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# Effect of Prostaglandin $E_2$ on the Ductus Arteriosus in the Newborn Rat. An Ultrastructural Study

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## Summary

A patent ductus arteriosus (DA) was maintained in newborn rats (Wistar strain) by administering prostaglandin $E_2$ (PG $E_2$) in doses of 15 $\mu g.kg^{-1}$ at 30 min intervals up to 300 min after birth. In the control animals, the DA was functionally closed 300 min after birth. The lumen was blocked by clustered endothelial cells at various stages of degeneration. Elastic membranes of the media had disintegrated into irregular fragments and the smooth muscle cells were contracted. Cytoplasm excrescences formed on their surface as a result of contraction protruded as hernias into adjacent muscle cells and into endothelial cells. The smooth muscle cells degenerated. The administration of PG $E_2$ inhibited contraction of the smooth muscle cells and so also the development of degenerative changes; 300 min after birth the DA was fully patent, the elastic membranes were structurally intact, regularly organized and continuous. The smooth muscle cells had the character of synthesizing cells with richly developed granular endoplasmic reticulum. The intima and its endothelial lining were likewise free from structural changes. The ultrastructural image of the wall of the DA correspondent to the state 10 min after birth, when the DA was fully patent. The administration of PG $E_2$ did not induce any ultrastructural changes indicative of injury to the wall of the DA.

**Key words**

Ductus arteriosus – Prostaglandins – Cardiac development – Rat heart

## Introduction

The ductus arteriosus (DA) is the distal part of the left sixth aortic arch which persists during intrauterine life as a shunt connecting the pulmonary trunk with the aorta. After birth it closes physiologically. Group E prostaglandins play a significant role in keeping the DA patency during prenatal development (Coceani and Olley 1973, 1980, Coceani *et al.* 1986, Sharpe and Larsoon 1975, Bernal *et al.* 1986).

The ability of prostaglandins $E_{1/2}$ (PG $E_{1/2}$) to prevent the DA closure or reopen the closing DA during the early postnatal period enables effective therapy for neonates with different cardiac malformations (Schöber *et al.* 1980). Unfortunately, the data on the incidence and degree of histopathological changes in association with the administration are contradictory (Haworth *et al.* 1980, Silver *et al.* 1981, Gittenberger-De Groot *et al.* 1980, Arnold *et al.* 1985).

In a previous study (Janatová *et al.* 1989) we demonstrated that the repeated administration of PG $E_2$ prevented the rat DA from closing within 300 min after birth, when its functional closure in the control animals had been completed. The closure mechanism and the sequence of changes in the wall of the DA during this process were studied at ultrastructural level (Jarkovská *et al.* 1989). It was found that physiological closure of the DA was accompanied by profound changes in the structure of its wall (in particular the intima and the media), starting with contraction of the smooth muscle cells. The findings were used as a basis for evaluating the effect of PG $E_2$ on the DA closure process. We were especially interested in whether the

ultrastructure of the wall of the treated DA would be different from the physiologically patent DA.

## Methods

The experiments were performed on 42 spontaneously born rats (Wistar strain) of both sexes. At 30 min intervals (which enabled to keep the DA open, Janatová *et al.* 1989) beginning with the 5th min after birth, the controls (n = 28) were given a subcutaneous injection of 20 $\mu$l of physiological saline while the experimental animals (n = 14) received prostaglandin $E_2$ (Léčiva, Prague) in a dose of 15 $\mu g.kg^{-1}$. Samples of DA were taken 10 and 300 min after birth from the controls and 300 min after birth from animals given PG $E_2$. The DA was fixed in two ways as described previously (Jarkovská *et al.* 1989). In one group it was carefully dissected out under a dissecting microscope and fixed by immersing it for 2 h in 6 % glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 at 0-4 °C; the samples were then rinsed in cacodylate buffer and postfixed for 90 min in 1 % $OsO_4$ in 0.1 M cacodylate buffer, pH 7.4 at the same temperature. The animals in the other groups were perfused after Buss (Rosenbauer and Kegel 1978) with 3 % glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. After perfusion, the DA was dissected out, fixed in the same glutaraldehyde solution, rinsed and postfixed for 90 min in 1 % $OsO_4$ solution in 0.1 M phosphate buffer pH 7.4. After routine dehydration, the samples were embedded in Durcupan ACM. Ultrathin sections for electron microscopy were cut on a Reichert OMU 3 ultramicrotome and stained with uranyl acetate and lead citrate (Reynolds 1963). Electron micrographs were taken with a Tesla BS 513 electron microscope and evaluated qualitatively.

