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Decreased cerebrospinal fluid flow through the central canal of the spinal cord of rats immunologically deprived of Reissner's fibre

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Abstract The subcommissural organ is an ependymal brain gland that secretes glycoproteins to the cerebrospinal fluid (CSF) of the thrid ventricle. They condense to form a fibre, Reissner's fibre (RF), that runs along the aqueduct and fourth ventricle and the central canal of the spinal cord. A single injection of an antibody against the secretory glycoproteins of RF into a lateral ventricle of adult rats results in animals permanently deprived of RF in the central canal and bearing a "short" RF extending only along the aqueduct and the fourth ventricle. These animals, together with untreated control animals were used to investigate the probable influence of RF in the circulation of CSF in the central canal of the spinal cord. For this purpose, two tracers (horseradish peroxidase and rabbit immunoglobulin) were injected into the ventricular CSF. The animals were killed 13, 20, 60, 120 and 240 min after the injection, and the amount of the tracers was estimated in tissue sections obtained at proximal, medial and distal levels of the spinal cord. In rats deprived of RF, a significant decrease in the amount of tracers present in the central canal was observed at all experimental intervals, being more evident at 20 min after the injection of the tracers. This suggests that lacking a RF in the central canal decreases the bulk flow of CSF along the central canal. Turbulences of the CSF at the entrance of the central canal of RF-deprived rats might explain the inability of the regenerating RF to progress along the central canal, as well as the reduced flow of CSF in the central canal of these animals.

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Introduction

The subcommissural organ (SCO) is a diencephalic ependymal gland that releases glycoproteins into the cerebrospinal fluid (CSF) of the third ventricle. Some of these glycoproteins condense to form a fibre, Reissner's fibre (RF) (Reissner 1860) that extends along the Sylvian aqueduct, 4th ventricle and the central canal of the spinal cord. By addition of newly released material to its proximal end, RF grows caudally at a rather fixed rate (Ermisch 1973; Herrera 1988). At the caudal end of the central canal of lower vertebrates, glycoproteins undergo certain chemical changes (Rodríguez et al. 1987) and gain access to the local blood vessels through ependymal openings (Hofer et al. 1984; Peruzzo et al. 1987). In mammals, the fate of RF material arriving at the caudalmost region of the central canal is not known. Although the SCO and the RF were discovered more than a century ago, the functional role of the SCO-RF complex remains unknown (see E.M. Rodríguez et al. 1992).

The mechanism underlying the assembly of the glycoproteins released by the SCO, first into a pre-RF (E.M. Rodríguez et al. 1986) and then into a RF, is not known (E.M. Rodríguez et al. 1990; Nualart et al. 1991). The participation of hydrodynamic factors of the CSF ciculation in this process has been suggested (Olsson 1958; Oksche 1961). Some experimental evidence speaks in favour of this hypothesis: induced postnatal hydrocephalus in rats leads to the disappearance of RF, although the SCO continues to release glycoproteins into the ventricle (Irigoin et al. 1990) and rat SCO grafted under the kidney capsule secretes glycoproteins into newly formed follicles cavities, but this material does not aggregate to form a RF (E.M. Rodríguez et al. 1989).

On the other hand, there are reports indicating an effect of SCO secretions on the dynamics of CSF. Overholser et al. (1954) suggested that the secretory activity of SCO during embryonic life prevents the closure of the Sylvian aqueduct, allowing the free circulation of CSF from the third to the fourth ventricle. A maldevelopment of the SCO during foetal life should lead to stenosis of the aqueduct and hydrocephalus (Overholser et al. 1954; Newberne 1962). Takeuchi and Takeuchi (1986) showed that alteration of the SCO development by X-irradiation precedes aqueductal stenosis and hydrocephalus. Moreover a mouse strain with spontaneous congenital hydrocephalus lacks a SCO (Takeuchi et al. 1987), and rat strains with the same syndrome showed a drastic reduction in the size of the SCO (Jones and Bucknall 1988; Takeuchi et al 1988). Thus a relationship between the SCO secretions and CSF circulation seems likely.

In a previous investigation, we showed that a single injection of an antiserum against RF glycoproteins into the ventricular CSF of adult rats leads to a fragmentation of RF followed by the formation of a new RF. The latter, however, although growing along the Sylvian aqueduct and fourth ventricle, does not proceed along the central canal (S. Rodríguez et al. 1990). These authors (S. Rodríguez et al. 1993) extended the period of observation to several months after the intraventricular injection of the anti-RF serum and found that these rats were permanently devoid of RF in their central canal, thus becoming a valuable experimental model. In the present investigation, we used this model to investigate whether or not RF has an influence in the circulation along the central canal of the spinal cord of tracers injected into the ventricular CSF. Recently we have used the same tracers to investigate the circulation of CSF along the central canal of the spinal cord of the normal rat (Cifuentes et al. 1992).

Materials and methods

Animals

Male Sprague Dawley rats (body weight 250–300 g) were used in the present study. The animals were kept under a photoperiod of LD 12:12 h and an environmental temperature of about 25° C. They were fed ad libitum with rodent food. Handling, care and processing of the control and experimental animals were carried out according to principles approved by the council of the American Physiological Society and national laws (B.O.E. 67, 1988, Spain). All animals were anaesthetised with ether.

RF-deprived rats

Forty-one rats deprived of RF in the central canal of the spinal cord were obtained by following the schedule designed by S. Rodríguez et al. (1990). Briefly, the animals were subperfused (2 µl/min) through a lateral ventricle with 20 µl of PBS pH 7.4, containing the IgG fraction (10 µg protein/µl) of an antiserum raised in rabbits against the constituent glycoproteins of the bovine RF (AFRU, antiserum against fibre of Reissner extracted in medium containing urea; cf. Rodríguez et al. 1984). These rats lacks RF in the central canal, even 6 months after the injection of AFRU (cf. S. Rodríguez et al. 1990, 1993).

