

Interarterial Coronary Anastomoses in Neonatal Pigs.* (26457)

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(Introduced by Alfred J. Weil)

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The hearts of human neonates born at and beyond term, are endowed with interarterial coronary anastomoses in a high percentage of cases, as demonstrated by a technic of post-mortem arteriography based upon unilateral injection of the right or left coronary artery with a radiopaque mass(1). With this technic it was disclosed that interarterial coronary anastomoses are not to be viewed as an all-or-none phenomenon but rather as a spectrum of vascular communications which could be graded on a scale of from 0 to 4+ depending on the arteriographic richness of vascular filling in the contralateral arborization. Collaterals of any grade were found in 4/5 of all cases; fully one-third exhibited 4+ collaterals. The question was raised whether these findings were characteristic of the neonatal state of man or whether they also applied to other mammals. To this end, the hearts of neonatal pigs were examined.

Materials and methods. Twenty-nine newborn and young suckling pigs were obtained from 3 different sources in Westchester and Dutchess Co., New York. Of these, 7 were stillborn having died presumably at or near term as judged by their birth weights; the remainder died within 2 hours after birth or were killed during the first 12 postnatal days by ether inhalation or by a blow on the head. In Table I the animals are listed according to sex, age and weight of body and heart. The 29 animals belonged to 17 litters, 4 of these litters being represented by 2, 2, 4 and 8 animals, respectively. All the animals were offspring of primiparous sows bred soon after attainment of sexual maturity at the age of 8-9 months and approximately 1 year of age at time of delivery. (Gestation in the pig according to the mnemonics of breeders takes 3 months + 3 weeks + 3 days).

Apart from atresia ani in a few of the ani-

mals no developmental abnormalities were noted. Two animals showed fibrous adhesions between loops of the small intestine.

The technic of arterial injection was identical with that employed in the study of human hearts(1). In short, it comprised the following steps: 1) Cannulation of right and left coronary arteries with polyethylene tubing; 2) Flushing of both coronary arteries with 1-2 cc of physiological (0.9%) saline solution by a hand-operated syringe; 3) Unilateral injection of either right (13 cases) or left (16 cases) coronary artery with a highly viscous, radiopaque mass (barium sulfate in gelatine at pH 6.2)(2) for 10 minutes and at a pressure which was gradually raised to 150 mm Hg; 4) "Unrolling" of the heart (3); 5) Stereoscopic x-ray photography.

Results. In all the hearts the arborization injected showed optimum filling as judged by the appearance on X-ray films. However, in none of the hearts was any injection mass seen in the contralateral arborization. The injection mass here employed advances quite regularly into vessels having a diameter of about 40 μ (2) and irregularly into still smaller vessels (as measured in paraffin sections after formalin fixation of the tissues), without entering capillaries or crossing over into veins. Thus, the findings indicate the absence of interarterial coronary anastomoses of about 40 μ or larger in the hearts of neonatal pigs, under the conditions of the experiments. These findings are essentially identical with those of a previous series in which the above technic was applied to hearts of 18 adult pigs of unknown sex and age. Of these 18 hearts, 17 showed no collateral flow and 1 showed 1+.

Discussion. Postmortem injection of hearts of human neonates previously reported has revealed the existence of interarterial coronary anastomoses in a high percentage of those born at or beyond term, in-

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TABLE I. Distribution of Neonatal Pigs by Sex, Age and Weights of Body and Heart.

Age	No. of cases	Sex		Wt (g) of	
		♂	♀	Body	Heart
<i>Stillborn</i>					
—	7	4	3	930-1170	7.5-10.5
<i>Liveborn</i>					
2 hr	10	6	4	590-1220	6.0- 8.5
1- 3 days	6	4	2	830-1360	7.5-12.5
4-12 "	6	4	2	2010-2940	15.0-35.0

cidence and degree of such collaterals increasing with weights of body, brain and heart. In analogy to the observations of Zoll *et al.* (4) in adults the possibility was considered that development of collaterals in term and overdue babies was mediated by intrauterine hypoxia including that due to deterioration of fetal oxygen supply which, according to Walker & Turnbull(5) occurs in late and especially in postmature stages of human pregnancy, as well as in pre-eclampsia and other abnormal states. Interarterial coronary anastomoses were observed also in premature neonates but, for undetermined reasons, only in those born of mothers 23 years of age or under. In some of these premature cases, development of collaterals may have been related to maternal toxemia; in some others, coexistent congenital heart disease in the neonate may have played a role.

