The Action of Rifampicin on Stabilized Cell Lines HEp-2 and HeLa

Rifampicin, a semisynthetic derivative which belongs to the group of rifamycines, is a highly effective antibiotic of wide spectrum, exhibiting an apparent action on the synthesis of bacterial RNA, especially on DNA-dependent RNA polymerase[1,2]. This new antibiotic may inhibit the transfer of genetic information exactly in a locus of RNA synthesis in the DNA matrix (a blockade of transcription I.); with bacteria, this phenomenon occurs already at very low concentrations (1 μg/ml) contrary to mammalian cells which are often considered as intact ones[3,4], or affected by high doses (100 μg/ml)[1,2,5,6]. Toxicity of rifampicin for mammalian cells has been described by BERGAMINI and FOWST[7] as very low. Antiviral[8] and antitumor actions[9] of rifampicin are promining; nevertheless its teratogenic effect is also to be born in mind[5].

Our investigations are focussed on the study of the action of higher concentrations of rifampicin (from 75 μg/ml) on cellular morphology and physiology, clarifying firstly the thesis that in mammalian cells a rifampicin blockade of RNA synthesis is not involved, and secondly the thesis on rifampicin action on the chromosomal composition of mammalian cells. For these experiments, HEp-2 and HeLa cell lines of human origin were used[10].

Microcinematographical short-time examination of HEp-2 cells showed a slight inhibiting effect of rifampicin at 78 μg per ml concentration, reflected in specific reversible alternations in size and structure of the nucleoli. The nucleoli which are of predominant interest play a significant role in cellular division, in the synthesis of RNA and proteins[11]; their physiological state is reflected in cell morphology. Variability occurring in their structure, number, size, and consistency is an evaluable criterion of experimental affectation[12,13]. Under our conditions, the changes were detected even during the course of the first hour. Most remarkable was the occurrence of centric clearing of the nucleoli (Figure 1). However, the cells continued to divide and no significant degenerative changes were observed. After the addition of 10 volume portions of calf serum to the synthetic cultivation medium ÚSOL[14] instead of 5, the action of rifampicin was clearly eliminated (Figure 2).

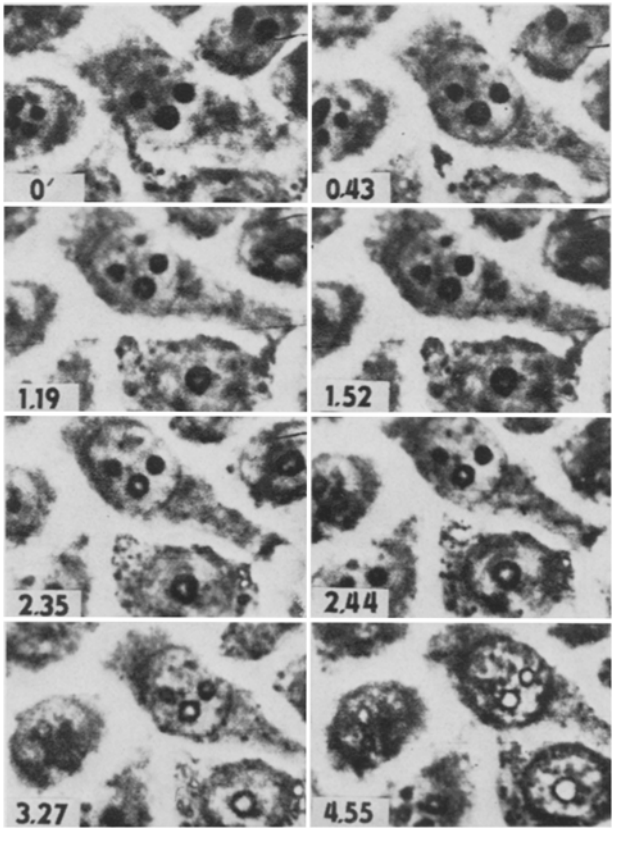

Fig. 1. Dynamics of the changes of nucleoli in HEp-2 cells (synthetic medium ÚSOL+5% of calf serum, 78 μg/ml rifampicin) observed by microcinematographic technique. Time course of the evolution of centric clearing of nucleoli is shown.