Four of the RF-deprived rats were left to survive for 1 month. They were then anaesthetised and fixed by vascular perfusion with Bouin's fluid. The brain, together with the proximal cervical segments of the spinal cord, was cut sagittally; blocks containing all other segments of the spinal cord were transversally cut. The sections were processed for immunostaining according to the method of Sternberger et al. (1970). Two primary antibodies were used: (1) AFRU; (2) the same immunogen was used to raise antibodies in rats (RAFRU S. Rodríguez et al. 1990). Both antisera were used at a dilution of 1:1000 and incubation lasted 18 h. Anti-rabbit IgG developed in goat and a rabbit-PAP were employed when AFRU was used; anti-rat IgG developed in rabbit and a rat-PAP were used for sections incubated in RAFRU.

The remaining 37 rats deprived of RF were used for the experimental study 11 days after AFRU injection as follows.

Experimental groups

Normal and RF-deprived rats were anaesthetised and perfused, using a pump, via a canula stereotaxically positioned in the right lateral ventricle (0.5 mm posterior from Bregma, 1.5 mm lateral from saggital suture and 4.0 mm ventral from dura) with one of the solutions containing the tracers: (1) horseradish peroxidase (HRP Sigma, Type IV, Madrid, España), (2) rabbit IgG (Sigma). In cases with a post-injection interval exceeding 1 h, the animals were placed back into their cages and kept under the same environmental conditions and with free access to food and water. Five groups of rats were prepared:

Group 1: Intact rats injected with peroxidase

These rats were perfused into a lateral ventricle with 30 µl 3% HRP, dissolved in 0.9% NaCl, at a rate of 3 µl/min. The central nervous system was fixed 13 min ($n=6$), 20 min ($n=8$), 1 h ($n=5$), 2 h ($n=3$) and 4 h ($n=3$) after starting the HRP perfusion.

Group 2: Intact rats injected with rabbit IgG

These rats were perfused with 20 µl of PBS, pH 7.4, containing rabbit IgG (10 µg/µl), at a rate of 2 µl/min. The central nervous system was fixed 13 min ($n=4$), 20 min ($n=9$) and 1 h ($n=4$) after starting the perfusion.

Group 3: RF-deprived rats injected with peroxidase

These rats were injected with HRP in the same manner as the rats from group 1. The central nervous system was fixed 13 min ($n=5$), 20 min ($n=8$) and 1 h ($n=4$) after starting the HRP perfusion.

Group 4: RF-deprived rats injected with rabbit IgG

These rats were injected with rabbit IgG in the same manner as the rats from group 2. The central nervous system was fixed 13 min ($n=4$), 20 min ($n=8$) and 1 h ($n=4$) after initiation of perfusion.

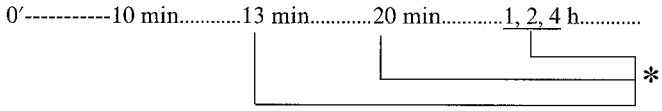
Group 5: Control rats

Two intact rats and two RF-deprived rats were perfused with 30 µl of 0.9% NaCl at a rate of 3 µl/min, killed 20 min after perfusion started and processed for the demonstration of peroxidase. Two intact rats and two RF-deprived rats were perfused with 20 µl of PBS, pH 7.4, at a rate of 2 µl/min, killed 20 min after

perfusion started and processed for the demonstration of rabbit IgG.

The following diagram depicts the time course of events in the experimental protocol (the asterisk denotes vascular perfusion of fixative for 30 min):

Perfusion of tracer CSF



Processing of tissue samples

HRP-injected animals

Under ether anaesthesia, the animals were transcardially perfused first with 0.1 M phosphate buffer, pH 7.4, and then with the fixative (1% glutaraldehyde, 1% paraformaldehyde in 0.1 M phosphate buffer). Vascular perfusion of the fixation lasted for 30 min and was initiated 13 min, 20 min, 1 h, 2 h and 4 h after perfusion of HRP into the ventricle had started (see diagram). After perfusion, the brain and the whole spinal cord were dissected out. The spinal cord was divided into five fragments: (1) *cervical* included cervical segments C1–C6; (2) *proximal thoracic* included thoracic segments T1–T4; (3) *medial thoracic* (T5–T8); (4) *distal thoracic* (T9–T12); (5) lumbo-sacral included segments L1 to the filum terminale. The brain and all pieces of the spinal cord were left immersed in the same fixative overnight at 4° C. Vibratome sections about 40 µm thick were obtained from all tissue blocks. The brain was cut transversally; each one of the five fragments into which the spinal cord was cut was, in turn, subdivided into two segments: the distal half was cut transversally and the proximal half was cut horizontally. Peroxidase was visualised according to the method of Graham and Karnovsky (1966), using 0.1% 3,3' diaminobenzidine (DAB, Sigma) and 0.03% H_2O_2 (Merck, Darmstadt, Germany) in TRIS-phosphate buffered saline (0.05 M, pH 7.8). After being rinsed in the same buffer for 30 min, the sections were mounted in glycerol or in Aquatex (Merck) for light microscopy.

IgG-injected animals

Under ether anaesthesia, the animals were transcardially perfused with a washing solution containing 0.9% NaCl, 0.8% sucrose and 0.4% glucose. Then, Bouin fixative was perfused for 30 min. After perfusion, the brain and the whole spinal cord were dissected out and divided into five fragments as described above. The brain and all pieces of the spinal cord were left immersed in the same fixative for 2 days. Then the pieces were dehydrated and embedded in paraffin. Brains were cut either in transverse or sagittal sections. Spinal cord blocks were cut transversely. To visualise the rabbit IgG injected as a tracer, the sections were submitted to an "incomplete immunostaining", i.e. they were directly incubated in an anti-rabbit IgG serum developed in goat (from E.M. Rodríguez, Valdivia, Chile) (1:30) for 30 min. After washing in Tris-PBS, the sections were incubated in PAP complex (1:75) (Sigma P2030) for 30 min at 22° C. DAB was used as the electron donor. The antisera and the PAP complex were diluted in 0.1 M TRIS buffer, pH 7.8, containing 0.7% lambda carrageenan (Sigma).

