Ontogenic development of the human subcommissural organ

A. Castañeyra-Perdomo[1,2], E.M. Carmona-Calero[1,2], H. Pérez-González[1], I. Martínez-Peña y Valenzuela[1], P. Plaza-Moreno[1], C. Ormazabal-Ramos[3], I. González-Marrero[2], A. Trujillano-Dorado[2] and R. Ferres-Torres[1]

1- *Departamento de Anatomía, Facultad de Medicina, Universidad de La Laguna, La Laguna, Tenerife, Islas Canarias, Spain*
2- *Departamento de Biotecnología, Instituto de Investigación y Ciencias de Puerto del Rosario, Puerto del Rosario, Fuerteventura, Islas Canarias, Spain*
3- *Departamento de Pediatría, Facultadl de Medicina, Universidad de La Laguna, La Laguna, Tenerife, Islas Canarias, Spain*

SUMMARY

The structure of the human subcommissural organ during its ontogenic development in 24 human embryos and foetuses ranging from 6 to 40 weeks of gestation (WG), and three adult human brains from 27-, 65- and 70-year old subjects was investigated using both qualitative and quantitative methods. Concurrently, the appearance of the subcommissural organ, pineal gland and mesocoelic recess was determined by studying their structure, length and volume. The human SCO appears at the beginning of 8th WG, which confirms previous results; the complete maturation of the SCO occurs at the 15th WG and the following three parts can be distinguished: the precommissural part, located in the rostral zone of the posterior commissure (PC) and extending to the pineal recess; the subcommissural part, located under the PC, and the retrocommissural part, located in the caudal zone of the PC, in the mesocoelic recess and at the beginning of the Sylvian aqueduct. The reduction in size of the SCO begins after the 17th WG and this decrease in size begins in the precommissural, continues in the subcommissural, and finishes in the retrocommissural part. The regression and atrophies of the SCO begin after birth, and the SCO disappears completely after the age of 30. The mesocoelic recess starts to form at the beginning of the 10th WG, and is completely formed by the 14th WG and this is where the retrocommissural part of the SCO is located. In the 40th WG the regression of the mesocoelic recess begins and this takes place at the same time as the regression of the SCO. A parallel development between the SCO and the pineal was found. Thus, we observed the first appearance of the pineal recess in the 7-8th WG; during the 10th WG a compact mass of cells appeared in the rostral part of pineal recess and by the 15th WG the pineal gland (PG) had acquired an almost definitive aspect.

Key words: Subcommissural organ – Human – Ontogeny – Development – Pineal gland

INTRODUCTION

The subcommissural organ (SCO) is a cerebral gland that is formed by secretory cells that cover the lower part and penetrate the posterior commissure (PC). These cells are specialized ependymocytes and hypendymocytes which are elements derived from the ependyma of neuroepithelial origin. In humans this cellular complex, which forms the SCO, protrudes in the posterior part of the ceiling of the third ventricle and is located caudal to the pineal gland, framing the entrance to the Sylvian aqueduct (Castañeyra-Perdomo et al., 1985; Rodriguez et al., 1992). Therefore, since the SCO is located on the border between the diencephalon (epithalamus) and the mesencephalon (pretectal areas), and

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Correspondence to:
Dr. Agustín Castañeyra Perdomo. Departamento de Anatomía, Facultad de Medicina, Universidad de La Laguna, 38071 Tenerife, Spain.
Tel.: 34 922 319352; Fax: 34 922 660253. E-mail: acastane@ull.es

since it is in contact with the third ventricle and is surrounded by a well developed vascular system, this structure is considered to be one of the circumventricular organs (CVO) (Hofer, 1959).

The SCO has been differentiated into two parts (Krabbe, 1925): an epithelial stratified and/or pseudostratified part, and another part composed of a long network of glial and nerve fibres, blood vessels, a basal stratum of epithelial fibres, glial cells and secretory cells, which as a whole forms the hypendymal layer. It is therefore possible to affirm that the SCO is formed by two populations of secretory cells, which in most species are organized into two layers: ependyma and hypendyma (Talanti, 1958; Oksche, 1961a). The degree of development of both layers varies enormously throughout vertebrate ontogeny and phylogeny. (Oksche, 1961b; Castañeyra-Perdomo et al., 1980, 1983a). Although the hypendymal layer was described by Krabbe (1925), Oksche (1961a) characterized and named all the secretory cells located outside the ependymal layer as hypendymal cells.

The SCO receives monoaminergic fibres. Fluorescence, autoradiographic, and immunohistochemical studies have established the existence of a large plexus of serotonergic fibers in the basal portion of the SCO (Fuxe et al., 1968; Björklund and Owman, 1972; Mollgart and Wiklund, 1979; Takeuchi and Sano, 1983; Bouchaud and Bosler, 1986). Autoradiographic and immunohistochemical studies have also demonstrated the presence of GABA in the ependymocytes of the SCO, as well as in fibers and nerve terminals (Ganrani et al., 1981, Rodriguez and Bouchaud, 1996). Thus, two types of nerve terminals can be found in the SCO, serotonergic and GABAergic, which come from neurons located in the nuclei of the mesencephalic raphe. In addition, it has been suggested that serotonergic innervations influence the secretory activity of SCO cells and the metabolic state of GABAergic elements (Mollgard et al., 1978, 1979). Studies using morphological techniques and different experimental conditions have demonstrated that serotonergic innervation and GABA produce an inhibition of the secretory activity of the SCO (Mollgard et al., 1978.1979; Lerger et al., 1983; Bouchaud, 1993; Rodriguez and Bouchaud, 1996).

The SCO and the Reissner's Fiber (RF) have been associated with a multitude of normal and pathological functions, among which the following should be emphasized: a sensitive receptor to variations in the volume of cerebrospinal fluid (CSF); the detoxifying function of the CSF; the role in the homeostasis of salt and water (Dundore et al., 1984; Palkovits, 1987; Rodriguez et al., 1992; Carmona-Calero et al., 1996); a potential role in the physiology of reproduction (Limonta et al., 1982; Castañeyra-Perdomo et al., 1983b, 1983c); the dream mechanism (Sallanon et al., 1984); hypothyroidism (Ferres-Torres et al., 1985), arterial hypertension (Cuevas et al., 1996; Castañeyra.Perdomo et al., 1998), and hydrocephalus (Rodriguez et al., 1992; Castañeyra-Perdomo et al., 1994; Perez-Fiagares et al., 1998).

