to see differential regulation of homologous sex determination genes[21]. Such regulation may be accompanied by a parent-of-origin effect such as that seen among X-linked genes in marsupials[28,29] and in the *IGF2/H19* complex[27] in humans, or be without it, as among X-linked genes in eutherians. On the other hand, in case of genes on the single X chromosome of *E. lutescens*, such silencing could be parent-of-origin dependent, but occurring in one sex and not in the other. The effective dosage of X-linked genes so silenced would then become reduced from one to zero. In embryos developing into females, one would expect to find genes concerned with maleness to be imprinted, and in those developing into males, genes for femaleness are expected to be among those imprinted. On the basis of what is known in coccid genetic systems, one other expectation may be stated: the sex ratio in *E. lutescens* is likely to be variable and subject to environmental influences, especially those acting on the mother.

There may be difficulties associated with testing this hypothesis in *E. lutescens*. For instance, genetic variation in this rodent is poorly documented. Identification of imprinted genes by pedigree analysis is hampered by the fact that these animals breed poorly in captivity. CpG methylation could be an alternative criterion for identifying imprinted genes. Two genes, *Dax1* and *Sox9*, show evidence of dosage-dependent roles in mouse sex determination[18,19]. *DAX1* and *SOX9* have similar effects on human sex determination[19,20]. *DAX1/Dax1* is of particular interest because it is X-linked, and subject to inactivation. Its dose-dependent regulation can therefore be regarded as a product of X-chromosome imprinting[17]. When abnormally present in two copies in XY individuals, it overrides the effects of *SRY/Sry* and causes sex reversal. *E. lutescens* equivalents of these genes, and that of *Sox3* (ref. 30) – the closest known relative of *Sry*-, may be good candidates for studying whether there are such epigenetic differences between male and female voles.

1. Lederberg, J., *Genetics*, 1989, **121**, 395–399.
2. Vogel, W., Jainta, S., Rau, W., Geerkens, C., Baumstark, A., Correa-Cerro, L. S., Ebenhoch, C. and Just, W., *Cytogenet. Cell Genet.*, 1998, **80**, 214–221.
3. Matthey, R., *Arch. Klaus-Stift VererbForsch.*, 1953, **28**, 271–280.
4. Ohno, S., Becak, W. and Becak, M. L., *Cytogenet. Cell Genet.*, 1964, **15**, 14–30.
5. Lyapunova, E. A., Vorontsov, N. N. and Zakarjan, G. G., *Experientia*, 1975, **31**, 417–418.
6. Matthey, R., *Rev. Suisse Zool.*, 1964, **71**, 410.
7. Just, W., Rau, W., Vogel, W., Akhverdian, M., Fredga, K., Graves, J. A. M. and Lyapunova, E., *Nature Genet.*, 1995, **11**, 117–118.
8. Fredga, K., in *The Differences Between the Sexes* (eds Short, R. V. and Balaban, E.), Cambridge Univ. Press, UK, 1994, pp. 419–431.
9. Metz, C., *Am. Nat.*, 1938, **72**, 485–520.
10. Brown, S. W. and Nelson-Rees, W. A., *Genetics*, 1961, **46**, 983–1007.
11. Chandra, H. S., *Chromosoma*, 1963, **14**, 330–346.
12. Nur, U., *Chromosoma*, 1963, **14**, 123–139.
13. Chandra, H. S., *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 6947–6949.
14. Chandra, H. S., *Nature*, 1986, **319**, 18.
15. Chandra, H. S. and Nanjundaiah, V., *Development* (Suppl.), 1991, 47–53.
16. Chandra, H. S., XVI International Congress of Biochemistry & Molecular Biology, New Delhi, 1994, vol. III, Abst, P2–104, p. 14.
17. Brown, S. W. and Chandra, H. S., *Proc. Natl. Acad. Sci. USA*, 1973, **70**, 195–199.
18. Ramkissoon, Y. and Goodfellow, P., *Curr. Op. Genet. Dev.*, 1996, **6**, 316–321.
19. Greenfield, A. and Koopman, P., *Curr. Top. Dev. Biol.*, 1996, **34**, 1–23.
20. Capel, B., *Annu. Rev. Physiol.*, 1998, **60**, 497–523.
21. Brown, S. W. and Chandra, H. S., in *Cell Biology: A Comprehensive Treatise* (eds Goldstein, L. and Prescott, D. M.), 1977, vol. 1, pp. 109–189.
22. Brown, S. W. and Bennett, F. D., *Genetics*, 1957, **42**, 510–523.
23. Chandra, H. S. and Brown, S. W., *Nature*, 1975, **253**, 165–169.
24. Kitchin, R. M., *Chromosoma*, 1970, **31**, 165–197.
25. Nur, U., *Chromosoma*, 1972, **39**, 381–401.
26. Nur, U., *Am. Zool.*, 1971, **11**, 301–308.
27. Morison, I. M. and Reeve, A. E., *Hum. Mol. Genet.*, 1998, **7**, 1599–1609.
28. Cooper, D. W., VandeBerg, J. L., Sharman, G. B. and Poole, W. E., *Nature New Biol.*, 1971, **230**, 155–157.
29. Graves, J. A. M., *Annu. Rev. Genet.*, 1996, **30**, 233–260.
30. Foster, J. W. and Graves, J. A. M., *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 1927–1931.

