A new class of nonlinear optical materials based on push–pull quinonoid molecules

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Diamino-substituted dicyanoquinodimethane molecules are shown to be promising new candidates for nonlinear optical materials. The molecular hyperpolarizabilities are large and the materials are nearly transparent in the visible region; they are also chemically and thermally stable. Suitable strategies can be used to induce noncentrosymmetry and, consequently, capability for second-harmonic generation.

ORGANIC molecules with large first-order hyperpolarizability, β, and polymers with large second-order hyperpolarizability, γ are of great interest in the fabrication of nonlinear optical (NLO) materials[1]. The former, when assembled into a noncentrosymmetric crystal lattice are useful for applications like second-harmonic generation (SHG), electrooptic modulation, frequency mixing and optical parametric oscillation[2]. Design of molecules with large β and transparency in the visible range along with acceptable thermal and chemical stability is an interesting and challenging problem. Obtaining bulk nonlinear optical effects from these molecules continues to be an even more difficult proposition.

We have examined a class of push–pull quinonoid compounds which appear to be promising candidates to develop second-order NLO materials. The prototypical systems **1**, **2** and **3** (Figure 1) and some derivatives were prepared by a Du Pont group[3] and electric-field-induced second-harmonic generation studies (EFISHG) in solution indicated unexpectedly large β in one of these compounds[4], namely, **1**. We have computed the β values of several of such molecules and found them to be quite large, considering the short extent of conjugation present in them. We have also synthesized several new members in this class of molecules and present in this paper three typical cases (**4**, **5**, **6**) Molecule **4** and **5** did not show any SHG in the solid state, since they formed centrosymmetric crystal lattices. Since a centre of symmetry (S_2 operation) would necessarily be absent in the crystal lattice of a pure enantiomer, we prepared **6** to provide a noncentric crystal. This strategy[1] led to a material with moderate SHG. These studies are expected to initiate investigations of push–pull quinonoid molecules for the fabrication of novel nonlinear optical organic materials.

The molecular structure of all the compounds were optimized using the AM1 semiempirical method. The dipole moments of ground and excited states and hyperpolarizabilities were computed using the SCAMP routine[5]. This program provides theoretical estimates of β in good agreement with experiments; in the case of **1** the reported experimental value[4] of $\beta(\hbar\omega = 1.17\,\text{eV})$ is $-(245 \pm 60) \times 10^{-30}$ esu and the value calculated (as discussed below) using SCAMP is -243×10^{-30} esu. Typically, all single and pair excitations within the manifold of 12 molecular orbitals bracketing the HOMO–LUMO were included in the CI scheme. Hyperpolarizabilities presented in this paper are at zero excitation energies, calculated as the projection of the β tensor on the strongest dipole axis. The $\beta(\hbar\omega = 0\,\text{eV})$ is particularly relevant since it avoids any resonance enhancement from absorption.

The computational results for six typical molecules from our studies are provided in Table 1. The ground state dipole moments are quite large. These arise from the zwitterionic character due to the intramolecular charge transfer from the diaminomethylene end to the dicyanomethylene end. On excitation the back charge transfer leads to decreased dipole moments. The negative $\Delta\mu$ is supported by the negative solvatochromism observed in these molecules using a procedure we have proposed recently[6].

The calculations indicated notable oscillator strengths only for two excited states in all the molecules, one in the visible range with low oscillator strength (≈ 0.2) and the other in the UV with high oscillator strength (≈ 0.6). The $\beta(\hbar\omega \approx 0\,\text{eV})$ are quite appreciable in these systems in view of the fact that these do not have very extended π electron conjugation paths (*p*-nitroaniline, for example[5], has a $\beta(0)$ value of