Foldvari and Palkovits (1964) found alterations in the volume of the SCO in animals fed with sodium- and potassium-deficient diets, but these findings have not been confirmed by other authors. For example, no alterations in the secretion in the SCO of animals fed on diets rich in salt but low in water have been reported (Rodriguez et al., 1992). Despite this, some works continue to associate the SCO with sodium homeostasis (Severs et al., 1993) and that of potassium, since alterations in the overall volume of the SCO have been described in animals with potassium loss via the kidney as a result of treatment with captopril (an angiotensin converting enzyme inhibitor, ACEI), (Carmona-Calero et al., 1996). On the other hand, bands of angiotensin II in the SCO have been described (Ghiani et al., 1988), providing further evidence of its possible role in the salt/water balance and arterial pressure. In addition, hypertension produces alterations in the expression of the fibroblasts growth factor in the SCO (Cuevas et al., 1996).

The relationship of the SCO and the RF with the circulation and composition of the CSF has been reported by several authors such as Sever et al. (1993), who have suggested that the SCO, by means of a specific ion regulation, controls electrolyte distribution in and the pressure mechanisms of the CSF. Also, alterations of the SCO in hydrocephalus have been described. Takeuchi and Takeuchi (1986) reported the agenesis of the SCO and PC in hydrocephalic mice, alterations in the glycoprotein secretion of the SCO have been described in rats with hydrocephalus induced by kaolin injection in the cisterna magna (Irigoin et al., 1990; Rodriguez et al., 1992) and the alteration in glycoprotein precedes aqueduct closure and ventricular dilatation in H-Tx rat hydrocephalus (Somera and Jones, 2004). Aplasia or hypoplasia of the SCO have reported in the mice with partial or complete deficiency in a brain-specific transcript variant of the winged helix RFX4, which is probably the cause of congenital hydrocephalus, because it interferes with the flow of CSF in the rostral part of the Sylvian aqueduct (Blackshear et al., 2003; Cifuentes et al., 1994; Perez-Figares et al., 2001). In addition, in a previous work (Castañeyra-Perdomo et al., 1994) we described alterations in the SCO of hydrocephalic human foetuses, where the affectation of SCO was different, depending on the type of hydrocephalus, which is why we described large cytological and structural alterations of the SCO in the hydrocephalic foetus that were accompanied by other corporal malformations. By contrast, only one precocious atrophy of the

SCO in a hydrocephalic foetus with no other pathological manifestation was observed. The relevance of the SCO in the neurosurgical scenario has also been suggested (Galarza, 2002).

In 1981, Sterba et al. obtained antibodies against a watery extract of bovine RF, and only immunostained secretory cells of the SCO of the rat. In a later study (Sterba et al., 1982), in many species with the exception of the sprocket wheel, ependymal and hypendymal cells were marked. These studies by Sterba were completed by those of Rodriguez et al. (1984a,b) who obtained an excellent anti-bovine RF antibody dissolved in a buffer containing urea. In that work Rodriguez et al. observed that the immunoreactive material (IRM) was located in the SCO of many species, including some monkeys, but they did not find IRM in SCO of either the human or the anthropomorphic monkey.

Meiniel et al. (1988) produced several monoclonal antibodies against extracts of the bovine SCO. One of these antibodies recognized a glycoprotein secreted in the ventricle by the ependyma of the bovine SCO, which also marked the glycoprotein inside the hypendymal cells.

Work using immunohistochemical and ultra structural methods with anti-RF, have also show that the immunoreactive material is located exclusively in the cisternae of the endoplasmic reticulum (ER) and grains concentrated near the ventricular surface of the ependymal cells of the SCO (Lösecke et al., 1984; Rodriguez et al., 1986, 1987a,b; Peruzzo et al., 1990). Furthermore, immunoreactive material was detected in two extracellular locations: material released to the ventricle and condensed in the form of pre-RF and/or RF; and material accumulated in the intercellular spaces located in the vicinity of the perivascular hollows (Rodriguez et al., 1986, 1987a,b).

The SCO has mainly been described in human foetuses, which according to most authors is when it reaches its highest level of development (Dendy and Nichols, 1910; Puusepp and Voss, 1924; Pesonen, 1940). However, the most complete and systematic studies of the SCO are those of Oksche (1956, 1961a) and of Wislocki and Roth (1958) in foetuses 72 and 70 mm long and, specifically, Rakic (1965) who studied the organ in human foetuses ranging from 20 to 43 weeks of gestation (WG). In a previous work (Castañeyra-Perdomo et al., 1985) we studied the development of the SCO of human embryos and foetuses ranging from 10mm (7GW) to 100 mm (15WG) and provided evidence of the human SCO appearing at the end of the embryonic period (8 WG), corresponding to the end of second month of gestation, and of its appearance concurring with the pineal recess. In this work, we observed that the ependymal cells of the SCO were high ependymocytes, with a nucleus in the basal position, that were clearly different from the underlying ependymal cells. These results were confirmed in 1988 by O'Rahilly et al., who described the appearance of the human SCO at the beginning of the eighth week of gestation. These first stages of the development of the human SCO are similar to those of other animal species, in which the SCO does not disappear after the postnatal life, as can be seen on comparing the illustrations of Ariens Kappers (1960) and Castañeyra-Perdomo et al. (1983a,b; 1985). There is secretory activity in the human SCO during the first half of pregnancy, after which regressive changes occur until it completely disappears in adulthood (Oksche, 1956, 1961a,b; Wislocki and Roth, 1958; Wildi and Frauchiger, 1965).

It should be emphasized that all standardised and successfully used antibodies in the SCO of different animal species always produce negative results in the humans (Rodriguez et al., 1992). In the few immunohistochemical studies on the human SCO in foetuses, Rodriguez et al. (1990, 1993) using anti-RF and lectins, demonstrated that the human SCO showed immunoreactive lectin similar to those of the SCO of other mammals, but with a different protein part, since the human SCO is always negative to antiserum against the different components from bovine RF. An attempt has also been made to demonstrate that the human SCO secretes substances that dissolve in the CSF, since it does not produce RF (Rodriguez et al., 1993, 2001). In those works, Rodriguez et al. (1993, 2001) prepared antibodies against different protein fractions from the CSF of a one-month old hydrocephalic male that were not present in the CSF of a normal adult subject. The result was as follows; among all the antibodies obtained, only two antibodies marked the choroid plexus and the SCO of a human foetus of 13 WG and one of them, anti-150 kDa, also marked the rat SCO.

Although the structure and origin of the subcommissural organ are well documented in different animal species, work on the overall morphologic variations of the human SCO during its ontogeny are scarce, since no precise information exists on the morphology and size of the human SCO at the different stages of its development, such as when it makes its first appearance, develops, and begins its regression until its possible atrophy. In the present work, using quantitative and qualitative methods, we attempted to define the cellular and global structure of the human SCO during its ontogenic development in order to establish a reference for future research into the role of the SCO in a physiopathological process such as hydrocephalus.

