290 synapses were counted in each cat. Details of the procedure have been previously described[14]. No corrections were made for shrinkage and Holmes effects.

In order to calculate the number of synapses per unit volume of molecular layer (N_v) in the absence of an accurate determination for form and size distribution of synapses, we considered that the synaptic contacts could be compared to flat circular surfaces[13]. This assumption renders the use of the average length of the synaptic contacts in the calculation of the area of the referred synaptic circles ($A=\pi(0.5\ L_{sin})^2$) possible. Serial sections were used to measure the greatest tangential diameter of 30 synapses at a final magnification of $\times 60{,}000$. The value thus obtained was compared with the average length of synaptic contacts, calculated according to the method of Vrensen and De Groot[13], in order to evaluate the underestimation caused by this method.

Assuming that there is no overlap between adjoining dendritic trees[5,8,15], multiplication of synaptic surface and number of synapses per unit volume of molecular layer by the volume of molecular layer occupied by each dendritic tree (540,000 μm^3, according to Eccles et al.[5]), gives the synaptic surface and the number of molecular layer synapses per Purkinje cell (S_p and N_p, respectively).

Results and discussion. The results obtained are summarized in the table. The average length ($\pm$SD) of synapses was 0.316 ± 0.150 µm (1153 synapses were counted in the 4 cats). In order to calculate the number of parallel fibre-Purkinje dendrite synapses from the number of molecular layer synapses per Purkinje cell that we obtained (table), 2 kinds of corrections appeared necessary. 1. We used an underestimated value for the synaptic circle; from the value that we obtained in serial tangential sections (0.397 ± 0.062 µm), we estimated such error to be about 37%. 2. It must be considered that we counted all the synapses in the molecular layer, thus including other than parallel fibre-Purkinje dendrite synapses. According to Palkovits et al.[6], this procedure gives an error of about 6%. The number of cat parallel fibre-Purkinje dendrite synapses is slightly over 200,000 (202,000), when those corrections are made. This number is in keeping with Eccles et al.[5] who state that 209,000 parallel fibres cross and establish synaptic contacts with each cat Purkinje cell dendritic tree, and is much greater than that found by Palkovits et al.[6], who estimated that only 80,000 of the 400,000 parallel fibres which cross each cat Purkinje cell dendritic tree establish synaptic contacts with dendritic spines.

Our results support Palay and Chan-Palay[8] criticism regarding the accuracy of techniques currently employed in calculating the number of cerebellar synapses, and point to the usefulness of morphometric methods for such calculations. In fact, ultrastructural morphometry not only facilitates direct counting of synapses, but also allows the evaluation of their number per unit volume of tissue.

Ultrastructural morphometric results of molecular layer synaptic contacts

Synaptic surface per unit volume of molecular layer* S_v ($\mu m^2/1000\ \mu m^3$)	Synapses per unit volume of molecular layer* N_v (No./$1000\ \mu m^3$)	Synaptic surface per Purkinje cell** S_P (μm^2)	No. of synapses per Purkinje cell** N_P
49.2 ± 4.7	628 ± 60	26,568	339,120

* Results are expressed in mean$\pm$SE (80 micrographs were analyzed in 4 cats). ** The volume of cat Purkinje cell dendritic tree was assumed to be 540,000 μm^3, according to the values reported by Eccles et al.[5].

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Thoughts about high chlorine peaks in X-ray microanalysis

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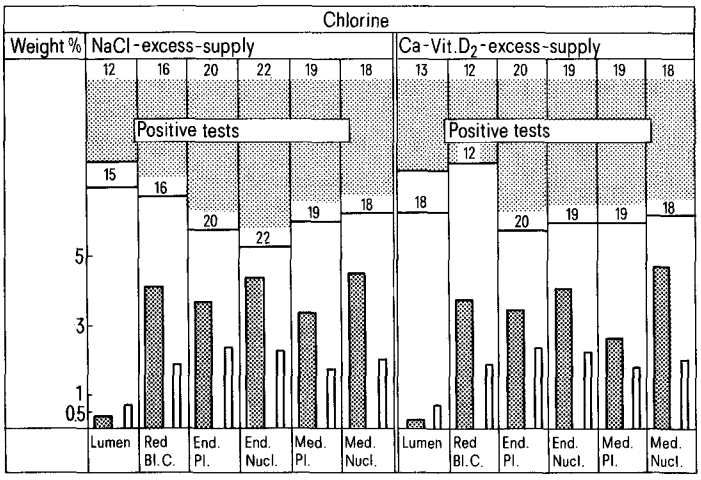
Summary. In X-ray microanalysis of biological tissues, high Cl-peaks are constantly observable. Higher Cl-peaks in the tissue than in the epon environment suggest that most Cl originates from the tissue. Therefore rough regional estimations of the Cl level could be possible.

