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.

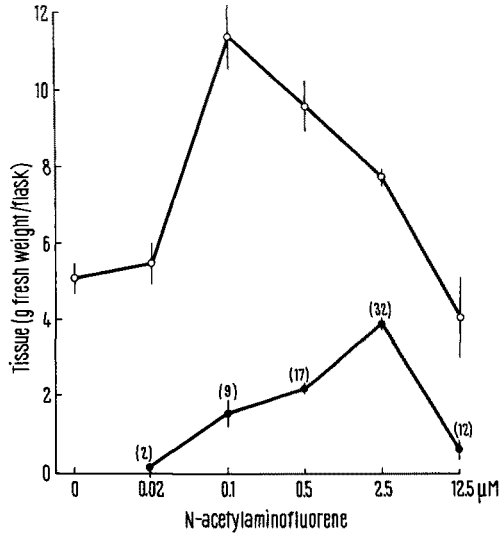

Fig. 2. The yield of callus and cytokinin independent tissues at increasing levels of N-acetylaminofluorene. (-○-), callus; (-●-) nodule tissue. The vertical lines indicate the standard error of the mean of 4 replicate cultures. The numbers in parenthesis above the nodule tissue curve represent the average 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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[5] Acknowledgements. We thank Miss R. SORG and Miss N. SCHUETTE for their very able technical assistance. This work was supported by the Milwaukee Division of the American Cancer Society.

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

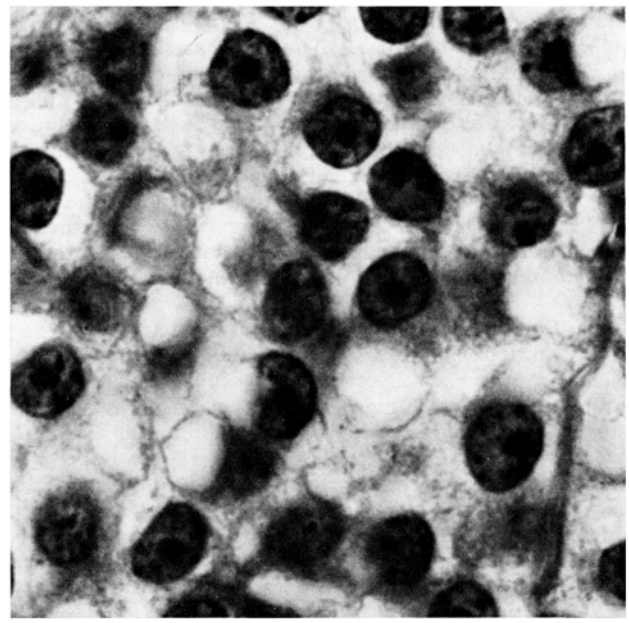

Fig. 1. Interrenal tissue of adult *Lampestra fluviatilis*, ×866. Fixation in Bouin's fluid; staining by Masson's trichrome.