Material and methods

We used a total of 24 human embryos and foetuses ranging from 6 to 40 weeks of gestation (WG) and 3 adult human brains from 27-, 65- and 70-year old subjects (Table 1). The embryonic and foetal material mainly came from the collection of the Department of Anatomy of the University of La Laguna, and from the Collection of the Port-Royal Hospital in Paris (Dr J.C. Larroche). The adult human material came from the collection of the Department of Anatomy of University of La Laguna. The material from the Department of Anatomy of the University of La Laguna, was processed using the following standardized form: fixation in formol, postfixation in Bouin for 24 hours, dehydration, and paraffin embedding. The brains were cut in three (A, B, and C) coronal and sagittal sections 10 microns thick. The A series was stained with Cresyl Violet (V), Haematoxylin Eosin (H-E) or Klüver-Barrera (K-B) (Table 1). The material from the collection from the Port-Royal Hospital in Paris was processed as described by Feess-Higgins and Larroche, (1987).

Table 1.

AGE	vertex-coccyx length	section	staining
6 WG	7mm	c	H-E
7 WG	10mm	s	H-E
7 WG	15mm	c	V
8 WG	21mm	s	H-E
8 WG	27mm	c	V
9 WG	29mm	s	H-E
10 WG	34mm	c	H-E
10 WG	32mm	s	H-E
11 WG	45mm	s	V,H-E
12 WG	53mm	c	H-E
13 WG	59mm	c	H-E
15 WG	100mm	c	H-E
16 WG	113mm	s	H-E
17 WG	120mm	s	H-E
20 WG	158mm	c	V
22 WG	183mm	c	H-E
22-23 WG	189mm	s	V
23 WG	180mm	s	V
26 WG	220mm	s	V
27-28 WG	231mm	c	V
32 WG	276mm	s	V
36 WG	310mm	s	V
36-37 WG	340mm	c	V
40 WG	360mm	s	V
27 Y		s	K-B
65 Y		s	K-B
70 Y		s	H-E

c = coronal
s = sagittal
H-E = Haematoxylin eosin
V = cresyl-violet
K-B = Klüver-Barrera

The length and the volume of the SCO were calculated with the aid of a three-dimensional reconstruction system: "The Eutectic SSRS-system" (Eutectic Electronics, Inc. 8608 Jersey Court, Raleigh, NC 27613, USA) and the artistic 3D representation with the ADOBE photoshop 5.0. Statistical analysis was performed by means of analysis of variance (ANOVA) with the Bonferroni post-hoc test and the Kruskal-Wallis non-parametric test.

Results

We observed that, in the most immature stages (embryo 6 WG), the posterior commissure was not present, and that the ventricular wall had a uniformly proliferative structure in the chronological development of the diencephalon-mesencephalon transition zone of the human embryo. The posterior commissure (PC) was recognized for the first time in the 7th WG, forming fine fibers that crossed the dorsorostral part of the mesencephalic plate. During this stage, the cells located underneath the PC were identical to those of the adjacent ependymal regions and displayed images of mitosis.

The subcommissural organ was observed during the 8th WG for the first time. The cells of the dorsocaudal part of the third ventricle under the PC were clearly different from those of the adjacent regions (Figs. 1A, 4A). The cells of the subcommissural organ had a high columnar pseudostratified aspect, with an extended perikaryon. The apical pole was in contact with the ventricle and the nuclei were located at different levels on the basal position (Fig. 1A). Few mitoses in the SCO were observed, although they persisted in the underlying ependyma. During these first stages (8-9-10 WG), the SCO covered the inferior part of the PC from the pineal recess to its caudal part, extending 757 μm and with a volume of 114.10^6 μm^3. At these ages, the PC showed few and light folds (Table 2, Graphs 1, 2).

During the 11th-12th weeks of gestation the PC folds increased in size and the formation of a recess caudal to the PC or mesocoelic recess (MR) was observed (Figs.1C, 1C', 4B). The SCO was adapted to the PC folds and it was possible to distinguish the following parts: a) a first or precommissural part (RSCO) occupying the whole of anterior part of the PC until the pineal recess (PR), b) a second or subcommissural part (SSCO), which occupied the whole of the inferior part of the PC, c) a third or postcommissural part (CSCO), which occupied the caudal part of the PC and the mesocoelic recess (Figs.1C, 1C', 4B). Therefore, the SCO had increased in extension and volume. Thus, at 11-12 WG it had a length of 988 μm and a volu-

me of 125.10^6 μm^3 (Table 2, Graphs 1, 2). Simultaneously, the cells increased in height and their nuclei were clearly found in a basal position.

At 13 WG the PC had acquired its definitive curvatures, and during the 13th, 15th, 16th and 17th WG the SCO was fully developed (Figs.1B, 1D, 4C, 5A, 5C) with an extension of 1287 μm and a volume of 181.10^6 μm^3 (Table 2, Graphs 1, 2). At these ages their cells were completely developed and formed a high columnar epithelium, and their nuclei had some folds or invagination in their membrane (Figs. 5B, 5D).

At the same time as the pineal gland (PG) was being formed, the first appearance of the pineal recess was observed in 7th-8th WG (Figs. 1A, 4A) and by 11 WG a dual component of the PG, located rostral of pineal recess, was observed (Figs. 1C, 1C', 4B); a rostral pineal part (RP), composed of a compact mass of cells localized dorsorostral to the habenular commissure (HC), and a posterior pineal part (PP), structurally formed by a flexuous laminar epithelial structure located dorsocaudal to the HC (Figs. 1C, 1C', 4B). At 16 WG (Figs. 1B, 4C), the dual pineal gland component was clearly separated by a deep pineal invagination (PI). At 22 WG, the two pineal areas had fused and the PG had acquired an almost definitive aspect (Figs. 2A, 4D).

By 20, 22 and 22- 23 WG (Figs. 2A, 2C, 4D) the SCO had begun to decrease in size (1108 μm in length and 162.10^6 μm^3 in volume) (Table 2, Graphs 1, 2), since the precommissural part of the organ had begun to disappear (Figs. 2A, 4D). From the 17th WG, the SCO initiated a decrease in length and volume in such a way that at 26, 27-28 and 32 WG the SCO had an extension of 957 μm and a volume of 145.10^6 of μm^3 (Table 2, Graphs 1, 2); the rostral zone of the precommissural part of the organ had also disappeared.

Table 2.

