When the lengths of the chromosomes were compared, P. palmas consistently had longer metaphase chromosomes than C. calabaricus. In a typical metaphase spread of P. palmas, the smallest chromosome averaged 2.3  $\mu$ m while the longest was 11.2  $\mu$ m (Figure 1). In C. calabaricus, the smallest chromosome averaged 1.8  $\mu$ m while the longest was 7.8  $\mu$ m (Figure 2). Thus, it seems that P. palmas has more chromosome material than C. calabaricus.

Seven of the 105 metaphase cells analyzed for *C. calabaricus* had a chromosome fragment that resembled a small acrocentric chromosome. Since it did not stain as well as the surrounding autosomes, it was considered to contain mostly heterochromatin. No chromosome fragments were found in *P. palmas*. However, in 20 of 43 metaphase cells examined in *P. palmas*, a distinctive chromatid gap was found in a member of chromosome pair number 4 (Figure 1). No gaps were found in *C. calabaricus*. The occurrence of the chromatid gap and the occasional chromosome fragment suggests that some mechanism of karyotypic change is still operating in this group. Except for these two conditions, all karyotype preparations were consistent in both number and structure for both genera.

Most present day classification schemes place the polypterids in the subclass Actinopterygii, superorder Chondrostei, along with the sturgeons and paddlefishes. However, a comparison of polypterid chromosomes with those of the shovelnose sturgeon, Scaphirhynchus platorhynchus  $^{16}$ , shows great dissimilarities (Table III). The most obvious differences are the high 2n number of 112 and the presence of 48 microchromosomes in the sturgeon. Microchromosomes are characteristic of the sturgeon, gar, reptiles and birds  $^{16}$ , and their absence in polypterids is certainly noteworthy. In addition, the longest chromosome of the sturgeon is only 4  $\mu m$  while the longest chromosome in polypterids is  $12~\mu m$ .

Polypterid chromosomes are also very distinct from those reported for the holostean gar, Lepisosteus productus (= oculatus) and bowfin, Amia calva  $^{16}$  (Table III). The gar, like the sturgeon, has microchromosomes, a much higher 2n number, and smaller-sized chromosomes. Thus, the gar and sturgeon are much closer in chromosome morphology than either are the polypterids. The bowfin, like the polypterids, lacks microchromosomes but has a higher 2n number (46) and the largest chromosome is only about  $3 \mu m^{16}$ .

As already indicated, polypterids have many morphological features in common with fishes of the crossopterygian line and were considered by a few early workers to belong to that group. Unfortunately, the chromosomes of the 2 groups cannot be compared as there have been no chromosome studies done on Latimeria, the only living crossopterygian. However, a comparison of polypterid chromosomes with those of Australian, African, and South American dipneustans shows many striking similarities (Table III). The 2n numbers range from 32 to 38 for these dipneustans and is in conformity with the 2n of 36 for polypterids. In addition, all of the dipneustans have biarmed chromosomes which give a fundamental number of 64-76 which is in agreement with that of 72 in polypterids. The only dissimilarity seems to be in the length of the chromosomes which is up to 30  $\mu m$  in dipneustans 14, 16, 17, but only 12 µm in polypterids.

Thus, based upon chromosome morphology as well as body structure, the polypterids seem to have more in common with the dipneustan lungfishes than with the Actinopterygians. However, polypterids differ significantly enough from the dipneustans in chromosome size, route of pulmonary circulation, fin structure, etc., to avoid their placement into this group. Thus, based upon our chromosome data, we agree with others <sup>11–13</sup>, that it is justifiable to remove the polypterids from the superorder Chondrostei, subclass Actinopterygii, and place them in a separate group by themselves, the subclass Brachiopterygii. Surely they are distinctive from both groups and seem to occupy a somewhat intermediate phylogenetic position.

Résumé. Une étude de chromosomes des poissons polyptères africains, Polypterus palmas et Calamoichthys calabricus révèle un nombre diploïde de 36. Ce caryotype a été comparé à celui du Dipneustes, du Holostéens et du Chondrostéens. Il ressort de cette analyse que les Polyptères occupent une situation intermédiaire entre celles des Actinopterygii et des Dipnoi, ce qui légitime le point de vue selon lequel ils doivent être placées dans une sousclasse distincte, les Branchiopterygii.

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## Testing of Rifampicin on Possible Genetic Effects on *Drosophila melanogaster* and Human Leukocyte Chromosomes in vitro

Rifampicin, belonging to the rifamycin antibiotics, is the most widely used compound of this class for both clinical and biochemical purposes. Clinically, rifampicin proved to be very helpful, especially for the treatment of tuberculosis. The antibacterial activity of the rifamycin group is due to specific inhibition of bacterial DNA-dependent RNA polymerase. For details on the chemical structures and the actions of the rifamycins see ref. 1 and 2.

Because of the widespread clinical use of rifampicin, we were interested in any mutagenic effects of this substance on *Drosophila* and human leukocyte chromosomes. *Drosophila*: In order to study a possible genetic effect of rifampicin on *Drosophila*, the Basc-technique for the determination of recessive X-chromosome lethals was applied. The sodium salt of rifampicin was dissolved in 5% sucrose solution containing phosphate buffer to keep

the pH at 7.0. Berlin wild K males, 1–2 days old, were fed with the test solution (concentration 0.5 mg/ml rifamycin) for 3 days, using the adult feeding method described elsewhere  $^{3,4}$ . Each P-male was crossed to two  $sc^{81}$  In S B  $w^a$   $sc^8$  females (genetic symbols see ref. 5). The Basctechnique was performed in the usual way with 3 successive broods, each for 3 day's duration. Progeny obtained from

<sup>&</sup>lt;sup>1</sup> G. BINDA, E. DOMENICHINI, A. GOTTARDI, B. ORLANDI, E. ORTELLI, B. PACINI and G. Fowst, Arzneimittelforschung (Drug Res.) 21, 1907 (1971).

