As far as the pylorus is concerned, in our study far fewer HRP-positive neurons were found in the nodose ganglia than were reported in a previous study on the guinea pig [19]. The discrepancy can be only partially explained by the species difference. In the study on the guinea pig, HRP injections were made into the whole of the pyloric sphincter, which could have damaged the nearby pancreas, making it easier for tracer to be taken up from nerve terminals of this gland, which is richly innervated by the vagus nerve [14]. Furthermore, the high concentration of HRP used in that study could also have facilitated a backward spreading of the tracer in large quantities, labeling the surrounding tissues and so giving false positive results [20]. With a lower concentration of HRP injected only into the ventral side of the pylorus we believe we have overcome the problem of mislabeling due to diffusion from the injection site. Our results on vagal afferent innervation of the pylorus are of course limited to one side of this sphincter only, but are more reliable.

The number of HRP-positive neurons calculated from our experiments on the vagal gastric afferents is similar to that found in the nodose ganglia of guinea pigs and cats [21]. This study was also one in which mislabeling was avoided, by using a very low concentration of HRP. A study in the rat carried out by injecting a large amount of HRP into the stomach wall resulted in the labeling of the majority of the ganglion cells [11]. Unfortunately, the number of HRP-positive neurons was not specified, so we cannot make a relative comparison; also our investigation was in fact limited to the gastric antrum.

In the rat, the extent of labeling in the nodose ganglia found by studying the vagal afferent innervation of the gastric antrum and pylorus probably indicates that both vagi participate in the reflex regulation of gastric function and reflex control of the pyloric region. In contrast, the vagal afferents are scarcely present in the upper part of the small intestine. Keeping in mind that in the proximal small intestine the vagal efferent projection is also sparse and is limited to the dorsal motor nucleus [11], it is reasonable to suppose that the intrinsic innervation plays a principal role in duodenal function, compared to the extrinsic one of the vagus nerve.

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Vagal afferent innervation in regenerated rat liver

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Summary. The possible presence of neural sprouting in the afferent neurons of regenerated rat liver after hepatectomy was investigated by retrograde transport of horseradish peroxidase. This experiment was carried out to see if the increase in hepatic parenchyma could provide an adequate stimulus for the sprouting process. The study was limited to the vagal afferents, particularly the left ones, because they are the principal contributors to hepatic afferent innervation in the rat. The results show that neural sprouting does not occur in regenerated rat liver after 3 weeks. In fact, the number of intensely labeled neurons in the left nodose ganglia of hepatectomized rats was significantly smaller than in controls. This could be due to a lessened availability of horseradish peroxidase to nerve terminals because of the increased non-innervated hepatic mass. There was no difference between right nodose ganglia neurons in hepatectomized and control animals. This could be a consequence of their possible distribution in hepatic areas not involved in the regenerative process.
Key words. Hepatectomy; neural sprouting; nodose ganglion; horseradish peroxidase.

Recent studies have clearly demonstrated the presence of tonic afferent discharges from the liver, which can be modulated by various stimuli and can also determine reflex responses aimed at homeostatic control[1,2]. In order to perform such functions, nerve sprouting of the afferent fibers would have to occur in the regenerated liver so as to make it comparable to a normal one.

The aim of this study was to investigate afferent innervation in the rat liver after regeneration and to demonstrate the presence of nerve sprouting if this occurred, by retrograde transport of horseradish peroxidase (HRP). Research was limited to the vagal afferents as the supply to the rat liver originates mainly from the nodose ganglia of both vagus nerves, whereas the contribution of the dorsal root ganglia (Th7–Th12) is minor[3,4]. Since the amount of HRP granules present in a cell body can be related to the number of nerve terminals in the injected area[5,6], we compared the ratio of intensely labeled neurons to total HRP-positive neurons in controls and in hepatectomized animals, to obtain an indication of the presence of sprouting in the afferent fibers of a regenerated liver. If the ratio in hepatectomized rats is equal to or higher than that in controls, this could indicate the presence of nervous sprouting. If new nerve terminals grow in regenerated liver in such a way that afferent innervation is similar to that of normal liver, they would be expected to take up as much HRP as controls, or even more if their localization in the new hepatic parenchyma is better with regard to availability of the neural tracer. In contrast, if the ratio is lower in hepatectomized rats than in controls, this shows that nervous sprouting has not taken place, thus demonstrating that the increased hepatic mass in the right and caudate lobes has reduced HRP access to nerve terminals.

The possible presence of nervous sprouting in the afferent fibers was investigated three weeks after hepatectomy, bearing in mind the evolution time of monoaminergic fiber regeneration in the rat liver[7,8], and also the fact that three weeks after hepatectomy changes in the population of cells other than the parenchymal cells, such as those of the bile duct, have already occurred[9].

Materials and methods

18 male Wistar rats (average b.wt 250 g) were used; 12 were hepatectomized and 6 were used as controls. Partial hepatectomy was carried out by almost complete excision of the left and median lobes after ligation, according to the method of Higgins and Anderson[10]. The bile duct was first ligated with linen immediately above the confluence of the right lobe duct. Controls were also subjected to this ligation before being treated with HRP. The injected material was distributed only to the right and caudate lobes of the liver[11]. This experimental maneuver was clearly necessary in the controls to prevent sensory afferents of the left and median lobes from being labeled by HRP, while in hepatectomized rats it allowed the exclusion of bile ducts still present after resection of the left and median lobes.

