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Attempt to Visualise the Ventricular Conduction System Intravitam

Radiologic in vitro Visualisation of the Left Ventricular Conduction System in Cow, Calf and Sheep Hearts

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Summary. A radiologic method for the visualisation of the left ventricular portion of the conducting system using cow, calf and sheep hearts which can be repeatedly reproduced in vitro is presented. The results of the experiments were documented by macro- and microphotograms.

Critical analysis of a further in vivo study of this method is also discussed.

Zusammenfassung. Es wird eine röntgenologische, in vitro an Rinder-, Kälber- und Schafsherzen erprobte und beliebig oft reproduzierbare Methode zur Visualisation linksventrikulärer Anteile des Reizleitungssystems vorgestellt. Die Ergebnisse der Untersuchungen werden anhand von Makro- und Mikrophotogrammen dokumentiert.

Zu der in einer weiteren Studie geplanten in vivo-Testung der Methode wird kritisch Stellung genommen.

The possibility to visualise the ventricular conduction system of the human heart intravitam is not only of theoretical or academic interest, she also can be of practical and clinical importance.

The main motivation for undertaking the effort required by this research are the repeated observations of iatrogenic injury to the main branches and bundles of the specialised musculature since the beginning of the era of open-heart surgery. Especially endangered are the central portions of the ventricular conducion system during correction of a high-placed ventricular septum defect, or by closure of an ostium primum defect, by the implantation of prosthetic aortic valves, during an infundibulum resection of the right ventricular outflow tract and during excision of the myocardial cushion seen in IHSS (Sazaki, 1958; Bristol, 1960; Bristow, 1960; Lillehei, 1963; Gerbode, 1963; Titus, 1963; Gadboys, 1964; McGoon, 1964; Kulbertus, 1969; Knieriem, 1966 and 1969; Bekier, 1971).

In addition to these injuries due to direct trauma to the atrioventricular bundle, the postoperative subendocardial hemorrhagic edema also significantly affects conduction capacity.

Although modern embryology and anatomic dissection efforts have pinpointed the location and course of the conduction system in the ventricular plane both in the normal as well as in the defective heart (Uher, 1936; Reemtsma, 1958; M. Lev, 1958, 1959, 1960 and 1964; Richter, 1960; Kl. Goerttler, 1960; Schiebler and Doerr, 1963; Doerr and Schiebler, 1963; Titus, 1963; Doerr, 1967

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and 1969) and despite the most refined suture techniques employed in the various corrections (Gall and Cooley, 1961) the incidence of "surgical heart block" in the Mayo Clinic in 1964 was still 0.9 per cent (McGoon, 1964). Compared with the observations in 1960 when in the above clinic this complication arose in 30 per cent of all operative open-heart procedures (Lauer *et al.*, 1960), this is an enormous decrease in the heart block risk. However we hope for an even further improvement in the technical presuppositions of cardiac surgery when the possibility for an intraoperative visualisation of the conduction system has been perfected.

Morphologic Preliminary Remarks

Since the reports of Purkinje (1845), W. His jr. (1890) and Tawara (1906) and supplementary publications from Keith and Flack (1906/07), the stimulating and conduction structures in the mammalian heart together with their vessel-connective tissue apparatus are considered a "system with special functional importance" (Schiebler and Doerr, 1963).

The specific elements of the ventricular conduction system which also differ morphologically from normal contractile myocardium in many ways are enveloped in a discreet, loose connective tissue sheath separating it from the surrounding tissue. This is especially well-developed in the ungulates. The "fluid spaces" coursing within this connective tissue sheath were initially observed by Eberth and Belajeff in 1866. In connection with their research on the lymph vessels of the heart they injected them with dye solutions and visualised them in their subendocardial course. The authors then drew the false conclusion that these connective tissue spaces, subsequent named after them, were true lymph vessels. This has since histological been proven wrong. The close topographical relationship of their "lymph channels" with the specific fibers of the conduction system was not recognized by Eberth and Belajeff.

