Table I. Morphometric basic values

Symbols	Control rats	S.E.	Penicillamine treated rats	S.E.	Probability
N_{ANH}	4.366	0.344	3.880	0.087	not significant
V_{VNH}	0.025	0.002	0.013	0.007	not significant
N_{AM}	17.656	1.395	19.370	0.537	not significant
I_{LMO}	7.245	0.802	11.987	0.304	< 0.01
I_{LMC}	4.599	0.674	8.477	0.325	< 0.005
N_{AMB}	3.645	0.198	4.777	0.501	not significant
V_{VMB}	0.013	0.007	0.019	0.001	< 0.025
V_{VRER}	0.201	0.015	0.179	0.029	not significant
V_{VSER}	0.140	0.018	0.100	0.018	not significant
V_{VGF}	0.004	0.001	0.005	0.002	not significant
V_{VH}	0.862	0.012	0.839	0.013	not significant

Table II. Correlated values

Symbols	Control rats	Penicillamine treated rats
N_{VNH} ($\times 10^6$)	0.000180	0.000154
V_{VH}/N_{VNH} (μ^3)	4791.255	5451.261
V_{VNH}/N_{VNH} (cm^3)	291.883	328.792
V_{VNH}/V_{VH} (cm^3/cm^3)	0.060920	0.060307
V_{VM}/V_{VH} (cm^3/cm^3)	0.149665	0.186710
V_{VM}/N_{VNH} (cm^3)	0.519412	1.069232
N_{VM} ($\times 10^{12}$)	0.198452	0.192141
V_{VM}/N_{VM} (μ^3)	0.754162	0.971734
N_{VM}/N_{VNH}	1102.511	1247.668
S_{VMC} (m^2/cm^3)	0.845760	1.488171*
S_{VMC}/N_{VNH} (μ^2)	4698.666	9663.448*
S_{VMO} (m^2/cm^3)	0.372643	0.616182*
S_{VMO}/N_{VNH} (μ^2)	2070.238	4001.181*
S_{VMO}/N_{VM} (μ^2)	1.877758	3.206942*
N_{VMB}	0.066937	0.080569
V_{VMB}/N_{VMB} (μ^2)	0.173013	0.197768*
N_{VMB}/N_{VNH}	371.872	523.175
V_{VMB}/V_{VH} (cm^3/cm^3)	0.013428	0.018978*
V_{VMB}/N_{VNH} (μ^3)	64.338	103.467*
V_{VLY}/V_{VH} (cm^3/cm^3)	0.003701	0.005397
V_{VRER}/V_{VH} (cm^3/cm^3)	0.205552	0.186108
V_{VRER}/N_{VNH} (μ^3)	984.855	1014.655
V_{VRIBO}/N_{VNH} (μ^3)	286.661	427.766
V_{VSER}/V_{VH} (cm^3/cm^3)	0.143172	0.103618
V_{VSER}/N_{VNH} (μ^3)	685.977	564.922
V_{VGLY}/V_{VH} (cm^3/cm^3)	0.176923	0.129044
V_{VGLY}/N_{VNH} (μ^3)	847.683	703.545
V_{VGF}/V_{VH} (cm^3/cm^3)	0.004609	0.005827
V_{VGF}/N_{VNH} (μ^3)	22.083	31.772
V_{VFAT}/V_{VH} (cm^3/cm^3)	0.003838	0.003296
V_{VFAT}/N_{VNH} (μ^3)	18.388	17.974

* Correlated values resulting from statistically significant basic values.

lamine application, however, this mitochondrial transformation cannot be detected. The mechanisms responsible for the increase of mitochondrial cristae are not yet understood. WILSON and LEDUC[21] interpreted the mitochondrial enlargement in essential fatty acid deficiency as a negative feed-back mechanism: The altered molecular architecture of mitochondrial membranes is presumed to be due to a replacement of the lacking essential fatty acids by unsaturated ones. As a result, the oxidative phosphorylation, and consequently the production of ATP, would be diminished. Thus, the main source of cell energy would be exhausted. This lack of energy would then act as a trigger for the growth of mitochondrial substance forming additional cristae.

