150 μg/ml rifampicin; only minor interkaryotype redistribution concerning from akro- to telocentric chromosomes occurs. No induction of numerical or structural aberrations after rifampicin treatment was found. Some results were obtained in our earlier experiments dealing with long cultivation (140 days) of HeLa cells with rifampicin (150 μg/ml)[16]. In agreement with them, the results presented 1. indicate the possibility of cell cultivation (human malignant stabilized lines) even in media with a high content of rifampicin (150 μg/ml); this circumstance may be of great importance for virologists because a set of viruses may be totally inhibited by rifampicin at a concentration of 100 μg per ml[5,8]; 2. focus attention on the possible protective effect of calf serum of higher concentrations; 3. confirm that in mammalian cells chromosomal aberrations are not induced by even high concentrations of rifampicin; 4. contribute to the elucidation of rifampicin action on the mammalian cell by the observation that the RNA synthesis is inhibited.

Zusammenfassung. Nachweis, dass Rifampicin (Lepetit, Milano) als hochwirksames Antibiotikum im HEp-2- und HeLa-Zellversuch Viren in Ausbreitung und Entwicklung weitgehend zu hemmen vermag. Chromosomenaberrationen wurden keine beobachtet, hingegen wird die RNA-Synthese gehemmt.

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[16] R. KLEN, H. SKALSKÁ, V. SRB and J. HEGER, Folia biol., Praha, in press (1973).

Mutagenicity and Infertility Following Administration of Lead Sub-Acetate to Swiss Male Mice

Of all the heavy metals that contaminate the environment and pose a potential hazard to public health, lead appears to be of major concern. It is a metabolic poison, inhibits the formation of hemoglobin by interacting with-SH group, and interferes with other enzymatic processes.

Man retains approximately 200 to 400 μg/day of lead[1]. In the U.S., the main concentration of lead has been estimated to be 0.25 mg/kg[2]. Concentration of lead in blood excess of 0.8 mg/kg causes lead poisoning[3]. In children, symptoms of mental retardation, cerebral atrophy[4] and brain damage, due to changes in the gray and white matter[5] have been attributed to lead. Women exposed to lead have 3 times more abortions than those not exposed[6]. Tumors of the kidney were induced in rats following chronic administration of lead in their drinking water[7]. Very little is known about the genetic damage which is caused by lead. Genetic effects are important because these may be latent for several generations and in addition, the damage may be permanent. The present study was undertaken to determine mutagenicity following ingestion of lead.

14 Swiss male mice (Charles River Breeding Labs, Wilmington, Mass.), 8 weeks old, were fed 2% aqueous solution of lead sub-acetate in drinking water for 28 days; 5 additional male mice drank tap water and served as concurrent controls. During the treatment period, their

[1] R. A. KEHOE, J. R. Inst. Publ. Health Hyg. *24*, 81 (1961).
[2] C. C. PATTERSON, Arch. envir. Health *24*, 88 (1961).
[3] R. A. KEHOE, Publ. No. 1440, U. S. Publ. Health Service, Washington, D.C. (1966).
[4] J. M. BERG and M. ZAPELLA, J. ment. Deficiency Res. *8*, 44 (1964).
[5] T. J. HALEY, Clin. Toxic. *4*, 11 (1971).
[6] J. BOURRET and J. MEHL, Arch. Madies Profess. Med. Trav. Sec. sociale *27* (1966).
[7] G. J. VAN ESCH and R. KROES, Br. J. Cancer *23*, 765 (1970).

Incidence of mutagenicity and infertility following exposure to 2% lead sub-acetate orally to Swiss male mice

	Group	1	2	3	4	5	6	Overal
No. of females exposed	T	39	39	38	37	39	33	225
	C	12	12	12	12	12	12	72
No. of pregnant	T	9	9	11	12	8	13	62
	C	2	7	6	11	3	9	38
Percent pregnant	T	23.0	23.0	28.2	32.4	20.5	39.4	27.6
	C	16.6	58.3	50.0	91.6	24.9	75.0	52.7
No. early embryonic deaths	T	1	1	9	3	0	13	
	C	1	2	0	1	0	2	
No. of total implants	T	57	72	96	119	72	125[d]	541
	C	12	61	54	101	27	81	336
No. of total implants/pregnancy	T	6.3	8.0	8.7	9.9	8.0	9.6	8.7
	C	6.0	8.71	9.0	9.18	9.0	9.0	8.8
Mutagenicity Index[c]	T	1.75	1.39	9.4[a]	2.5	0	10.4[b]	
	C	8.34	1.64	0	0.99	0	2.98	

T, treated group; C, control group. [a] $\chi^2 = 5.38$ ($P \geq 0.01$). [b] $\chi^2 = 10.4$ ($P \geq 0.05$). [c] Mutagenicity Index = (No. of early fetal deaths/Total implants) · 100. [d] Includes 1 late embryonic death.

daily intake of fluid was recorded. At the end of the treatment period each test and control male was then individually caged for 1 week with 3 untreated virgin 8-week-old females. Females were replaced weekly and consecutively with fresh animals for a total of 6 weeks. During the mating period, mice were fed laboratory chow and had access to tap water ad libitum. All females were autopsied on day 13–15 of their exposure to males. They were scored for pregnancy and implants comprising of normal living implants, late fetal deaths, and early fetal deaths; the latter appeared as black deciduomata. Details of the assay system and technique[8,9] and satistical anylsis have been reported[10].