The first detailed reports on the perifascicular connective tissue sheath were published in 1893 by Renaut (cit. Aagaart and Hall, 1914) and in 1909/10 by E. J. Curran. The latter described this sheath as a "constant bursa in relation with the bundle of HIS" and ascribed it a protective function which "reduces the friction of the conduction fibers during ventricular contraction". Similarly, Doerr understands the Curran connective tissue lamella to be a gliding surface between the contractile myocardium and the less contracting fibers of the specific musculature.

Further injection experiments were performed in later years to explain various questions (Lhamon, 1912; Cohn, 1913; Aagaart and Hall, 1914; Chr. Korth, 1961; A. Geiss, 1970). Most researchers used ungulate hearts because of the especially distinctive character of the connective tissue sheath.

The injection into the left ventricular perifascicular connective tissue space is usually quite easy in contrast to the difficulty reported by most authors in the area of the crus dextrum. Apparently, caliber differences in the Curran connective tissue sheaths exist between the right and left atrioventricular bundle, or, the expansion of the right ventricular Eberth-Belajeff "lymph spaces" is retarded by the intramyocardial course and the unyielding muscular envelopment of the bundle.

Presently the Eberth-Belajeff spaces of the conduction system are generally regarded as facultative complementary spaces without endothelium or basal membrane. Parallel morphology can be drawn to the Disse spaces in the liver, the Cruenhagen-Mingazzini spaces in the intestinal mucosa and the Virchow-Robin spaces in the brain (Chr. Korth, 1961). These structures all have in common that they are either difficult or impossible to see under normal conditions but under pathological circumstances expand and become visible.

The question whether the Eberth-Belajeff spaces physiologically contain a fluid is still controversial. The presence of a flow is nowhere mentioned in the literature cited.

The starting point for the method employed for radiological visualisation of the conduction system was the idea that "lymph spaces" lend themselves to "lymphography".

The earlier dye solution injection experiments into the Eberth-Belajeff "lymph spaces" to visualise the conduction system had the disadvantage that only the immediate subendocardial portions of the system were made visible. All those conduction elements covered by muscle layers remained hidden. This can be rectified by employing radiologic visualisation.

The injection of X-ray contrast medium, especially when it is of higher viscosity, depends upon the presence of a Curran connective tissue sheath of adequate caliber just as is the case with normal dye solutions. According to the morphological situation it seems that the visualisation of the right ventricular components of the conduction system is more of a problem in comparison to the crus sinistrum.

Direct dye methods for delineation of the specific musculature using the high glycogen content of the conduction structures is theoretically possible (Schiebler, 1953; Uhley, 1959 and 1960), but appear impracticle in vivo due to the subendocardial and intramyocardial location.

Materials and Methods

After preliminary macropreparation and injection exercises with black ink on two cow hearts, 15 cow, 5 calf and 10 sheep hearts were used for the injection of X-ray contrast medium into the Eberth-Belajeff spaces of the left ventricular Curran connective tissue sheath.

On seven cow hearts the injection into the connective tissue lamella of the left and the right atrioventricular bundle was attempted.

The best left ventricular injection location were "false chordae tendineae" which extend into the bases of the anterior and posterior papillary muscle. Injection into the right ventricular Eberth-Belajeff spaces was attempted from the moderator band.

Among the injection solutions employed were: ordinary water-soluable X-ray contrast mediums (Angiografin, Urografin, Conray EV, Uromiro 380)[1] and tantalum suspensions (dissolution of tantalum powder[2], particle size under 1 micron, in a 10% sorbit solution) in varying concentrations (25 gm tantalum in 100 ml and 200 ml sorbit solutions).

The injections were carried out using a no. 17 or 20 cannula on a 2 ml Rekord barrel either immediatly after excising the heart from the killed animal or just prior to X-ray exposure.

The ordinary water-soluable contrast mediums demanded immediate post-injection X-raying due to the rapid diffusion of the medium out of the connective tissue sheath into the

1 The employed X-ray contrast mediums were kindly made available from the manufactuering firms as physician samples.

2 I thank Prof. Dr. med. F. Huth, Department of Pathology of the University of Düsseldorf for a portion of tantalum powder.