GROUP Nº		AGE	LENGTH	MEAN SD	VOLUME	MEAN SD
		WG	in mm		in μm^3 x 1000	
	1	8	600		87.901	
	2	8	758		111.182	
8	3	9	810	757+/-56	117.201	114.792+/-6.8
	4	10	838		137.741	
	5	10	792		118.259	
	6	11	967		114.667	
12	7	12	1010	988+/-125	136.317	125.492+/-8.0
	8	13	1100		160.457	
	9	15	1300		180.357	
15	10	16	1410	1287+/-190	206.573	181.076+/-18.0
	11	17	1340		176.918	
	12	20	1000		139.583	
	13	22	990		147.824	
22	14	22-23	1163	1108+/-150	173.656	162.147+/-15.0
	15	23	1280		187527	
	16	26	938		149.317	
28	17	27-28	1038	957+/-113	152.073	145.109+/-12.2
	18	32	897		133.937	
	19	36	639		69.413	
38	20	36-37	728	650+/-26	90.665	78.497+/-2.3
	21	40	583		75.413	
27*	22	27[a]	390		37.137	

KRUSKAL-WALLIS TESTS (non parametric) independent variable age in week of gestation (WG)

DEPENDENT VARIABLE	H	P
LENGHT	17.635	<0.0034
VOLUME	16.776	<0.0049

ANOVA independent variable age in week of gestation (WG)

DEPENDENT VARIABLE	MSS	F	P
LENGHT	202340	18.099	<0.0001
VOLUME	46.9x10E8	15.645	<0.0001

* One human of 27 years old, out of statistic study.

ABBREVIATIONS FOR ALL FIGURES:

* = Islet of the SCO parenchyma
➨ = limit between the SCO part and adjacent ependyma
C = caudal
CSCO = postcommissural part of the SCO
D = dorsal
E = ependyma
H = hypendymal cells
HC = habenular commissure
MR = mesocoelic recess
PC = posterior commissure
PG = Pineal gland
PI = pineal invagination
PP = posterior part of pineal gland
PR = pineal recess
RP = rostral part of pineal gland
RSCO = precommissural part of the SCO
SA = Sylvian aqueduct
SCO = subcommissural organ
SSCO = subcommissural part of the SCO
V = ventricle

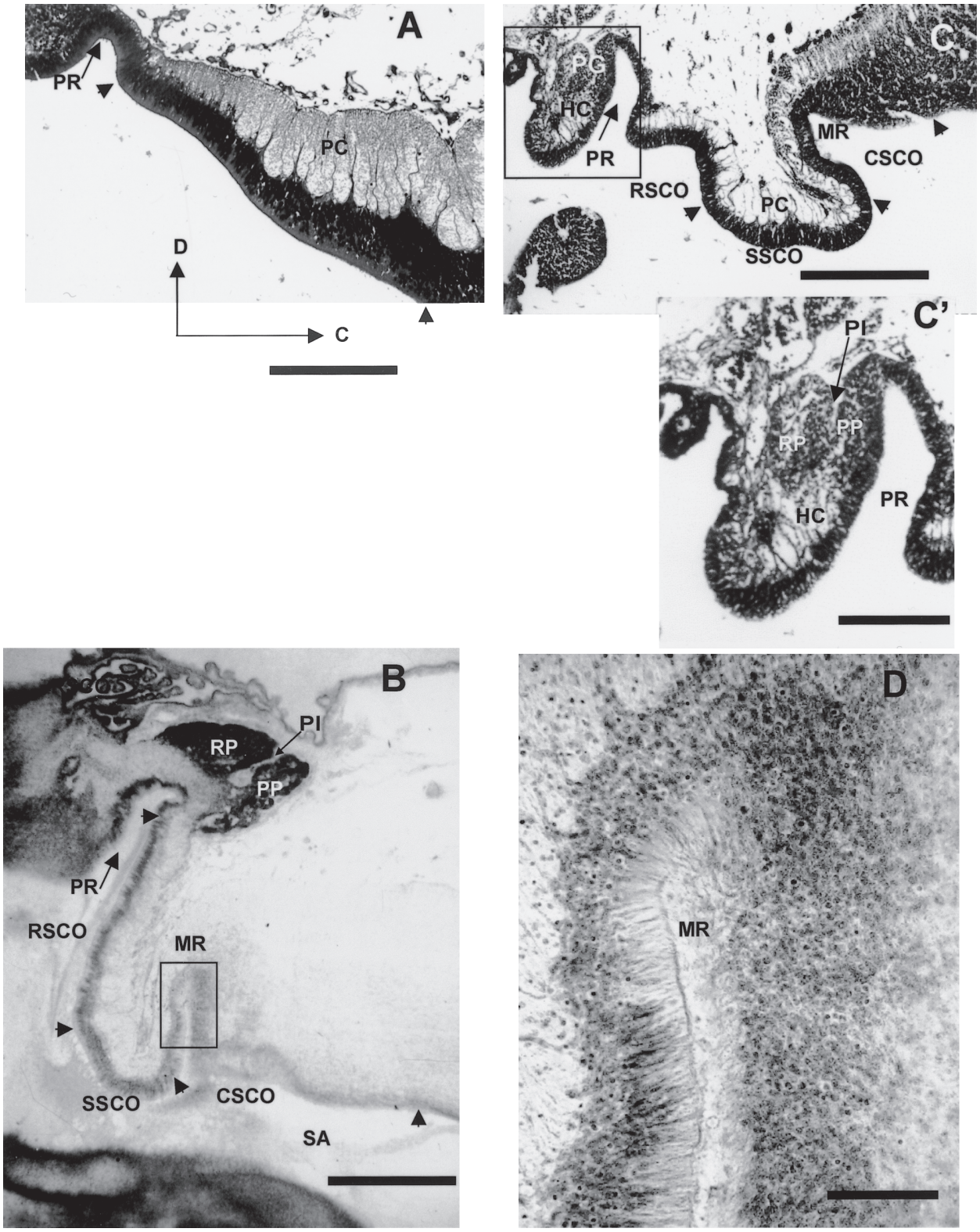


Fig. 1.- Sagittal sections of the human SCO and adjacent structures. **A**: SCO and pineal recess at 8WG, bar = 250 μm. **B**: SCO and pineal gland at 16WG, bar = 200 μm. **C**: SCO and pineal gland at 11WG, bar = 250 μm. **C'**: (frame of C) pineal recess, pineal gland and habenular commissure, bar = 110 μm. **D**: (frame of B) mesocoelic recess at 16WG, bar = 75 μm.

