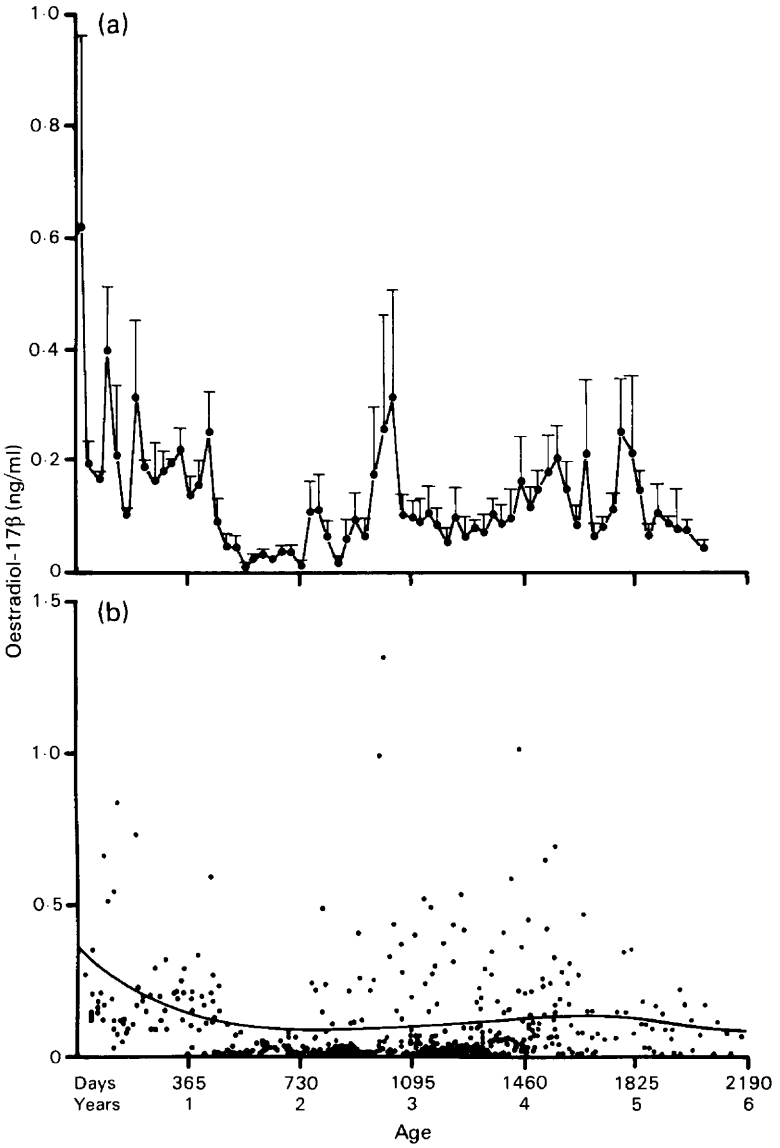
In the postnatal male crab-eating monkey (*M. fascicularis*), the pattern of plasma testosterone concentrations was composed of four phases, corresponding closely to those distinguished in an histological study that included the morphometric analysis of testicular cell populations (Fouquet *et al.*, 1984). The individual values of plasma testosterone and the Leydig cell population in that study showed a positive and significant correlation with the total volume and number of Leydig cells. The correlation with variations in cell volume was even more significant. There was a good parallel between variations in smooth endoplasmic reticulum (SER) and fluctuations in the plasma



Text-fig. 5. Profile of changes in plasma dehydroepiandrosterone concentrations: (a) monthly means ± s.e.m.; (b) individual values. The derivation of the polynomial curve is given by the equation: $y = 12{\cdot}7 + 2{\cdot}5x - 4{\cdot}1x^2$ with x = age (days)/1000 and y = plasma concentration (ng/ml).

testosterone level from birth to puberty, individual testosterone values being significantly correlated with the volume density and the absolute volume of SER. There was no significant correlation between testosterone and the volume density of mitochondria and lipids at any time during development, except a negative and significant correlation between Leydig cell lipids and testosterone from the onset of spermiogenesis to spermiation.