Analogously to this hypothesis, Penicillamine-induced increase in mitochondrial cristae could be interpreted as a defective compensation of inefficient membrane-bound enzymes.

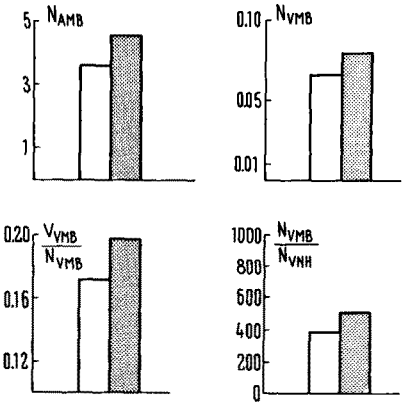

Fig. 5. N_{AMB}, number of microbodies within unit area. N_{VMB}, number of microbodies per unit volume liver tissue: No significant change after Penicillamine treatment (black column). White column = control. V_{VMB}/N_{VMB}, single volume of microbodies significantly increased after Penicillamine treatment (black column). White column = control. N_{VMB}/N_{VNH}, number of microbodies per hepatocyte: No significant change after Penicillamine treatment (black column).

Zusammenfassung. D-Penicillamin als Kupferchelator bewirkt im Langzeitversuch eine isolierte Veränderung der stereologischen Parameter der Mitochondrien und der «microbodies». Zusammenhänge mit der Aktivitätshemmung kupferhaltiger Oxydasen in den beiden Zellorganellen werden vermutet.

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An Electrophysiological Study of Human Foetal Cardiac Muscle

Although extensive information is available on the transmembrane action potential of heart muscle from many species, the findings are of limited value in assessing the response to disease or drugs in man. The study of human transmembrane action potentials has been restricted to records obtained with flexible microelectrodes in situ during cardiac surgery[1,2] and in vitro using pieces of atrium or ventricle excized during cardiac surgery[3-7]. These studies have been somewhat limited, since the heart is often hypothermic at surgery for operative reasons and biopsy specimens are usually obtained from hearts damaged by rheumatic fever or other diseases.

The transmembrane potential of human foetal tissue is of great interest because it differs from the adult in showing widespread pacemaker activity which is only found in the sinoatrial node and specialized Purkinje tissue at later stages of development. Foetal tissue has been found to be more sensitive in its response to cardiac anti-arrhythmic drugs (propranolol)[8] than the adult human tissue[9] and less sensitive in its inotropic response to carbamylcholine. Thus, human foetal myocardium would seem to be a suitable tissue for electrophysiological studies as it provides normal myocardium which might be expected to show a specific human response. To date, however, there is no report of the electrophysiology of the human foetal myocardium.

Fourteen hearts have been studied from human foetuses of 12–22 weeks gestation. The whole heart or double atria was mounted in a large chamber constantly perfused with well oxygenated (95% O_2 – 5% CO_2) Krebs-bicarbonate solution at 32 °C. The preparation was usually stimulated with platinum wire electrodes at a frequency 10% above the spontaneous rate. The tissue was found to be more difficult to work with than cardiac tissue of other species. The major difficulty was that the tissue was soft and tended to tear when subjected to mechanical stress, such as an excessive resting tension. These tissues were able to maintain their contractile responses for many hours so that they were considered to be in a viable condition. Membrane potentials were measured with glass microelectrodes filled with 3M KCl which had a resistance between 10–25 megohms. A negative capacitance cathode follower and Tektronix 502 oscilloscope were used to record potentials, and a RCA 5734 transducer tube used to measure contractions. Inotropic dose-response relationships were determined with carbamylcholine in 3 electrically driven atrial preparations with simultaneously recorded action potentials and in 2 preparations without electrophysiological recordings. Concentrations of carbamylcholine are expressed as g of the salt per ml of media in the chamber.