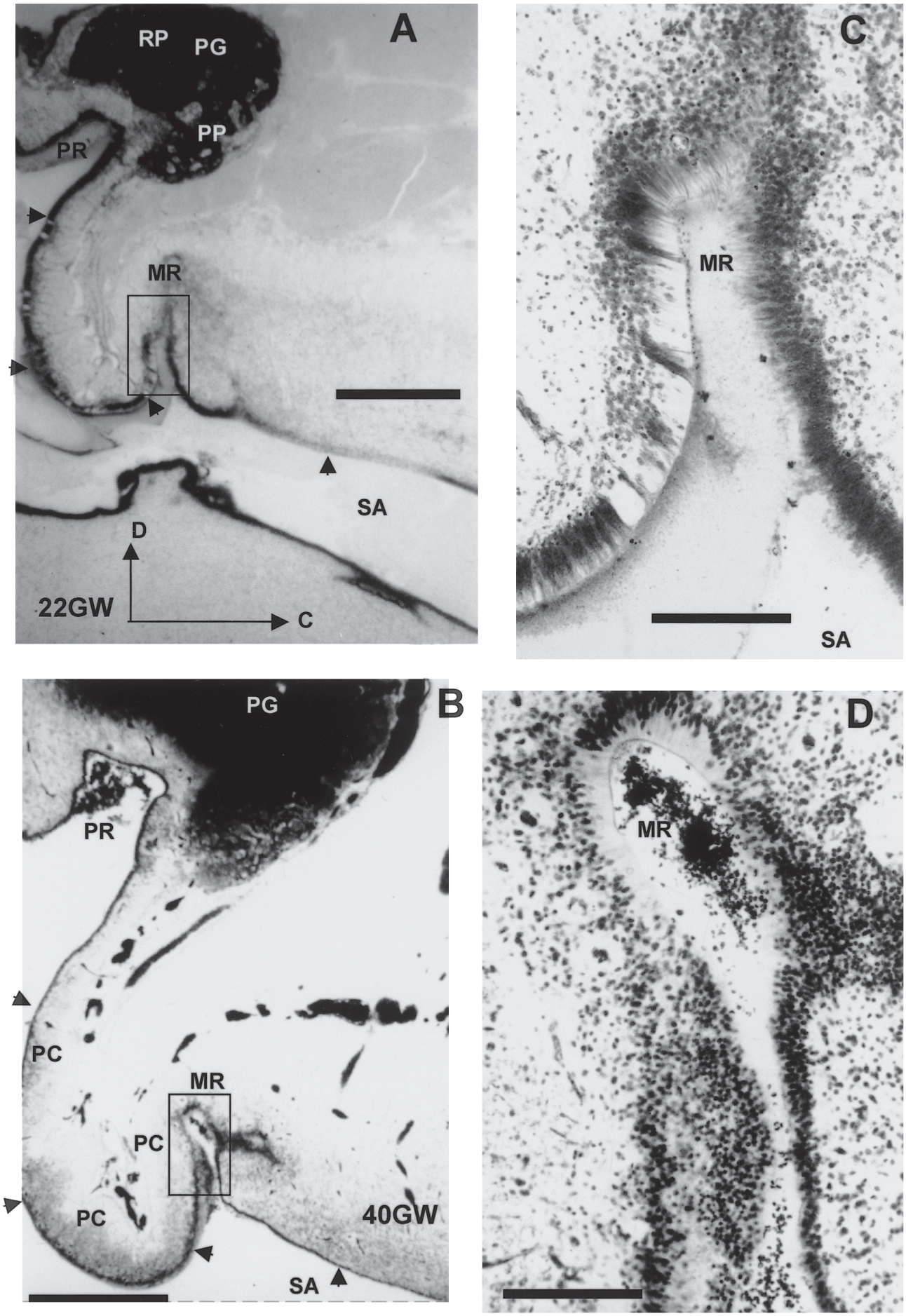


Fig. 2.- Sagittal sections of the human SCO and adjacent structures. **A**: SCO and pineal gland at 22WG, bar = 220 μm. **B**: SCO and pineal gland at 40WG, bar = 280 μm. **C**: (frame of A) mesocoelic recess at 22WG, bar = 50 μm. **D**: (frame of B) mesocoelic recess at 40 WG, bar = 50 μm.

In the two last months of intrauterine life (36, 36-37 and 40 WG) the SCO only occupied one zone of the subcommissural part, the postcommissural part and mesocoelic recess (Figs. 2B, 2D, 4E), which had also diminished in size, with an extension of 650 μm and a volume of 78.10^6 μm^3 (Table 2, Graphs 1, 2). Their cells remained columnar but were not as high as during the previous ages and the oval nuclei were clearly in basal position and different from subjacent ependyma. At 40 WG (Figs. 2B, 2D), the precommissural part of the SCO showed a strong reduction of the ependymal and hypendymal layer and only some islets of the SCO parenchyma could be observed.

In the 27-years old human adult, remains of the mesocoelic recess could still be recognized in the retrocommissural zone, specifically in some SCO ependymal cells that appeared as a complex of high columnar pseudostratified ependymal cells (Figs. 3A, 3B, 3C, 4F) that had conserved their round or oval nuclei in a basal position. However, several groups of cells with atrophic signs such as hyperpyknotic nuclei (Fig. 3C) could also be observed. At this age the only case analysed had an extension of 390 μm and a volume of 37.10^6 μm^3 (Table 2). The rest of the adult human material did not present SCO, and SCO remains in the form of small and scarcity groups of high columnar cells were only observed in the depths of the MR.