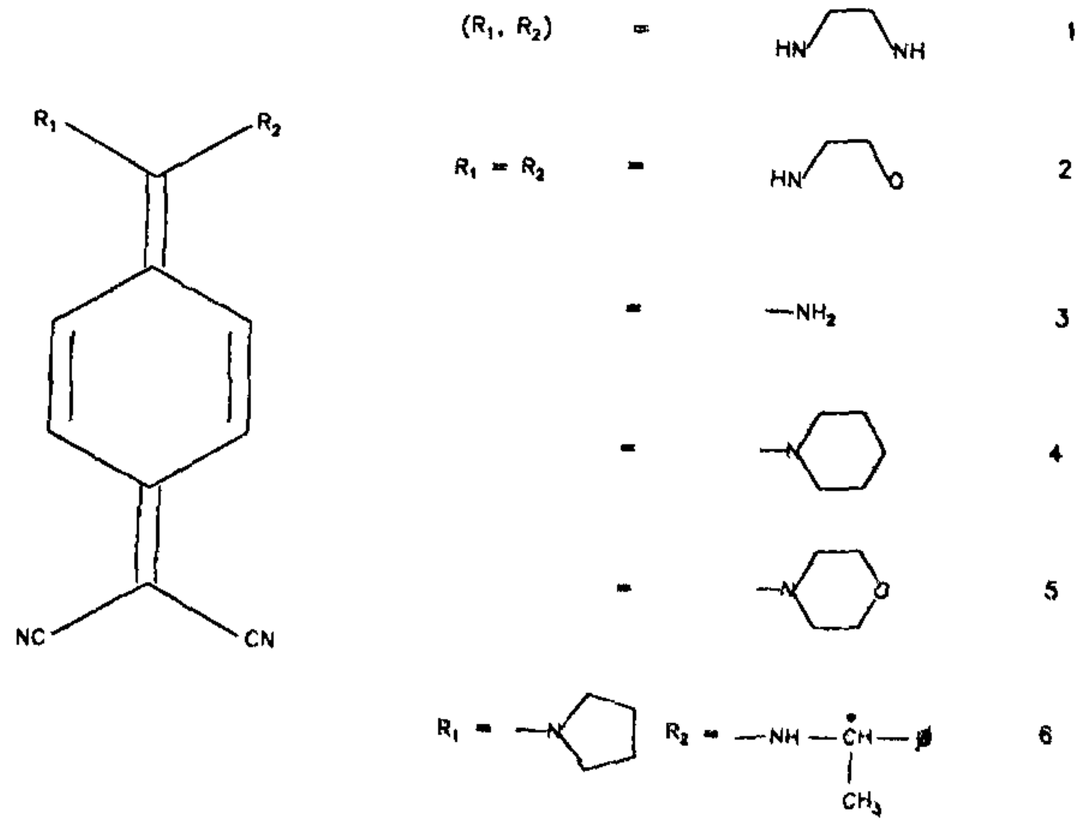

Figure 1. Molecules considered in the study

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Table 1. The calculated ground state dipole moment (μ_g), dipole moment change on excitation ($\Delta\mu$) and the static hyperpolarizability ($\beta(0)$) as well as the experimental absorption maxima and melting points of compounds **1–6**

Molecule	$\mu_g(D)$	$\Delta\mu$(D)	β (0)†	λ^*_{max}	m.p. (°C)
1	14.8	−9.2	−57.2	405	>350
2	12.5	−4.5	−29.0	370	>350
3	14.5	−10.2	−58.6	403	>350
4	15.0	−8.0	−73.3	410	302
5	12.5	−9.3	−67.7	420	>350
6	12.5	−3.0	−31.5	368	245

†In units of 10^{-30} esu
*In acetonitrile solution (in nm).

-9.1×10^{-30} esu). This seems to arise from the large $\Delta\mu$ associated with the electronic excitations. A detailed theoretical analysis we have recently carried out[7] on 7,7′-diamino-8,8′-dicyanoquinodimethane (**3**) indicates that the usual two-level approximation[8] to β reproduces only 50% of the full β and that appreciable contributions from several excited states lead to large values of β in these systems. The negative effects of the rather large excitation energies with strong oscillator strengths in these molecules are offset by these large $\Delta\mu$ contributions.