Semiquantitative studies

Only the sections from rats injected with HRP have been quantitatively analysed. An image analyser IBAS-2000 (Kontron, Germany) provided with a program IPS (image processing system) was used. By means of a video camera connected to a light microscope, the image of a frame containing the central canal was digitalised as a matrix of 100×100 points. A value representing the relative optic density (ROD) on a grey scale from 0 (white) to 255 (black) was assigned to each point. This value was automatically corrected with respect to the background density of an area of the section apparently devoid of peroxidase reaction product. At least three transverse sections from each of the five fragments in which the spinal cord had been divided were used for the analysis. The value given to a section of the central canal represented the mean of those values produced by the 10,000 points, with the exception of those points having a value lower than the background unstained tissue. The value for a given fragment of the spinal cord was the mean of the values recorded for the three sections; the value ascribed to an experimental group of animals represented the mean of values estimated for the same segments corresponding to animals of such a group.

The data of ROD obtained for the different experimental groups and segments of the spinal cord were submitted to the Kolmogorov-Smirnov test ($P < 0.05$). The data were then submitted to an analysis of variance for the three variables, namely, the time after perfusion of peroxidase, the anatomical region, and the presence or absence of RF.

Results

Animals without RF

According to the report of S. Rodríguez et al. (1990), a single injection of AFRU triggers an immunological reaction that leads to fragmentation and dissappearance of the RF and formation and growth of a new RF. In the current study the observation period extended to 8 days after the administration of the antibody. At this postinjection interval RF had grown to reach the fourth ventricle.

One month after the injection of AFRU in the CSF, the rats bore a RF with normal appearance and immunoreactivity, but that only extended along the aqueduct, fourth ventricle and a few micrometres into the central canal (Figs. 1, 4). At this point the RF material formed a folded and twisted structure that occupied most of the lumen of the central canal (Figs. 2, 3). This structure was strongly immunoreactive to RAFRU and resembled the massa caudalis present in the ampulla caudalis of lower vertebrates (cf. Olsson 1958). At the level of this massa rostralis, an immunoreactive material was seen within the ependyma lining the dorsal wall of the central canal and extending some microns into the underlying neuropil (Fig. 3). The exact subcellular location of this material and its fate could not be established.

Comparative analysis of the distribution of HRP in normal and RF-deprived animals

In the region of the central canal of the spinal cord, the peroxidase reaction product was seen in the following locations (Fig. 5): adhered to the luminal surface of the ependymal cells and to RF; in the intercellular spaces among the ependymal cells; in a system of labyrinths of basement membranes occurring among the basal portions of the ependymal cells; in the subependymal neu-