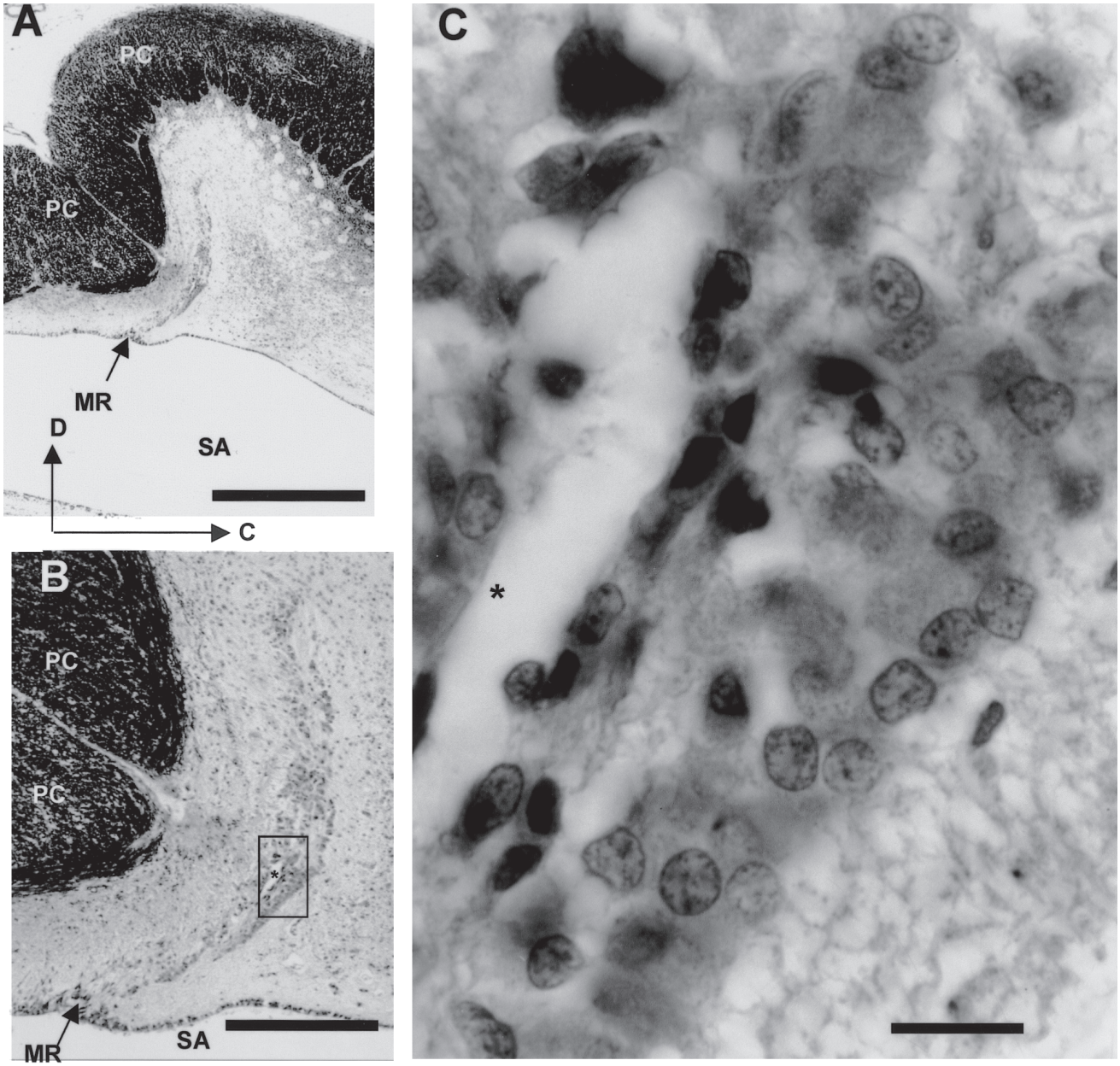


Fig. 3.- Sagittal sections of the posterior commissure, SCO rest and MR of a human of 27 years old. **A**: posterior commissure and MR, bar =300 μm. **B**: MR, bar = 100 μm. **C**: (frame of B), bar = 20 μm.

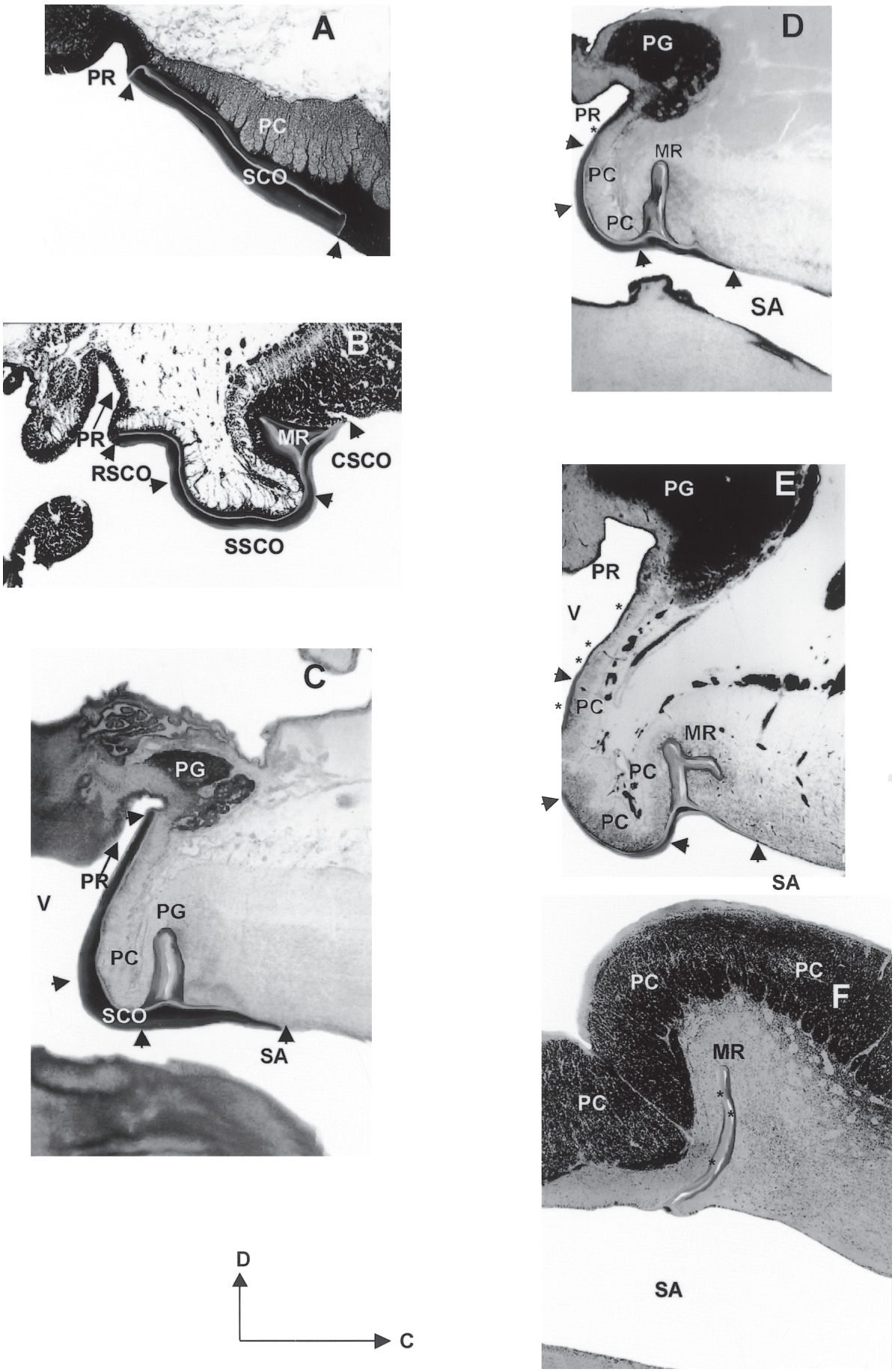


Fig. 4.- Sagittal view of the human SCO, adjacent structures and artistic representation SCO. **A**: a computer of the treated photograph of the SCO and pineal recess at 8 WG. **B**: a computer of the treated photograph of the SCO, pineal gland and pineal recess at 11 WG. **C**: a computer of the treated photograph of the SCO and pineal gland at 16 WG. **D**: a computer of the treated photograph of the SCO and pineal gland at 22 WG. **E**: a computer of the treated photograph of the SCO, pineal gland and pineal recess at 40 WG. **F**: a computer of the treated photography of the MR remnants of a human of 27 year old.