Three weeks after the operation, 40 µl of 21% HRP (sigma type VI) dissolved in H_2O were slowly delivered to the liver parenchyma, followed by 400 µl of saline solution to wash the dead spaces, by retrograde injection through the common bile duct cannulated with a fine-tipped polyethylene tube. All operations were carried out with the animals under ether anesthesia.

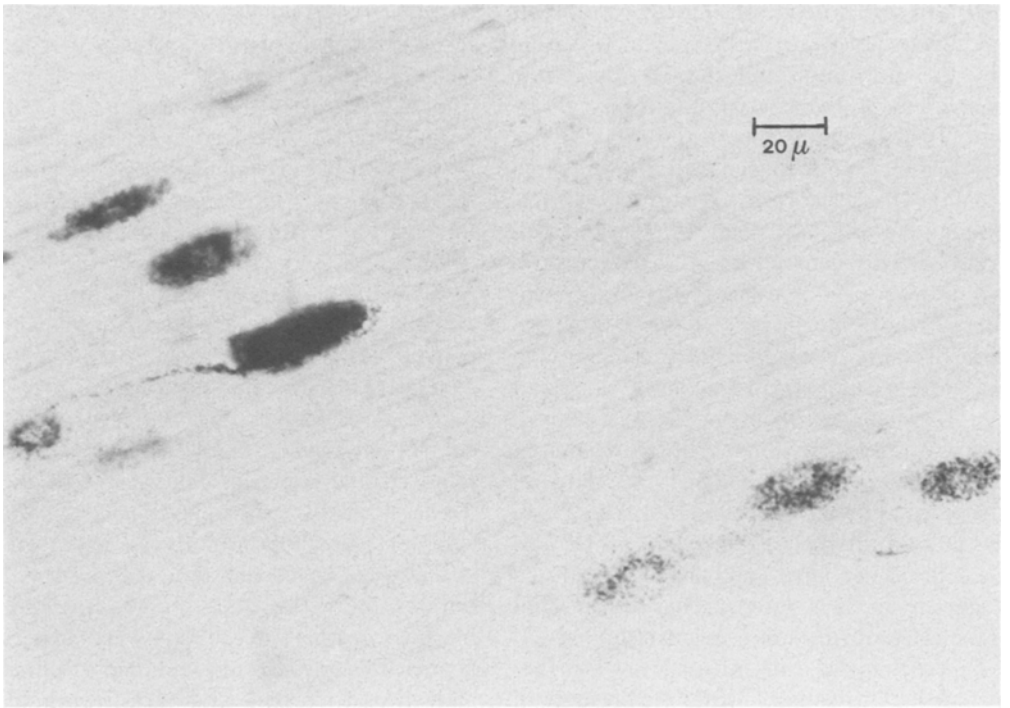
After 48 h the rats were reanesthetized and transcardially perfused with 50 ml saline solution, followed by 250 ml of fixative (1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4) and finally by cold (4 °C) sucrose in the same buffer. The liver was removed and weighed. The weight of the regenerated liver was nearly the original value, showing an almost complete regeneration. Both vagi with their sensory ganglia were dissected out and, after 24 h in cold sucrose-buffer solution, were cut into 40-µm sections by a freezing microtome. The sections were processed for HRP histochemistry following Mesulam's technique[12] and examined under bright- and dark-field illumination without counterstaining.

Only the neurons showing a nucleus in the plane of the section were counted, and distinguished as being either intensely or lightly labeled. The degree of labeling of the neuronal cell bodies varies (fig.). The reaction product is deposited in the form of granules that can be sparsely (lightly labeled neurons) or densely packed (intensely labeled neurons). A variety of factors contribute to the apparent density of perikaryal labeling[5,13] but under the same experimental conditions this can be mainly due to the extent of the terminal arborization which has access to HRP.

Results

The relative frequency of intensely labeled neurons to total HRP-positive neurons in hepatectomized and control rats was compared. No significant difference was found in the right vagi ($u = 0.09$); on the contrary, the number of intensely labeled neurons in the left vagi compared to the total number of HRP-positive neurons was significantly lower in the hepatectomized animals than in controls ($u = 3.31$; $0.01 > p > 0.001$).

In order to analyze the data better, it seemed useful to calculate, for each vagus, the ratio of intensely labeled neurons (I) to total labeled neurons (T) as the arc sine $\sqrt{I/T}$, to stabilize the variance[14]. When the mean values of these ratios between hepatectomized and control rats were compared in the right vagi the difference was still not statistically significant (using Student's test, $t = 0.13$), whereas in the left vagi it was statistically significant at less than 0.01 ($t = 2.33$). For complete data, see table.



A photomicrograph of HRP-positive neurons in the left nodose ganglion of a control animal. A heavily labeled cell can be seen at the lower left.

