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

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 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[6].

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 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.[8]). The aberrations show no dose effect relationships and are not elevated over the base line found with this test system[9,10]. As in the *Drosophila*-test in the leukocyte-test also rifampicin shows no genetic activity.

Zusammenfassung. Mutagenitätsuntersuchungen von Rifampicin an *Drosophila melanogaster* (*X*-chromosomale rezessive Letalmutationen) und an menschlichen Leukozytenchromosomen in vitro ergaben keine Anhaltspunkte für eine genetische Wirksamkeit dieser Substanz.

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

Rifampicin concentration mg/ml	No. of cells analyzed	Achromatic lesions (AL) Percent of cells	Achromatic lesions (AL) Number per cell	Chromatid breaks (B') Percent of cells	Chromatid breaks (B') Number per cell	Isochromatid breaks (B'') Percent of cells	Isochromatid breaks (B'') Number per cell
0.019	400	7.00 ± 1.28	0.078 ± 0.015	8.25 ± 1.38	0.098 ± 0.018	0.25	0.003
0.037	400	4.75 ± 1.06	0.053 ± 0.012	3.50 ± 0.92	0.040 ± 0.011		
0.055	400	5.00 ± 1.09	0.060 ± 0.014	5.00 ± 1.09	0.058 ± 0.013		
0.073	360	5.00 ± 1.15	0.075 ± 0.022	6.94 ± 1.34	0.108 ± 0.029	0.23	0.003

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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 carp[1]. 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 loudspeaker 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[2]. 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[3]

[1] F. Schmeing-Engberding, Z. Fisch. *2*, NF, 125 (1953).
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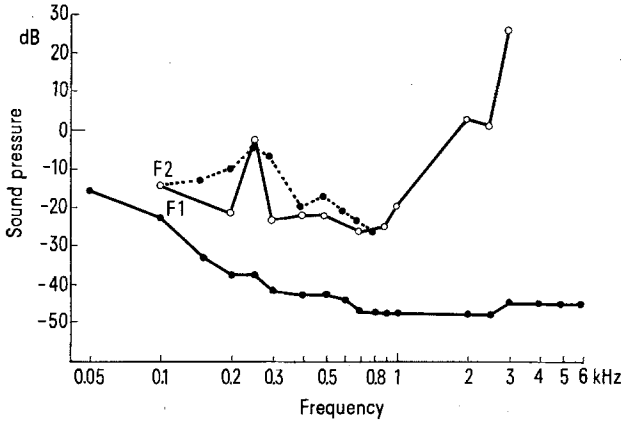
was taken into account. The fish had to cross into the other part of the test tank within 10 sec after resounding a tone of 1,000 Hz (50 dB). When not doing so, the carp received an electric shock of 4 V. (50 Hz, A. C.). The experiments were performed in the afternoon from the end of March till the beginning of May. The threshold determination was accomplished by means of the audiometric staircase method in steps of 5 dB.

Result. Two animals reached the criterion of 80% avoidance after 200 and 450 trials. Earlier attempts with a testing temperature of 13°C and a barrier that the fish had to swim over failed. In this case the animals did not reach the criterion, even after 775 trials. The Figure shows the threshold curves of the 2 carp and the lower one represents the band width levels of the background noise that occurred during the experiments. The frequency of 3,000 Hz (26 dB) was determined as the upper threshold limit. The sensitivity steadily increased up to 1,000 Hz (–20 dB) and the range from 900 Hz (–25 dB) to 400 Hz (–22 dB) is the most sensitive of the tested carp. This range is followed by a slight decrease of the threshold and at 250 Hz (–2 dB) a peak decrease can be noticed for fish 1. The curves of both fish end at 100 Hz (–14 dB) but the lower limit is certainly not reached at this frequency level. Positive responses were obtained up to 75 Hz. Unfortunately the sound pressure at this frequency could not be measured. For the second carp in general the same results were obtained. Even the peak at 250 Hz (–4 dB) was again noticeable, only the decresae from the most sensitive region up to this peak was more gradual. This fish could only be tested up to 800 Hz (–26 dB) because it became neurotic.