Encouraged by the theoretical results, we have synthesized several compounds in this class with a view to investigating, in particular, the NLO properties of the bulk materials, which are practically more relevant than the molecular properties like β. The synthesis is quite simple and follows the general procedure outlined in ref. 3. In the case of molecule with two different donor amines, the monopyrrolidine- substituted 7,7′,8,8′-tetracyanoquinodimethane was prepared initially, followed by the replacement of the second cyano group by the appropriate second amine. Table 1 provides the experimental absorption wavelengths of the six molecules recorded in acetonitrile solution. In every molecule only a single absorption is seen, which appears close to the UV-visible interface or outside the visible window. The solid materials are all either colourless or pale yellow. The melting points provided in Table 1 point to the general thermal stability of these compounds, showing that poling techniques can be applied on them and that laser irradiation damage will be minimal. We present below the preliminary studies on **4**, **5** and **6**.

The *bis*-piperidine and *bis*-morpholine derivatives (**4**, **5**) gave crystals suitable for X-ray analysis from acetonitrile; the crystal structure analysis[9] of these two compounds indicated centric space groups $P\bar{1}$ and $P2_1/n$, respectively. This was not unexpected as the dipole moment of these compounds is very large. As indicated by the space group symmetry, no SHG was detected in these compounds.

Since chirality in the molecule forces the crystal packing to be noncentric, we have prepared the S(−)-α-methylbenzylamine derivative (**6**). Crystals grown from acetonitrile were protected from solvent loss and structure analysis was carried out[9]. The noncentric space group

