concentration of the mitochondrial and lysosomal enzymes. This observation points to the presence of severe cellular damage and breakdown. It should be mentioned that 2 h clamping of renal artery leads in anaesthaetized dogs to severe but usually reversible renal insufficiency[15], in unanaesthaetized animals this injury is fatal[16].

Zusammenfassung. Nachweis, dass 30-minutige Nierenischämie zum Austritt von Enzymen des Zellplasmas aus den Zellen führt. Nach 120 min Nierenischämie kommt es auch zum Austritt von lysosomalen und mitochondrialen Enzymen aus den Zellen.

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Importance of Reverse Transcriptase in Plant Tumour Tissue of Viral Origin Cultivated in vitro

The wound tumour virus is known to be the causative agent of *Rumex acetosa* tumour[1-3]. Gentile[4] and Raychaudhuri[5] reported that the virus is not present in the tumour tissue of *R. acetosa* maintained in vitro over long periods. We report in this communication certain tumour-specific characteristics in the in vitro cultivated tissue of *Rumex acetosa*, and the possible role of the *reverse transcriptase*[6] in their inheritability in tissue devoid of the original double stranded RNA virus.

Materials and methods. The normal and the tumour tissue was kindly supplied by Dr. S. K. Srivastava, M. S. University, Baroda, India, and was maintained under 12-hourly dark and illumination rhythms on the medium described by Gentile[7]. Generally 15-day-old tissue was used for investigation.

The extracts of the tissue were prepared in each case by grinding 1 part by weight of fresh tissue with 1 part by volume of 0.9% sodium chloride solution in cold, and after centrifugation at about 1,100 *g*, the supernatants were examined after dise electrophoresis[8] for NADP dependant isocitric dehydrogenase by the method of Fine and Costello[9] and peroxidase isozymes by the method of Hirschfeld[10]. NADP dependant isocitric dehydrogenase was estimated by the method of Ochoa[11], peroxidase by the method of Lück[12] and protein by the method of Lowry et al[13]. Subcellular fractions were prepared as described by Peacock and Dingman[14].

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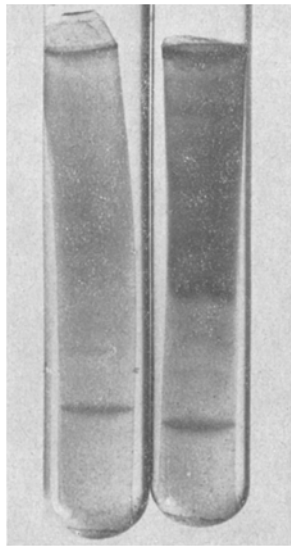

Fig. 1. Protein components in the supernatant fractions (20,000 g) of normal (1) and tumour (2) tissue of *R. acetosa* after disc electrophoresis under identical conditions.

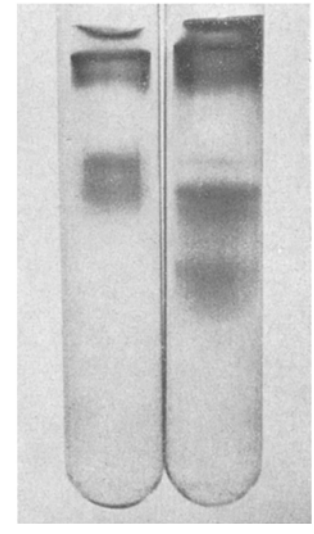

Fig. 2. Differences in peroxidase isozymes between normal (1) and tumour (2) tissue of *R. acetosa* after disc electrophoresis.

Results. Under comparable conditions, the tumour tissue extracts had $1^1/_2$ to 2 times the protein content of the normal tissue extracts. The disc electrophoretic analysis revealed the difference to be mainly a quantitative one, mostly in the supernatants at 20,000 *g* (Figure 1). The specific activity of the tumour tissue isocitric dehydrogenase was about twice that of the normal tissue. Similar results were also obtained in the disc electrophoretic analysis. The isocitric dehydrogenase had a well defined pH optimum of 7.6, while consistently higher activity over the pH range 7.2–9.2 was obtained for the tumour tissue. The bulk of the isocitric dehydrogenase activity in both the tissues was localized in the supernatant at 20,000 *g*. There was an important qualitative difference between the 2 tissues with respect to the peroxidase isozymes (Figure 2.) This was further confirmed by the pH optima for peroxidase; being 5.2, 6.0 and 7.2 for the normal tissue and 5.2 and 6.4 for the tumour tissue.

