These observations establish the prevalence of RNA-rich RB in both zygotic and androgenic embryogenesis of *Datura*. Most importantly, such bodies observed in all the pollen embryos, irrespective of the particular androgenic pathway, followed (table, pathways A, B and C, etc.) showing that their formation occurs with any of the different nuclear types. The phenomenon is therefore characteristic of early embryogenesis in whatever form. Moreover, it does not seem to be related to any stress resulting from prior cold treatment or centrifugation[8].

RB were not observed in pollen before embryogenesis in vitro nor during normal gametophytic development in vivo[8]. They appeared only when pollen advanced towards embryo formation. To our knowledge, this is the 1st report of ribosomal-body formation in plant embryology, whether androgenic or zygotic. In contrast, the origin, role and function of the RNA-rich ribosomal body are well described in animal embryology, particularly in that of the mollusc, *Lymnaea stagnalis*[14–16]. It has also been suggested that the RB are an important site of enzyme formation necessary for the induction and development of animal embryogenesis, and they are very active in the diffused state[14].

Consequently, the deviation from the gametophytic to the sporophytic pathway in higher plants like *Datura*, could be better understood by determining 1. the type of enzymes or proteins synthesized in the RB, and 2. whether their state is the condensed or diffused one.

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Cholinergic nerves in the rat portal vein

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Summary. The innervation of the rat portal vein was studied using the cholinesterase histochemical method. 2 plexuses of cholinergic nerve fibers were found; an external plexus localized at the level of the outer adventitial layers and an inner plexus at the level of the adventitial-medial transitional zone and in the outer layers of the media.

A detailed knowledge of portal vein innervation is of particular interest since this vessel is widely used as a model for studies on the influence of various substances on vascular smooth muscle cells[3].

Histochemical studies demonstrate that the portal vein is provided with a rich adrenergic innervation[4–8] and in the rabbit with a purinergic innervation[8].

The available information about the cholinergic innervation of the portal vein is very poor, and limited to the results of Mootz[9] and Booz[5] who described the presence of few adventitial cholinergic nerve fibers (CNF) in the rat, and to the results of Ungvary et al.[6] and Burnstock et al.[8]. These authors[6,8] did not observe any appreciable cholinergic innervation of the portal vein in guinea-pig and rabbit.

The finding that there is a very poor cholinergic innervation in the rat portal vein (see Booz[5] and Mootz[9]) does not seem to be in agreement with the results of Reilly et al.[10] who observed a moderately rich cholinergic innervation of the intrahepatic branches of the portal vein in the same species.

With the purpose of clarifying the problem of the extent of the cholinergic innervation to extraparenchymal branches of the rat portal vein we have carried out the present experiments.

Methods. 15 young rats (1–3 months of age) were used in our study. 5 animals were chemically sympathectomized using an i.p. injection of 6-hydroxydopamine (6-OHDA, for references see Burnstock et al.[8]). The drug was injected in 2 doses of 200 mg/kg, the second was given 24 h after the first. The 6-OHDA (HCl salt, K.E.K., USA) was dissolved in 0,9% w/v saline containing 0.1 mg/ml ascorbic acid. 10 rats were used as a control and received 2 injections of a saline solution.

All animals were sacrificed under ether anaesthesia. The abdominal cavity was rapidly opened and the portal vein was dissected and washed in a Krebs solution at 4 °C. The pieces were divided into laminae and stretched flat on slides, or cut transversely on a cryotome (sections of 10–30 µm in thickness). The tissues were dried over P_2O_5 for 60–90 min and then processed for the histochemical detection of acetylcholinesterase activity as previously described[11]. The incubation was accomplished at room temperature for 2–6 h in a medium containing 10^{-5} M iso-OMPA to inhibit nonspecific cholinesterases[11].