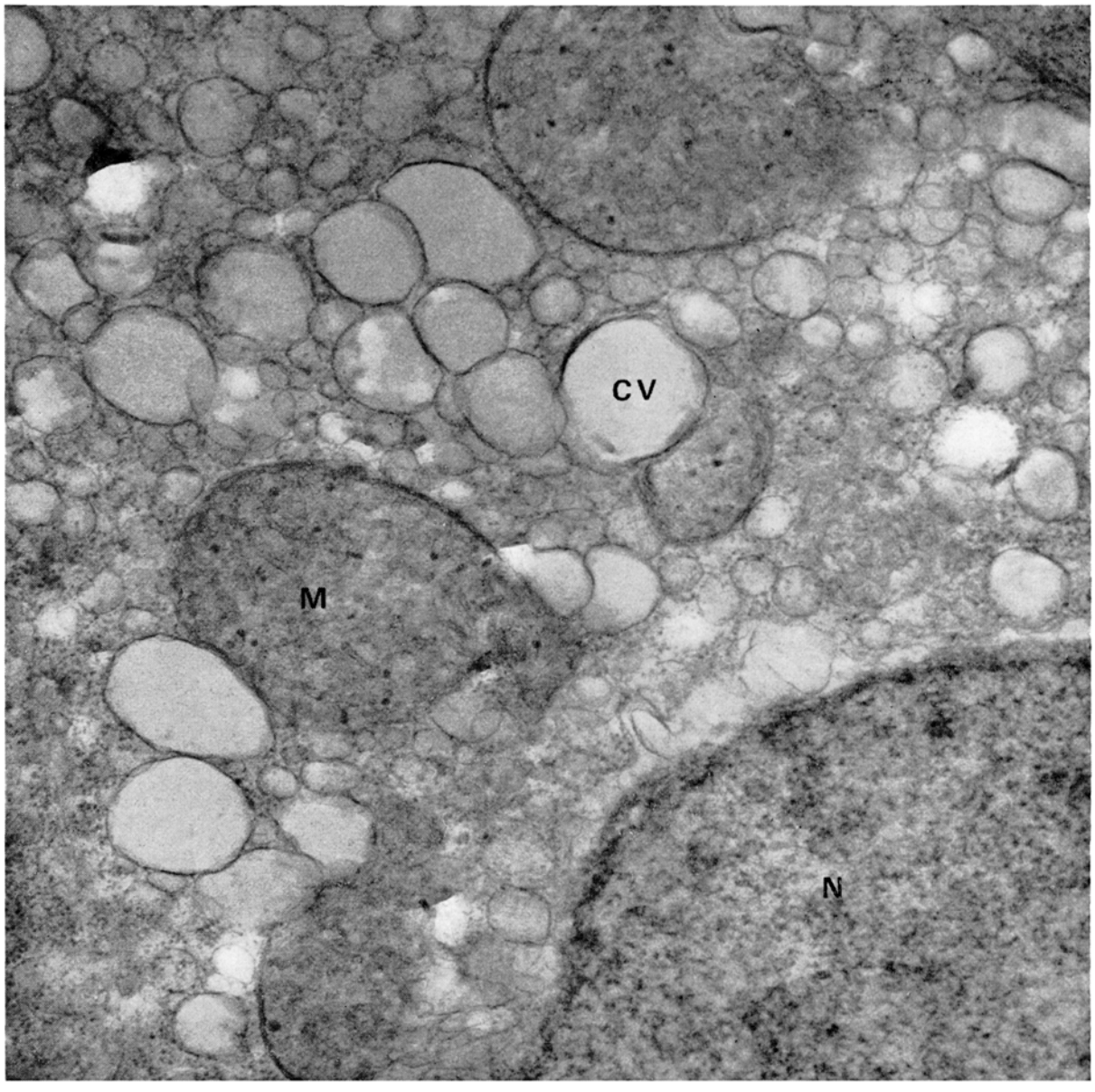

Fig. 2. Interrenal cell from an ammocoete of *Lampetra planeri*, × 30,000.

the animals were perfused with phosphate buffered glutaraldehyde, allowing sufficient time to dissect out the areas containing interrenal tissue under a dissecting microscope. After further fixation in glutaraldehyde, the tissue was postfixed in osmium tetroxide, dehydrated in alcohol, stained with phosphotungstic acid and embedded in Epon.

A typical light microscope image of interrenal cells of an adult of *L. fluviatilis* (Figure 1) shows highly vacuolated cells, with rounded nuclei and a single conspicuous nucleolus. Where, as in this case, large masses of cells are present, they may often show a cord like arrangement, with intervening septae of collagen fibres. In the electron microscope, the interrenal cells of the larval *Lampetra planeri* show large vacuoles, measuring up to 5 µm in diameter which are seen to be liposomes, in some examples showing a collapsed inner membrane; in others they may have either a well defined, or a diffuse osmiophilic outer zone (Figure 3) due to the incomplete extraction of lipid during dehydration[5]. As in the adrenal cortex and other steroidogenic tissues, there appear to be light and dark cell types[6–9], but in both cases the endoplasmic reticulum is of the smooth types, with an abundance of free ribosomes strewn throughout the cytoplasmic matrix. In the dark cell the reticulum is tubular; in the light cell type there is an abundance of vesicles (Figures 2 and 3) ranging