Elevated serum testosterone concentrations, observed at the beginning of postnatal life, have indicated early testicular activity in males of several species (guinea-pig: Rigaudière, Pelardy, Robert & Delost, 1976; rat: Döhler & Wuttke, 1975; boar: Meusy-Dessolle, 1975; Colenbrander, de Jong & Wensing, 1978; pig-tailed macaque: Robinson & Bridson, 1978; rhesus monkey: Robinson & Bridson, 1978; Huhtaniemi *et al.*, 1979a; Huhtaniemi, Korenbrot, Lautala & Jaffe, 1979b; Frawley & Neill, 1979; crab-eating macaque: Steiner & Bremner, 1981; man: Forest, Cathiard & Bertrand, 1973; Faiman, Reyes & Winter, 1974; Forest, Sizonenko, Cathiard & Bertrand, 1974; Forest & Cathiard, 1975; Forest, de Peretti & Bertrand, 1976; Forest, Lecoq, Salle & Bertrand, 1981; Bidlingmaier, Dörr, Eisenmenger, Kühnle & Knorr, 1983).



Text-fig. 6. Profile of changes in circulating oestradiol-17β concentrations during postnatal life: (a) monthly means ± s.e.m.; (b) individual values. The derivation of the polynomial curve is given by the equation: $y = 0{\cdot}4 - x + x^2 - 0{\cdot}3x^3$ with x = age (days)/1000 and y = plasma concentration (ng/ml).

In the present study of crab-eating macaques, the testosterone concentrations, which were high in intact males during the first 3–4 months of postnatal life, were significantly higher than in castrates or in females. Since there was no evidence of an increase in adrenal steroidogenesis during this period, it seems likely that the high testosterone values were the result of testicular steroidogenic activity. Huhtaniemi *et al.* (1979b) have shown that basal testosterone concentrations reach maximal values in rhesus monkeys between 2 weeks and 3 months of age. During that period, GnRH administration stimulated an increase in both the sensitivity and the levels of circulating testosterone. Huhtaniemi *et al.* (1979b) also demonstrated that, during the second half of gestation, the pituitary–testicular axis was responsive to hypothalamic stimulation, but that this axis was less

sensitive to GnRH *in utero* than immediately after birth. The response increased temporarily for 3 months, then gradually disappeared; this decline was a major factor in the low activity of the prepubertal testis. Bercu *et al.* (1983a, b) have demonstrated pulses of testosterone in rhesus and crab-eating monkeys. They report 'macropulses' with amplitudes as high as 3 ng/ml in young postnatal animals, 'micropulses' during infancy, daily pulses during the prepubertal phase which increase in number and amplitude and, lastly, in the pubertal and postpubertal periods, 'macropulses' of great amplitude, often reaching values of 12 ng/ml. These findings, together with the inevitable individual variability, could explain the dispersion of the experimental points on the curve of our study (Text-fig. 1), especially in the neonatal and pubertal periods. Steiner & Bremner (1981) observed that serum testosterone concentration in infant, juvenile, prepubertal and adult crab-eating macaques was episodic, pulses occurring only in the evening between 19:00 and 23:00 h, i.e. after the bleeding period in the present study.

A comparison of testosterone and DHT patterns shows that their curves are almost parallel, except at the end of the pubertal phase when DHT tended to stabilize at about 1·5 ng/ml from 4·5 years of age, whereas testosterone continued to increase up to adult levels. Testosterone appeared to be metabolized into DHT at a relatively constant rate from birth to puberty and, after that, this rate slows down. It would be necessary to assay 5α-reductase and 3α-hydroxysteroid dehydrogenase to determine whether there are wide variations (1) in the activation of this metabolization and (2) in the inactivation of DHT through its conversion into androstanediol when puberty occurs.

