A Non-Thermal Effect of Millimeter Wave Radiation on the Puffing of Giant Chromosomes

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A non-thermal influence of millimeter wave radiation (swept in frequency from 64.1 GHz to 69.1 GHz, sweep-time 6 s, and with stabilized frequencies of 67.200 ± 0.001 GHz and 68.200 ± 0.001 GHz, power density $\leqq 6$ mW/cm^2) on the puffing of giant chromosomes of the midge *Acricotopus lucidus* (Diptera, Chironomidae) was found. The effect is manifested as a reduction in size of a specific puff that expresses genes for a secretory protein. The non-thermal nature of the effect was proved by experiments in which the sham-exposed sample was warmed up by 2.5 °C which is more than the eight-fold microwave induced temperature increase of $\leqq 0.3$ °C. Concerning the very low photon energy of mm-waves compared to the thermal energy kT, it seems likely that the coherence of the radiation is essential for the observed effect.

The non-thermal influence of millimeter wave radiation on biological systems is a topic of considerable importance not only for the understanding of the mechanisms of interaction between electromagnetic radiation and living systems [1–2] but also for the establishment of microwave safety standards. Several biological effects of low-intensity millimeter wave radiation have been reported [3–7], among them genetic effects in *Drosophila melanogaster* [4, 6]. We have now found an influence of millimeter waves on the activity of a certain gene locus (Balbianiring BR2) in giant chromosomes from salivary glands of the midge *Acricotopus lucidus* (Diptera, Chironomidae) [8].

For the experiment larvae in their fourth larval instar of *Acricotopus lucidus* were dissected. Their paired salivary glands were placed in a sample container which consisted of a fused silica plate with an indented circle of radius 40 mm and depth 0.2 ± 0.02 mm. In its centre two further circular incisions of diameter 2 mm and depth 0.3 ± 0.02 mm were prepared. One salivary gland was put in each of these last indentations. Cannon's medium [9] was added and then the sample container was covered by an oxygen-permeable membrane. The glands are sac-like and have a diameter of about 0.25–30 mm. Thus the layer of aqueous medium between the membrane and the glands was at least 0.2 mm thick. The sample container was positioned on a fused silica temperature controlled dish (9.0 ± 0.1 °C). An identical dish was mounted in the control chamber. The temperature of the sample was measured with a micro-miniature thermal probe (diameter 0.2 mm, thermal rise-time: 3 ms). The microwave induced temperature increase was found to be less than 0.3 °C at 20 mW forward power.

The frequency was swept between 6.41×10^{10} Hz and 6.91×10^{10} Hz (Sweep time: 6 s). For the experiments with single stabilized frequencies of $6.72 \pm 0.0001 \times 10^{10}$ Hz and $6.82 \pm 0.0001 \times 10^{10}$ Hz a source-locking counter was employed. The forward power was measured as 20 ± 2 mW and the power reflected by the sample container as 2 ± 0.5 mW. Thus a power of 18 ± 2 mW entered the sample container. After passing through the 200 µm thick medium layer the power was reduced to 4.5 ± 0.5 mW (calculated using the absorption coefficient $\alpha = 70$ cm^{-1} of water [10] at 7×10^{10} Hz for the medium). This resulted in a power density of less than 6 mW/cm^2 (horn area 1.6 cm^2).

Each salivary gland is composed of two clearly differentiated cell-types. These cell-types correspond to morphologically distinct lobes which are characterized by a specific pattern of predominant puffs (Balbianirings) [11–15] and are designated main lobe and anterior lobe. Each main lobe consists of about 50–60 cells whereas the smaller anterior lobe has 12–20 cells. The nuclei of all cells contain three polytene chromosomes. In the cells of the main lobe two cell-type specific Balbianirings (BR1 and BR2) are developed in chromosomes I and II, respectively. Since in preceeding experiments no obvious reactions at other Balbianiring sites had been observed, only the BR2 was microscopically examined.