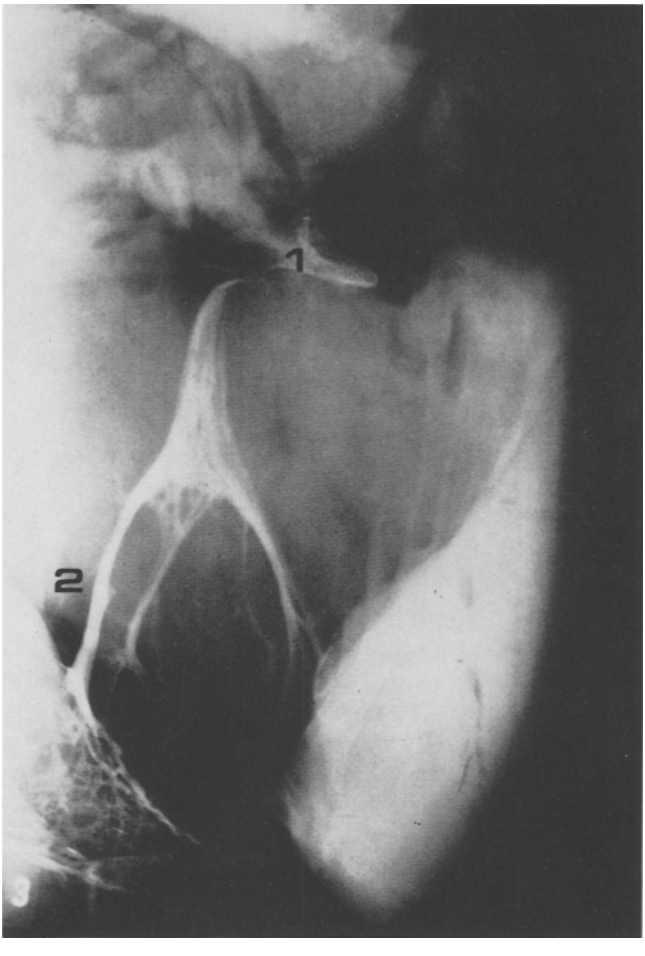

Fig. 1. Radiologic visualisation of the bundle of His and the left av-bundle on a cow heart. Injection of the contrast medium (Conray) into a "false chorda tendinea" which extends to the base of the anterior papillary muscle (*2*). *1* The cardiac bone correspondents to the trigonum fibrosum dextrum and the topografic location of the Aschoff-Tawara-node

surrounding tissue, creating unsharp pictures. The metal particles in the tantalum suspensions remain at the injection site allowing a longer period of time to elapse before X-raying without a reduction in picture quality.

Should the time between excision of the heart and injection be prolonged, the heart was placed in physiologic salt solution at 37°C to prevent rigor which would have made injection impossible[3].

The X-ray pictures were taken with the Mammomat from the Siemens Firm[4].

3 I thank the veterinarians of Heidelberg and Mannheim slaughterhouses for making the hearts available.

4 I thank Prof. Dr. med. Gerhard and Dr. med. Schröder from the University of Heidelberg Surgery Clinic, Department of Radiology, for the technical assistence in producing the X-ray pictures.

