density of the rod material, is not available and quite difficult to measure. However, some reasonable assumptions can be made in this regard since the mass density and the elastic properties of some common materials such as aluminium and quartz on one side and certain woods on the other are well known[8]. Since the range of sound velocities for most of the solids are covered by these materials[8], it is expected that the material properties of trichobothria fall somewhere between those of quartz and oak. The boundary conditions of the trichobothria, i e. whether the ends are fixed or free, are also known from the scanning electron micrograph. Since one end of the hair is attached to the bottom of the lower socket and the other end is free (Figure 1 *b*), the boundary conditions for the vibrations of the trichobothrium appear to be closest to 'fixed–free' conditions.

The natural resonant frequency of the lower socket chamber can be estimated by utilizing the theory of the Helmholtz resonator[8]. The chamber can act as a simple Helmholtz resonator provided its linear dimension is small compared to the wavelength of sound in air. The acoustic wavelength in air (20°C) at $f = 1 \times 10^5$ Hz is[8] 34.7 μm. Since the greatest linear dimension of the lower socket chamber is 18 μm, the wavelength of sound anywhere in this frequency range is much larger than the largest linear dimension of the lower socket chamber. Thus, the lower socket chamber can act as a simple Helmholtz resonator.

Pertinent here is to mention that a similar type of very long double-chambered sensilla inserted in a bed of microtrichia as reported in the complex sensory apparatus, the 'sensillium', of some flea[9]. On the basis of the scanning electron microscopic data on the length and width of the trichobothria, volume and dimensions of the upper and lower socket chambers, and with some reasonable assumption on the material properties of the trichobothria, it was reported that in flea the calculated resonant frequencies for the trichobothria (1.49×10^5–1.7×10^7 Hz) and for lower socket chamber (4.77×10^6–6.44×10^6 Hz) overlap, which gives further support to the view that the sensilla indeed functions as ultrasonic receptors[9]. Since the scanning electron microscopic data for the trichobothria and the sockets in the bat fly are more or less similar to the data reported in flea, similar assumptions on involvement of the structure in ultrasonic reception can be made. However, experiments involving sound waves in the frequency range 1.49×10^5–1.7×10^7 Hz would be required to verify the hypothetical conclusion that the fly uses ultrasound as a means of communication.

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Zinc therapy?

Zinc is known to be a crucial element for the basic function of life and is present at a relatively high concentration in the cells of most organisms. Among all the 3d" elements of the periodic table, Zn is only second to Fe in terms of its concentration available in any biological species[1]. Thus, at every stage its functional importance has been emphasized in life processes.

The body of an adult human contains about 2 g of Zn. However, as its concentration is low in most of the body cells, its importance has not been fully realized. Zn deficiency can lead to reduction in normal growth, impaired bone development, hindered maturation and function of reproductive organs as well as impaired protein and carbohydrate metabolism. The metal is also thought to be involved in wound healing and photochemistry of vision[2].

Although the essentiality of Zn for cell growth, development and differentiation has been well established[3,4], the biochemical mechanisms by which Zn exerts its effects are still not well-understood. Within the last two decades, Zn has been shown to be a functionally essential component of more than 250 enzymes[5]. However, the first observation of Zn metalloenzyme was by Keilin and Mann[6] in 1940, who showed that carbonic anhydrase from mammalian red blood cells contains 33% of Zn essential for its activity.

Zn enzymes encompass all known classes of enzymes which participate in synthetic or degradative metabolic processes of biological macromolecules. Thus, they form an integral part of oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases[5]. However, the structure–function relationship of a class of Zn metalloenzyme that has caught the widespread attention of the researchers are the enzymes involved in the biochemistry of nucleic acids or regulation of gene expression. It is interesting that almost all nucleic-acid-binding enzymes have stoichiometric Zn as an integral part performing dual functions, that is either involved in catalytic function of the enzyme or in maintaining its structural integrity. In this article we emphasize the role of Zn in metabolic processes involving nucleic acids.

Perhaps the importance of Zn in nucleic acid chemistry lies in its unique electronic configuration ($3d^{10}$). Zn is neither a very reactive metal, because of its filled-up d orbitals, nor very inert due to its position among group II b metals of the periodic table. The participation of Zn in catalytic mechanism

of nucleic-acid-synthesizing enzymes depends largely on its ability to act as a hard acid. During the polymerization of nucleotides, Zn is thought to generate a nucleophilic centre at the hydroxyl group of ribose sugar by abstracting a proton from it[7].