The audiogram of 2 juvenile carp (upper curves) and the band-width noise level (lower curve). Note the threshold decrease at 250 Hz. All pressure values are given in dB re 1. μbar.

Discussion. Despite the new barrier that tried to consider the escaping behaviour in carp, it seemed difficult for the animals to learn to avoid the electric shock. Such difficulties are also reported from the crucian carp[4]. Even some marine species could not be trained by this method[5]. Before the conditioned reaction was stabilized, a suppression of respiration could be observed during the first 10 sec. This short suppression of respiration could still be noticed after stabilizing the avoidance behaviour, actually before crossing into the other half of the tank. FAY[6] conditioned this reaction in as little as 10 trials. The striking threshold decrease at 250 Hz might separate the sensitivity of the lateral line organ from that of the inner ear. The phenomenon may be caused by the high far-field background noises at the lower frequencies. The noises injured the perceptivity of the inner ear and that was the reason why the fish had to use the displacement receptor, the lateral line, in order to perceive the acoustic stimulus. For the lateral line of the goldfish, WEISS[7] has found an upper frequency limit at 200 Hz. At the frequency range from 100 to 400 Hz, other authors [5,8] also found an intersection between the sensitivity of both sense organs. No startle reactions, as described for the goldfish[9] and *Leucaspius delineatus*[10], could be observed at the lower frequencies in the present study.

The high upper frequency limit (22 kHz), as mentioned by ROUGH[11], could not be found. It may be that he had used higher sound pressures in his experiments but unfortunately he did not give any suggestions about it. STEPANEK[12] received positive responses from 50 Hz to 11 kHz in his experiments but within this range there were frequencies (1, 5–7 kHz) to which the carp did not respond. But these results are difficult to compare because for the determination he used the spontaneous behaviour of fish to sound, and, on the other hand, an air loudspeaker as sound source. In this way there were other acoustical conditions, and this method also required relative high sound pressures. If we consider the upper frequency limits in other cyprinids, we can see that *Carassius auratus*[6,9,13] ends at 3,000 Hz, too. In the midbrain of *Tinca tinca* responses were obtained up to 3,500 Hz[14]. In *Idus melanotus* (5,524 Hz)[15], *Semotilus a. atromaculatus* (5,700–7,750 Hz)[16], *Phoxinus phoxinus* (5,000–7,000 Hz)[17], *Carassius carassius* (8,000 Hz)[4], and *Leucaspius delineatus* (13,000 Hz)[10] higher limits were found. The peak sensitivity of the carp is within the region found in other cyprinids (from about 300 to 1,000 Hz). The thresholds can be compared to those of the tench[14], the crucian carp[4], the goldfish[9], and *Leucaspius*[10], but for the goldfish also lower thresholds (about 20 dB lower) were found[6,13]. As to the relation of sound production to hearing in carp, it can be seen that the feeding sounds in carp are mainly in the region of the obtained audiogram. The sounds range from 50 to 5,000 Hz without any special frequency peaks[18].

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Zusammenfassung. Mittels des Meideverhaltens wurde die Hörkurve einsömmriger Karpfen bestimmt. Als obere Hörgrenze wurden 3000 Hz (26 dB) und als «untere» 75 Hz erhalten. Zwischen 900 Hz (–25 dB) und 400 Hz (–22 dB) lag das Perzeptionsoptimum. Bei 250 Hz war eine auffällige Empfindlichkeitsverringerung zu beobachten.

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Metamorphic Changes in the Intracerebral Neurosecretory Pathways in the Lemon-Butterfly, *Papilio demoleus* L. (Lepidoptera)