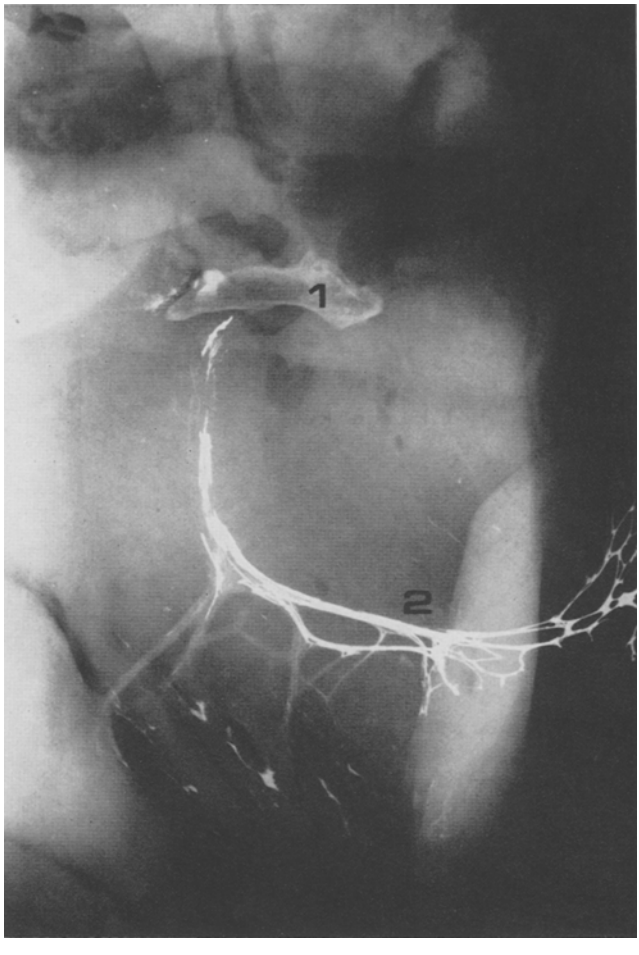

Fig. 2. Radiologic visualisation of the bundle of His and the left av-bundle on a cow heart. X-ray contrast medium is a tantalum suspension. *1* Cardiac bone (corr. av-node). *2* Injection into the posterior "false chorda tendinea"

After X-ray examination, biopsies from the corresponding areas of the interventricular septum of the tantalum-injected hearts were taken for histological investigation. These sections were prepared with hematoxilin-eosin dye. In addition, equivalent histological sections were taken from uninjected hearts.

Results

Photograms 1 to 4 and 5 to 6 are representative documentation of the total results of the experiments performed. These concern the macroscopic-radiologic presentation of the left ventricular portion of the conducting system (partially including the bundle of His) in cow, calf and sheep hearts with various contrast dyes and corresponding histological sections for identifying the localisation of the injected material into the Eberth-Belajeff spaces.

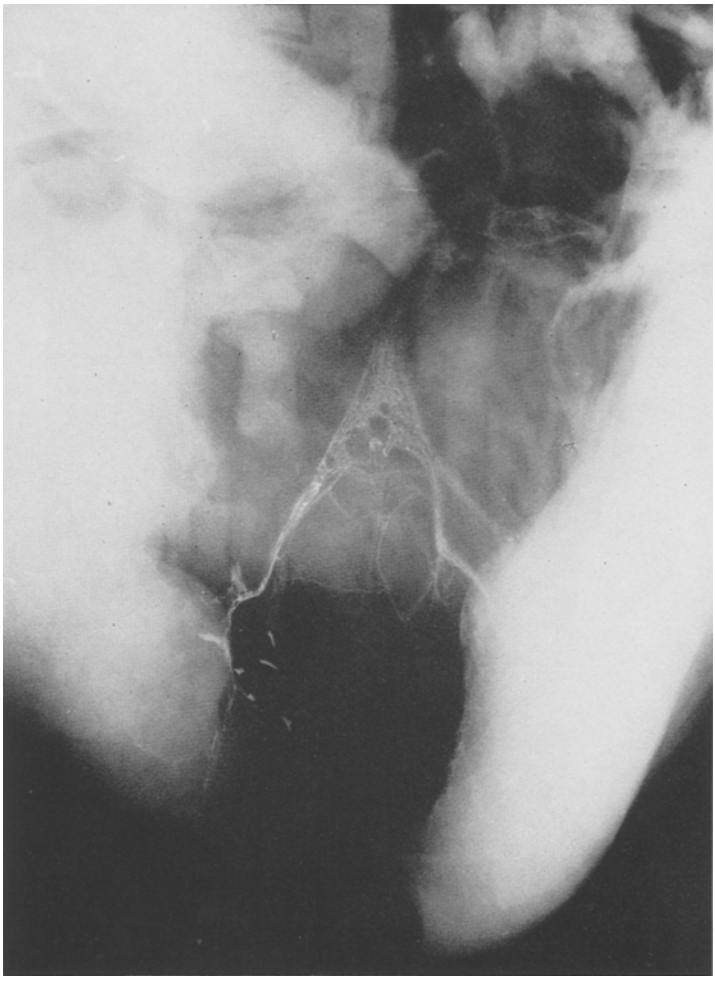

Fig. 3. Radiologic visualisation of the left ventricular av-bundle on a calf heart. X-ray contrast medium is a tantalum suspension

Comparison of the microphotograms of the injected and uninjected hearts (see Fig. 5, 6 and 7) shows the Ebert-Belajeff spaces to be facultative which expand after dye injection and are not visible in uninjected preparations. This negates the possibility of a fluid content or flow in these spaces.