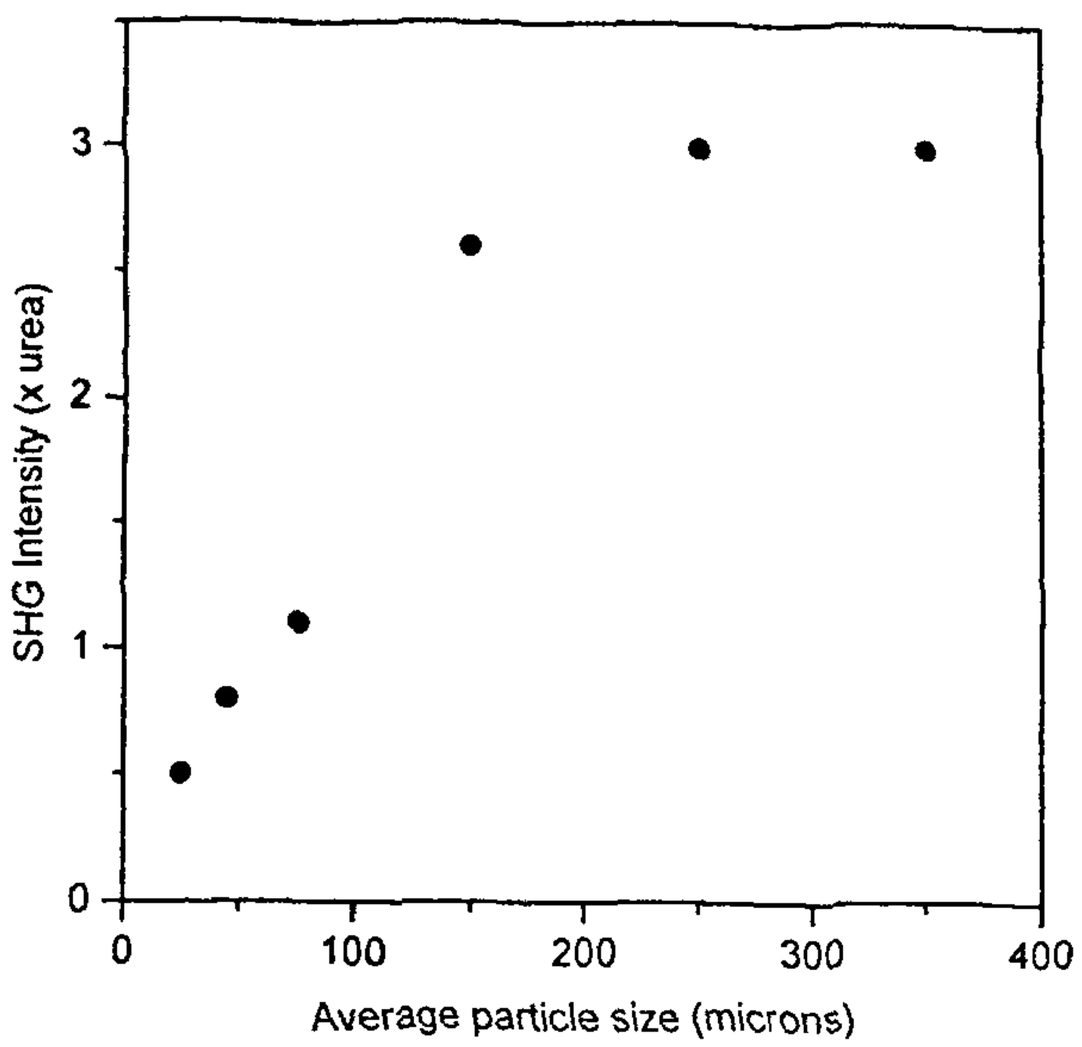

Figure 2. Variation of the powder SHG intensity with particle size of **6**

$P2_1$ was observed. SHG studies were carried out using a Nd : YAG laser with 1064 nm radiation. The samples were loaded between glass plates with uniform teflon sheets to control the sample thickness. Powder material of different particle sizes were selected by grading with standard sieves. Particle size dependence of the SHG (Figure 2) was analysed following the Kurtz and Perry method[10], and **6** showed a phase-matchable SHG three times stronger than urea at particle sizes above 150 μm. Crystals also produced the same moderate SHG. It is noteworthy that this compound shows an absorption at about 368 nm in acetonitrile solution and is light yellow in colour in the solid state. The material is stable to extended laser irradiation (6 ns and 10 Hz) at 1 GW/cm^2 and has a melting point (m.p.) of 245°C. It holds promise for applications involving visible light, especially in view of the phase-matchability of the SHG observed.

We have presented theoretical results on hyperpolarizabilities of a new class of push–pull quinonoid systems. The β values are very promising for these molecules, which also show only weak absorption in the visible region. Experimental studies indicate that compounds of this group are easily synthesized and are chemically and thermally very stable. They are also amenable to a variety of chemical modifications. For example, introduction of chirality is shown to produce noncentric crystal structures and, consequently SHG activity. Crystal structures and NLO studies on these and other systems will be published later. Experimental determination of molecular hyperpolarizabilities are also being planned.

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Testosterone metabolism by rhesus monkey spermatozoa: Effects of antifertility agents

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Androstenedione was the major metabolite when ejaculated rhesus monkey spermatozoa were incubated with ^{14}C-testosterone. Ejaculated human spermatozoa also showed a similar pattern of conversion of testosterone to mainly androstenedione. The percentage of androstenedione formed by rhesus monkey spermatozoa did not show any statistical difference during different months of the year, indicating that this parameter may be useful in evaluating the effect of contraceptive agents in rhesus monkeys. This was tested in *in vitro* studies using α-chlorohydrin or cyproterone acetate. The results of these experiments did not show any direct effect of the antifertility agents on testosterone-metabolizing capacity of rhesus monkey spermatozoa, indicating the possibility that these antifertility agents exert their effects on spermatozoa by alteration of epididymal function.

A SUBHUMAN primate model to test the action of antifertility agents, specifically on epididymal spermatozoa, is of urgent need. The rhesus monkey has been extensively used in biomedical research, particularly in testing the mode of action of new fertility-regulating agents. The parameters available today for assessing the action of such drugs are mainly subjective[1] or time-consuming and require sophisticated laboratory equipment[2]. We have been involved in developing alternative biochemical parameters to assess sperm function. Our studies have shown that caudal or ejaculated spermatozoa of a number of species, including primates, metabolize steroids[3,4]. It was therefore, of interest to determine if testosterone-metabolizing capacity of rhesus monkey spermatozoa could be used as a useful biochemical index to assess sperm function. But, since rhesus monkey is a seasonal animal, it was first necessary to assess the changes, if any, in the metabolism of testosterone during the different months of the year. This paper reports the changes in testosterone metabolism of rhesus monkey spermatozoa during different months of a two-year period and also the effects of known male antifertility agents on this parameter.