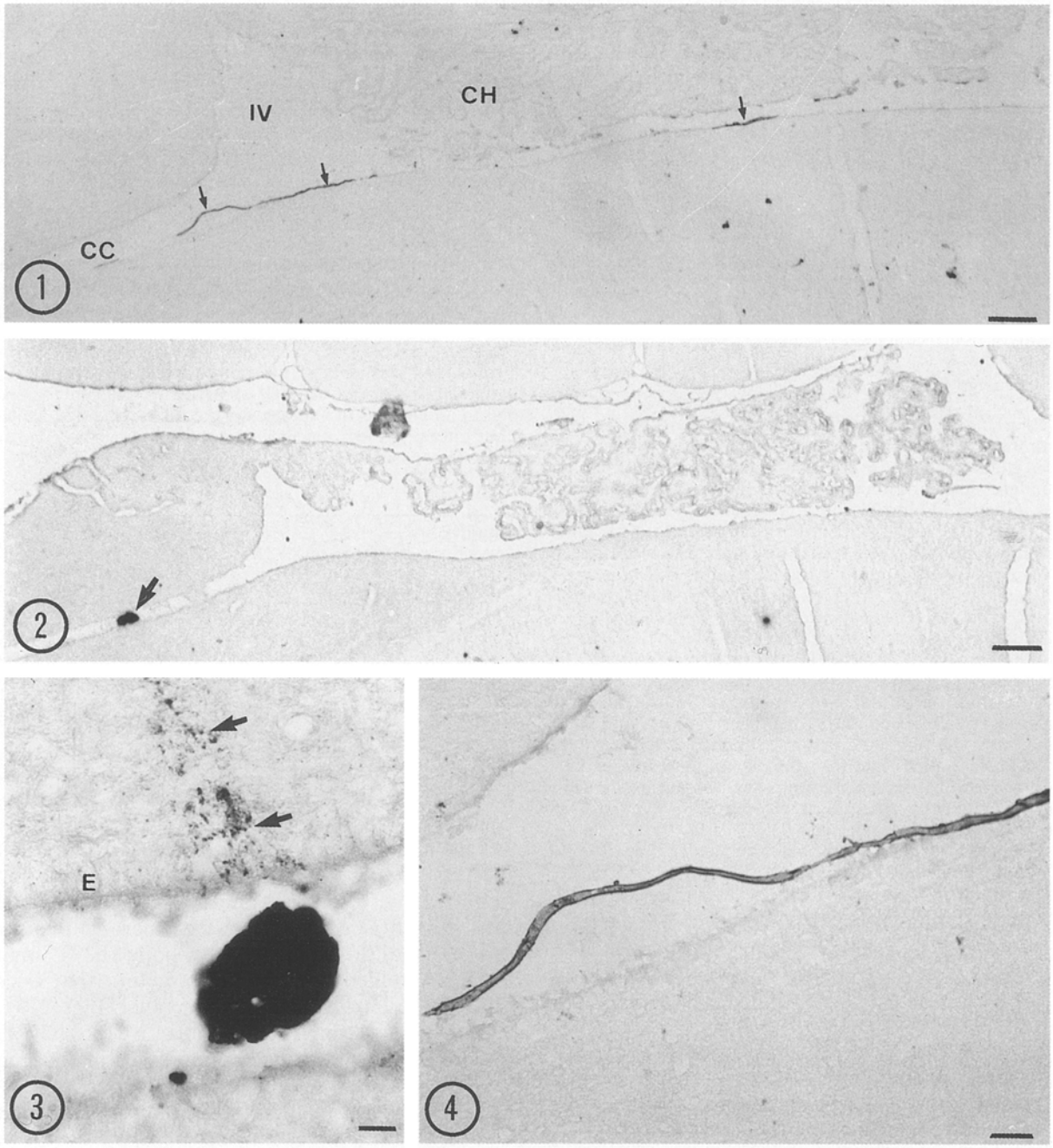


Fig. 1–4 Sagittal sections through the rhombencephalon of a rat sacrificed 1 month after intracerebroventricular injection of the IgG fraction of an antiserum against bovine Reissner's fibre developed in rabbit. The sections were immunocytochemically stained with an anti-bovine RF developed in rat (RAFRU). **1** The RF (*arrows*) appears along the fourth ventricle (*IV*) under the choroid plexus (*CH*) and reaches the entrance of the central canal (*CC*). (A detail of RF is shown in **4**. **2** An adjacent section to **1**, showing a mass of RAFRU-positive material at the entrance of the central canal (*arrow*). **3** A detail of this mass (*arrow*). Note strands of RAFRU-positive material in the ependyma (*E*) and subependymal area (*arrows*) of the dorsal region of the central canal. *Bars*: **1**, **2** 100 μm; **3**, **4** 10 μm

ropil; and in the wall of the neighbouring blood vessels (cf. Cifuentes et al. 1992).

In normal rats sacrificed 13 min after HRP injection, the tracer was visualised throughout the whole length of the central canal. The tracer appeared bound to the ependymal surface and to RF (Fig. 7). Traces of HRP were also seen among the ependymal cells and labelling a few labyrinths. The rats killed 20 min after the HRP injection showed the highest amount of the tracer in their central canal (Fig. 8), being accumulated in the five compartments described above. In RF-deprived rats killed 20 min after the HRP injection, a distinct labelling of the luminal surface of the central canal was seen; however, the actual wall of the canal and the un-

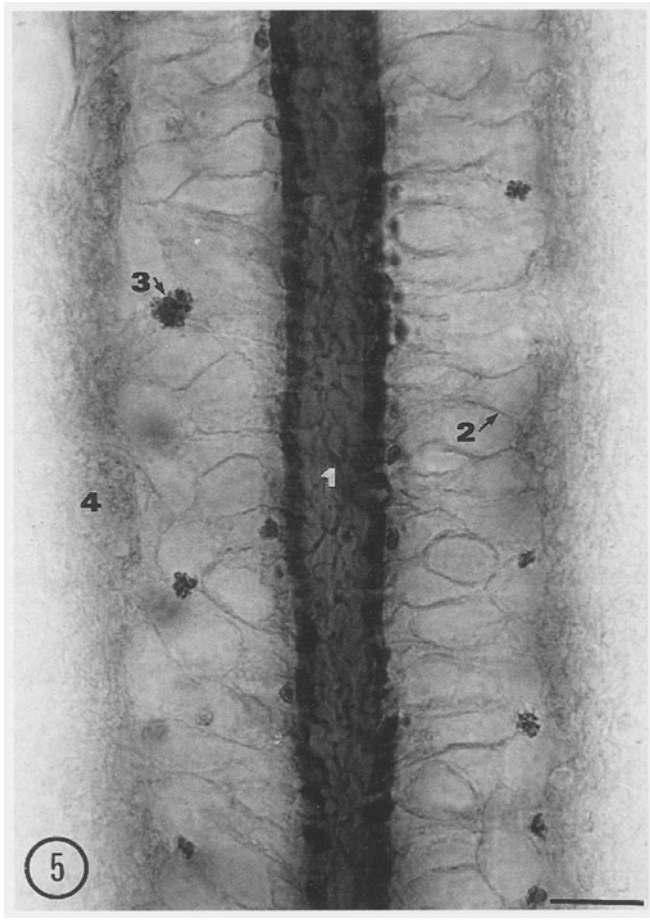


Fig. 5 Horizontal section through the central canal of the thoracic spinal cord. Normal rat sacrificed 20 min after perfusion of peroxidase into a lateral ventricle. Peroxidase reaction product is visualized: *1* on the surface of the ependymal cells; *2* in the intercellular space of the ependymal lining; *3* in the labyrinths of basement membranes; *4* in the subependymal neuropil. *Bar* 10 μm

Fig. 6 A, B Plots representing the relative optic density (*R.O.D.*) of peroxidase reaction product in the central canal region of the thoracic spinal cord. Normal (**A**) and RF-deprived (**B**) rats were sacrified 13, 20 and 60 min after the perfusion of peroxidase into a lateral ventricle. *Vertical bars* standard error, *asterisks* significant differences between control and experimental groups ($P<0.01$)

derlying neuropil was virtually devoid of HRP (compare Figs. 8 and 10). The amount and distribution of HRP in RF-deprived rats sacrificed at 20 min resembled that of normal rats sacrificed at 13 min (compare Figs. 7 and 10).