The intramyocardial portion of the conduction system which is usually not visualised with simple dye solutions can be demonstrated using the radiologic method. This includes deeper-lying structures such as the intramural Purkinje fibers and the central portion of the left ventricular conduction system up to the bundle of His. The Aschoff-Tawara node was never visualised.

The results of the attempt to radiologically define the right ventricular atrio-ventricular-bundle (anatomic preparation see Fig. 8) are unsatisfactory. In only

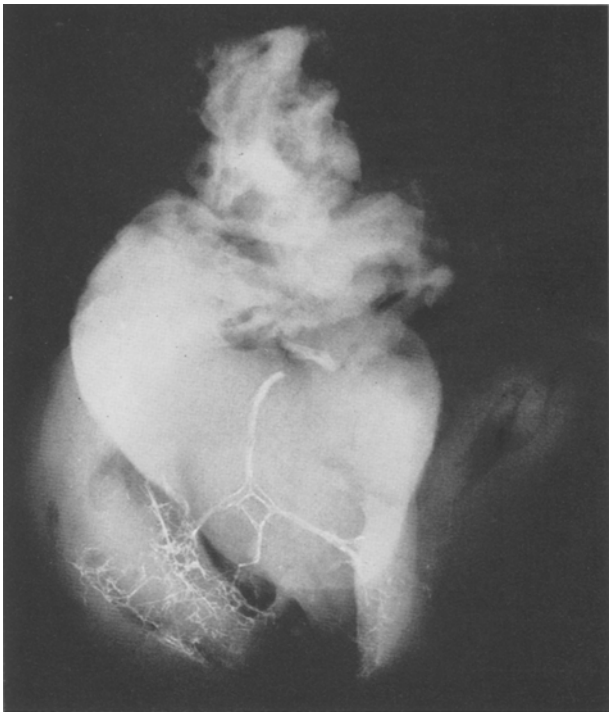

Fig. 4. Radiologic visualisation of the bundle of His and the left ventricular av-bundle on a sheep heart. X-ray contrast medium is a tantalum suspension

one instance the crus dextrum of a cow heart was visualised by retrograde filling the connective tissue sheath via the bundle of His during the injection of the left av-bundle (see Fig. 9). All experiments employing direct injection of the crus dextrum were unsuccessful. Either a continuing spread of the contrast dye in the area of the moderator band resulted in a stagnation at the septal origin of the trabecula despite pressure increase or the contrast medium extravasated immediately. The latter is seen as unsharp, polygonal, diffuse spots. Since the histological sections of uninjected hearts demonstrate no principle difference between the left and the right ventricular Curran sheath, it can be logically concluded that the intramyocardial course of the right sided connective tissue spaces with their inherent less yielding qualities prevent their expansion.

Discussion

The advantages of the above-mentioned radiologic visualisation of the conduction system over the injection of dyes or fluorescent substances into the perifascicular connective tissue spaces are quite evident by the gain in visual information. Up until now, macroscopic presentation of intramural paths of the av-system has not been possible.