Nonradioactive steroids of chromatographic grade from Sigma Chemical Co. (St. Louis, USA) were used. ^{14}C-testosterone (specific activity 58 mCi/mmol) was purchased from Amersham International plc (Buckinghamshire, UK); its purity was checked in methylene chloride : diethyl ether (4 : 1) on thin layer chromatograms. All solvents were distilled before use. RSα-chlorohydrin was generously supplied by Dr G. M. H. Waites, World Health Organization, Geneva.

To compare the ability of ejaculated human (n = 6) and rhesus monkey (n = 6) spermatozoa to metabolize ^{14}C-testosterone, spermatozoa were collected from men of proven fertility by masturbation and from rhesus monkey by electroejaculation[5] and processed as described earlier[4]. Semen analyses were done according to WHO manual[1].

The experiments to assess seasonal effects, if any, on the capacity of rhesus monkey ejaculated spermatozoa to metabolize ^{4}C-testosterone were done in 1984 and 1985 using 3 animals per year in order to take into account any vagaries in weather during one single year. Spermatozoa were collected by electroejaculation[5] during the first week of each month and processed as described earlier[4]. Two concentrations of spermatozoa, 50×10^6/ml and 5×10^6/ml, were used per animal.

Ejaculated monkey spermatozoa (50×10^6/ml) were suspended in phosphate-buffered saline (PBS) containing 100 mM NaCl, 3 mM KCl, 6.5 mM Na_2HPO_4, 1.3 mM KH_2PO_4, 0.8 mM $CaCl_2$ and 0.5 mM $MgCl_2$ and preincubated at 34°C for 30 min with 1, 5 or 10 mM RSα-chlorohydrin before addition of ^{14}C-testosterone. Control samples were run simultaneously without preincubation with α-chlorohydrin. Incubation medium contained either (i) no substrate, or (ii) 5 mM D-glucose, or (iii) 10 mM lactate + 1 mM pyruvate or (iv) 5 mM glucose + 10 mM lactate + 1 mM pyruvate. The concentrations of these substrates were based on earlier studies using ram spermatozoa[6].

Ejaculated monkey spermatozoa (50×10^6/ml) were preincubated with 100 ng/ml cyproterone acetate (CPA) *in vitro* in Eagle's medium supplemented with 0.1% bovine serum albumin, 20 mM D-glucose, 15 mg streptomycin sulphate and 15 mg penicillin per 100 ml for 60 min prior to addition of ^{14}C-testosterone.

Incubations were performed in airtight vials that were silanized using 2% dichloromethylsilane. The mass of ^{14}C-testosterone added to vials was 1.25 ng. ^{14}C-testosterone in benzene was added to vials and benzene evaporated under nitrogen prior to the addition of 1 ml of sperm suspension in enriched Eagle's medium[4]. All incubations were done in a metabolic shaker at 34°C for 18 h in an atmosphere of 95 O_2 : 5 CO_2. Control vials without spermatozoa and containing 1 ml Eagle's medium were run simultaneously in all experiments and the background values obtained were deducted from experimental samples. On termination of incubation, the vials were rapidly frozen after adding 0.1 ml ethyl acetate containing a mixture of the following 7 nonradioactive androgens at 20 mg each: androstenedione (5α-androstane-3,17-dione), androstanedione (4-androstene-3,17-dione), dihydrotestosterone (17β-hydroxy, 5α-androstane-3-one), testosterone (17β-hydroxy-4-androsten-3-one), androsterone (3-α-hydroxy-5α-androstan-17-one), 5α-androstane-3α-17β-diol and 5α-androstane-3β-17β-diol.

The samples were thawed and radioactive metabolites were extracted thrice with five volumes of diethyl ether. The aqueous phase was frozen and the ether phase decanted and evaporated under nitrogen. The radioactive metabolites were resuspended in vials in minimal volumes of ethyl acetate before application to thin-layer chromatograms.

Thin-layer chromatography separation of metabolites and recrystallization of major metabolites to constant specific activity were done as described earlier[4].