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Ivermectin: Effect on sugar metabolism in *Acanthocheilonema viteae*, a rodent filarial parasite

Lymphatic filariasis is a major public health problem in several tropical countries including India[1]. Despite serious efforts made worldwide, no suitable control measure against adult worms has been developed. Hence, there exists a persistent need for developing an effective and safe macrofilaricide. Ivermectin (Mectizan), which kills microfilariae of a wide range of filarial species, is not lethal to adult worms[2]. Recently, at higher doses, this drug has been shown to produce adulticidal action against *Acanthocheilonema viteae* and *Brugia malayi* in Mastomys[3]. It is therefore of

Table 1. Effect of ivermectin on some parameters and enzymes of sugar metabolism in *A. viteae*

Parameter/enzyme	Control	Treated	Change (%)
Glucose uptake*	2.14 ± 0.06	1.45 ± 0.11	+ 27.2[a]
Lactate production*	2.20 ± 0.13	0.62 ± 0.76	– 35.0[b]
Lactate/glucose	1.94	0.43	
ATP content**	38.8 ± 0.04	21.1 ± 0.16	– 45.6[a]
PEP content**	121.1 ± 1.02	83.3 ± 0.58	– 31.2[a]
Hexokinase (HK)⁺	120.6 ± 0.08	58.4 ± 0.54	– 51.6[b]
Phosphoglucoisomerase (PGI)⁺	422.0 ± 0.08	276.9 ± 0.98	– 34.4[b]
Aldolase⁺	198.1 ± 0.98	64.1 ± 0.64	– 67.7[a]
Phosphofructokinase (PFK)⁺	129.2 ± 1.45	35.8 ± 1.10	– 72.3[a]
Enolase⁺	116.3 ± 0.06	62.2 ± 0.04	– 46.5[b]
Lactate dehydrogenase (LDH)⁺	163.6 ± 1.01	33.9 ± 0.10	– 79.3[a]
Pyruvate kinase (PK)⁺	155.0 ± 0.01	54.6 ± 0.06	– 64.7[a]

The data are means ± SD of 3 experiments.
*μmol/mg worm/4 h.
**pmol/mg protein.
⁺nmol/min/mg protein.
P values: [a]< 0.001 and [b]< 0.005.

interest to elucidate the effect of this compound on the filariids in order to delineate its possible mechanism of action. The present report deals with alterations in glucose uptake and glycolytic enzymes of *A. viteae* isolated from ivermectin-treated Mastomys.

The studies were carried out in the experimentally-induced *A. viteae* infection in the rodent host *M. coucha*[4]. Animals at 75 days post inoculation with infective larvae showing progressive rise in microfilariaemia were divided into two groups. One group was treated orally with the drug at 250 μg/kg for 5 consecutive days, while the other was kept as the untreated control. After 16 days of therapy, the animals were killed by deep anaesthesia and the worms were recovered for investigation. The whole study was repeated thrice.

20–30 mg intact, motile worms were incubated in Hank's balanced salt solution (pH 7.4) containing 0.5% glucose. After 4 h the worms were removed and the medium was assayed for glucose[5] and lactate[6] contents. For enzyme assays 50–70 mg of the worms were homogenized (5% w/v) in 0.15 M KCl. The homogenate was centrifuged at 900 *g* for 10 min and the supernatant was sonicated. The sonicate was centrifuged at 105,000 *g* for 30 min and the supernate was used for the assay of enzyme activities as described elsewhere[7]. Protein content was measured according to Lowry *et al.*[8] using bovine serum albumin as a standard. Adenosine triphosphate (ATP) and phosphoenol pyruvate (PEP) contents of the worm extract were determined by spectrophotometric methods[9,10].