But at the moment a degree of sceptisism concerning the intravitam use of this method is reasonable. It is a speacially problematic whether such an injection

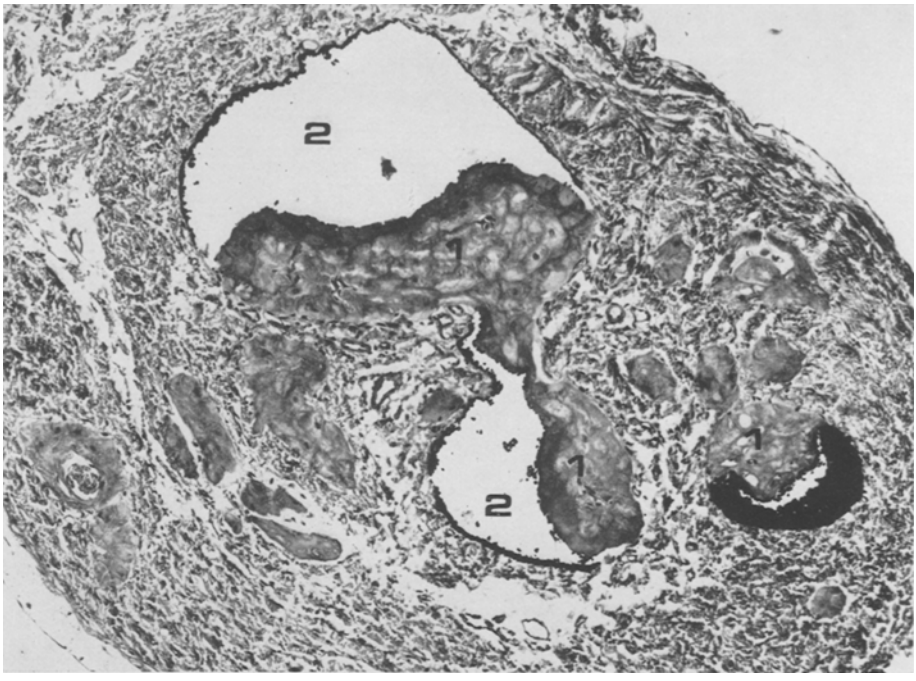

Fig. 5

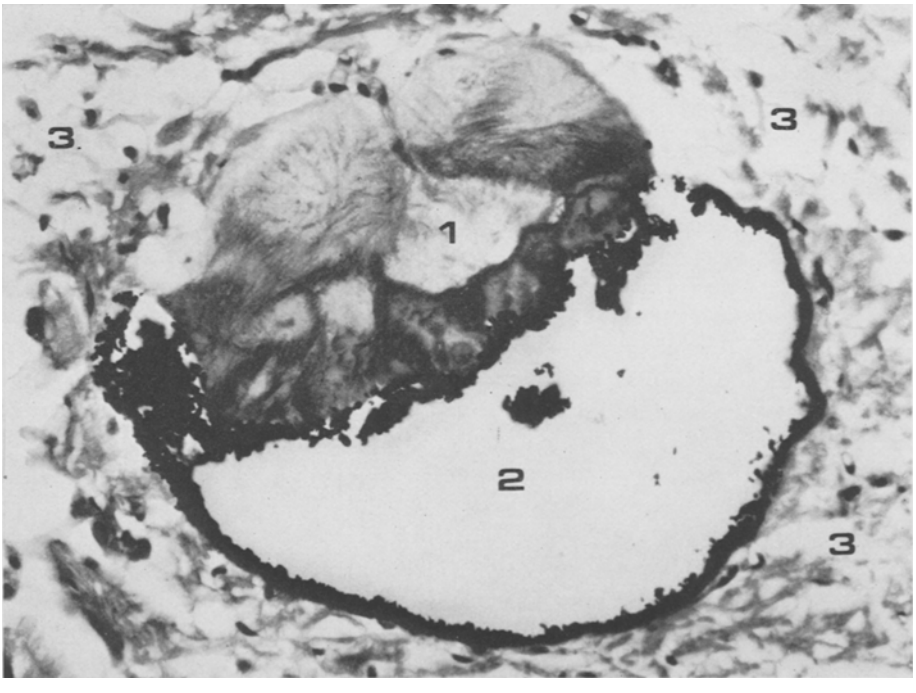

Fig. 6

Figs. 5 and 6. Histological preparation of the specific musculature (*1*), the expanded Eberth-Belajeff connective tissue spaces (*2*) and the Curran connective tissue sheath (*3*) of a cow heart following injection of a tantalum suspension. The tantalum particles are easily seen in the connectiv tissue soaces. In Fig. 5 a "false chorda tendinea" is cut