It is now known since some time that in the case of *Euglena gracilis* the deficiency of Zn in the growth medium leads to a drop in the protein and RNA content of the cell and also the growth is arrested at a stage where the DNA content of each cell is doubled[8]. It has also been reported almost a decade ago that when *Euglena* was grown in a Zn-depleted medium, the organization of histones and their synthesis were greatly altered[9].

Surprisingly, the literature accumulated over the last ten years shows that almost all the DNA-binding proteins are Zn-containing metalloproteins where Zn is involved both in catalytic and structural functions[10,11]. At least in a few cases it has been conclusively demonstrated that the removal and readdition of Zn or other divalent cations makes the enzyme to go through denaturation and reactivation cycle without any loss of structural integrity[12].

Although there exists some controversy regarding the status of Zn in DNA polymerases[13], the catalytic and structural role of Zn during transcription has been unambiguously demonstrated[12]. One of us has shown in the past[10] that the intrinsic Zn in *E. coli* RNA polymerase is responsible for substrate selection. However, its structural role in maintaining the overall conformation of the enzyme is yet to be demonstrated. Similarly, RNA polymerases from higher organisms are also Zn-containing metalloproteins and, at least in yeast, the removal of Zn damages the enzyme irreversibly (D Chatterji, unpublished observation).

It is now well-established that the most common chelating motifs for Zn in any DNA-binding proteins are Zn finger motifs[14] and many of the transcription factors are known to have these motifs with uniquely placed 'Cys' and 'His' amino acid residues to chelate the Zn atom in tetrahedral configuration. Such a ligand geometry raises two interesting possibilities. Firstly, either acidic pH below the pK_a of 'His' or a reducing environment in the medium which would in turn keep the free SH's of 'Cys' in reduced form can remove Zn reversibly from the protein, destroying its DNA-binding ability. On the other hand, a more potential ligand can competitively chelate out Zn from its Zn finger environment, rendering the protein ineffective in DNA recognition. An interesting question thus arises whether it is at all possible to inhibit selectively a transcription factor, or for that matter any DNA-binding protein, with the ultimate aim of controlling the regulation of gene expression where the protein plays an obligatory role.

Retroviral nucleocapsid and *gag* protein from all retroviruses contain one or two copies of a Zn finger motif, Cys–X_2–Cys–X_4–His–X_4–Cys[15,16]. They bind Zn on maturation. However, if 'Cys' or 'His' residues are modified, defective packaging of genomic viral RNA results, with the concommitant formation of noninfective virus particles. Rice *et al*[17] have recently been able to destabilize Zn finger motifs in poly(ADP-ribose) polymerase and inhibit the HIV-1 infectivity following the same strategy, thus opening up an interesting possibility of a new kind of antiviral therapy.

Retroviral Zn fingers with the sequence shown above bind Zn stoichiometrically and with high affinity[20] ($K_d \sim 10^{-12}$ M). However, under close-to physiological conditions of ionic strength and pH, a 10-fold excess of EDTA removes only 50% of the Zn from the N-terminal CCHC Zn finger domain of the HIV-1 nucleocapsid protein[16]. On the contrary, a potential Zn ligand, 3-nitrosobenzamide (NOBA), is capable of ejecting Zn from retroviral-type Zn fingers. NMR studies carried out by Rice *et al.*[17] showed that upon addition of NOBA, signals due to Zn-bound 'His' are lost, with the concommitant appearance of signals due to Zn-free 'His'. It was also observed that NOBA reacts stoichiometrically with Zn finger in the capsid protein. Spectral changes identical to those observed in NMR would require at least a 50-fold molar excess of EDTA.

The HIV-1 infection is impaired by specific HIV-1 protease inhibitors, suggesting that protein processing by the viral protease might be a required event in the early phase of the viral replication cycle[18]. A target of such protease-mediated processing might be the nucleocapsid protein of HIV-1 itself, which is a major component of the viral core. However, along with protease inhibitors, NOBA also restricts processes relating to steps early in infection, indicating that Zn ion may be involved in the overall viral infectivity. Recently, Wondrak *et al.*[19] have shown that removal of Zn from finger motif in nucleocapsid results in oxidation of free cysteines and the nucleocapsid protein becomes resistant to cleavage.