The cofactors and coupling enzymes used for various determinations were obtained either from Sigma Chemical Co., USA or SISCO Research Labs., Bombay. Ivermectin was obtained as a generous gift from Merck, Sharp and Dohme Research Lab., Rahway, USA.

Ivermectin-treated *A. viteae* filariids utilized 27% more sugar but produced 35% lesser lactate as compared to the untreated worms (Table 1). This resulted in a drop of *L*/*G* ratio from 1.94 to 0.43. The treated filariids expressed lower contents of ATP and PEP. As far as glycolytic enzymes are concerned, ivermectin-treated parasites showed marked depression in the activities of all enzymes studied (Table 1). Lactate dehydrogenase (LDH) and phosphofructokinase (PFK) showed maximum depression while phosphoglucoisomerase (PGI) registered the least change.

Ivermectin (Mectizan), a macrocyclic lactone, is an effective microfilaricidal agent. It is the current drug of choice for treating patients infected with *O. volvulus*[11]. The drug is also effective against the mf. of *B. malayi* and *W. bancrofti*, the causative agents of lymphatic filariasis[12]. Ivermectin has no advantage over diethylcarbamazine (DEC) as far as the treatment of lymphatic filariasis is concerned. However, according to a recent report, ivermectin at a higher dose (200 mg/kg for 5 days) than required for microfilaricidal action, kills 74% of the adult worms of *A. viteae* in Mastomys[3]. These observations warrant for extensive search on its adulticidal property and the possible mechanism of action. Since glucose serves as the principal source of energy and glycolysis as the predominant pathway, the investigations were initiated by observing the effect of ivermectin on the above-mentioned activities of *A. viteae*.

The drug-treated *A. viteae* exhibit greater uptake of glucose but reduced rate of lactate production as compared to the untreated worms (Table 1). This indicates that ivermectin inhibits glucose metabolism by glycolysis but not its uptake. This notion gets support from the observation concerning significant drop in the activities of all the enzymes of the glycolytic pathway included in the study. The subdued combustion of glucose appears to result in the lower production of energy (ATP) and its glycolytic precursor PEP. The parasite in an attempt to meet its energy demand utilizes more glucose.

Since in the present study the level of all the enzymes examined was found to be markedly low (Table 1), this appears to be the result rather than the cause of the adulticidal action. It is not very surprising since in this study the worms were removed from the host on the 16th day of drug treatment while the effect of the drug was noticed much earlier in the form of microfilaricidal action. It is possible that some kind of effect on adults initiates much earlier but the cumulative alterations in the form of biochemical parameters become noticeable later. The initial action of a candidate macrofilaricidal agent (CDRI compd. 82/437), has been found to be its interference with antioxidant defence[13,14] rather than on energy metabolism or glycolysis. A similar type of situation in the initiation of action of ivermectin may not be denied since GABA receptor is already known to be a potential target for the drug's microfilaricidal activity[2]. Therefore, the observed interference with the sugar metabolism leads to the lower production of ATP and the energy deprived worms ultimately die.