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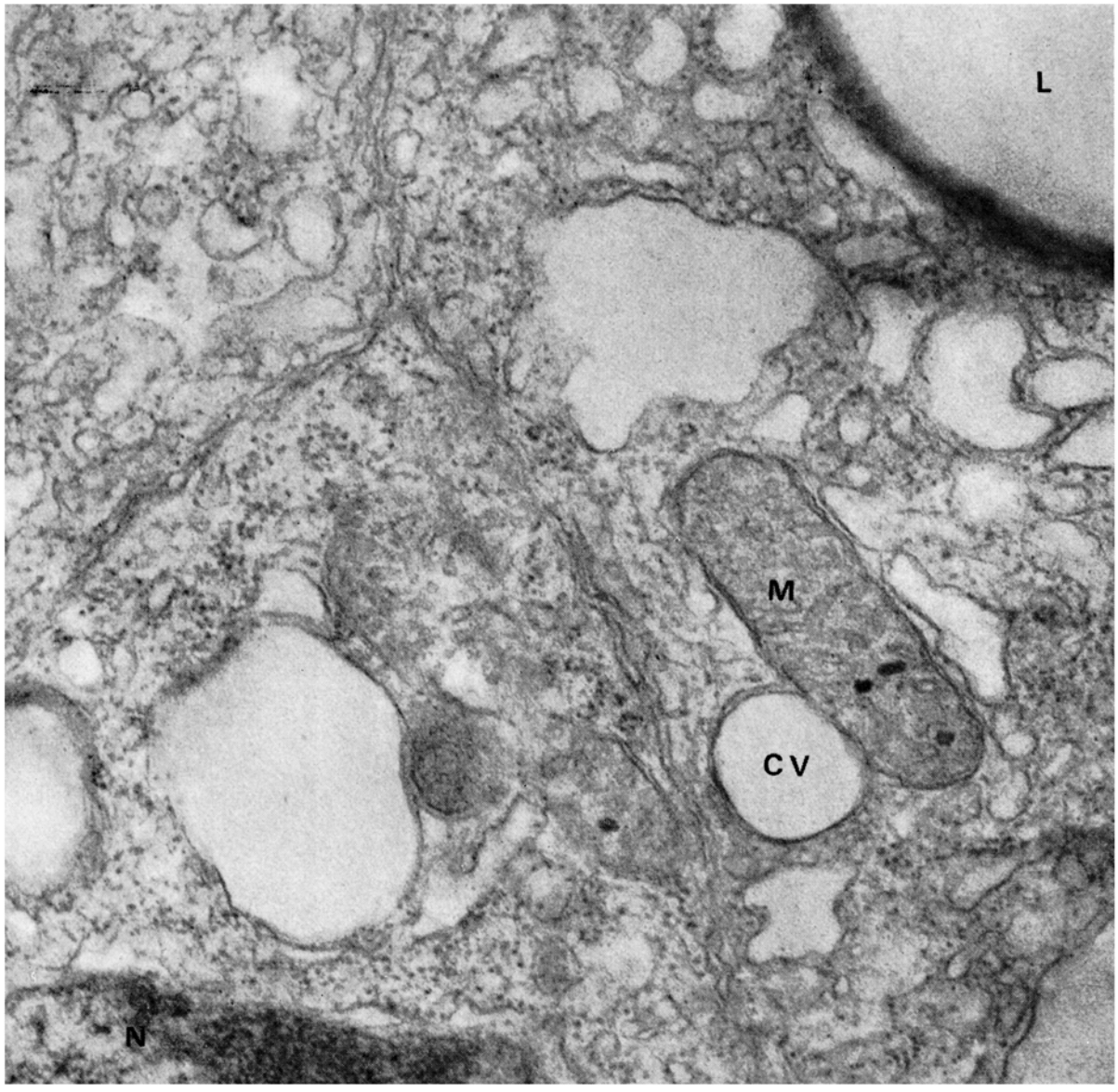

Fig. 3. Interrenal cell from *L. planeri*, ×45,000. CV, cytoplasmic vesicles; l, liposome; M, mitochondria; N, nucleus.

in diameter up to 0.5 μm. Vesicular protrusions of the nuclear envelope are frequently observed. The Golgi zone is inconspicuous or absent.

The numerous mitochondria vary considerably in shape, size and internal structure. In some examples, the cristae consist of narrow straight and parallel tubules which may be restricted to only a small region of the mitochondrion. This bears some resemblance to the type of structure which has been reported in the glomerulosa of the mammalian cortex[10]. In others, the cristae are of an irregular tubulo-vesicular type and the matrix is usually electron dense (Figure 3). Small osmiophilic inclusions are frequently observed within the mitochondria. The presence of 'open form' mitochondria with incomplete outer membranes and the frequent associations between mitochondria and cytoplasmic vesicles or liposomes (Figure 3) recall the observations that have been made in mammals[10,11] and in a teleost[12]. These relationships have been interpreted in terms of the participation of these organelles in the various stages of steroid biosynthesis, but their elucidation in our material must await the results of more detailed cytological and experimental studies on the interrenal tissue of the larval and adult lamprey in relation to the various phases of the life cycle.

Nevertheless, these preliminary studies support the view that this tissue is indeed steroidogenic, showing the major ultrastructural features that are regarded as characteristic of vertebrate steroid secreting cells. These are: the abundance of lipid inclusions, a well developed smooth endoplasmic reticulum and dispersed polysomes, numerous cytoplasmic vesicles, and mitochondria with tubular or vesicular cristae. These same features have also been reported in the Leydig cell homologues of the lampreys

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testis[13,14]. In considering their histochemical evidence, SEILER et al.[3] have suggested that the interrenal tissue of the lamprey may bear a closer resemblance to the Stannius corpuscles of the teleosts than to the interrenal cells of the higher vertebrates. However, in view of the ultrastructural evidence presented here and the well developed rough endoplasmic reticulum and Golgi of the corpuscles of Stannius[15,16] (features that are usually associated with protein secretion) this point of view must almost certainly be rejected[17].