The absence of interarterial coronary anastomoses in hearts of neonatal (term) pigs indicates that the factor or factors which in human neonates govern development of collaterals in general, and of high-grade collaterals (3+ and 4+), in particular, do not operate in the pig. The difference between the 2 species may be an organ characteristic since under natural conditions collaterals are absent not only in the newborn pig but with rare exceptions also in the adult animal whereas in normal hearts of man (low-grade) collaterals are a common finding throughout postnatal life. On the other hand, the difference between the 2 species may be secondary to specifics of the intrauterine environment including differences in degree and duration of hypoxia, in adaptation on the part of the fetus or in both. Since interarterial coronary anastomoses in human neo-

nates, especially those of high grade, might be looked upon as altogether pathological and the result of such states of pregnancy as are also associated with increased perinatal mortality, it is perhaps significant that no collaterals had developed even in the pig fetuses which had died either *in utero* or shortly after delivery.

Differences of placental structure(6) probably played no role since the hemochorial placenta of man would be expected, if anything, to provide a better oxygen supply to the fetus than the epithelial-chorial placenta of the pig. In the former, maternal blood circulates freely in the intervillous space and oxygen has to travel through only 3 anatomic layers (villous epithelium, villous stroma and endothelium of fetal capillaries) whereas in the latter, 6 layers intervene between maternal and fetal blood (endothelium, stroma, and epithelium on the maternal side and, in reverse order, on the fetal side). However, Dempsey(7) has pointed out that "the placental type does not accurately describe the distance across which metabolites must be transferred" and that it is therefore unjustified to draw physiological inference from anatomic schemes. Amoroso(8) reported that of all the placentas examined (by Louis Flexner) only the placenta of the pig failed to show a terminal decline of sodium transfer, that "it is the only one which remains functionally active to term and even seems to improve with age" and that it does not display the senescent changes seen in placentas of most other mammals. The possible relevance of these remarks to the current topic is obvious and calls for investigation of interarterial coronary anastomoses in other mammalian species.

Summary. The right or left coronary arteries of the hearts of neonatal pigs were injected with a highly viscous barium sulfate-gelatin mass which quite regularly fills vessels down to a diameter of about 40 μ and which fails to enter capillaries or cross over into veins. No anastomoses were demonstrated in 29 hearts examined. This is in contrast to the findings in hearts of human term neonates which are endowed with collaterals of varying grades in most cases. The

differences between the 2 species indicate that interarterial coronary anastomoses in hearts of neonates are not a general characteristic of the mammalian phylum but rather of man. Whether they are unique to the latter requires the study of neonatal hearts in other species.

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Allergic Reactions to Hyaluronidase.* (26458)

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During the past several years a number of reports of allergic reactions to hyaluronidase have been published(1-5). Holborrow and Keech(1) believed that the occurrence of hypersensitivity reactions in their patients was probably due to presence of protein impurities in the hyaluronidase preparations employed. Although the purity of more recent preparations has been emphasized(2), the data to follow will indicate that a highly purified commercial hyaluronidase extracted from bull testes was found to contain considerable bovine serum proteins. Such impurities could easily have been responsible for the reported allergic reactions to hyaluronidase.

Experimental. An adult albino rabbit was injected subcutaneously once or twice weekly with 1-4 mg of hyaluronidase (Wydase)[†] in Freund's adjuvant. A total of 16 mg was administered over 5 weeks. Five days after the last injection the animal was bled and its serum tested for antibodies to bovine serum albumin (BSA) and bovine gamma globulin (BGG) by the hemagglutination technic of Stavitsky and Arquilla(6). BSA or BGG coupled to rabbit red blood cells with bis-diazotized benzidine served as antigens.

Table I shows that the serum of the rabbit injected with hyaluronidase had a titer of 1:10,240 to BSA and a titer of 1:10,240 to BGG. Pre-incubation of this serum with 1 mg of hyaluronidase reduced the titer to zero. In the next experiment a series of dilutions of a rabbit anti-BSA serum (titer 1:25,600) was incubated with various amounts of BSA or hyaluronidase prior to addition of BSA coupled to red cells. Table II demonstrates that 1 mg of hyaluronidase reduced the anti-BSA serum titer to zero and 0.1 mg lowered the titer to 3200; .01 mg of hyaluronidase had no inhibitory effect. Comparison of the above inhibition with that produced by BSA (Table II) suggests that the hyaluronidase preparation employed had 1/100 to 1/1000 the inhibiting activity of BSA. Pre-incubation of rabbit anti-rye grass serum with 1 mg of hyaluronidase did not lower the titer of this serum to rye grass pollen (Table II).

In a third experiment mice were sensitized to BSA by intraperitoneal injection of 5 billion *Bordetella pertussis* organisms and 4 mg of BSA(7). Ten days later the animals were challenged intravenously with various amounts of BSA or hyaluronidase. Due to the small amounts of material used in challenge, fatal anaphylaxis did not take place

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