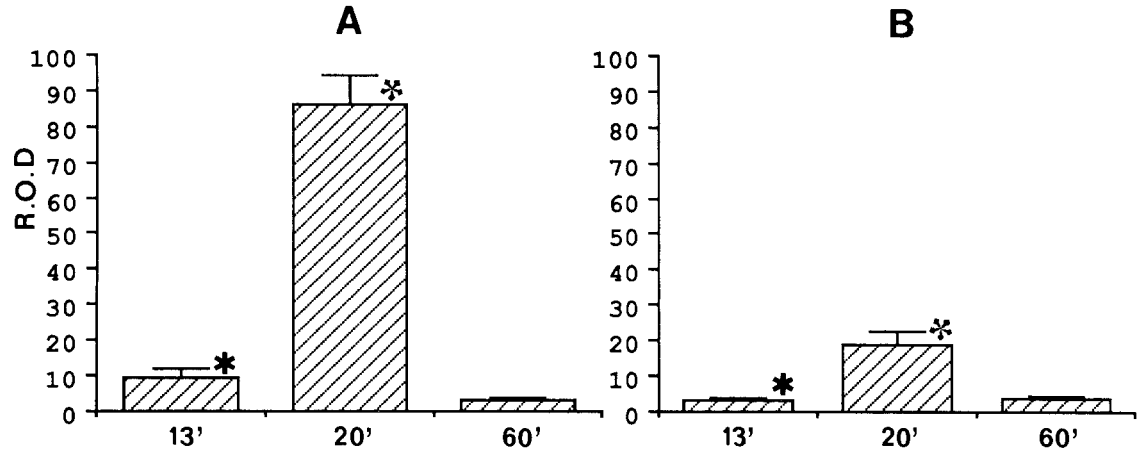
In both normal and RF-deprived rats sacrificed 60 min after the HRP injection, a small amount of tracer was visualised bound to the apical surface of the ependyma and in some labyrinths; at postinjection intervals of 2 h and 4 h HRP was undetectable in the central canal. In all normal and RF-deprived rats sacrificed 20 and 60 min after the administration of HRP, the amount of tracer in the caudalmost levels of the central canal was higher than in the thoracic and cervical segments.

The quantitative analysis of the amount of HRP in the central canal revealed differences between normal and RF-deprived rats at all levels of the spinal cord. However, these differences were more evident at the caudalmost levels. Figure 6 shows the results obtained at the caudal thoracic level. At the postinjection intervals of 13 min and 20 min the amount of HRP in the central canal area was significantly lower in RF-deprived rats as compared to intact rats (Figs. 7–10). The pattern of time course variations in the amount of HRP in the central canal area, i.e. maximum amount at 20 min and minimum amount at 60 min, is similar for the control and RF-deprived rats.

Analysis of the distribution of immunoglobulins

The injected IgG did not penetrate the intercellular spaces of the ependymal lining, neither in the brain ventricles nor in the central canal of the spinal cord. In the latter, the injected IgG was detected exclusively on the luminal surface and within the ependymal cells (Fig. 11).

Although a quantitative analysis was not performed in the IgG experiment, distinct qualitative differences between the different experimental groups were detected. At post-injection intervals of 13 min, normal rats displayed IgG only over the luminal surface of the ependyma, while at the 20 min interval the tracer was