## Results

*Ductus arteriosus ten minutes after birth (Fig. 1)*

The fixatives caused slight contraction of the wall of the DA. The wavy inner surface of the tunica intima was bordered by endothelial cells protruding slightly into the blood vessel lumen. The cells contained richly developed granular endoplasmic reticulum, mitochondria and Weibel-Palade bodies. Large numbers of pinocytotic vesicles were present on both the luminal and the abluminal surface.

The inner elastic membrane corresponded structurally to the elastic membranes in the tunica media. The tunica media consisted of regular, concentrically organized layers of smooth muscle cells and fenestrated elastic membranes. The few collagen fibrils accompanied the elastic membranes in the form of thin bundles. The elastic membranes were slightly wavy and their surface was covered by fine, unorganized elastic microfibrils. The same microfibrillar material was also found in the intercellular space, where it occurred diffusely or in the form of clusters of different size unassociated with the elastic membranes. These microfibrils showed a tendency to be oriented parallel to the cell surface. Here and there, extracellular vesicular structures occurred in the intercellular space. The muscle cells were bordered by a continuous basement membrane, which was absent only at sites of intercellular contacts. Most of the smooth muscle cells were rich in granular endoplasmic reticulum and elements of the Golgi complex and many of them also contained lysosomes. In this synthesizing type of the smooth muscle cell rich in organelles, the contractile myofibrillar apparatus was confined to the peripheral zone of the cytoplasm. Here there were normally developed dense bodies and fusiform densities. The tunica adventitia of the DA mainly contained fibroblasts with slender processes accompanied by bundles of collagen fibrils.

*Ductus arteriosus 300 minutes after birth (the control group – Fig. 2)*

Changes associated with physiological closure had taken place in the wall of the DA. The lumen was virtually blocked by clustered endothelial cells, which were seen to be at various stages of degeneration, from swelling of the mitochondria to disintegration of the organelles and pycnosis of the nucleus.

The inner elastic membrane was fragmented and the tunica media contracted. The most striking feature of contraction of the smooth muscle cells were cytoplasmic hernias – excrescences which bulged into neighbouring muscle cells or adjacent endothelial cells. The excrescences were of different sizes and were often multiple. They contained oedematous cytoplasm, and sometimes also a small amount of vesicular material or myelin figures. Host cells containing large hernias often had a deformed nucleus and were generally at an advanced stage of degeneration.

The elastic membranes in the inner layers of the media persisted only as fragments grouped together in random clusters between the layers of smooth muscle cells. In the outer layer of the media the elastic membranes were still continuous, but highly wavy. In addition to elastic microfibrils and collagen fibrils, profiles of the processes of smooth muscle cells were found in the extracellular space of the media. According to their internal structure, some of them were sections of excrescences and some were true processes in which enhanced pinocytotic activity could be observed. Extracellular vesicular structures were also present.

The tunica adventitia contained abundant fibroblasts with richly branching processes accompanied by bundles of collagen fibrils. Their