One-way analysis of variance was used and the presence of any significant differences was checked by Neuman Keul's multiple range test.

Ejaculated human spermatozoa (50×10^6/vial) converted testosterone mainly to androstenedione (37.9 ± 5.2%) and to a lesser extent (6.8 ± 1.8%) to dihydrotestosterone (DHT). The other metabolites formed were androstanediols (0.8 ± 0.2%) and androstanedione (0.3 ± 0.2%). The percentage of unutilized testosterone was 48.4 ± 3.8%. Rhesus monkey spermatozoa (50×10^6/vial) incubated with ^{14}C-testosterone gave a similar pattern of conversion and the percentages of metabolites formed were as follows: androstenedione 32.3 ± 6.5, DHT 1.3 ± 0.9, androstanedione 1.5 ± 0.9 and androstanediols 1.3 ± 0.5. The percentage of unmetabolized testosterone was 58.2 ± 5.6%.

The data obtained for the two consecutive years were pooled and statistically evaluated. The percentage of androstenedione formed was higher with 50×10^6 sperms per incubation vial than with sperm counts of 5×10^6/vial. The percentages of androstenedione formed during the different months with 50×10^6 sperms/vial (January 26.4 ± 6.3, February 41.7 ± 8.0, March 24.3 ± 4.1, April 36.0 ± 8.0, May 36.5 ± 4.7, June 48.4 ± 9.7, July 51.6 ± 11.3, August 46.9 ± 7.0, September 41.7 ± 12.2, October 47.9 ± 10.4, November 44.9 ± 9.5 and December 34.7 ± 6.3) were not significantly different. The percentages of DHT (range 1.2 ± 0.4–3.8 ± 1.4), androstanedione (range 1.0 ± 0.1–3.0 ± 0.9) and androstanediols (range 0.7 ± 0.1–2.7 ± 1.2) were very low. With 5×10^6 sperms/vial, a decrease in the percentage of androstenedione formed during different months was seen (Table 1). The decreases seen in January, March and December were statistically insignificant. The percentage of live spermatozoa did not show any seasonal change (92–96%). These results indicate the absence of seasonality in the conversion of testosterone to androstenedione at both concentrations of spermatozoa used in the study.

The percentage of androstenedione formed by the spermatozoa preincubated with α-chlorohydrin was not significantly different (Table 2) from control samples incubated in the presence of 5 mM D-glucose (26.8 ± 4.5%), 10 mM lactate + 1 mM pyruvate (18.6 ± 6.8%) or 10 mM lactate + 1 mM pyruvate + 5 mM glucose (20.4 ± 3.1%). The percentage of other metabolites was very low (< 2%).

Preincubation of monkey spermatozoa with the potent antiandrogen CPA did not alter significantly their testosterone-metabolizing capacity. The percentages of androstenedione (14.1 ± 4.9) and minor metabolites (DHT 2.9 ± 1.3, androstanediols 1.7 ± 0.3, androstanedione 0.3 ± 0.1) formed were similar to those seen in incubations without CPA (androstenedione 17.9 ± 5.5, DHT 1.4 ± 0.1, androstanediols 2.3 ± 1.3, androstanedione 0.4 ± 0.2).

Earlier observations showed the presence of 17β-hydroxysteroid dehydrogenase activity catalysing the conversion of testosterone to androstenedione by spermatozoa of different mammalian species[3,4,7–9]. The present study established that (a) the patterns of conversion of testosterone by rhesus and human spermatozoa are similar and (b) seasonal changes did not alter significantly the testosterone-metabolizing capacity of rhesus monkey sperm. The well-known male antifertility agent CPA did not show inhibition of conversion of testosterone to DHT by epididymis when given simultaneously with ^{3}H-testosterone or a little earlier[10], but when animals were exposed to chronic release of CPA from silastic capsule, testosterone-metabolizing (to DHT) capacity was inhibited[11]. It is likely that spermatozoa also may exhibit similar inhibition of testosterone-metabolizing capacity after chronic exposure to the antifertility agent and the alteration in the results obtained would indicate fertility mediated through epididymis.