<sup>&</sup>lt;sup>2</sup> W. Wehrli and M. Staehelin, Bact. Rev. 35, 290 (1971).

<sup>&</sup>lt;sup>3</sup> H. Lüers, Arch. Geschwulstforsch. 6, 77 (1953).

<sup>&</sup>lt;sup>4</sup> E. Vogel, Mut. Res. 11, 397 (1971).

<sup>&</sup>lt;sup>5</sup> D. L. LINDSLEY and E. H. GRELL, Carnegie Inst. Washington, Publ. No. 627 (1968).

Table I. Drosophila melanogaster. Induced frequence is of recessive lethals following treatment of Drosophila males with 0.5 mg/ml rifampicin

Brood	X-chromosomes tested	No. of recessive lethals	Recessive lethals (%)	
I	614	2	0.33	
II	613	1	0.16	
III	612 .	0	_	
I–III	1839	3	0.16	

found only achromatic lesions (AL), chromatid breaks (B'), and isochromatid breaks (B"). Chromatid translocations (RB') were never seen (for description of these aberration types see ref.<sup>8</sup>). The aberrations show no dose effect relationships and are not elevated over the base line found with this test system <sup>9,10</sup>. As in the *Drosophila*test in the leukocy'c-test also rifampicin shows no genetic activity.

Zusammenfassung. Mutagenitätsuntersuchungen von Rifampicin an Drosophila meianogas . (X-chromosomale rezessive Letalmutationen) und an meischlichen Leuko-

Table II. Chromatid aberrations produced by rifampicin in human leukocyte chromosomes in vitro

Rifampicin concentration mg/ml	No. of cells analyzed	Achromatic lesions (AL)		Chromatid breaks (B')		Isochromatid breaks (B")	
		Percent of cells	Number per cell	Percent of cells	Number per cell	Percent of cells	Number per cell
0.019	400	7.00	0.078	8.25	0.098	0.25	0,003
0.037	400	$\pm 1.28 \\ 4.75 \\ + 1.26$	$\pm 0.015$ $0.053$	± 1.38 3.50	$\pm 0.018$ $0.040$		
0.055	400	$\pm 1.06$ 5.00	$\pm 0.012$ $0.060$	士 0.92 5.00	$\pm 0.011 \\ 0.058$		
0.073	360	$egin{array}{c} \pm \ 1.09 \\ 5.00 \\ + \ 1.15 \end{array}$	$egin{array}{c} \pm \ 0.014 \ 0.075 \ + \ 0.022 \end{array}$	$egin{array}{c} \pm \ 1.09 \ 6.94 \ + \ 1.34 \end{array}$	$egin{array}{c} \pm \ 0.013 \ 0.108 \ + \ 0.029 \end{array}$	0.23	0.003

the first brood mainly represent the sensitivity of mature sperm; progeny obtained from brood II and III correspond to spermatids and spermatocytes, respectively. The results presented in Table I clearly demonstrate that rifampicin, even at such a high dose as  $0.5~\rm mg/ml$ , did not affect the frequencies of recessive lethals in Drosophila. Out of 4023~X-chromosomes tested in 3 broods, the spontaneous rate has been determined as 0.15% in the Berlin wild stock. Thus, rifampicin seems to be quite ineffective according to the incidence of recessive lethals in Drosophila that are mostly due to point mutations or small deletions after chemical treatment.

Human leukocyte chromosomes in vitro: To test a possible chromosome breaking activity of rifampicin in this test system, microcultures from the blood of a normal healthy man were set up 7. Twenty-four h before fixation, water solutions of rifampicin-Na were added to the cultures to final concentrations ranging from 0.019 mg/ml to 0.073 mg/ml. For each concentration, 4 cultures were set up and 60 to 100 mitoses were analyzed per culture. We

zytenchromosomen in vitro ergaben keine Anhaltspunkte für eine genetische Wirksamkeit dieser Substanz.

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## A Behavioural Audiogram of Juvenile Carp

For further investigations an audiogram of the carp was necessary, but surprisingly little could be found about hearing in carp. Therefore it seemed advisable to test the auditory capacity of this fish once more.

Material. The auditory threshold of newly captured 'mirror' carp (Cyprinus carpio) was established in the shuttle-box. The fish were 11 cm in length, and were housed in individual 50 l tanks. The temperature in these tanks was 21°C which represents the preferred temperature for carp¹. The 6 trained fish were fed with meat daily after the trials.

Method. The shuttle-box was 50 cm in length and 28 cm wide. In the swimming area the water level amounted to 11 cm. The testing temperature ranged from 19° to 22°C.

By a RC. decade generator (type PW-7, Zopan, Warsaw) the pure tones were generated. As an underwater loud-speaker the type Lp 2256 (R. Lausch KG, Leipzig) was used. It was calibrated by the sound level meter SDM 57 (Entwicklungswerk für Funkmechanik, Leipzig). For further details of the equipment see Wolff<sup>2</sup>. The shuttle-box was divided by a barrier that possessed an opening (8 cm high, 10 cm wide) 2 cm above the bottom. The width of this opening could be diminished. In this way the escaping behaviour turned towards the bottom<sup>3</sup>

<sup>&</sup>lt;sup>1</sup> F. Schmeing-Engberding, Z. Fisch. 2, NF, 125 (1953).

<sup>&</sup>lt;sup>2</sup> D. L. Wolff, Z. vergl. Physiol. 60, 14 (1968).