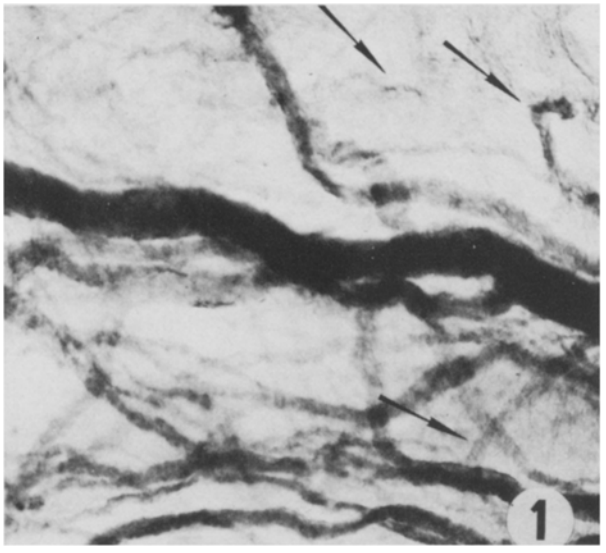

Figure 1. Rat portal vein. Acetylcholinesterase. Whole mount. Adventitial plexus. Cholinergic nerve fibers are organized in a plexus composed of thick nerve fibers parallel to the course of the vein and of thin nerve bundles (arrows) (× 400).

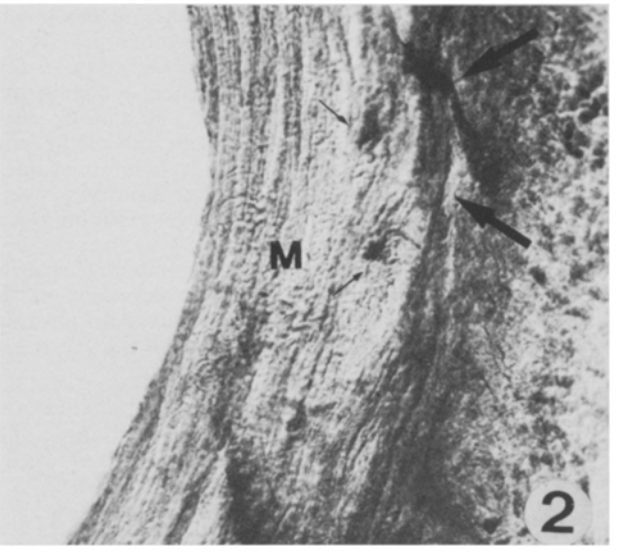

Figure 2. Rat portal vein. Acetylcholinesterase. Transverse section. The plexus localized at the adventitial-medial border can be seen (thick arrows). Nerve fibers originating from this plexus may reach the outer layers of the media (thin arrows). Observation carried out using phase contrast to improve the contrast. M = media (× 480).

To ensure the effective degeneration of adrenergic nerves after 6-OHDA treatment some specimens were exposed to gaseous formaldehyde according to the Falck-Hillarp method[12].

Results. Stretched specimens and transverse sections show the presence of a cholinergic innervation in the rat portal vein. We do not observe any alteration in the pattern of cholinergic innervation of the portal vein after chemical sympathectomy, while adrenergic nerve fibers disappear completely after 6-OHDA treatment.

The rat portal vein is provided with 2 plexuses of CNF. The 1st plexus is localized in the outer adventitial layers (fig. 1). The 2nd plexus was found in the adventitial-medial transitional zone (fig. 2). We do not observe any topographic difference in the pattern along the portal vein, since the density of CNF is the same in specimens of the vein taken from the origin of the blood vessel or from its entrance in the liver.

The external plexus is composed of thick nerve fibers (18–25 μm in thickness), longitudinally arranged, parallel to the course of the vein and consisting of few nerve bundles. These nerve fibers form a sheet-like plexus.

The adventitial-medial plexus is composed of thin nerve fibers organized in a large-meshed network. The nerve fibers of this plexus reach the media only rarely (fig. 2).

Discussion and conclusions. The present results provide evidence that the rat portal vein has a cholinergic innervation. The finding that chemical sympathectomy does not alter the pattern of the cholinergic innervation of the portal vein indicates that stained nerve fibers are independent of the sympathetic (adrenergic) nervous system.

In the portal vein 2 plexuses of CNF exist, a first more external, and a second localized just outside the media. The acetylcholine released from cholinergic nerve terminals of the adventitial-medial transitional zone reaches the cholinergic receptors localized in the wall of the portal vein (see Hugher and Vane[13]), probably through a diffusion mechanism[14], and presumably counterbalances the effects of catecholamines.

As to the function of the external plexus, the arrangement of its fibers parallel to the course of the vein supports Mitchell's hypothesis[15] that autonomic nerves also reach the liver along the portal vein.

Further studies are in progress in our laboratory to elucidate the origin of cholinergic (presumably parasympathetic) nerves observed in the portal vein.

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