1. Ottesen, E. A. and Ramachandran, C. P., *Parasitol. Today*, 1995, **11**, 129–131.
2. Goa, K. L., McTavish, D. and Clissold, S. P., *Drugs*, 1991, **42**, 640–658.
3. Fatma, N., Murthy, P. K. and Chatterjee, R. K., in *Tropical Diseases: Molecular Biology and Control Strategies* (eds Kumar, S., Sen, A. K., Dutta, G. P. and Sharma, P. N.), PID, CSIR, New Delhi, 1994, pp. 55–64.
4. Singh, D. P., Misra, S. and Chatterjee, R. K., *Indian J. Parasitol.*, 1989, **13**, 101–110.
5. Burliegh, I. G. and Schimke, R. T., *Biochem. Biophys. Res. Commun.*, 1968, **31**, 831–836.
6. Barker, B. S. and Summerson, W. H., *J. Biol. Chem.*, 1941, **138**, 535–554.
7. Marcus, R. E. and Srivastava, V. M. L., *Proc. Soc. Exp. Biol. Med.*, 1973, **143**, 488–491.
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265–275.
9. Lamprecht, W. and Trautschold, J. V., in *Methods of Biochemical Analysis* (ed. Bergmeyer, H. U.), Academic Press, New York, 1963, pp. 453–551.
10. Kornberg, A., *Methods Enzymol.*, 1955, 441.
11. Boatin, B. A., Hougard, J. M., Alley, E. S. *et al.*, *Ann. Trop. Med. Parasitol.*, 1998, **92**, S47–S60.
12. Campbell, W. C., *Med. Res. Rev.*, 1993, **13**, 61–79.
13. Batra, S., Chatterjee, R. K. and Srivastava, V. M. L., *Biochem. Pharmacol.*, 1990, **40**, 2363–2369.
14. Batra, S., Singh, S. P., Fatma, N., *et al.*, *Biochem. Pharmacol.*, 1992, **44**, 727–731.

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Mercury accumulation in selected plant species exposed to cement dust pollution

Mercury is a typical toxic trace metal pollutant. Bio-accumulation of mercury in plants and its entry into the food chain resulting in long-term health hazards, is of major concern. Since the beginning of the industrial era, anthropogenic activity made a substantial contribution to the mercury adulteration of the atmosphere. Increased mining, high rate of fossil fuel burning, widespread use of raw materials containing mercury in the industry are some of the sources of mercury, creating a vitiated environment.

The cement manufacturing process is one of the chief sources of mercury in the environment. The present study focuses on the analysis of accumulation levels of mercury in selected plant species around a cement factory.

The cement factory under study was Panyam Cements and Mineral Industries Ltd., located at Bugganipalli near Bethamcharla, Kurnool district, Andhra Pradesh, India. The area about ± 5 km around the factory is considered as polluted and that about ± 12 km away as control. Among the plant species encountered around the factory, *Tephrosia purpurea* and *Cassia auriculata* are abundant both in polluted and control areas, and hence were selected for experimentation. Besides, *Arachis hypogaea*, a crop plant species, common to both areas was also selected for the study.

The wild plant samples were collected at distances of 1, 2, 5 and 12 km and crop plant samples were collected at 5 and 12 km, as the crop is cultivated only from 5 km onwards. To study seasonal variations and to see the impact of wind direction on mercury accumulation, the samples were collected twice, with a gap of three months (September and January), from both the east and west directions. The plant samples were packed in air tight polythene covers and preserved in a freezer until further analysis. The mercury accumulation levels are estimated individually in the root, stem and leaf.

Wet digestion or acid digestion method was employed for quantitative analysis of mercury[1]. To 0.5 g of plant tissue (root, stem, leaf), 5:1:1 ml of HNO_3, $HClO_4$ and H_2SO_4 were added in a teflon bomb and heated on a mantle at 80°C for 7 h. The digest is diluted to 25 ml with double distilled deionized water and taken for analysis. Cold vapour atomic absorption spectrometry (CV-AAS) is adopted to analyse the plant samples[2] using a mercury analyser (MA 5800 E of ECIL) with mercury levels expressed in ng/mg.

The amounts of mercury in *Tephrosia purpurea* and *Cassia auriculata* compared with *Arachis hypogaea*, where the higher levels of mercury were noticed are shown in Table 1. This may be found to be more in the leaves than in the stem and root. However, in *Arachis hypogaea*, roots showed higher levels of mercury compared to the stem and leaves at both 5 and 12 km.

The uptake and accumulation of chemicals by plants may prove to be the most important aspect of pollution dynamics[3]. It is interesting to note that the elements like cadmium and mercury are more easily bio-accumulated than other elements[4].

The results obtained in this study reveal that the accumulation levels are not uniform, either within or among the plant species. They support the fact that many factors like the fluctuating environment (such as temperature, soil pH, soil aeration, soil moisture), the root system, availability of element in the soil, plant energy supply to all parts, etc. influence the uptake and accumulation of the metal[5]. It was observed that the root accumulated comparatively less amounts of mercury than the stem and leaf in *Tephrosia purpurea* and *Cassia auriculata*. On the other hand, *Arachis hypogaea* showed higher accumulation levels in the root. This may be partly due to plant varieties and their ability to absorb and accumulate heavy metals in their tissues[5].