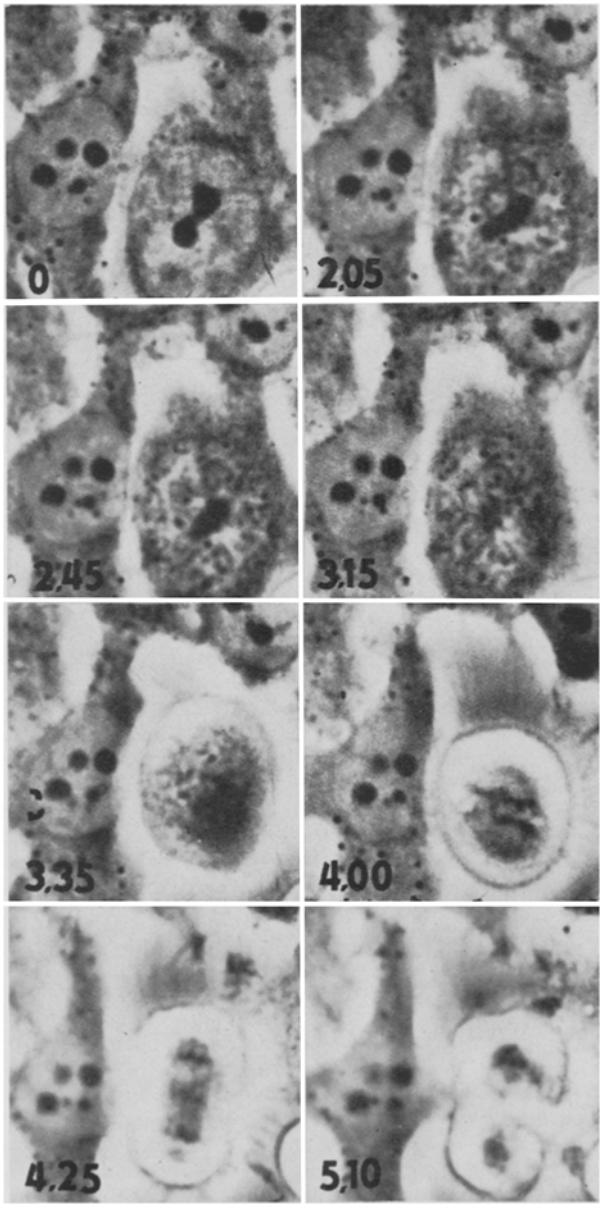

Fig. 2. HEp-2 cells (synthetic medium ÚSOL+10% of calf serum, 78 μg/ml rifampicin) observed by microcinematographic technique. The course of mitotic division of 1 cell is demonstrated.

Effect of rifampicin (Lepetit Milano) on the chromosomal composition of stabilized cell line HEp-2

Determination	Control (K)	Rifampicin (RFP) (150 μg/ml)	Statistically significant difference (p)
Modal chromosome number (n = 115)	70	70	0
Average chromosome number $\bar{x} \pm s_{\bar{x}}$ (n = 115)	70.29 ± 0.44	70.02 ± 0.37	0
Cells having 70 chromosomes in the set	24 (= 21%)	24 (= 21%)	0
Number of chromosomal arms in the set $\bar{x} \pm s_{\bar{x}}$ (n = 26)	126.46 ± 0.12	131.92 ± 0.17	<0.01
Type of chromosomes occurring in the set most often	Submetacentrics ($^1/_3$)	Submetacentrics ($^1/_3$)	0
Statistically significant difference between K and RFP cells in representation of chromosomal type	Acrocentrics+telocentrics	Acrocentrics+telocentrics	<0.05
Cells with numerical aberrations (n = 200)	2 Polyploid (= 1%)	2 Polyploid (= 1%)	0
Number of structural aberrations	16	38	0
Chromosome aberrations (%)	1.45 (n = 1098 chromosomes)	3.47 (n = 1094 chromosomes)	0