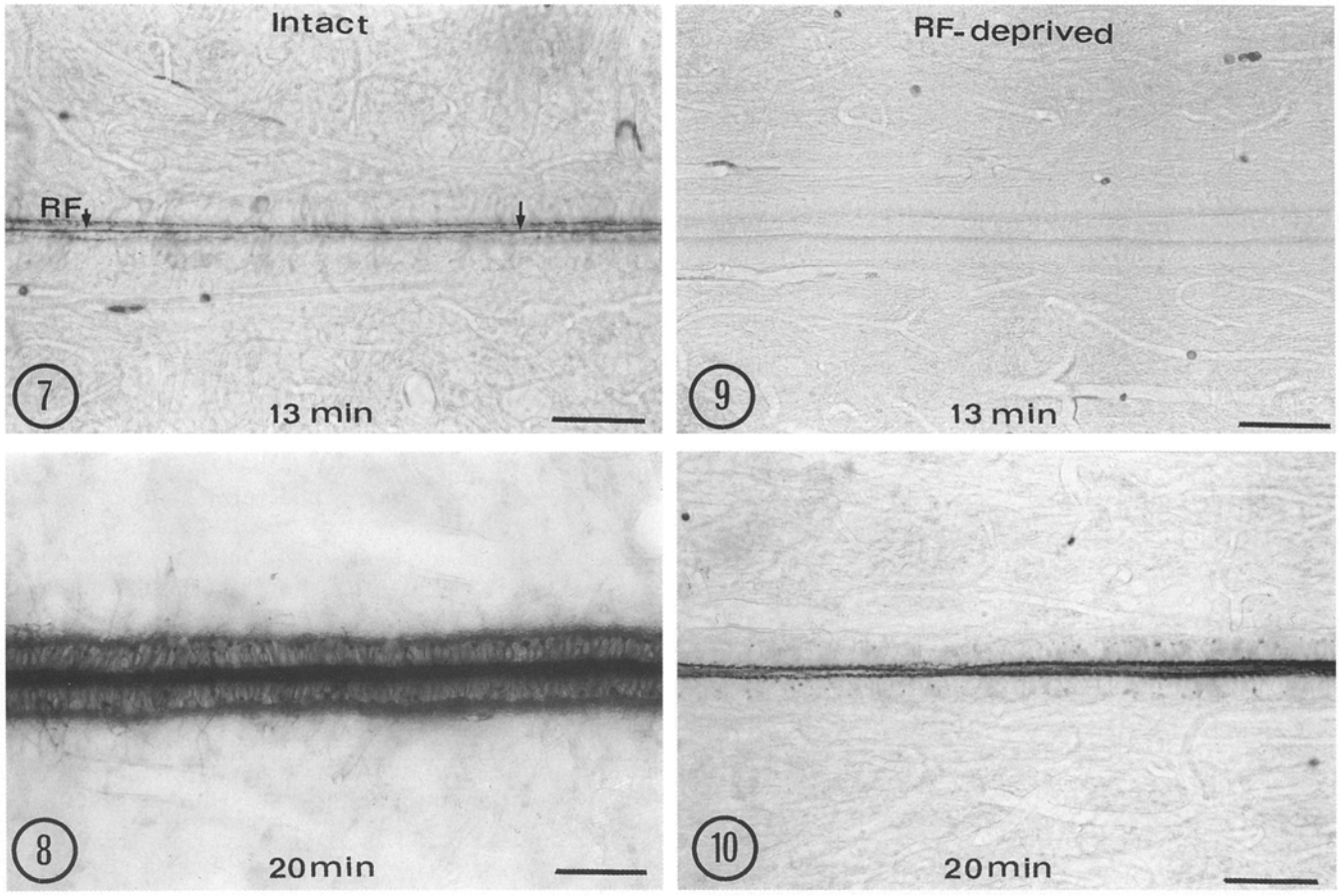


Fig. 7–10 Horizontal sections through the thoracic spinal cord of normal (**7, 8**) and RF-deprived (**9, 10**) rats sacrificed 13 min (**7, 9**) and 20 min (**8, 10**) after perfusion of peroxidase into a lateral ventricle. Note the decreased amount of peroxidase reaction product in the rats without RF. *Bars* 50 μm

present within the ependymal cells (Fig. 11). At the 60 min interval, a small amount of tracer appeared on the surface of the ependymal lining (Fig. 12) and only few ependymal cells appeared labelled. RF-deprived rats injected with rabbit IgG did not display this label along the central canal at any of the post-injection intervals studied (Fig. 14). Only a minute amount of the injected IgG was detected on the luminal ependymal surface of rats killed 20 min after the injection (Fig. 13).

Discussion

The tracers

HRP is an exogenous glycoprotein of 40 kDa and diameter 500 nm that has been widely used in the central nervous system for tracing intracellular and extracellular pathways (Wagner et al. 1974; Warr et al. 1981; Krisch et al. 1984). Although HRP is moderately toxic when injected intravenously, no serious damage has been reported when the tracer is administered into the CSF at low concentrations; there is no cell death and HRP is efficiently cleared by specific cells (Wagner et al. 1974). Furthermore, the availability of sensitive histochemical method to detect HRP (Graham and Karnovsky 1966), makes this molecule a suitable tracer for certain studies performed in the CNS. The amount of HRP injected in the present study is considerably lower than those used in studies by Wagner et al. (1974) and Krisch et al. (1984). In the present investigation, HRP was used following the same protocol applied to study the distribution and flow of this tracer in the central canal of intact normal rats (Cifuentes et al. 1992).

IgG is a molecule of 146 KDa and a diameter of about 1000 nm. Since, under normal conditions, IgG is present in the cerebrospinal fluid (CSF) (Davson et al. 1987), this molecule could be regarded as a physiological marker. The use of antibodies to reveal the injected IgG has resulted in an appropiate method to detect small amounts of this tracer (cf. S. Rodríguez et al. 1990). IgG, at variance with HRP or ferritin (Brightman 1965; Wagner et al. 1974; Cifuentes et al. 1992), does not penetrate the intercellular spaces of the ependymal lining, but it is incorporated by the ependymal cells lining the brain ventricles and the central canal. The possibility that the ependymal cells have specific receptors for IgG at their apical plasma membrane that are able to recognise even heterologous IgG (rat ependymal cells

