Discussion. Dietary selenium deficiency was found to aggravate the arachidonate-induced respiratory distress. Since the respiratory distress is due to platelet thrombi in the microvascular system of the lung[1], platelet function may possibly be altered in selenium deficient mice. In fact, platelet aggregation by ADP, collagen and arachidonate was stronger in the selenium deficient rats than in the selenium supplemented rats. This indicates that platelet aggregability is enhanced in selenium deficient rats. According to Bryant and Bailey[5], selenium deficient platelets showed a marked alteration in the lipoxygenase metabolism of arachidonate. In their experiment, while the conversion of L-12-hydroperoxy-5,8,10,14-eicosatetraenoic acid to L-12-hydroxy-5,8,10,14-eicosatetraenoic acid was slightly suppressed, the conversion to trihydroxy fatty acids, 8,9,12-trihydroxy-5,10,14-eicosatrienoic acid and 8,11,12-trihydroxy-5,9,14-eicosatrienoic acid was increased 3-4-fold in selenium deficient platelets as compared to selenium supplemented ones. From the recent findings that lipoxygenase metabolites may also play an important role in platelet aggregation[9], the alteration of the lipoxygenase pathway seems to contribute to the enhanced platelet aggregation in selenium deficient rats. As another mechanism of aggravated vascular thrombosis in selenium deficient mice, the formation of PGI_2 with a potent anti-aggregatory and vasodilating activity may be suppressed in the blood vessel. In the present study, the formation of PGI_2-like substances was markedly suppressed in the aorta of selenium deficient rats. Biosynthesis of PGI_2 is known to be inhibited by hydroperoxide derivatives of arachidonate[10-12], which was recently suggested to exist in vascular tissue[13]. Lipid peroxide estimated by the amounts of MDA was increased in the aorta from selenium deficient rats. Further, under the present experimental conditions, glutathione peroxidase, a seleno-enzyme[7], in the platelets and aorta of selenium deficient rats decreased to about 10% of the level found in selenium supplemented rats (data not shown). These findings suggest that lipid peroxide accumulated in the arterial wall owing to severe depletion of glutathione peroxidase suppresses the formation of PGI_2. The present findings suggest that selenium may function in vascular hemostasis and thrombosis by maintaining the metabolism of arachidonate. In the present study, however, the effect of selenium on the metabolism of endogenous arachidonate is obscure, because we did not determined the levels of arachidonate and its metabolites in the tissues. Further investigations are necessary to evaluate the role of selenium in the regulation of arachidonate metabolism.

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Electron microscopic stereology of constitutive heterochromatin in *Rhinanthus minor*

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Summary. The chromocenter heterochromatin of cell nuclei in the Scrophulariacean plant, *Rhinanthus minor*, was reconstructed from ultrathin serial sections by various methods. The chromocenters are irregularly shaped blocks through which runs a ramified anastomosing system of channels. No higher order structure of chromatin organization could be recognized.

Heterochromatin remains in the condensed state through interphase[2]. Therefore, it may serve as a model system in the analysis of the higher order structure of chromatin, which is yet poorly understood. The cell nuclei of *Rhinanthus* exhibit large chromocenters, which are associated with the nucleolus, within a diffuse background composed of euchromatin and nucleolymph[3]. We have chosen this species to reconstruct the organization of heterochromatin from ultrathin sections.

Material and methods. Various tissues of *Rhinanthus minor* were fixed with glutaraldehyde (6.25%) and osmium tetroxide (1%), both in PIPES buffer, pH 7.3. After dehydration the material was embedded according to Spurr[4]. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 10 electron microscope. Serial sections were picked up with one-hole grids. Reconstruction of 17 chromocenters was made from electron micrographs of serial sections. Four chromocenters were reconstructed by the aid of an automatic image analyzing system at the Unit of Data Recording at the German Cancer Research Center, Heidelberg. The system stores the images of the micrographs, digitized into 256×256 grey level points; the connected VAX-11/780 computer and RAMEX color display unit allow visualization of the

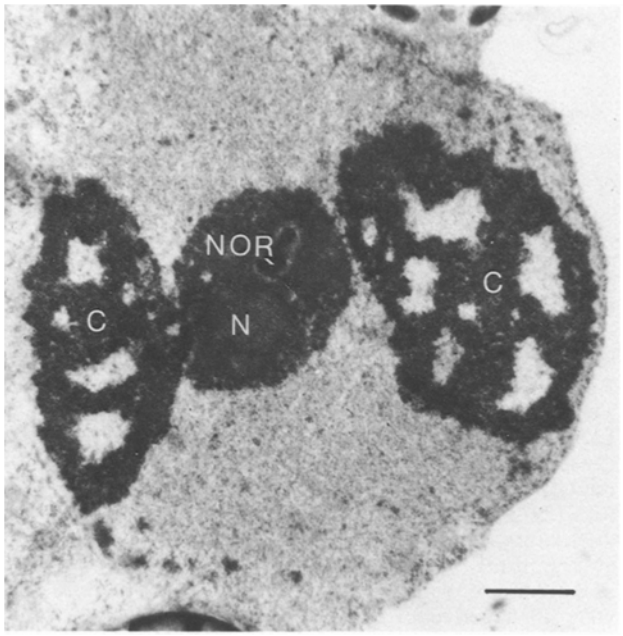

Figure 1. Electron micrograph of an interstitial section (No. 28) of a serially cut nucleus from *Rhinanthus minor* ovular tissue. C, chromocenters; N, nucleolus; NOR, part of nucleolus organizing region. × 11,330. (The bar represents 1 µm.)