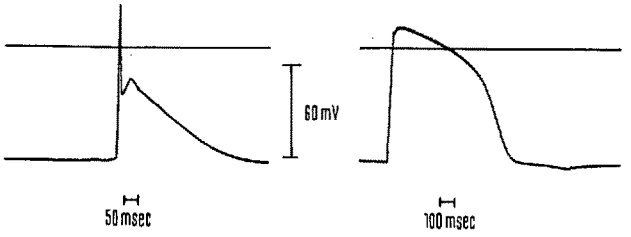

Fig. 1. Recordings of human foetal action potentials in a spontaneously beating atrium (Left) and ventricle (Right). The horizontal line indicates zero potential.

The electrophysiological values of resting and action potential amplitude, duration of the action potential (time to 10% and 90% repolarization) and maximal rate of depolarization for foetal atria and ventricle are summarized in Table I, with typical recordings shown in Figure 1. The values for resting and action potential amplitude were less than those recorded in adult human atria[3,4,6] and ventricle[1–3,5,7] as well as those recorded from other species[4,10]. The relative prolongation of the foetal action potential when compared to those from mature and foetal tissues of other species[10] would appear to be characteristic of the human myocardium, as it is also seen in adult human tissue[3–7].

A 20-week-old foetal heart contracted at 142 beats per min at 37 °C which is similar to the expected pacemaker rate of 140 beats per min in vivo at this stage of gestation. The rate progressively slows as the embryo approaches term, presumably due, at least in part, to an increase in vagal tone.

Carbamylcholine decreased contractile responses in each preparation studied by an average of 44.9% at a

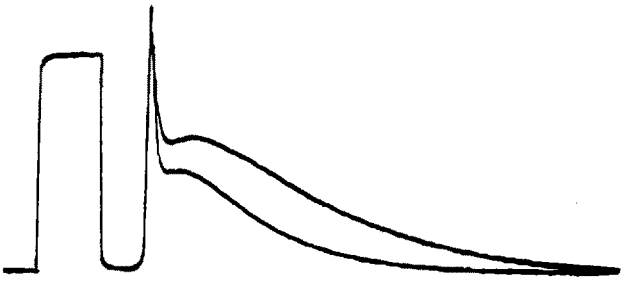

Fig. 2. Superimposed recordings of the action potential of a human foetal atrial fibre from a 16-week-old embryo before and after (lower tracing) carbamylcholine 10^{-5} g/ml. The calibration signal represents 60 mV (vertically) and 50 msec (horizontally).

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Table I. Electrophysiological values of human foetal myocardium

Tissue	Resting potential (mV)	Amplitude of action potential (mV)	Maximal rate of depolarization (V/sec)	Time to repolarise (msec) 10%	90%
Spontaneously beating double atria[a]	63.8 ± 3.0	75.8 ± 2.3	84.8 ± 7.0	9.1 ± 0.4	308 ± 16.2
Electrically driven atria[b]	66.6 ± 1.3	80.0 ± 1.9	42.2 ± 2.6	6.6 ± 0.4	245.7 ± 14.5
Ventricular fibers of spontaneously beating heart[c]	75.3 ± 1.7	95.1 ± 2.5	121 ± 10.2	125.5 ± 19.0	729 ± 35
Ventricular fibers of electrically driven heart[d]	66.7 ± 4.7	85.6 ± 6.0	105 ± 9.8	27.0 ± 0.4	726.8 ± 36

[a] N = 44–54 ± S.E. from 7 hearts. Heart rate averaged 52 beats/min (range 32–72). [b] N = 21–34 ± S.E. from 3 hearts. [c] N = 18–25 ± S.E. from 2 hearts. [d] N = 12–18 ± S.E. from 3 hearts.