The amazingly high level of the very unstable element chlorine (Cl) found by X-ray microanalysis is well known and has been mentioned in most papers dealing with this subject. Not only conventionally prepared tissues for morphological investigations in the transmission electron microscope - having the chance to acquire their Cl from the epon impregnation - show abundant Cl, but also other methods like freeze-drying, for example, yields high Cl-peaks.

Our results too have always provided evidence for a 4-8-fold higher yield of Cl compared with the other elements analyzed in epon-embedded vascular tissues from different organs[1]. Comparing control animals with rats, exposed to experimental metabolic disorder by overdoses of sodium



The hatched columns show the number of positive results of total measurements. Black and white columns at the bottom show semiquantitative values in weight percent. Treated rats: black, controls: white. End. Pl., endothelial plasma; Nucl., nucleus, Med., mediocyte, Bl.C., blood cells.

chloride and calcium-vitamin D_2, the latter showed 2-fold higher Cl-values in the vessel wall and the red blood cells (figure). It is of interest that the abundance of Cl in the lumen, which holds a significantly lower level than in the wall of the same vessel, in controls, conversely, were 2-fold higher than in the treated rats.

These results caused us to believe in the real existence of Cl in the tissues even after conventional fixation (glutaraldehyde), washing (distilled water or several puffer solutions), dehydration (ethanol), and embedding (epon 812). The often stressed statement that Cl in epon-impregnated biological tissues has its origin in the epon itself, while the tissue-chlorine has been washed out quantitatively or is removed by the medium, cannot be accepted, mainly for 2 reasons.

Firstly, the amount of Cl in the measured area is usually 4 times higher in tissues than in the tissue-free epon environment. And secondly, we observed differences as already mentioned between controls and rats with metabolic disorder.

Thus it seems that the very labile Cl-ion, though not fixed at its intracellular sites and floating during the preparational procedure, will remain in remarkable amounts in the competent tissue region, i.e. in our case in the vascular wall. Thus for rough comparisons between vessels with similar parameters, it should be possible to find out true differences after experimentally changing a factor like blood pressure. In this connection, the observation of Läuchli et al.[2] must be mentioned, who after osmium fixation, washing, dehydration and Spurr-embedding with radiochemical measurement missed only 4% of the Cl that they had found before fixation.

These results should be verified with the generally accepted freezing methods. They are encouraging in so far as it seems that, even with conventional preparative work, regional differences in the rough element distribution can be estimated.

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The localization of mutarotase in rat kidney*

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Summary. The localization of mutarotase in rat kidney was investigated by fluorescein-labelled and peroxidase-labelled antibody techniques, and by method of isolation of the nuclei and cytoplasm in non-aqueous solvents. In these immunohistochemical studies, mutarotase was almost exclusively recognized in the nuclei of epithelial cells of renal tubules and glomeruli in rat. The specific activity of mutarotase was found to be 1.5 times higher in the nuclei (122 units/g dry wt) than that in the cytoplasm (80 units/g dry wt) isolated with non-aqueous solvents. These results suggest that mutarotase may be involved in the metabolism of D-glucose in nuclei.

Mutarotase [aldose 1-epimerase, EC 5.1.3.3] catalyzes the interconversion of D-glucose and other sugars, and is known to be present in high activity in the kidney, liver, and small intestine of higher animals[2-5]. Any definite function of this enzyme has not yet been clarified, but it has been postulated that the enzyme may be involved in the transport of sugars[2,6] or in D-glucose metabolism[7,8]. To find a clue to the role of the enzyme, we attempted to determine the localization of the enzyme in rat kidney cells using the fluorescein-labelled and peroxidase-labelled antibody techniques, and the method of isolation of nuclei and cytoplasm in non-aqueous solvents. Since we have recently found that the kidney mutarotase appears in both serum and urine of patients[9] and rats[10] with nephrotic syndrome, it is also of interest to determine the localization of the enzyme.

Materials and methods. Purification of mutarotase from rat kidney. Mutarotase was purified as reported previously[11]. 4 different forms of mutarotase were found to exist in rat kidney, of which a major form (type II) was purified to homogeneity. We used the mutarotase type II for the preparation of anti-mutarotase antibody.