Several authors have described the neurosecretory cells (NSC) and their pathways in Lepidoptera[1–8]. But they have not reported any difference in their arrangement in the pupal and adult brains. During our investigations on the postembryonic changes in the neurosecretory system of *Papilio demoleus*, we found a considerable topographic variation in the NSC and their intracerebral pathways in the pupal and adult brains. This has been made possible by the use of the recently developed in situ staining technique of DOGRA and TANDAN[9]. This technique makes it possible to observe the complete neurosecretory pathway even within a single preparation. Three groups of NSC stain in the pupal brain of this insect: a median group, a lateral group and an anterior group. The median group of NSC is located in the median region of the pars intercerebralis close to the intercerebral midline, the lateral group, on the mid-dorsal region of the protocerebrum and the anterior group, on the anterior margin of the pars intercerebralis (Figure 1). In other lepidopterous insects this last mentioned group is reported on the posterior margin of the brain and has been considered characteristic for the order Lepidoptera[10–13]. The median NSC give rise to 2 sets of axons: those that run outwards constituting the lateral neurosecretory pathway (LNSP) and those that run inwards and forwards constituting the median neurosecretory pathway (MNSP) (Figure 1). The distal portion of the LNSP is not stainable, possibly because it takes a deeper course inside the brain and is not available to the stain in bulk-preparations. On the other hand, the MNSP, being superficial, is fully stainable and could be traced from its origin to emergence from the brain. In addition to the axons of the median NSC, the MNSP is also joined by the axons of the lateral NSC (Figure 1, extreme left, broken arrow), a feature not common in other insects and also whose implication is not immediately clear. A thick band of axons, thus formed, runs inwards and forwards on the pars intercerebralis and decussates with its counterpart from the other side to form the chiasma. Thereafter, the fibres cross into opposite hemispheres and run in close proximity upto the anterior NSC. At the level of the anterior NSC, they make a bend (Figure 2, solid arrows) and possibly, accompanied by the axons of these cells, run backwards traversing the entire length of the protocerebrum before emerging from the brain as the nervi corporis cardiaci I (NCC I) (Figure 2, broken arrow). The portion of the MNSP between the median and anterior NSC is reckoned as the ascending limb (Figure 1) and

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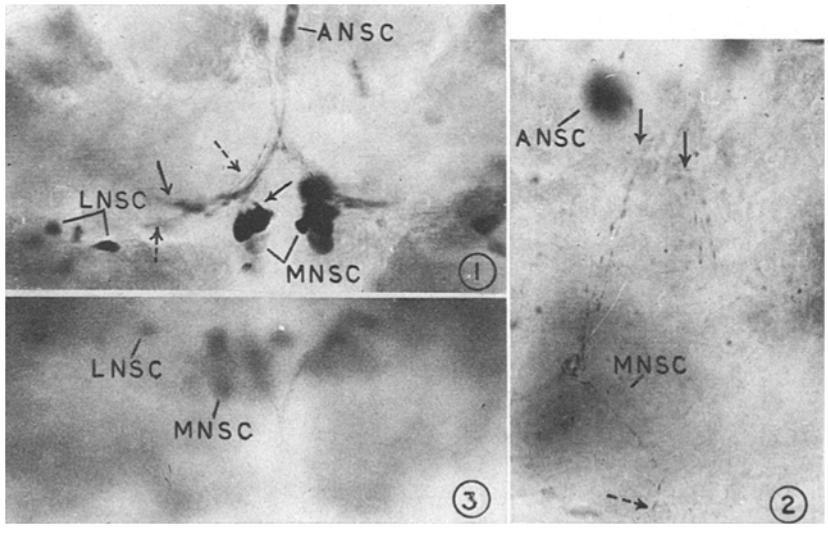

Fig. 1. Bulk-stained preparation of the pupal brain showing 3 groups of NSC and their axons. Solid arrows indicate outwardly directed axons of the LNSP and the broken arrows, inwardly running fibres of the MNSP (median NSC, contributing to MNSP in the left hemisphere, are unstained), ANSC, LNSC, MNSC, anterior, lateral and median groups of NSC, respectively. AF, ×180.

Fig. 2. Bulk-stained preparation of the pupal brain showing descending limbs of the MNSP in the 2 hemispheres (ascending limbs being more superficial are out of focus). Solid arrows point to the bend in the pathway and broken arrow to the emergence of NCC I. AF, ×400.

Fig. 3. Bulk-stained preparation of the adult brain showing the formation of the chiasma by the descending limb of the MNSP (whose post-chiasmatic portion unstained). AF, ×150.