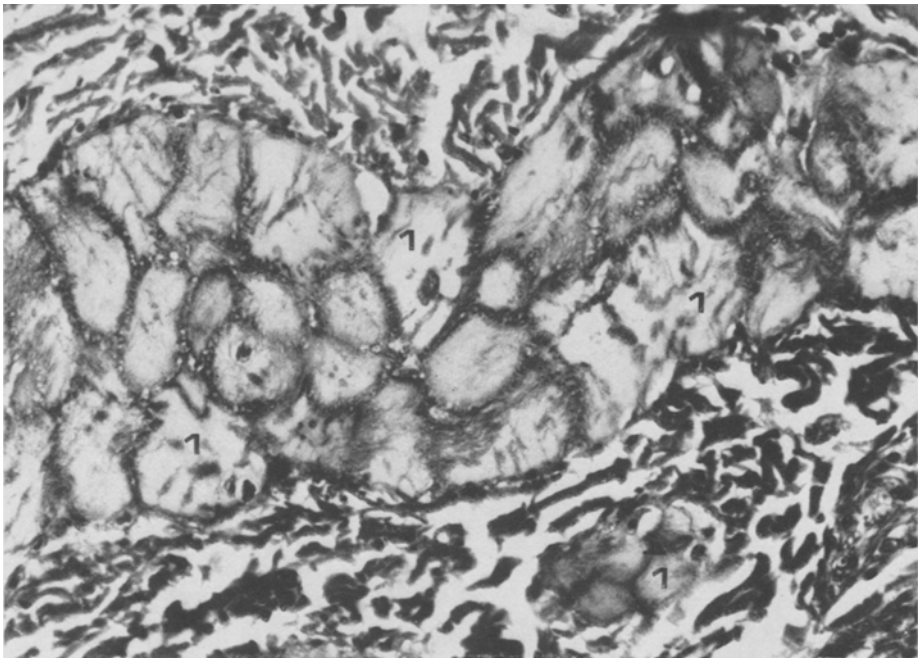

Fig. 7. Histological preparation of the left ventricular specific musculature without previous contrast substance injection. *1* Specific muscle fibers surrounded by the Curran connective tissue sheath. The Eberth-Belajeff spaces are hidden

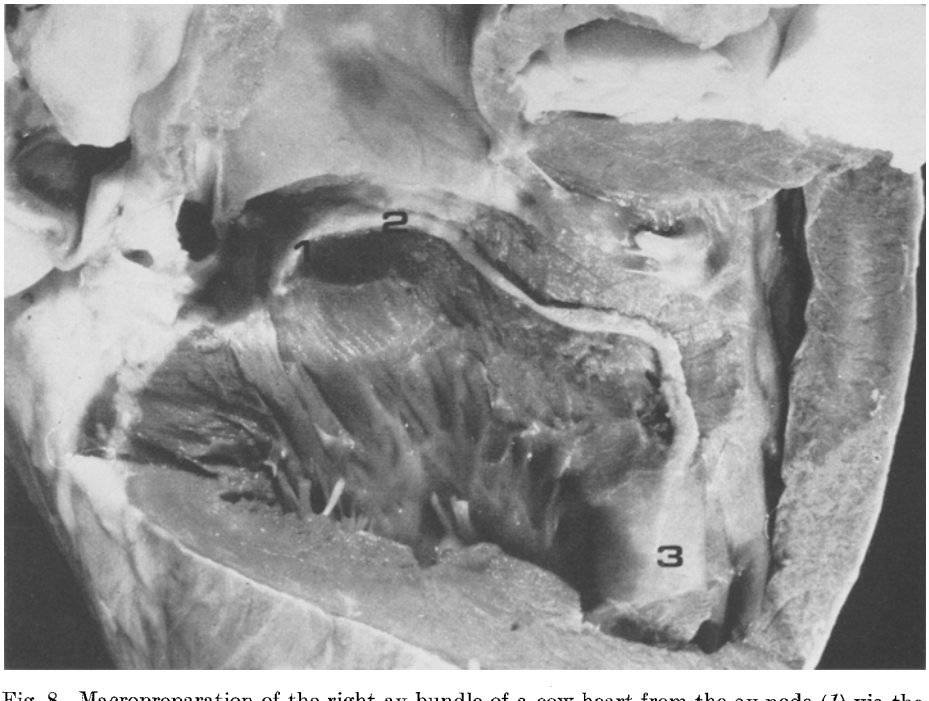

Fig. 8. Macropreparation of the right av-bundle of a cow heart from the av-node (*1*) via the bundle of His (*2*) to its extension into the moderator band (*3*). Portions of the parietal ventricular wall, the crista supraventricularis, the whole tricuspid valve with chordae tendineae are removed

