observed with higher probability among cancer patients. Since the body pool of selenium is essentially determined by the dietary selenium intake, the published selenium concentrations in grain and forage crops of the US[12] provide an approximate indication of the dietary selenium supply. If selenium has a cancer protecting effect an inverse correlation between the cancer mortality and the selenium content of forage crops and grains may be expected. Using published[13] cancer mortality rates and the selenium distribution map of the US one finds that 21 States are located in low to very low selenium areas. Of these 17 or 81% show the highest cancer mortality, the remaining 4 are in the second highest rank. Of 30 states situated in areas with adequate to high or excessive supply of selenium 10 or 33% show the lowest cancer mortality rate, 15 or 50% the second highest and only 5 or 17% the highest mortality rates (Table II). It would seem for this reason that the possible cancer protecting effect of selenium merits further attention. The role of selenium as a biological antioxidant may be connected with this function[14].

Zusammenfassung. Der früher zur Krebsdiagnose vorgeschlagene Plasma-Methylenblau-Entfärbungstest nach SAVIGNAC[2] und BLACK[3] spricht im wesentlichen auf die Plasma-Selenkonzentration an. Daraus ergeben sich Hinweise für die möglicherweise krebsschützende Wirkung des Selens, die durch statistische Daten gestützt wird.

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The Potency of N-acetylaminofluorene in the Production of Cytokinin Autonomous Tobacco Tissues in vitro

In a previous communication[1] we reported the induction of cytokinin autonomous nodules in cultured tobacco tissues by several substituted fluorenes. The experiments showed small amounts of substituted fluorenes in the presence of 0.1 to 12.5 μM kinetin and 10 μM indoleacetic acid (IAA) produced significant quantities of cytokinin autonomous nodule tissue. The nodules upon subculture behaved like the hormone-dependent plant tumours described by BRAUN[2]. These tissues have lost their exogenous cytokinin requirement, but still need an auxin such as IAA for growth in vitro. The purpose of this study was to quantitatively define the levels at which one of the more potent fluorenes, acetylaminofluorene (AAF), induced the formation of cytokinin independent tobacco tissue under standardized conditions.

Materials and methods. The medium and general procedures were those described for use in cytokinin bioassays[3]. The tobacco stock callus requiring both an auxin and a cytokinin for growth in vitro was obtained from the pith of *Nicotiana tabacum* Wis. No. 38 and grown on medium containing < 0.15 μM kinetin and 10 μM IAA. Some stock callus was routinely subcultured on kinetin and/or IAA free media to detect any spontaneous occurrance of auxin and/or cytokinin independent tissues. No hormone autonomous tissues were observed in stock or control cultures. The experimental series contained kinetin at a concentration of 0.5 μM with IAA maintained at 10 μM. The AAF solution was filter sterilized and added to the autoclaved medium at concentration between 0.02 and 12.5 μM. 3 small (about 20 mg fresh weight each) pieces of friable stock callus were planted on 50 ml medium solidified with 1% agar in 125 ml erlenmeyer flasks. 4 replicate flasks of each treatment were incubated at 28 °C. Illumination was furnished by standard cool-white fluorescent lamps producing 4500 $\pm$ 500 ergs $cm^{-2} sec^{-1}$ incident upon the tissues. After 58 days the tissues were harvested. The firm white nodules were counted, separated from the moderately friable callus and weighed independently.

Results and discussion. The appearance of the 2 kinds of tissues produced in the presence of AAF is illustrated in Figure 1. The tissue shown consists of a white compact nodule composed of cytokinin autonomous cells and darker green friable callus which is still cytokinin dependent upon subculture. The potency of AAF in producing these nodules and its effect on the growth of callus are presented in Figure 2. The yield of the cytokinin independent tissue increased with increasing concentrations of AAF between 0.02 and 2.5 μM. Concentrations of AAF above 5.0 μM showed a strong inhibitory effect on the growth of both callus and nodule tissues. The greatest yield of nodule tissue was obtained at concentrations of 0.5 and 2.5 μM

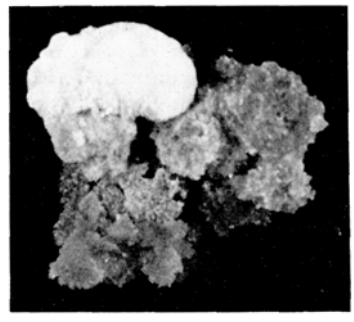

Fig. 1. Cultured friable tobacco callus with a large nodule composed of cytokinin-independent cells.

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AAF, treatments in which 18.7% and 33.7% of the total tissue present were cytokinin autonomous. The average number of nodules per flask also increased with the dose of AAF up to growth limiting concentrations; however, this occured at the expense of their relative size at levels above 0.1 μM. The enhancement of callus growth by small amounts of AAF is an effect only seen in tissues previously grown on low cytokinin concentrations. However, the growth of callus from both, low and high ($> 0.2\ \mu M$) kinetin pretreated tissues was inhibited by AAF concentrations greater than 5.0 μM.