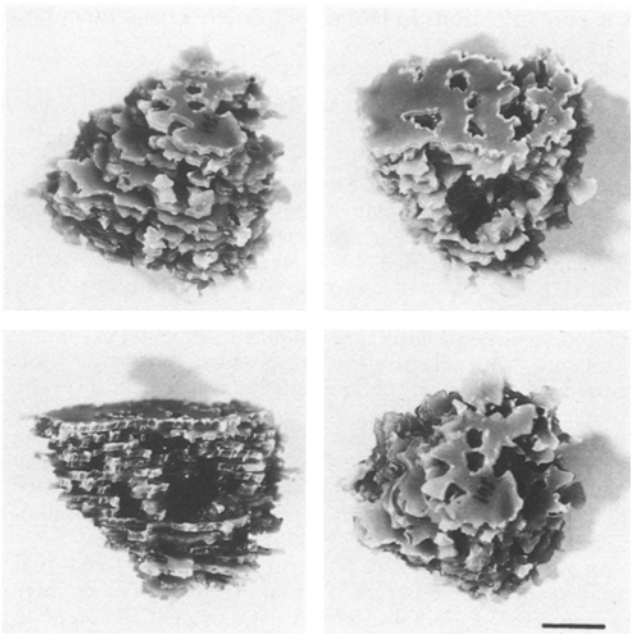

Figure 2. Wax model of one half of a chromocenter as seen from different sides (reconstruction from serial section). Note the tunnelling and the openings. The bar represents 1 µm.

3-dimensional architecture, and any section through it can be displayed. From other chromocenters models were made with dental wax and plastic plates.

Results. About 9% of the nuclear cavity is occupied by heterochromatin. If the volume amount that is occupied by the nucleolus and the protein crystal (which is present in many nuclei) is eliminated from the nuclear area, the percentage of heterochromatin is 13.5. This value fits the regression line between the 2C DNA amount and the percentage of condensed chromatin in plant nuclei (reviewed by Nagl[5]). Figure 1 shows a thin section of a typical nucleus; note the 'holes' in the chromocenters.

The 3-dimensional architecture of the chromocenters (i.e. the masses of constitutive heterochromatin) was reconstructed from serial sections of ovule nuclei. In principle, the surface of the chromocenters often shows many protrusions and invaginations, and their body is pervaded by a ramified tunnel system (fig. 2). These tubes communicate with each other, and many have openings at the surface. While some of the chromocenters are simply discus-like, most exhibit a bizarre morphology and are the result of fusion between 2 or more or even all of them.

Of the 17 chromocenters sectioned, 4 were reconstructed using the image analysing computer system, and 4 were reconstructed using wax or plastic models. The other serial sectiwns were used to follow up the 'holes'. Four chromocenter types could be distinguished. Type I represents chromocenters with a very irregular surface and morphology and mainly thin tunnels. The size of the chromocenters is approximately $3 \times 2 \times 2$ µm, and they have a horn-like protrusion into the nucleolus, evidently part of the nucleolus organizing region (NOR). Type II comprises discus-like chromocenters with a rather smooth surface, a few large tunnels, and an average size of $4.5 \times 3.6 \times 1.8$ µm. Type III represents collective (fused) chromocenters. They are larger than the others (e.g. $5.5 \times 5.0 \times 2.5$ µm, $6.7 \times 3.8 \times 2.5$ µm) and their tunnelling is heavy and irregular. The NOR forms a twisted strand between 2 parts of such a collective chromocenter. In many nuclei all chromocenters are fused and may form a horseshoe-like structure.

Discussion. Constitutive heterochromatin is thought to remain in a mitotically (metaphasic) condensed state. This is, however, an over-simplification. Investigations on telophase nuclei in semi-thin and ultra-thin sections have clearly shown that at least 1 level of condensation is lost in heterochromatin and that the telophase chromosomes appear like tubes in cross-sections. Also interphase heterochromatin often exhibits a hole in cross-section, suggesting a telophase-like state of constitutive heterochromatin. Our chromocenter reconstructions, however, reveal that the organization of heterochromatin is more complex, and, in the case of *Rhinanthus*, resembles a sponge- rather than tube-like structure. It is not clear whether this structure is built up by coiling or bundling of the chromatin fiber as suggested by different recent models. While the basic organization of chromatin, the nucleosome chain, has been demonstrated in plants[6], the higher order structures of condensed chromatin are still poorly understood. Serial sectioning of heterochromatin partially decondensed by decondensing compounds may help to fill this gap.

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