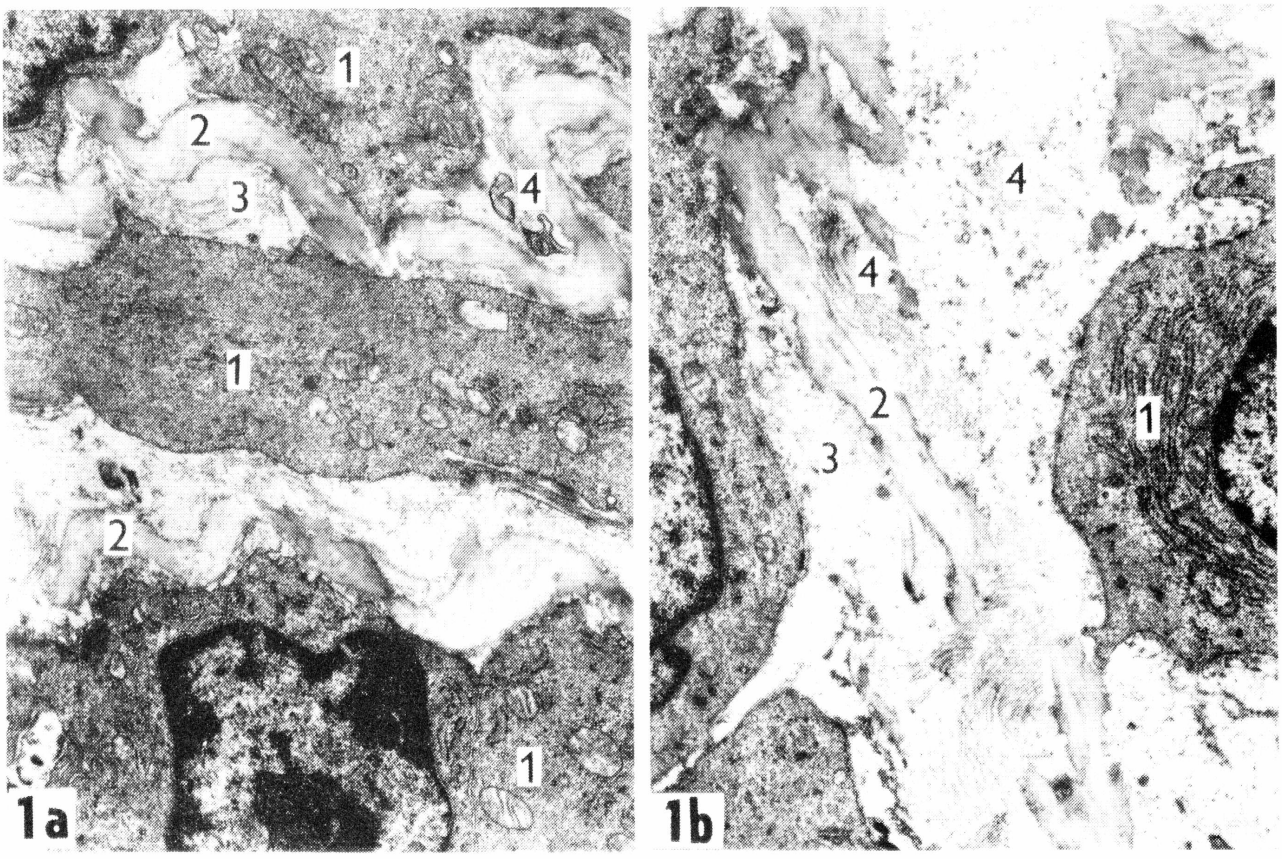



**Fig. 1 a,b**

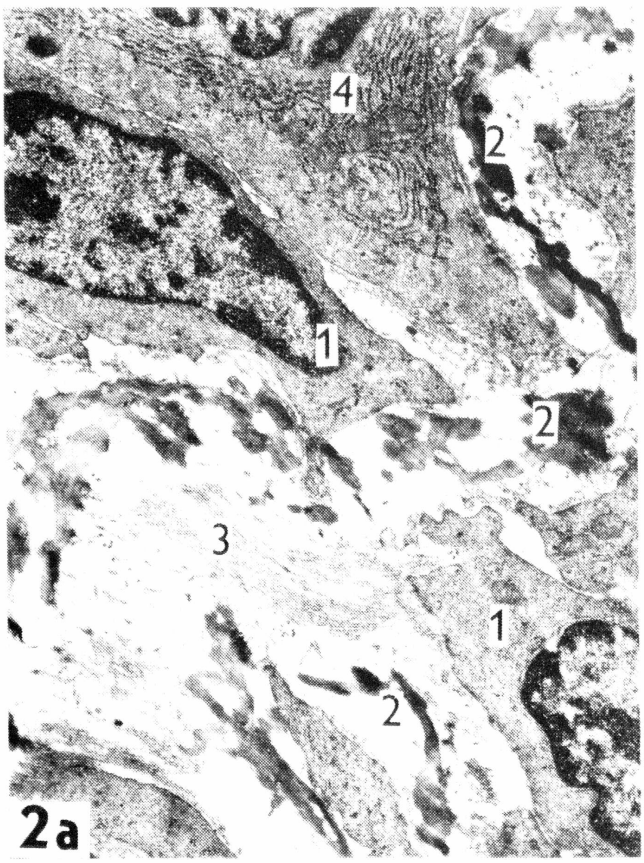
Tunica media of the DA, 10 min after birth. a – between the concentric layers of smooth muscle cells (1) there are mildly waved elastic membranes (2), fine bundles of collagen fibrils (3) and occasional extracellular vesicular structures (4). x 14 000. b – large nuumbers of smooth muscle cells have the character of synthesizing cells with richly developed endoplasmic reticulum (1). The extracellular space contains typical elastic membranes (2) accompanied by fine networks of elastic microfibrils (3) and thin bundles of collagen fibrils (4). x 14 000