The mean fluid intake of lead sub-acetate solution for the entire treatment period was 107.4 ml/mouse ranging from 207 to 220 ml/mouse; total mean intake of lead amounted to 1.64 g. Body weights of the treated and control groups were similar at the end of the treatment period. In the treated group there was no mortality during the treatment period, but 1 male died during the 4th week and another died in the 6th week of mating; 1 control male died in the 2nd week; the cause of death of these males was not attributed to treatment.

The overall incidence of pregnancy, indicative of fertility, was 52.7% in control group, as compared to 27.6% in the treated group (Table). The data of the individual weeks also showed that fertility of the treated males was consistently lower, except in the 1st week which was perhaps due to sexual inexperience of the males. Fertility was lowest in the 4th week. Thus, the treatment with lead reduced the fertility of the males by 50%. Testicular degeneration and oligospermia based on histological exmination in the rat has been reported following repeated administration of lead acetate[11].

Number of total implants per pregnancy in the treated group when compared with the corresponding value in the controls did not indicate any systematic variation; the overall mean values were 8.7 and 8.8 respectively. Thus, there were no preimplantation losses either due to paternal or maternal causes.

Statistical analysis of the differences in mutagenicity index between the test and controls were highly significant ($p \geq 0.1$) in the 3rd week and were significantly different ($p \geq 0.05$) in the 6th week; this index was not statistically significant in the 1st week. Chromosomal aberrations in leukocytes cultured in vitro have been reported in mice fed with led acetate in their diet[12].

The study indicates that lead caused infertility in mice as evidenced by reduced pregnancy rates among the females. In addition to this, perhaps of greater importance is the genetically-related mutagenicity that was also detected. Undoubtedly more extensive dose-response study is required to establish the threshold limits for human exposures.

Zusammenfassung. Nach 28tägiger Verabreichung von 2% Blei-Acetat im Trinkwasser wurden männliche Mäuse mit Gruppen unbehandelter Weibchen wöchentlich über 6 aufeinanderfolgende Wochen gepaart und die Weibchen in der mittleren Gestationsperiode seziert und untersucht. Das Gesamtvorkommen von Schwangerschaften war 27,6% und 52,7% in behandelten, bzw. unbehandelten Tieren. Die Fruchtbarkeit war in der 4. Woche am geringsten und der Mutagenitäsindex zeigte in der 3. und 6. Woche statistisch signifikante Differenzen.

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Bio-Environmental Engineering and Sciences Research Laboratory, Department of Civil Engineering, Washington (D.C. 20001, USA), 26 October 1973.

[8] S. S. Epstein, E. Arnold, J. Andrea, W. Bass and Y. Bishop, Toxic. appl. Pharmac. *23*, 288 (1972).
[9] S. R. Joshi, S. S. Epstein and Y. Bishop, Experientia, *29*, 1253 (1973).
[10] J. Kruger, *Chemical Mutagenesis in Mammals and Man* (Springer-Verlag, New York 1970).
[11] E. Golubovich, M. M. Avkimenko and E. M. Chirkova, Toksik. Khim. Veshchestv. *10*, 64 (1968).
[12] L. A. Muro and R. A. Goyer, A.M.A. Arch. of Pathology. *87*, 660 (1969).
[13] This work was supported in part by grant No. WP-178-05 from the Environmental Protection Agency; some mice were donated by Meloy Labs., Springfield, Va.

DNA Renaturation Kinetics in Some Paedogenetic Urodeles

Paedogenesis, namely the persistence of larval characters throughout life, has played an important role in the evolution of the Amphibia *Caudata*[1,2]. 4 out of the 8 living families of this order include only paedogenetic forms, either permanently larval (Proteids and Sirenids) or semi-larval (Cryptobranchids and Amphiumids)[3]. These last families show the same karyotype morphology (though with larger chromosomes) of the families from which they presumably originated: in fact the Cryptobranchids ($2n = 60$–64) have karyotypes similar to those of the primitive Hynobiids, and the Amphiumids ($2n = 28$) have the same karyotypes as the ambystomatoid stock (Ambystomatids and Plethodontids). The Proteids ($2n = 38$) have karyotypes intermediate between those of the Hynobiids and the ones of the more advanced ambystomatoid stock while the still problematic Sirenids show peculiar characters in their karyotypes which are possibly originated by polyploidization[4,5].

Considering the DNA content per nucleus, all paedogenetic species are characterized by very large amounts in the nuclear DNA, the largest among the other families of the same order and, together with the Dipnoi among all vertebrates; this amount is very likely to be of secondary origin since none of these species is basic in the phylogeny of the Urodeles[4,6].

It would have been of interest to find out possible correlations between the large genomes of paedogenetic species and their content in highly repetitive DNA, and to have some indications on the genome complexity (the amount of diverse DNA sequences)[7] of species from different evolutionary stages within the order. To these

[1] I. I. Schmalhausen, *Factors of Evolution* (Blakiston, Philadelphia and Toronto 1949).
[2] D. B. Wake, Mem. South Calif. Acad. Sci. *4*, 1 (1966).
[3] G. K. Noble, *The Biology of the Amphibia* (Mc Graw-Hill Book Co., New York 1931).
[4] A. Morescalchi, in *Cytotaxanomy and Vertebrate Evolution* (Eds. A. B. Chiarelli and E. Capanna; Academic Press, London 1973).
[5] A. Morescalchi and E. Olmo, Experientia *30*, in press (1974).
[6] A. Morescalchi, Boll. Zool. *37*, 1 (1970).
[7] R. J. Britten and E. H. Davidson, Q. Rev. Biol. *46*, 111 (1971).