Both the nucleolar index (number of the nucleoli in the nucleus) and the mitotic activity (evaluation by mitotic index calculated from a set of 1000 cells) determined at HEp-2 cell line (occurring in the logarithmic phase of growth) after 6, 12 and 24 h cultivation of the cells in the medium containing 150 μg/ml rifampicin, confirmed an inhibition of the antibiotic mentioned. The use of demonstrated concentration prolonged the course of presynthetic G_1 phase and slowed down the beginning of the S-phase of the cellular cycle. It is known that the nuclei containing a high amount of nucleoli, are characteristic for the G_1-phase, and that, in a sense of S- and G_2-phases, the nucleolar index is of decreasing value[12, 13, 15]. After 12 and 24 h, this index was found to be statistically higher in the culture administered with rifampicin, as compared with the non-affected control sample (especially in the nuclei with 3–4 nucleoli examined after a 12 h interval). Elongation of the state of the cells in pre-synthetic phase necessarily leads to a delayed beginning of cellular division; evidence may be found in the low mitotic index which is most remarkable in the period from 12 to 24 h of the cultivation with rifampicin ($MI_c = 120$, $MI_{Rif} = 20$).

The morphological characteristics presented (quantitative and qualitative), demonstrating rifampicin blockade of the RNA synthesis in human cells cultivated in vitro, were confirmed by autoradiography. During a 24 h period (2, 6, 12, and 24 h), the RNA synthesis observed in HeLa cells after application of 150 μg/ml rifampicin by means of ^{3}H-uridine incorporation was inhibited[10] (Figure 3). Even though the mechanism of rifampicin inhibition of RNA synthesis in HeLa cells could not be clearly elucidated by autoradiographic technique, we may suppose that the inhibition of DNA-dependent RNA polymerase occurs.

The chromosome number of the HEp-2 cells (Table) was statistically unchanged after a 24 h application of

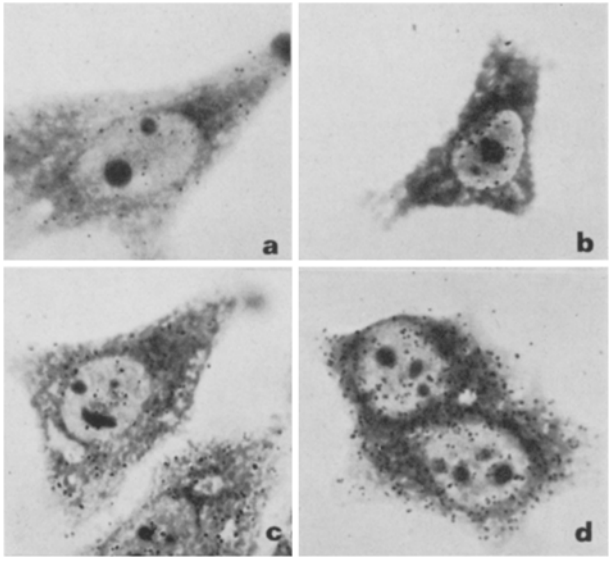

Fig. 3. Incorporation of ^{3}H-uridine into HeLa cells. Cells were preincubated for 12([a]), 6([b]), or 2([c]) h in rifampicin-containing medium (150 μg/ml), pulse labelled by ^{3}H-uridine (30 min o.3 μCi per ml, spec. activity 14.2 Ci/M) and chased for 6 h in the medium with non-active uridine. In the samples (a, b, c), radio-activity of ^{3}H-RNA is remarkably lower, as compared with the controls (d).

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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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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

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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.