**Fig. 2 a,b**

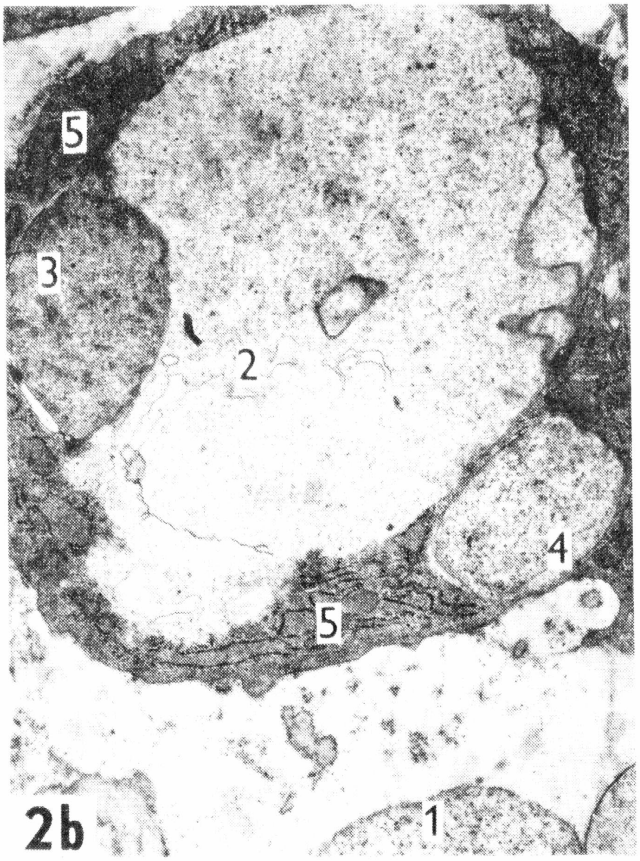
Tunica media of the DA, controls, 300 min after birth. a – intercellular spaces between the smooth muscle cells (1) contain elastic membrane fragments of varying density (2) accompanied by clusters of elastic microfibrils (3). Cells with an intact ultrastructure are mainly of the synthesizing type, with numerous granular endoplasmic reticulum profiles (4). x 14 000. b – the high degree of continued contraction of the smooth muscle cells is manifested in the formation of cytoplasmic excrescences whose profiles are to be found in the intercellular space (1) and as hernias in the cytoplasm of adjancent cells (2,3,4). The host cell displays signs of advanced degeneration, in particular dilatation of the cisternae of the granular endoplasmic reticulum (5). x 14 000

**Fig. 3 a,b**

Tunica media of the DA, group with PG $E_2$, 300 min after birth. a – a longitudinal section of the DA shows layers of transversally sectioned smooth muscle cells (1) alternating with profiles of elastic membranes (2). x 14 000. b – a cross section showing characteristic waving of elastic membranes of an intact structure (1) (cf. Fig. 1). Smooth muscle cells of the synthesizing type with abundant profiles of granular endoplasmic reticulum (2) and signs of high pinocytotic activity (3). 4 - a lysosome. x 14 000




