removed, weighed, and ACh extracted by a formic acid-acetone method[14], with the exception that the final volume was adjusted to 3 ml with distilled water. These concentrated samples containing ACh were diluted with buffer and bioassayed for ACh activity as described above.

Results and discussion. Effect of LiCl on ACh release from the longitudinal muscle is shown in table 1. There were no differences in spontaneous or evoked release of ACh between acute and chronic treatments for either the NaCl group or the LiCl group. Therefore, data for acute and chronic treatments were pooled for each group and the data presented in table 1 analyzed via Student's t-test. Values for either spontaneous or evoked release of ACh were not different for LiCl treated animals compared to NaCl treated animals.

The data of table 2, analyzed by a Student's t-test, show no significant differences in brain levels of ACh between the treatment groups. Once again there were no differences between acute and chronic treatments; therefore, the data of table 2 are the result of pooling across injection schedules. Flame photometric analysis of serum samples showed LiCl levels to be within the clinical range (0.5–1.5 mEq/l)[15].

Table 1. Spontaneous and evoked release* of acetylcholine from longitudinal muscle-myenteric plexus of guinea-pig ileum*

	NaCl	LiCl
Spontaneous	0.0317 ±0.01 n=4	0.0569 ±0.0137 n=10
Evoked**	0.0682 ±0.0287 n=4	0.1261 ±0.0316 n=10

* All values are expressed as μg/min/g tissue ± SEM. ** 0.4 msec duration; 0.3 Hz frequency; supramaximal voltage, 30 min.

Table 2. Whole brain* level** of acetylcholine

NaCl	LiCl
2.317 ±0.226 n=6	2.283 ±0.156 n=12

* Except cerebellum. ** All values are expressed as μg/g tissue ± SEM.

The results of the present study indicate that acute or chronic LiCl treatments in vivo have no effect on the spontaneous or evoked release of ACh at neuroeffector junction or on whole brain levels of ACh.

Although we have found that whole brain shows no effects of LiCl treatment in terms of level of ACh, this does not eliminate the fact that changes in specific brain areas may be taking place. This was noted by Ronai and Vizi[10], who showed a significant fall in ACh content of medulla oblongata-pons-mesencephalic area in rats treated with lithium.

In summary, we conclude that in vivo administration of LiCl has no effect on release of ACh from nerve terminals in myenteric plexus or on whole brain levels of ACh compared to NaCl treated animals.

These data suggest that LiCl therapeutic effect is not the result of a presynaptic interaction on cholinergic sites to alter release of the transmitter. This, of course, does not eliminate other mechanisms of interaction such as post-synaptic changes[16] or block of synthesis of ACh by LiCl[8]. Further evidence concerning these latter possibilities could provide meaningful insight into LiCl therapeutic effects.

1 Present address: Department of Pharmacology, State University of New York, Downstate Medical Center, Brooklyn (NY 11203).
2 Present address: Department of Pharmacology, University of Health Sciences, The Chicago Medical School, 2020 West Ogden Avenue, Chicago (IL 60612).
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Acetylsalicylic acid-induced morphological changes in the ductus arteriosus of the chick embryo[1]

S. Ishikawa, M.O. Cheung, E.F. Gilbert and H.J. Bruyère, Jr

Department of Pathology, University of Wisconsin Medical School, Madison (Wisconsin 53706, USA), 27 February 1978

Summary. The effect of acetylsalicyclic acid upon the ducti arteriosi of the embryonic chick was studied. A spectrum of gross malformations and histological findings associated with premature closure of the right ductus arteriosus is presented.

It has recently been demonstrated that the ductus arteriosus (DA) can be chemically manipulated in patients with congenital heart disease[2,3]. Prostaglandins, in addition to indomethacin and acetylsalicylic acid (ASA) (inhibitors of prostaglandin synthesis), have been employed both experimentally[4] and clinically[5] due to the physiological and morphological changes which these agents induce in the DA. It has been suggested that one of several pharmacological mechanisms of indomethacin[6,7] and ASA[8], namely inhibition of endogenous prostaglandin synthesis and subsequent increased catecholamine release[9], may relate to changes in vasomotor tone and ultimately closure of the DA. Although many studies have demonstrated closure of the DA with ASA and/or indomethacin, detailed morphological changes in the DA have not been documented.

The purpose of this report is to delineate changes in a) the DA proximal and distal to the right pulmonary artery (RPA) and b) in the pulmonary artery itself, and in this manner to suggest a morphological mechanism involved in premature closure of the DA.

Chick embryos (exclusively developmental stage 26)[10] were used in this study. In the avian embryo, the ventral-dorsal extension of the 6th aortic arch is known as the ductus arteriosus[11]. The right DA is a right-sided secondary flow

channel to the dorsal aorta and runs parallel with the descending aorta. The left DA, however, provides exclusives left-sided flow to the dorsal aorta.

After the 6th day of incubation, when the dorsal aorta begins to undergo a secondary division anteriorly, the DA is lengthened by the addition of a newly differentiated segment of the dorsal aorta. The entire DA becomes a solid cord of tissue shortly following the onset of pulmonary respiration[11].