α-Chlorohydrin, even at low doses, induces reversible

Table 1. Percentages of metabolites of ^{14}C-testosterone by rhesus monkey spermatozoa ($X \pm SE$)

Month	Androstanediols	Testosterone	DHT	Androstenedione	Androstanedione
Jan.	1.96 ± 2.4	70 22 ± 7 55	2 11 ± 0 70	9.32 ± 2.4	1.28 ± 0 30
Feb.	1.59 ± 0.43	62 26 ± 5 62	1 46 ± 0.53	22 24 ± 4.37	1 41 ± 0 35
March	5.38 ± 2 21	59 11 ± 10 15	1.36 ± 0.29	9.73 ± 3 25	1 54 ± 0 40
April	1.31 ± 0 42	71.29 ± 5.31	1 49 ± 0.32	17 05 ± 4 12	1.43 ± 0 45
May	2.0 ± 0.72	65.21 ± 4 93	1.38 ± 0.36	21.56 ± 5.2	1 29 ± 0 37
June	0 62 ± 0 07	66 34 ± 7 5	1 14 ± 0.50	22 73 ± 7.37	0 87 ± 0 16
July	0 69 ± 0.14	62 09 ± 6 54	0 80 ± 0 13	27.29 ± 6 03	0 69 ± 0 1
Aug	1.40 ± 0 83	68 79 ± 6.36	1 5 ± 0 39	17 18 ± 4 95	1.01 ± 0.23
Sept.	1 27 ± 0.50	66 98 ± 9 83	1 08 ± 0.50	16 68 ± 5 64	0.68 ± 0 31
Oct.	0.56 ± 0.16	65.34 ± 7 89	2 86 ± 1 92	15.75 ± 4.68	0.71 ± 0 25
Nov.	0 69 ± 0.13	68 63 ± 5.88	0 9 ± 0.21	21.86 ± 5.44	0 74 ± 0 31
Dec	1 20 ± 0 29	75.45 ± 3 03	0.76 ± 0 10	9 58 ± 1 05	0 87 ± 0 23

Note. 5×10^6 sperms/vial were used for incubation.

Table 2. Effect of α-chlorohydrin on androstenedione formation by monkey spermatozoa

Substrate	Concentration of α-chlorohydrin		
	1 mM	5 mM	10 mM
None	22 5 ± 3 6	23 3 ± 2 4	24 5 ± 4 3
5 mM glucose	12.2 ± 2 5	15 9 ± 1 9	12.9 ± 3 9
10 mM lactate + 1 mM pyruvate	16.6 ± 3 0	14.8 ± 3.2	18 6 ± 3 3
10 mM lactate + 1 mM pyruvate + 5 mM glucose	15.3 ± 3 8	19 5 ± 2 1	14 1 ± 4 8

infertility in many species, including rhesus monkey[12-14]. It also inhibits glycolysis of spermatozoa in treated animals by inhibiting glyceraldehyde α-3-phosphate dehydrogenase[15, 16]. Spermatozoa exposed to α-chlorohydrin are motile for one hour in the absence of a substrate or in the presence of lactate/pyruvate but become rapidly immotile in the presence of glucose either alone or in combination with pyruvate and lactate[6]. The antiglycolytic action of α-chlorohydrin is due to the action of (s)-3-chlorolactaldehyde[17]. The present data using concentrations of substrates, as used in studies related to assessing the mode of action of α-chlorohydrin, did not show any direct effect of the drug on testosterone- metabolizing capacity of spermatozoa. CPA, which is progestational as well as an antiandrogen, inhibits various androgen-dependent or androgen-induced biological actions[18, 19]. Antiandrogenic action of CPA is due to competitive antagonism or its effect on testosterone biosynthesis in the testes[20]. It also has an inhibitory effect on the gonadotrophin release by the pituitary[21]. In the present investigation, preincubation of sperm *in vitro* with CPA did not alter the pattern of testosterone metabolism, indicating the possibility that these antifertility agents exert their effect on spermatozoa by altering epididymal function. Further studies are ongoing to study the pattern of testosterone metabolism in monkeys exposed *in vivo* to these antifertility agents.

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