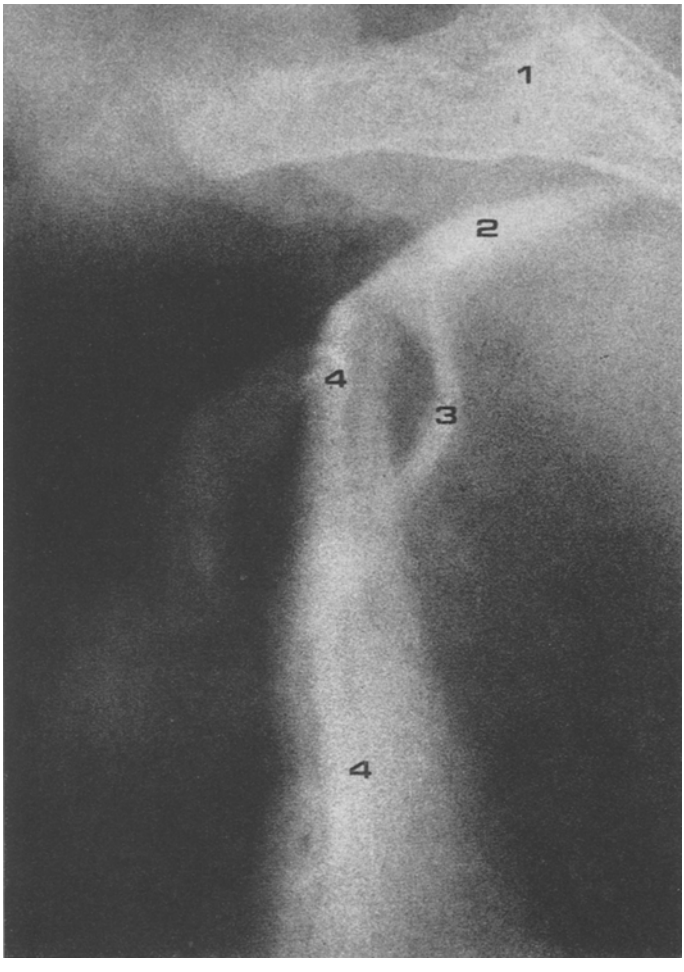

Fig. 9. X-ray picture of the entire ventricular conduction system on a cow heart via unintentional retrograde filling of the right bundle branch with contrast substance through the bundle of His following injection into a left ventricular "false chorda tendinea". *1* Heart bone (correspondents to the av-node), *2* bundle of His, *3* right bundle branch, *4* left bundle branch

into the Eberth-Belajeff spaces would be tolerated functionally. For one the injection necessarily causes a momentary increase in pressure on the fibers of the specific musculature with unknown results. Secondly, the ordinary X-ray contrast mediums are solutions which could have a negative effect upon the local ion balance and possibly cause a disruption of the membrane ion exchange. It is impossible to eliminate the chance that in vivo after injection electrophysiologic cardiac failure due to mechanical or physico-chemical occurances could result.

It is known from experiments with tantalum suspensions that the metal particles remain for an indefinite period of time. Although tantalum is a fully

inert metal and well tolerated by tissue (Davaris and Huth, 1971; Ulrich *et al.*, 1973), it would be interesting to investigate the alterations in the electrophysiologic reactions in the presence of this metal.

It is then logical to continue next with an in vivo tolerance study of the method described above before further technical problems (visualisation of the conductionssystem in smaller dimensioned infantile hearts with perhaps micropuncture; developement of a method for injection into the right av-bundles Curran sheath) are set about.

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