The mechanism responsible for the advent of puberty is still unknown. Some authors have advanced the hypothesis that adrenal steroids may be involved in the onset of puberty (Gorski & Lawton, 1973; Rappaport *et al.*, 1974). Castracane *et al.* (1981), studying several primate species, have shown that, as in humans, there is a marked increase of plasma androstenedione and DHA in chimpanzees 2 years before testosterone elevation that corresponds to the true definition of the adrenarche. On the other hand, plasma androstenedione values in baboons decline progressively during the first 3 years and increase between 3 and 4 years when gonadal maturation begins (increase of testicular volume, plasma testosterone and copulatory behaviour). In baboons, DHA concentration is high from birth to adulthood, exceeding 7 ng/ml at all ages. Rhesus monkeys exhibit high levels of androstenedione from birth to sexual maturity; DHA levels are relatively high during the first 6 months of life and then decrease and stabilize until puberty is achieved. The patterns are similar in pig-tailed macaques (*M. nemestrina*), but there is a significant rise of DHA and its sulphated form after puberty, as in dogs and rabbits (Cutler *et al.*, 1978), indicating a possible delayed adrenarche. Our results, which are close to those of Castracane *et al.* (1981) for rhesus and pig-tailed macaques and of Smail, Faiman, Hobson, Fuller & Winter (1982) for rhesus monkeys, suggest that a typical adrenarche cannot be found in crab-eating macaques. As concluded by Cutler *et al.* (1978), Cutler & Loriaux (1980), and Forest *et al.* (1982), and contrary to Ducharme *et al.* (1976), we believe that the adrenarche, when it appears, is an event independent of gonadal maturation, each being activated separately as seen in numerous pathological cases. The adrenarche appears to be a simple, non-obligatory index of corporal maturation, the physiological levels of adrenal androgens having no major influence on the onset of puberty, as reported previously by many authors.

Androstenedione may be secreted by the adrenal glands and the testis. In humans, this steroid is considered as essentially adrenal because it is not correlated to testosterone, but in baboons it is regarded as being of testicular origin since its levels rise at the onset of puberty. In the present study, a comparative examination of intact and precociously castrated crab-eating macaque males up to puberty showed no significant differences in androstenedione levels, the androstenedione : testosterone ratio always being higher than 1 in the castrates during this period. However, the values in castrated adults were significantly ($P < 0{\cdot}01$) lower than in normal animals, indicating that at least some of the androstenedione in adults originates in the testis. Androstenedione appeared to be the quantitatively predominant androgen in the peripheral plasma between 3–4 months of age and the onset of puberty.

The high levels of plasma oestradiol-17β from birth to 3 months of age would be induced by intense adrenal activity at the end of gestation, as in rhesus monkeys up to 7 weeks after birth (Resko, 1974), in neonatal rhesus monkeys and pigtail monkeys (Robinson & Bridson, 1978) and in neonatal humans (Bidlingmaier, Versmold & Knorr, 1974). Moreover, Kelch, Jenner, Weinstein, Kaplan & Grumbach (1972) have reported that the secretion of oestradiol or oestrogens by the testis is very low in rhesus monkeys, but that peripheral aromatization of androgens could be important. According to Franz & Longscope (1979), about 40–50% of the circulating oestrogens in rhesus monkeys arise from the peripheral conversion of androstenedione and testosterone; these data are very similar to those obtained for humans. We do not yet have any data on crab-eating macaques to indicate the aromatization rate of androgens into oestrogens during postnatal life and to understand the testicular, peripheral, cerebral and hypothalamic contribution to oestrogen production.

We conclude that crab-eating macaques could be very useful for studies of the mechanisms affecting the hypothalamo–hypophysial–gonadal system during infancy and at puberty, but could not be used for studies of the adrenarche which is not readily detectable.

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