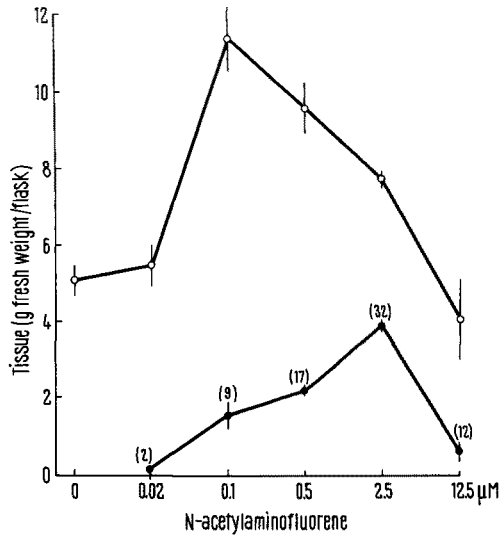

Fig. 2. The yield of callus and cytokinin independent tissues at increasing levels of N-acetylaminofluorene. (-○-), callus; (-●-) nodule tissue. The vertical lines indicate the standard error of the mean of 4 replicate cultures. The numbers in parenthesis above the nodule tissue curve represent the average number of nodules per flask.

This method provides a relatively simple, chemically defined system for the production of cytokinin autonomous tobacco tissues. Additional studies of these cells can furnish information on the role of AAF in the activation of the endogenous cytokinin synthesizing system and the part this event plays in the transformation of normal cells to tumor cells[4,5].

Zusammenfassung. Nachweis, dass *N*-Acetylaminofluoren in Cytokinin-Auxin-abhängigen Tabakgewebekulturen cytokininautonomes, knotenartiges Kallusgewebe zu bilden vermag.

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The Ultrastructure of a Cyclostome Interrenal

Although nearly 70 years have elapsed since the so-called interrenal cells of the lamprey were first described in detail by GIACOMINI[1], the physiological significance of this tissue is still uncertain. In its embryological origins there is little doubt that it resembles the interrenal and adrenocortical tissue of other vertebrates, but at the present time the only indication of a functional correspondence is the report that the interrenal tissue of the ammocoete of *Lampetra planeri* underwent hyperplasia after injections of mammalian ACTH[2]. After an extensive histochemical study of this tissue in larval, metamorphosing stages and adults of *L. planeri* and on a single specimen of *Petromyzon marinus*, SEILER, SEILER and STERBA[3] reported the presence in the interrenal tissue of unsaturated lipids, phospholipids, acetylphosphatide and cholesterol, but in common with experience in these laboratories, they were unable to obtain conclusive evidence for the presence of Δ^5-3β-hydroxysteroid dehydrogenase. On the other hand, quantitative studies on the interrenal tissue of upstream migrant stages of the river lamprey, *Lampetra fluviatilis* have demonstrated that these cells apparently respond to a variety of stress conditions[4], while a preliminary study of their ultrastructural features tends to support their steroidogenic character.

As previously described by STERBA[2], the main concentrations of interrenal tissue occur immediately above the pronephric funnels of the adult lamprey, although smaller islets are scattered in the walls of the Cardinal veins and the other great vessels of the pericardial regions. In the ammocoete on the other hand, they tend to occur in greatest numbers on the surface of the aorta and also amongst the pronephric tubules, which regress during metamorphosis. For electron microscopical investigations

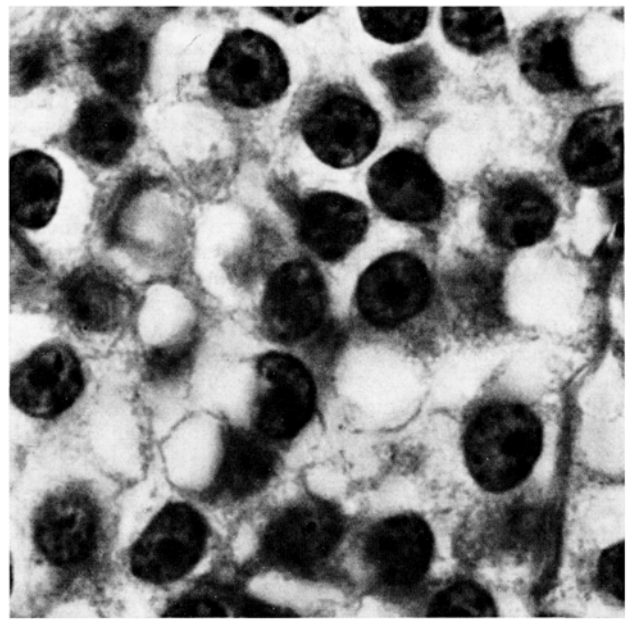

Fig. 1. Interrenal tissue of adult *Lampestra fluviatilis*, ×866. Fixation in Bouin's fluid; staining by Masson's trichrome.