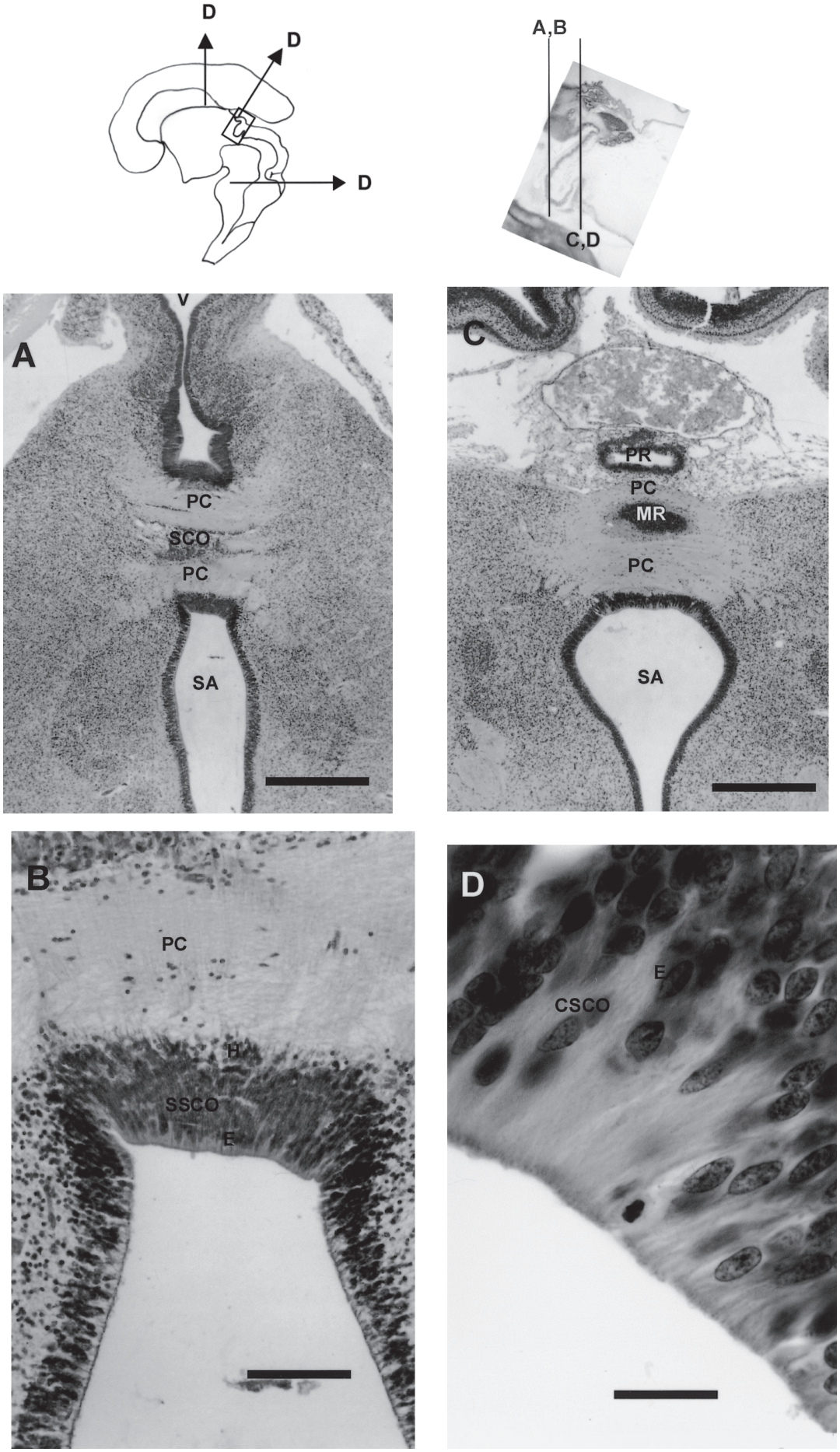
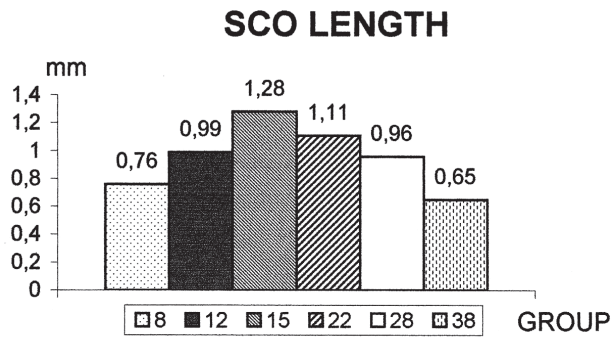
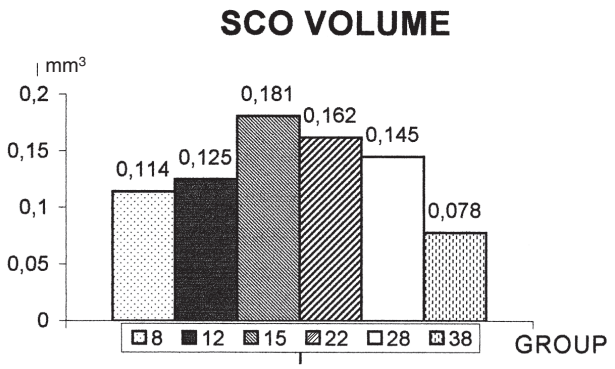


Fig. 5.- Frontal sections of the SCO and adjacent structures at 13 WG. **A**: panoramic view of the SCO and PC, bar = 300 μm. **B**: subcommissural part of the SCO and PC, bar= 150 μm. **C**: panoramic view of the SCO, PC, pineal recess and MR, bar = 300 μm. **D**: borderline between SCO and adjacent ependyma, bar = 20 μm.

Graphic 1.



Graphic 2.