Résumé. Après un exame au microscope électronique, le tissue interrénale de la lamproie, *Lampreta planeri*, montre tous les caractères essentiels d'un tissue stéroïdogénique, soit un reticulum lisse abondant, la présence de liposomes, de vésicules cytoplasmique très nombreuses et de mitochondries à crêtes tubulaires ou vésiculaires. Ces observations confirment l'idée que ces cellules correspondent au tissu interrénal ou adrénocortical des autres vertébrés.

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UV-induced Increase in Number of Periventricular 'Gomori-positive' Glial Cells in Brains of Mice

The brains of mammals, particularly rodents, contain a class of periventricularly localized glial cells with abundant cytoplasmic granulations showing strong affinity to Gomori's chrome haematoxylin and aldehyde fuchsin after permanganate oxidation[1-6]. The chrome haematoxylin-positive granules of the periventricular 'Gomori-positive' glia were shown to be large cytoplasmic organelles, unusually rich in thiol groups[7]. The number of the periventricular 'Gomori-positive' glial cells significantly increases in the brains of 800 R whole body X-ray irradiated animals[8]. A similar increase was observed after a local 3000 R and 4000 R irradiation of the head region[9].

In the present study, the periventricular 'Gomori-positive' glial cells were counted around the anterior part of the 3rd brain ventricle (Figure 1) in normal mice and those subjected to protracted UV-irradiation; 50 female white mice, 4 months old and weighing 25–30 g, were used. The animals were kept under standard laboratory conditions and fed a typical laboratory chow. The animals were divided into 4 experimental and 1 control group, each consisting of 10 individuals. The animals of the experimental groups were UV-irradiated at a spectral range of 254–405 nm, 68,000 erg/sec/cm^2. The irradiations were performed for 30 min on each consecutive day. Animals of the 1st experimental group were irradiated for 7 days, the 2nd group for 14 days, the 3rd for 21 days, and the 4th for 28 days. The irradiations were performed in a dark thermostat chamber at 10–12 °C.

The animals of the experimental and control groups were killed under ether anaesthesia, always at the same hour of the day. The brains were quickly dissected free, trimmed, fixed in Bouin's fluid, and stained with Gomori's chrome haematoxylin-phloxin in Bargmann's modification.

The 'Gomori-positive' periventricular glial cells were counted around the anterior part of the 3rd brain ventricle (Figure 1) under a 40× objective; 100 identical fields were scanned in each animal. The results were analysed statistically with Student's *t*-test.

The results are presented in the Table and in Figure 2. As can be seen, protracted UV-irradiation caused a

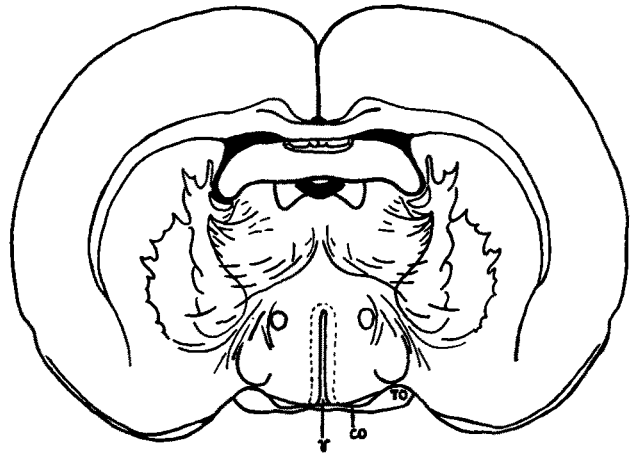

Fig. 1. Transverse section of mouse brain at the level of the optic chiasm. 'Gomori-positive' glial cells were counted in the area circumscribed by the broken line. V, third brain ventricle; CO, optic chiasm; TO, optic tract.

Group	No. of animals	Spectral range (nm)	Energy (erg/sec/cm^2)	Daily dose (min)	Total dose (min)	Mean number of cells	Standard deviation	Standard error	Student's *t*-test
Control	10	–	–	–	–	30.40	0.545	0.175	–
I. 7 days of UV	10	254–405	68,000	30	210	30.96	1.076	0.347	1.36
II. 14 days of UV	10	254–405	68,000	30	420	33.81	2.095	0.675	4.87
III. 21 days of UV	10	254–405	68,000	30	630	36.80	1.710	0.551	10.88
IV. 28 days of UV	10	254–405	68,000	30	840	43.53	6.456	2.082	6.40