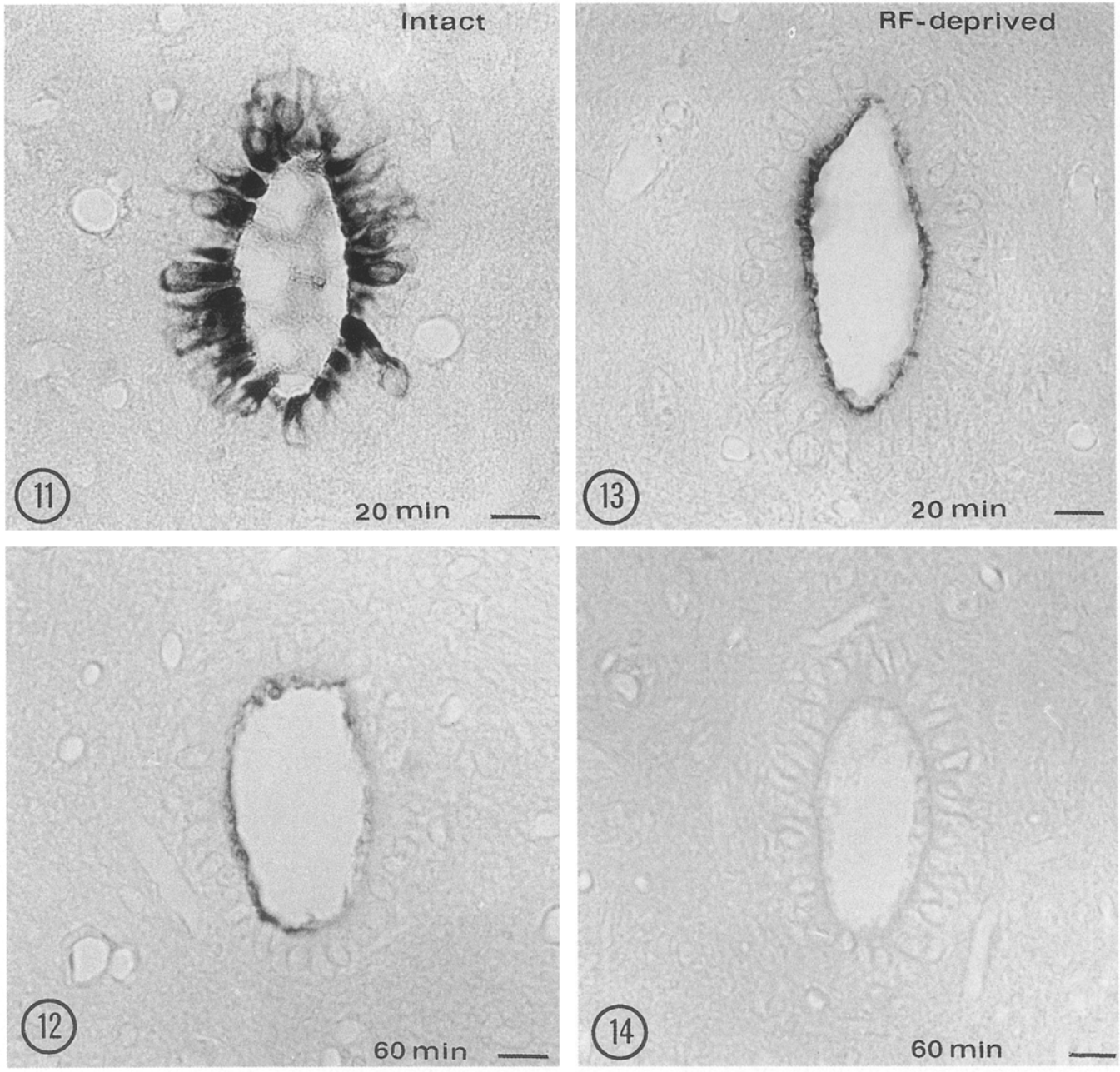


Fig. 11–14 Transverse sections through the thoracic spinal cord of normal (**11, 12**) and RF-deprived (**13, 14**) rats sacrificed 20 min (**11, 13**) and 60 min (**12, 14**) after perfusion of rabbit IgG into a lateral ventricle. The label is circumscribed to the surface and cytoplasm of the ependymal cells. Note the decreased amount of label in the rats without RF. IgG was immunocytochemicaly revealed. *Bars* 10 μm

receptors recognising rabbit IgG) should be considered. The presence of specific receptors for the Fc fraction of IgG has been reported to occur in the basolateral plasma membrane of the choroid plexus (Braathem et al. 1979). This question deserves further attention and may be of particular interest when studying the role that ependymal cells might play in immunological mechanisms of the central nervous system.

The animal model

Although the SCO is a brain gland discovered a century ago (cf. E.M. Rodríguez and Oksche 1993), its function is still unknown. To investigate the physiological role of a gland, animals deprived of such a gland are needed. In the case of the SCO, its deep location in the interface between the CSF and the neural tissue precludes its ablation or electrolytic destruction without damaging important neighbouring structures such as the habenula or the posterior commissure. Thus, other experimental

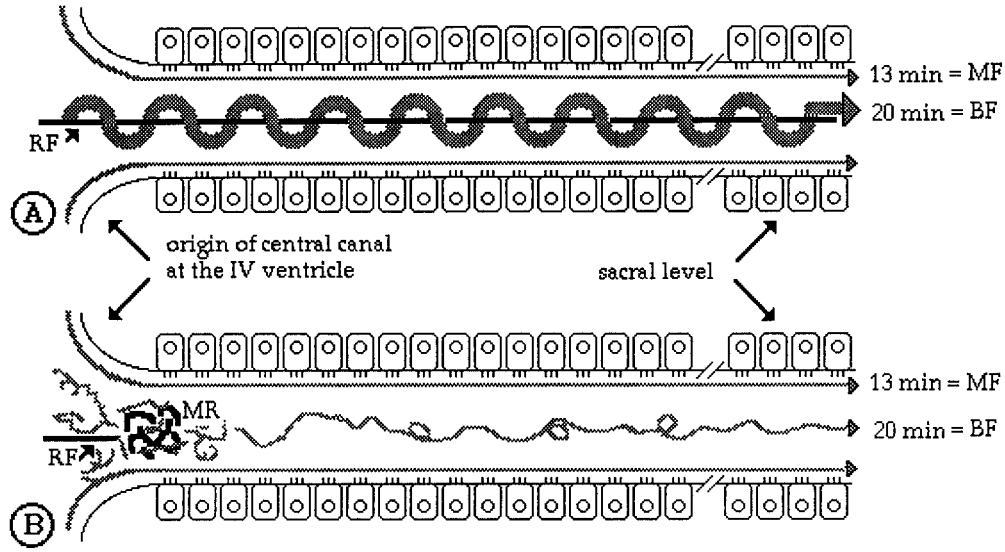


Fig. 15A, B Schematic representation of CSF circulation in the central canal of normal and RF-deprived rats. **A** Normal rats. The two proposed circulations of cerebrospinal fluid (*CSF*) along the central canal are represented: *1* at the periphery of the central canal, ciliary beats would rapidly transport (13 min) a small amount of the tracers throughout the central canal (*MF* marginal flow); *2* the main bulk of tracer (*thick wavy line* in central canal) is transported more slowly (20 min; *BF* bulk flow). **B** Rats without RF in the central canal: *1* the driving force originated by ciliary beats would be unaffected; *2* the driving force for the main bulk is drastically decreased so that the amount of tracer arriving at distal end of the central canal is much lower than in intact rats (*thinner wavy line* in central canal). The suggestion that in RF-deprived rats (**B**) turbulences at the entrance of the central canal (*lighter twisted line*) could be responsible for the decreased flow of CSF as well as for the twisting of RF (*solid twisted line*) is represented, as is the formation of a massa rostralis (*MR*)

approaches are needed to induce a selective destruction of the SCO or to selectively block its functional activity.