It appears, therefore, that Zn chelators like NOBA and many others which are yet to be tested may have great potential in therapeutic use, which can be termed as Zn therapy. It will be an interesting line of research in future which would tell whether the activity of any other DNA-binding enzymes or proteins can be controlled through the chemistry of Zn chelation.

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Reduction of liver folates in gossypol-treated rats

Gossypol is a yellowish phenolic compound occurring naturally in certain species of cotton plants of the family Malvaceae, mostly in the seeds and root bark. At one time it was considered only as a toxic waste in the processing of cotton seed products. However, following the initial observation of Liu in China in 1957, the pioneering work of other Chinese workers[2] in the mid-eighties and confirmation by several others established its role as a male antifertility agent both in laboratory animals and humans. There was a spurt of papers in the late eighties and early nineties on the antifertility effect of gossypol and WHO task force on male contraceptives even conducted extensive clinical trials on gossypol in China and elsewhere[3].

Though in several studies, its efficacy was well-proven with apparently no side-effects, in a couple of other studies toxic symptoms like anorexia, reduction in body weight, hypokalemia, etc., were observed even at low doses[2,4,5]. It was reported by Menaul[6] as early as 1923 that gossypol prevents liberation of oxygen from oxyhaemoglobin and has haemolytic effect on erythrocytes. In fact, studies by Danke and Tilman[7] showed that microcytic hypochromic anaemia occurs in rats when 10% cotton seed meal is fed for a period of 28 days. Since folic acid is involved in erythropoiesis and its deficiency leads to anaemia, we wanted to study the effect of gossypol at antifertility doses on liver folate levels in rats. Graded doses of gossypol acetic acid (Sigma Co. 20, 30 mg/kg body weight/day) were given through gavage for different durations (5, 7 weeks) to male rats of Wistar/Nin strain. The animals were fed stock colony diet with adequate levels of folic acid (1 mg/kg diet). At the end of treatment and after mating the animals with virgin females to assess the fertility, the animals were sacrificed. Liver was excised and weighed and a portion of the liver was processed to determine the folate levels as per the method of Lakshmaiah and Ramasastri[8].

The rats became totally infertile at the dose regimen of 30 mg/kg body weight/day for 7 weeks, as reported earlier[9]. Liver folate levels in gossypol-treated animals have not been reported so far and, to the best of our knowledge, this is the first study of the effect of gossypol on folic acid *in vivo*. The data are presented in Table 1. Liver weights were normal in all the treated groups. There was no difference in liver folate levels of treated rats at 20 mg regimen fed for 7 weeks. But at higher doses, i.e. 30 mg regimen, for 7 weeks there was significant reduction from control ($P < 0.001$) and this was seen as early as 5 weeks ($P < 0.05$). The dose effect was very evident, on comparing the 20 mg regimen with 30 mg regimen for 7 weeks ($P < 0.05$). Tissue distribution studies show that liver accumulates large amounts of gossypol[10] and because of its slow clearance from the body it is quite possible that over a prolonged period of ingestion, toxic effects may appear even at low antifertility doses. The reduction in liver folate levels in gossypol-treated animals may be due to the direct action of gossypol on liver, as some earlier studies[11,12] have shown hepatotoxic effect of gossypol. However, no apparent toxic symptoms were visible in these animals throughout the experimental period as the food intake was normal, and body weights were not affected.

Table 1. Changes in hepatic folic acid in gossypol-treated rats

Group	Duration of expt (weeks)	Gossypol acetic acid dosage (mg/kg body wt/day)	Liver wts (mean ± SE)	Liver folate µg/g liver (mean ± SE) (*L. casei* activity)
Control	5	–	10 9 ± 0.16 (4)	4 67 ± 0 04
Gossypol-treated	5	30	10 9 ± 0 42 (6)	2 5 ± 0.13*
Control	7	–	11 0 ± 0 82 (6)	4 5 ± 0 23[a]
Gossypol-treated	7	20	10 4 ± 0 33 (5)	3 8 ± 0 10[a, b]
Gossypol-treated	7	30	10 1 ± 0 30 (6)	2 9 ± 0 24[c]

Figures in parentheses indicate number of animals
*$P < 0$ 05 by modified t test, compared to control, 5 weeks Values not sharing common superscript were significantly different by analysis of variance, and studentized range test.
[a, c]$P < 0$ 001, [b, c]$P < 0$ 05.

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