Discussion. The higher isocitric dehydrogenase activity in the tumour tissue is quite significant, as MAINI, SRIVASTAVA and RAMAKRISHNAN[15] reported an NAD (NADP) glycohydrolase in the same tissue.

The present investigation has demonstrated quantitative (protein and -isocitric dehydrogenase) as well as qualitative (peroxidase) differences between the normal and the tumour tissue of *Rumex acetosa* maintained in vitro. The tumour specific characteristics are found to be transferred to the successive subcultures of the tissue indefinitely, and can be explained by the initial fixation of the information of the double stranded viral genome in the replicative DNA by the reverse transcriptase mechanism[6] which is transferred to the daughter cells via the daughter DNA and expressed as protein or isozyme characteristics even in the absence of the original virus[4,5], as observed in the present investigations.

Zusammenfassung. Es werden quantitative und qualitative tumorspezifische Merkmale von *Rumex acetosa* Geweben hinsichtlich Proteingehalt und Eigenschaften der NADP-abhängigen Isocitratdehydrogenase und der Peroxydas-Isoenzyme beschrieben. Das Weiterbestehen dieser Merkmale in den in vitro kultivierten Tumoren nach Verschwinden des Erreger-Virus wird auf die Aktivität einer reversen Transkriptase zurückgeführt.

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Depolarization by Acetylcholine (ACH Activation of Triphosphoinositide Phosphomonoesterase)

In cholinergic synapses, acetylcholine (ACH) generates postsynaptic depolarization by first forming a complex with its specific receptor located at the postsynaptic membrane. During this complex formation this specific receptor acquires the ability to initiate the processes that are needed for the execution of postsynaptic depolarization. The postsynaptic nicotinic receptor is the one involved in generation of fast bioelectric processes[1–4]. In in vitro biochemical experiments this nicotinic receptor was identified as the regulatory subunit of triphosphoinositide phosphomonoesterase (TPIPM)[5]. On combining with ACH, this regulatory subunit of TPIPM ceases to inhibit the enzymatic activity of the catalytic subunit of TPIPM and triphosphoinositide (TPI) becomes dephosphorylated to diphosphoinositide (DPI). This process initiates molecular reactions that control the local electric fields towards depolarization. The validity of the assumption that part of TPIPM functions as the specific nicotinic receptor of ACH at the postsynaptic membrane was here tested in vivo experiments.

Methods. The postsynaptic bioelectric processes of the superior cervical ganglion of the rabbit[6,7] were recorded with glass microelectrodes (0.5 μm tip diameter, 10 megaohm tip resistance, filled with 4 *M* K citrate), on a dual beam oscilloscope using a cathode ray follower. The presynaptic neuron was stimulated by constant voltage rectangular pulses (frequency 1/min) through platinum wire electrodes. At times hyperpolarizing currents were given through the intracellular microelectrodes. Intrapostsynaptic microinjections were given by combining pressure with iontophoresis (40 nA for 3–30 msec)[8]. The injected ACH, norepinephrine, and D-tubocurarine (DMTC) were commercially obtained, the subunits of TPIPM were prepared by the method described by TORDA[5]. 1 U of the catalytic subunit was arbitrarily chosen as the amount of enzyme that yielded 1 ng inorganic P/min. 1/2 U of the regulatory subunit caused 50% inhibition if incubated together with 1 U of the catalytic subunit. The subunits were injected in increasing amounts until bioelectric changes were observed. Less than 5 U always sufficed. Spontaneous recovery always occurred in less than 30 min.

Results. Near-synaptic microinjections of the catalytic subunit generated postsynaptic bioelectric processes (Figure) that were comparable to the effects of ACH (endogenous or exogenous). The endogenous ACH was released during presynaptic stimulation, the exogenous was delivered by near-synaptic microinjections. 1. The resting membrane resistance (aver. of 80 $\pm$ 1.8 mV) decreased in presence of subthreshold amounts of ACH or catalytic subunit. In both instances the equilibrium (reversal) potential averaged -7 mV. Postsynaptic EPSP and spiking did resemble. The average latency of EPSP was 2.8 $\pm$ 1.2 msec. The EPSP reached its summit (23 $\pm$ 2.4 mV) in an average of 4.4 $\pm$ 1.3 msec. The decay

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