2a

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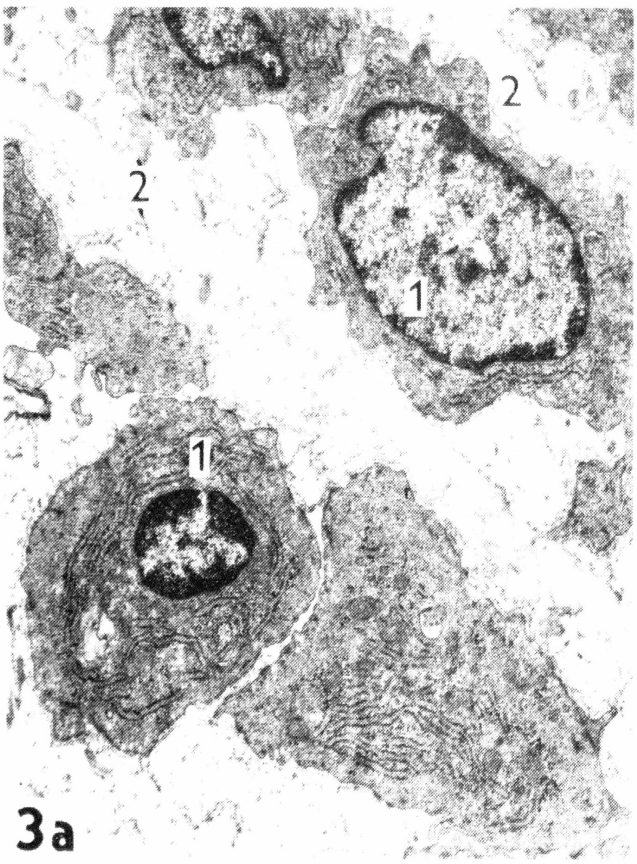
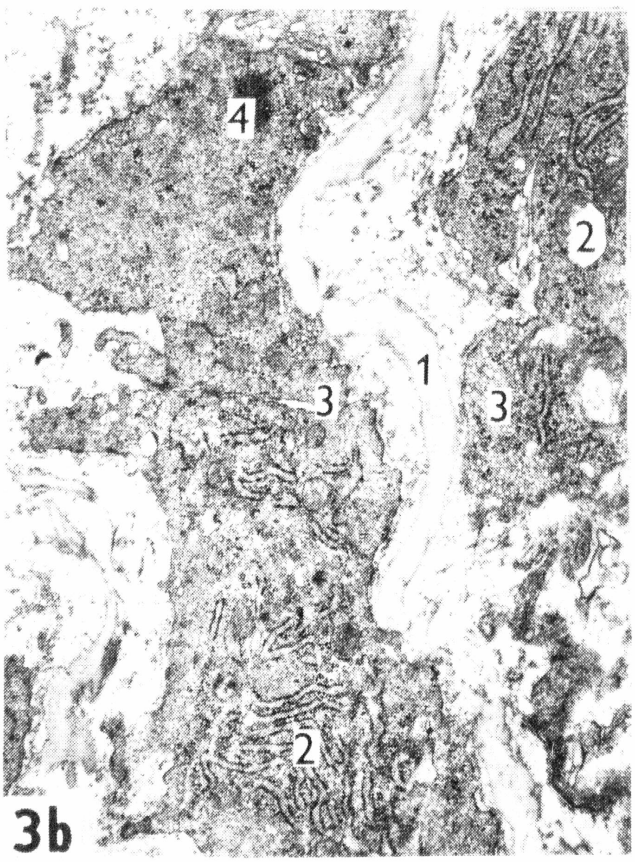



3a




3b

ultrastructure of the wall of the treated DA would be different from the physiologically patent DA.

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The tunica adventitia contained abundant fibroblasts with richly branching processes accompanied by bundles of collagen fibrils. Their

cytoplasm contained numerous profiles of granular endoplasmic reticulum and vacuoles with light contents.

*Ductus arteriosus 300 min after birth (PG E 2 – Fig. 3)*

The DA was patent, the inner elastic membrane mildly wavy. The endothelial cells were flat or protruding only slightly into the lumen. Their ultrastructure displayed no differences compared with endothelial cells in the samples taken 10 min after birth.

The muscle cells in the tunica media were structuraly comparable with those ten minutes after birth. No cytoplasmic excrescences were observed. Most of the smooth muscle cells had the character of synthesizing cells with richly developed organelles (especially granular endoplasmic reticulum). The mitochondria were generally normal in appearance. The cells displayed signs of marked pinocytotic activity.

The elastic membranes in the tunica media were continuous. Abundant microfibrillar material adhered partly to profiles of the elastic membranes and partly to the surface of smooth muscle cells, or else it was localized freely in the extracellular space, similar to the not very numerous collagen fibrils and extracellular vesicles, the numbers of which were slightly higher than at the 10 min interval. The adventitia had the same structure as in the patent DA.