Total labeled neurons (T) and intensely labeled neurons (I) in the right and left nodose ganglia of six controls (C) and twelve hepatectomized (H) rats after intrahepatic administration of 40 μl of 21% HRP.

	Controls		Hepatectomized		Comparison between C and H
	T	I	T	I	
Right nodose ganglion	15.6 ± 6.4	2.9 ± 1.2 (19%)°	23.9 ± 10.4	4.3 ± 2.9 (18%)°	NS
Left nodose ganglion	149.6 ± 56.3	35.7 ± 18.2 (23%)°	154.6 ± 59.5	28.8 ± 14.4 (18%)°	$p < 0.01$

° In parentheses, the percentage of I vs T.

Discussion

Recent studies have clearly shown that afferent hepatic nerves both in the isolated liver and in vivo modify their tonic discharges when mechanical, chemical, osmotic and thermal stimuli are applied to this organ[1,2]. Afferent discharges from the liver could thus monitor metabolic, hemodynamic or thermal changes and so promote reflex responses contributing to homeostatic control. In order to perform such functions, nerve sprouting of afferent fibers would have to take place in regenerated liver.

This study was undertaken to analyze such an event using HRP as neural tracer. In addition to considerable species-dependent differences, there are intra-species conflicting results about nerve distribution in the liver, particularly regarding termination of fibers on the hepatocytes. Some investigators believe that an extensive terminal arborization exists in contact with the hepatic cells[15]; others, on the contrary, believe that innervation is limited to hepatocytes located near the portal lamina[16,17]. Due to the marked variation in these observations and the lack of clear morphological evidence, retrograde transport of HRP was chosen for this study as it has already provided detailed anatomical evidence for the existence of afferent pathways originating from the rat liver[3,4,11].

Mature nerves sprout when adjacent ones are cut (denervation sprouting). There may also be sprouting in intact nerves when it is induced by nerve degeneration or by some influence from the target tissue[18–20]. The terminal nerve field thus results from a balance between the sprouting stimulus of a substance (a nerve growth factor) continually produced by the target tissue, and inhibition by neural factors released from the endings. Aguilar et al.[21] hypothesized that when this balance is disturbed either by elimination of the inhibiting neural factors from cut nerves or by the increase of the stimulating substance due to an increased target tissue, the intact nerves sprout until the new nerve terminals release enough of the neutralizing factors to restore the original equilibrium. This could explain nerve sprouting both after partial denervation and without denervation.

After hepatectomy, the hepatic parenchyma of the intact lobes readily grows to restore the original hepatic mass and this constitutes an adequate stimulus for the sprouting of monoaminergic nerves [7, 8].

Results obtained in this study would seem to indicate that nervous sprouting of vagal afferent fibers does not occur in regenerated rat liver during the experimental period investigated (until 3 weeks after hepatectomy). The decreased number of intensely labeled neurons in the left nodose ganglion of hepatectomized rats is probably due to a decreased availability of the neural tracer at the terminal endings due to the increased hepatic mass. The finding that the number of intensely labeled neurons in the right nodose ganglia is not significantly different from that of controls could be due to the fact that their afferent terminal fibers innervate some hepatic areas which are not involved in the regenerative process.

The behavior of afferent fibers in the regenerated liver seems to be quite different from that of the sympathetic efferent ones. Experiments regarding regeneration of monoaminergic nerves in the rat liver after partial hepatectomy [7, 8] have shown a characteristic hyperinnervation in the central area of the hepatic lobe at 7 days after surgery, whereas the innervation in the peripheral area was about 50% compared to controls. Two weeks after partial hepatectomy the hyperinnervation was still present in the central area of the lobe and there was a 70–85% innervation of the peripheral area. Liver innervation was complete by the sixth week.

Afferent hepatic fibers seem to behave differently. In fact, if hyperinnervation had occurred, the experimental results would have been completely different from those obtained; an increased, not a decreased number of intensely labeled neurons would have been found in the vagal nodose ganglia three weeks after regeneration.

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Location of an acoustic window in dolphins

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Summary. Auditory brainstem responses (ABR) to sound clicks from sources in different positions were recorded in dolphins *Inia geoffrensis*. The position of the acoustic window was determined by measurement of acoustic delays. The acoustic window was found to lie close to the auditory meatus and the bulla rather than on the lower jaw.
Key words. Auditory brainstem response (ABR); dolphins; hearing; acoustic window.

The mechanisms of sound transmission to the ear in aquatic mammals, particularly in dolphins, remain obscure. It has been suggested that sound reaches the inner ear directly via head tissues, or sound transmission involves the middle ear and the closed auditory meatus [2–4]. This assumption was supported by calculations indicating that the closed auditory meatus can serve as an effective acoustic transformer for the sounds to be transmitted to the middle ear [5].

On the other hand, there is the popular so-called mandibular hypothesis emphasizing the key role of the lower jaw with its peculiar fat body as a specific sound-conducting pathway [6, 7].

Sounds are believed to reach the fat body through a thin bone plate on the lateral mandibular surface and are transmitted further via the fat body to the bulla. Therefore, the hypothesis postulates the presence of an acoustic window on the lower jaw which allows for the percep-