Immediately after irradiation (2 h) each gland was fixed with ethanol-acetic acid (3:1) and stained for squash preparations. Then the number t of strongly reduced Balbianirings BR2 in m examined

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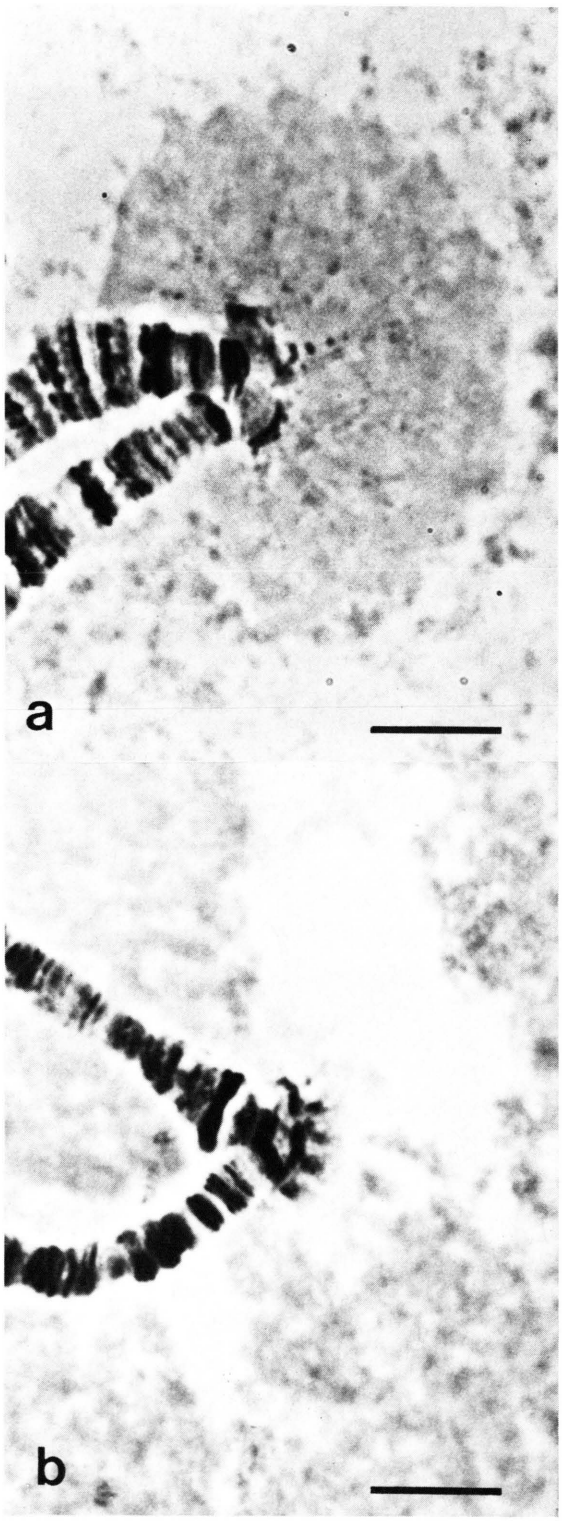

Fig. 1. a) Balbianiring BR2 (control), fully decondensed. b) BR2 locus after (2 h) irradiation with millimeter waves. The BR2 has regressed. The chromatine fibrils are totally condensed and the surrounding puff-material (ribonucleoprotein) has disappeared. Scale bars: 10 µm.

chromosomes of one gland was determined and the ratio $r = \frac{t}{m}$ was calculated for the sample in the irradiation (r^{irr}) and the control chamber (r^{contr}).

All experiments were carried out blind, *i.e.*, the examining biologist did not know which sample was irradiated and which had served as control. Furthermore without informing the biologist sham-exposure experiments were carried out (Fig. 2 and Table I). While for both sham-exposed series (I, II) no significant effect is observed, a highly significant effect is found when the mm-wave radiation is present (III). Notice that the mm-waves stabilized in frequency (IIIb and IIIc) seem to be more effective than swept frequencies (IIIa).