0.3 mg ASA (salicyclic acid, Sigma) in 0.1 ml 5.6% ethanol was topically administered to embryonic chicks in close proximity to the aortic arch system. Control embryos were exposed to 0.1 ml 5.6% ethanol. Following injection with ASA, eggs were reincubated until 17 days of incubation, at which time embryos were sacrificed and microdissected. Diameters of ducti arteriosi were measured with a vernier caliper. Specimens fixed in 10% formaldehyde and 2.5% phosphate-buffered glutaraldehyde were sectioned for histology and electron microscopy, respectively.

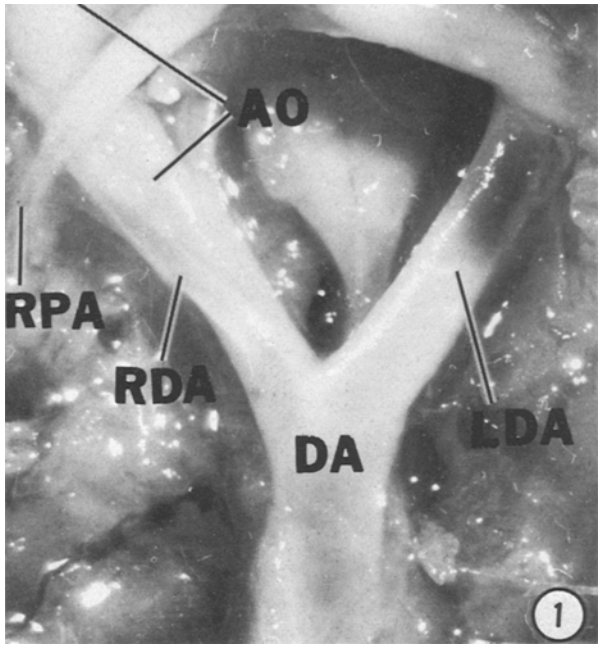

Fig. 1. ASA-induced constriction of the right ductus arteriosus (RDA) in a 17-day-old embryonic chick. Note the comparatively large size of the normal left ductus arteriosus (LDA).
MPA: main pulmonary artery; RPA: right pulmonary artery; LPA: left pulmonary artery; AO: aorta; DA: dorsal aorta.

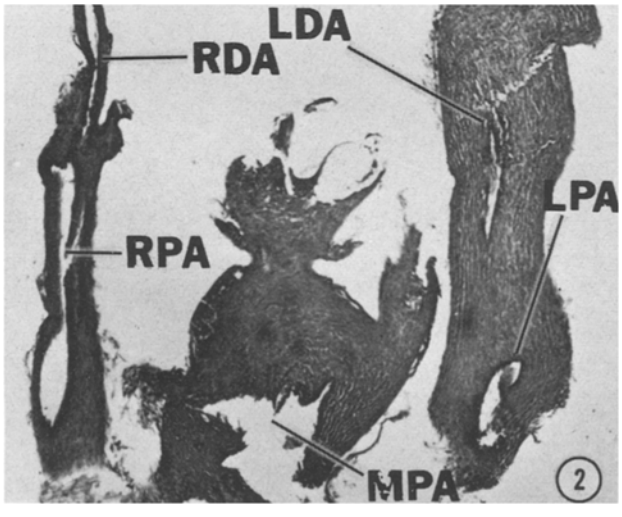

Fig. 2. X-section through aortic arch segment pictured in figure 1. The right ductus arteriosus is almost completely constricted.

Survival rates were 86% (43/50) in the ASA-injected group and 100% (30/30) in the control group. No cases of premature closure of the left ductus arteriosus were observed in the 73 specimens which survived.

The morphological results of the study were classified into three groups:

Group 1. 28% (12/43) of embryos to ASA demonstrated constriction of the RDA proximal to the RPA. The constricted portion measured 0.2–0.4 mm in diameter (normal, 1.0 mm). Light microscopy of the constricted area showed an endothelial proliferation so extensive that the lumen of the RDA was nearly obstructed. Electron microscopy revealed a proliferating tunica intima, abnormal configurations of intimal cells and an interrupted internal elastic lamina. The media was greatly compressed and cells demonstrated abnormal shapes. However, no degenerative changes were observed in the endothelial tissue or the tunica media.

Group 2. 9 of 43 cases (21%) demonstrated constriction along the entire length of the RDA. Histologically, there were no dramatic differences between findings in groups 1 and 2.

Group 3. 9% (4/43) demonstrated complete absence of the RDA associated with a hypoplastic RPA. The diameter of the RPA was $\frac{1}{5}$ that of a normal RPA. Light microscopy of the hypoplastic pulmonary artery revealed thinning of the media with normal endothelial tissue. Electron microscopy demonstrated degeneration of smooth muscle.

The literature is replete with studies which suggest that prostaglandins play an important role in the fate of the ductus arteriosus. Findings in this study convincingly indicate that endothelial proliferation is the major histological event during the closing process.

Furthermore, since other studies in this laboratory have demonstrated that catecholamines[12] and methylxanthines[13] induce similar closure in this system, it follows that factors currently recognized as critical in the regulation of contraction, transport and various metabolic activities, namely cyclic adenosine monophosphate and calcium, may play significant roles.

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