## Discussion

The most extensive information about the histopathology of the DA with and without treatment with PG $E_1$ was presented in the review by Gittenberger-de Groot and Stengers (1988). Having in mind the conflicting data in the literature (Haworth *et al.* 1980, Silver *et al.* 1981, Gittenberger-de Groot *et al.* 1980, Arnold *et al.* 1984), the present authors have restudied their rich material. They have evaluated the effects of PG $E_1$ on the DA wall as varying from minor histological changes to serious damage, but they could not detect "any relationship between dosage, route of administration, duration or start of treatment and the incidence of histopathology". Their conclusion that the administration of prostaglandin does not change the structure of the ductal wall specifically (see also Silver *et al.*, 1981) and that the adverse effects follow inital functional closure and sudden relaxation of the ischaemized duct are in good accordance with our conclusions. On the basis of our results, we suppose that changes of the DA wall can be abolished in case that PG $E_2$ is administered immediately after birth. In the rat, where we have not observed any changes preparing the DA for the closure in the neonate (Jarkovská *et al.* 1989), the absence of structural changes of the vessel wall during the regular PG $E_2$ application was not surprising.

Data on the ultrastructure of the DA during physiological closure are few and far-between (rat – Mato and Aikawa, 1968, Jarkovská *et al.* 1989, rabbit – Yoder *et al.* 1978, 1980, dog – Gittenberger–De Groot *et al.* 1985, man – Toda *et al.* 1980 and Silver *et al.* 1981). There are no substantial differences in the results of authors studying different animals and man. The main structural changes occurring in the wall of the DA during its closure appear to be similar and the description of the process differs only according to the special interest of the author in the individual structural details. Disunity appears in the question of the presence of the starting structural changes in the wall of DA before birth. In our experimental material, no degenerative changes have been observed in the DA of newborn rats.

Before proceeding to evaluate the ultrastructure of the wall of the DA influenced by PG $E_2$ treatment, we thoroughly studied the course of physiological closure of the DA of newborn rats in the electron microscope (Jarkovská *et al.* 1989). The key phenomena in this process are: a) the formation of cytoplasmic excrescences on the smooth muscle cells, their herniation into adjacent cells and progressive degeneration of the smooth muscle cells, and b) rapid disintegration of the elastic membranes of the media. The formation of cytoplasmic excrescences on the smooth muscle cells and the formation of hernias are classified in the literature dealing with the blood vessel wall as a morphological manifestation of intensified contraction (Wagenvoort *et al.* 1974, Dingemans and Wagenvoort 1976, Joris and Majno 1977). We attribute the disintegration of the elastic membranes in the physiologically closing DA to a disturbance of the balance between the synthetic activity of the myocytes and extracellular elastolysis. The myocytes, whose synthetic activity assures continuous renewal of the extracellular structures of the media – especially the elastic membranes (Kadar *et al.* 1971, Joris and Majno 1977) – degenerate as a result of continued intensified contraction and cease to fulfil this function. In agreement with Riede and Staubesand (1977), we consider the extracellularly occurring vesicular structures, which probably originate in the lysosomal apparatus of the myocytes, to be the morphological substrate of the elastolytic activity of the myocytes.

The time needed for functional closure of the DA varies considerably with the species and enviromental influences (Hörnblad 1967, Hörnblad and Larsson 1967 a,b). In the first phase of the closure, the main factor and the trigger mechanisms of structural changes is contraction of the smooth muscle cells in the media of the DA (Gittenberger–De Groot *et al.* 1985); this is in good agreement with our observation.

It may be concluded that the administration of PG $E_2$ in the used doses to newborn rats before the

onset of physiological closure of DA does not damage the DA wall in the given time. Our results cannot be taken as proof of the safety of PG $E_2$ administration in human neonates. We must keep in mind the differences in DA structure and in the time-course of its closure in individual animals (Hörnblad 1967, Olley and Coceani 1979). But the rapid development of irreversible structural damages during the process of the physiological closure of the DA observed in our material points to the possibility of negative consequences of the late pharmacological reopening of the DA in paediatric cardiology.

Another thing that needs to be determined is the length of time for which the DA can be kept patent by means of PG $E_2$ without the development of structural changes. Since we did not have a longlasting drug at our disposal, the above question still remains open.

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