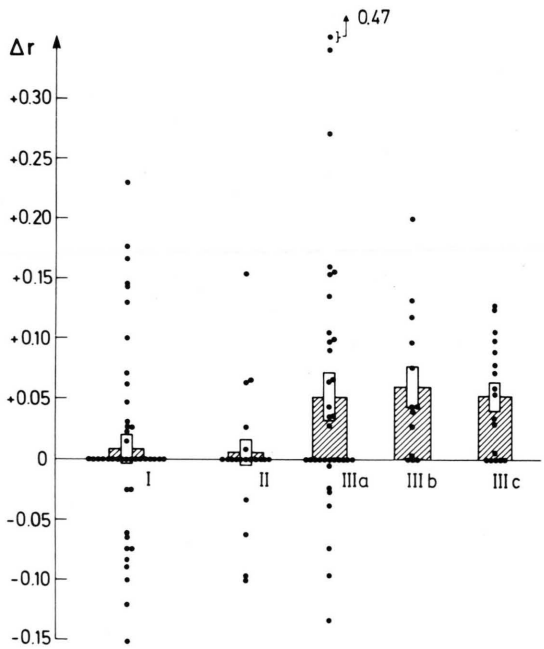

Fig. 2. The dots represent the difference $\Delta r = r^{irr} - r^{contr}$ for a gland pair in one of three experimental conditions: sham exposure (I); sham exposure with additional heating of 2.5 °C (II) and irradiation experiments (III). The shaded columns indicate the mean value of all Δr in one type of experiment, the empty columns the standard deviation of the mean value. IIIa: frequency swept between 64.1 GHz – 69.1 GHz, power density $\leqq 6$ mW/cm^2. IIIb: stabilized frequency 67.200 ± 0.001 GHz, power density $\leqq 6$ mW/cm^2. IIIc: stabilized frequency 68.200 ± 0.001 GHz, power density $\leqq 6$ mW/cm^2.

Table I. n: number of gland pairs (number of chromosomes in brackets) $\overline{\Delta r}$: mean value of Δr for the different types of experiments. s: standard deviation of the mean value. k: number of "successes" with $\Delta r > 0$. l: number of "failures" with $\Delta r < 0$; in cases of $\Delta r = 0$ we counted $k = l = 0.5$. P_1: probability in percent for k or more successes in n trials assuming a binomial distribution with $p = 1/2$. P_2: probability in percent that the experimental series (II, IIIa–IIIc) belong to the same distribution as sham exposed series (I) according to the U-Test of Mann-Whitney [16].

Type		n	$\overline{\Delta r}$	$\bar{s}$	k	l	P_1	P_2
I	sham-exposed	43 (2363)	0.0085	0.012	23.5	19.5	27.1	/
II	sham-exposed with additional heating of 2.5 °C	20 (1181)	0.0060	0.011	10.5	9.5	41.2	27.4
IIIa	64.1 GHz – 69.1 GHz Power: $\leqq 6\,mW/cm^2$	35 (2000)	0.0523	0.020	22.5	12.5	4.5	4.2
IIIb	67.200 ± 0.001 GHz Power: $\leqq 6\,mW/cm^2$	13 (707)	0.0606	0.017	11.5	1.5	0.2	0.5
IIIc	68.200 ± 0.001 GHz Power: $\leqq 6\,mW/cm^2$	17 (931)	0.0525	0.012	14.5	2.5	0.1	0.4

Since in the experiment of type II the sham-exposed sample was warmed up by more than the eight-fold microwave induced temperature increase, the failure to find a significant effect proves that the influence of the irradiation cannot be of thermal origin. It should be noted that a localized overheating of the sample above the temperature of the surrounding medium is not possible because of the facts that the sample itself contains about 90% water and that the mm-wave absorption of the protein material is about hundred-fold smaller than that of water [17, 18]. So the thermal properties of the sample can be considered as that of water while its mm-wave absorption cannot exceed that of the surrounding medium.

The energy of a mm-wave photon $h\nu$ (for $\nu = 7 \times 10^{10}$ Hz: 2.9×10^{-4} eV) corresponds to less than 1/200 of the thermal energy kT at 300 K. Therefore, a single photon process cannot explain the influence of the irradiation. So it seems that the coherence of the applied radiation is essential for the observed effect.

Our results could possibly be understood by H. Fröhlich's [1–2] conjecture of coherent electric vibrations in biological systems.

Our result might be of importance in the discussion of safety standards with regard to possible hazards from millimeter wave radiation. It is shown that millimeter waves of a power density less than $6\,mW/cm^2$ exert a non-thermal influence on the chromosomes of our eucaryotic system. This level is below the safety standard of $10\,mW/cm^2$ in most European countries and the USA.

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