By injecting specific antibodies against the glycoproteins constituent of the bovine RF (AFRU), S. Rodríguez et al. (1990) succeeded in reversibly blocking the products secreted by the rat SCO into the CSF. They studied the time course of events after a single injection of AFRU into the CSF. The antibodies bound to a film of aggregated secretory material covering the surface of the SCO (pre-RF) and to RF. After 4 h the RF detached from the SCO and fragmented. After 12 h immunocompetent cells infiltrated the clusters of RF fragments, and 12 h later RF disappeared from the central canal. One day after the injection of the antibody a new RF started to grow. This new fibre reached the fourth ventricle by the 8th day after the injection, after one month it had reached the entrance of the central canal in the cervical spinal cord. At this point the RF-material formed an irregular winding structure, resembling the massa caudalis, that occupied most of the lumen of the central canal [S. Rodríguez et al. (1993) and present report]. The new RF never proceeded beyond this point and was missing from the central canal of the spinal cord up to the end of the observation period (8 months).

The experimental rats used in the present tracing study had been injected with the AFRU 11 days before being perfused with the tracers. In these rats the newly formed RF was present in the fourth ventricle, but had not yet reached the entrance of the central canal.

Circulation of CSF through the central canal

CSF is being continuously produced by the choroid plexuses and the whole nervous parenchyma. The CSF circulates from the lateral ventricles caudalward to the third and fourth ventricles from where it passes to the outer meningeal spaces and is drained at the arachnoid villi (see Wood 1983; Davson et al. 1987). A part of the CSF enters the central canal and proceeds very rapidly (about 1 cm/min) caudalward (Bradbury and Lathem 1965; Nakayama 1976; Cifuentes et al. 1992). It is thought that the driving force for the circulation of the CSF through the ventricular cavities depends on the continuous production and drainage of CSF; an action of massage by the regular pulsations of the large arteries; and the activity of the cilia of the ependyma (Wright 1982; Davson et al. 1987). The relative contribution of each of these forces is still controversial.

Beating of the ependymal cilia seems to be a sine qua non condition for the maintenance of an adequate CSF flow (Worthington and Cathcart 1966; Yamadori and Nara 1979). This assumption is strongly supported by the demonstration that primary ciliary dyskinesia, a syndrome that impairs ciliary motility, leads to the development of hydrocephalus (Greenstone et al. 1984; Afzelius 1985). Shimizu and Koto (1992) suggested that immotility of cilia is of particular importance in narrow canals, such as the Sylvian aqueduct, for the development of hydrocephalus.

In the present investigation, HRP was detected in the caudal-most portions of the central canal 13 min after

the initiation of the perfusion of the tracer in both normal and RF-deprived animals. However, the bulk of tracer took 7 min more to reach the caudal end of the spinal cord. The estimated velocity for the CSF along the rat central canal is 1 cm/min (Cifuentes et al. 1992). This velocity agrees well with that estimated for the movement of particles in mucus-propelling, ciliated epithelia (Hill 1957). This leads us to suggest that the driving force accounting for the fast moving of an small part of the injected HRP is the ciliary activity of the ependymal cells of the central canal (cf. Cifuentes et al. 1992). It has been shown that ciliary beating participates in the flow of CSF over the walls of the ventricle and canals, and that the role played by cilia in the bulk flow is probably insignificant (Nelson and Wright 1974; Milhorat 1975; Wright 1978). The arrival of the main bulk of HRP to the caudalmost region of the central canal 7 min later might reflect the mass flow of CSF which is not dependent on ciliary activity. This hypothesis is represented in the drawing of Fig. 15A.

Decreased flow of CSF in RF-deprived animals

The main conclusion drawn from the present study is that the lack of RF in the central canal of the rat spinal cord co-exists with changes in the flow of CSF in this canal. In both normal and RF-deprived rats a small amount of HRP was consistently found at all spinal levels, even at the shortest post-injection interval of 13 min. However, at each of the experimental intervals investigated, significantly at 13 and 20 min, and in all segments of the spinal cord, the amount of HRP in the central canal region was lower in RF-deprived rats. These facts suggest the following: The velocity of circulation of a part of CSF close to the ependymal surface of the central canal, measured as the time required for any amount of a soluble tracer such as HRP to reach a certain distance, was unaffected by the absence of the RF. If, as proposed above, the force responsible for the rapid movement of HRP were the ependymal ciliary beats, then it can be concluded that the absence of RF does not affect the activity of the ependymal cilia. The bulk flow of CSF, considered as the total amount of tracer passing through a given section of the central canal at a given time, seems to be drastically decreased in the rats lacking a RF. It appears clear that the absence of RF from the central canal affects the movement of the main bulk of CSF along this canal.

The possibility should be considered that the injection of AFRU into the CSF could affect the pattern of fluid currents of the CSF in the brain ventricles and in the central canal. This possibility is supported by findings of S. Rodríguez et al. (1993) that, in AFRU injected rats, aggregates of RF-material appear in ectopic locations, such as the anterior region of third ventricle. The appearance of a mass of RF material at the entrance of the central canal could also be ascribed to a disturbance in the CSF circulation. We postulate that turbulences in the CSF circulation occurring in RF-deprived rats affect the normal flow of CSF from the fourth ventricle to the central canal of the spinal cord, so that when the newly growing RF reaches the entrance of the central canal it twists, forming a mass, instead of proceeding along a wide open central canal. Thus the mass of twisted RF seen in tissue sections would mirror the turbulences of the CSF at the entrance of the central canal. The reduced flow of CSF along the central canal of the spinal cord of RF-deprived rats could thus be a consequence of turbulences in the CSF at the site of origin of the central canal. This hypothesis is represented in Fig. 15B.

The drastic reduction in the labelling of the ependymal cells of the central canal of RF-deprived rats injected with rabbit IgG could be interpreted in two different ways. It could be that the reduced flow of CSF that appears to occur in the central canal of these rats would lower the amount of IgG made available to the ependymal cells. A most attractive alternative is that the presence in the central canal of RF glycoproteins is essential for the absorption of CSF-soluble molecules by the ependymal cells.

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