Discussion

The human subcommissural organ reaches its maximum development during foetal life (Dendy and Nichols, 1910; Oksche, 1956, 1961a,b,1993; Castañeyra-Perdomo et al., 1985; Rodriguez et al., 2001), begins its regression during the first moments of postnatal life, and is reduced to atrophic remains in the adult.(Pesonen, 1940; Oksche, 1961a,b, 1993; Rodriguez et al., 2001). In our material we observed that the human SCO was very well developed during foetal life and made its appearance at the beginning of 8th WG, which agrees with previous finding (Keene and Hewer, 1935; Pesonen, 1940; Castañeyra-Perdomo et al., 1985; O'Rahilly et al., 1988). Oksche (1993) and Rodriguez et al. (2001) reported that the human SCO shows a substantial development during the 3rd, 4th and even 5th month of foetal life (from 10th to 22nd WG), which is also confirmed in our results, although several small differences, since we found that the SCO reached its maximum development in 15th –16th WG with a tendency to stabilize but to diminish slightly in size by the 22nd WG. We also observed that during the first stages of its development, the SCO became adapted to the folds that were being formed in the PC in such a way that at the time of its maximum development the following parts could be distinguished in the human SCO: a precommissural part (rostral to the PC until the pineal recess), a subcommissural part (underneath the PC) and a retrocommissural part (occupying the caudal part of the PC, the mesocoelic recess and the beginning of the Sylvian aqueduct). Oksche (1969, 1993) reported that the human SCO initiates its regression during the second half of pregnancy. Indeed, we observed that as from the 17th WG the SCO started to decrease in size but we also found that this reduction began in the precommissural part, since by the 22nd WG the SCO had not reached the pineal recess and by the 28th WG it only occupied the subcommissural and retrocommissural parts and had diminished in volume by one third. Rodriguez et al. (2001) found that in 9 month old foetuses the regressive development of the SCO was already evident. We confirm this finding but, in addition we observed that at the end of pregnancy the SCO only occupied the retrocommissural part, since it was limited to the mesocoelic recess and its volume has been reduced by half. During post-natal life the SCO was progressively atrophied, Rodriguez et al. (2001) described that after one year old, the active parenchyma of the SCO was observed in form of islets and in the course of further development only remained small islets of characteristic parenchyma. However, the complete disappearance of the human SCO has been described at different ages; in the 6-year old (Oksche, 1993), in the puberty (Wildi and Frauchiger, 1965) and in the 30-year old (Palkovits, 1965). In a 34-year old man, only irregular, scattered islets formed by irrelevant SCO cells were found (Rodriguez et al., 2001). We believe that the reduction in size of the SCO begins in the 23rd WG, but complete regression probably varies greatly, depending on the individual, since in our material we found clear remains of the SCO in a 27-year old man, where the SCO was divided into atrophic and normal cell islets of the characteristic SCO parenchyma, which still occupied the mesocoelic recess with a volume of about 37.10^6 μm^3, i.e. nearly one quarter of its maximum size (22-23 GW). Finally, we observed that in the case of the 65- and 70-year old men the SCO had almost completely disappeared which agrees with the finding of Rodriguez et al. (2001) for an 85- year old man.

In a wide-ranging study on human mesocoelic recess (MR), Rakic in 1965 described it as the caudal part of the SCO. This was in conflict with the finding of Sargent (1904), who described the MR as being synonymous with the SCO. We observed that the MR initiated its formation during the 10th WG and was completely formed by 14th WG, with the retrocommissural part of the SCO in it. Rakic (1965) studied the development of the MR from the 20th WG to the age of 78-year old in one subject, and concluded that the MR gradually diminishes in size and disappears after birth or is reduced to isolated vestiges in some individuals. Rodriguez et al. (2001) also described the MR as a high columnar epithelium bipartite arrangement around the convexity of the posterior commissure of the

human foetus, and in the adult human as a high columnar cell around an irregular cavity (apparently they were remnants of the MR) that did not communicate with the light of the Sylvian aqueduct (Rodriguez et al., 2001). We observed that the MR increased in size almost throughout foetal life, although this is relative since it diminishes in size with respect to the increase in size of the PC and other adjacent structures. In agreement with Rakic, we also observed the regression of the MR at 40 WG and, in addition this coincided with the final regression of the SCO when its extension was limited to the size of the MR.

The early stages of the human SCO and pineal complex are similar to the embryonic stages of others species in which the SCO does not regress in postnatal life (Ariëns Kappers, 1960; Castañeyra-Perdomo et al., 1983a,b,c). We found a parallel development between the SCO and the pineal. Thus, we observed the first appearance of the pineal recess at 7-8 WG; a dual component of the PG, located rostral of pineal recess, was observed at 11 WG, and the pineal gland (PG) had already acquired its almost definitive aspect at 22 WG. The concurrent appearance of the SCO and PG does not imply a functional or morphogenetic relationship between these structures. Therefore, secretory pinealocytes are derivatives of pineal photoreceptors, primary sensory cells of neural character. In contrast to these neuron-like or paraneuronal elements, the secretor cells of the SCO are of ependymal origin (Oksche, 1988). However more recently, the relationships in the histogenesis and function of the SCO and PG has been suggested, e.g., Kasper (1992), because immunocytochemical cytokeratins have been found as immunoreactive material in the ependyma of the SCO, in the foetal pineal gland and other brain epithelia in man, and a concentration of atrial natriuretic factor (ANF)-like immunoreactive ANF-binding sites in both structures has been found (Matyh et al., 1987; Mathieu et al., 2001). On the other hand, a study using mice with a spontaneous point mutation of transcription factor Pax6 (Sey), which generates a non-functional Pax6 protein, concluded that the presence of Pax6 protein is required for the normal development of the SCO and PG (Estivil-Torrus et al., 2001). In spite of these findings the close vicinity during embryonic stages does not necessarily mean that there is a functional or morphogenetic relationship between these circumventricular organs, which in some species could be connected (Kelly, 1968).

We could conclude that the human SCO appears at the beginning of the 8th WG, which confirms previous results (Castañeyra-Perdomo et al., 1985; O'Rahilly et at., 1988). The complete maturation of the SCO occurs at 15 WG and it is possible to distinguish its three parts as follows: the precommissural part, located in the rostral zone of the PC until the pineal recess; the subcommissural part, located underneath the PC, and the retrocommissural part located in the caudal zone of the PC, in the mesocoelic recess, and at the beginning of the Sylvian aqueduct. The reduction in size of the SCO begins after the 17th WG, and this reduction begins in the precommissural part, continues in the subcommissural part, and finishes in the retrocommissural part. In concordance with Rodriguez et al. (2001), the regression and atrophies of the SCO begin at the end of the pregnancy and birth, and the SCO completely disappears after the age of 30 years. The mesocoelic recess initiates its formation at the beginning of the 10th WG, it is completely formed during the 14th WG, and the retrocommissural part of the SCO is located in it. In agreeing with Rakic (1965) we observed that the regression of the mesocoelic recess begins during the 40th WG, and this coincides with the regression of the SCO. A parallel development between the SCO and the pineal was found, since we observed the first appearance of the pineal recess and SCO at 7-8 WG and to all intents and purposes formed the pineal gland (PG) was during the 22nd WG: i.e., when the SCO reaches the complete maturation.

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