concentration of 10^{-5} g/ml (Table II). In the 12- and 13-week-old foetuses, there were no changes in the action potential after carbamylcholine (10^{-7}, 10^{-6}, 10^{-5}). In the 14-week-old foetus there was a 21% decrease in the time for 90% repolarization (285 msec to 225 msec) after carbamylcholine (10^{-5}). In the 16-week-old foetus there was no change in the time to 90% repolarisation at 10^{-7}, a 26.2% decrease at 10^{-6} and a 49.6% decrease at 10^{-5} (Figure 2). Contractions decreased by 10.5, 26.3 and 42.1% respectively in the preparation. A similar result was found in a 20-week-old foetus. No hyperpolarization was observed, as has been reported for adult human[3] and other species[10].

The adult human atria responds to acetylcholine in vitro by a shortening of the action potential[3,4] as does the 7-day-old chick heart[11]. HOFFMAN and SUCKLING[12] suggested that the insensitivity of dog ventricular tissue to acetylcholine was related to the absence of nervous fibers in the ventricle. This hypothesis was supported by the insensitivity of the aneural heart of Myxine to acetylcholine[13,14]. In the rat foetus, sensitivity to acetylcholine occurs at about the eleventh[15] or thirteenth[16] day of gestation. Since innervation occurs in the rat heart between 14 and 16 days of gestation, sensitivity to acetylcholine appears to precede innervation. Our results show that carbamylcholine decreases the contractile response by about 50% with no effect on the action potential of the 12- and 13-week-old human foetal myocardium. This indicates that inotropic responses to exogenous cholinergic agents develop before electrophysiological responses. The contractile mechanism in the foetus is less sensitive to exogenously administered cholinergic agents than adult tissue from human[3] and other species[10], as the foetal dose-response curve is shifted to the right. In addition, contractile responses were only decreased by 40–50% at 10^{-5} carbamylcholine in the foetal preparation, whereas contractions of adult atria from other species are abolished by even lower concentrations. Nerve cells and fibers have been found to be abundant in the 12–13-week-old human foetus[17]. In a 14-week-old foetus we found a limited electrophysiological response to carbamylcholine, an increased response was noted in a 16-week-old foetus with no further change in a foetus of 20 weeks. Hence, we conclude that electrophysiological responses of the human foetal myocardium to cholinergic agents are not developed until after innervation. A similar result has been reported for the effects of acetylcholine in the chick embryo[18,19].

Résumé. Dans le cœur du fœtus humain la réponse inotropique de la droge cholinergique ne se manifeste qu'après le développement des fibres nerveuses.

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Table II. The negative inotropic effect of carbamylcholine on the human foetal atria

Concentration	Decrease of contraction amplitude (%)		
	Left atrium[a]	Right atrium[b]	Double atria[c]
10^{-8}	0	0	–
3×10^{-8}	0	0	–
10^{-7}	15.8	16.7	6.0
3×10^{-7}	15.8	33.3	–
10^{-6}	38.6	41.6	23.5
3×10^{-6}	47.4	50.0	–
8×10^{-6}	47.4	50.0	–
10^{-5}	47.4	50.0	41.2

[a] The left atrium was electrically stimulated at 120/min. [b] The right atrium was spontaneously beating at 210/min. No change in heart rate was observed at any concentration. The right and left atria were from different foetuses. [c] The 2 double atrial preparations were electrically driven at 10% above their spontaneous rates.

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[19] Acknowledgments. We are grateful to Mr. D. FRASER and the Department of Obstetrics and Gynaecology for encouragement and supply of the surgical specimens and to Dr. J. HAMER for his guidance. We would also like to thank J. COBB for technical assistance.
[20] Dr. COLTART is the Mary Schlarieb Research Scholar of the University of London 1970/71 and the Cooper and Coventson Research Scholar of St. Bartholomew's Hospital 1970/71.

Functional Reinnervation of Cat Sympathetic Ganglia with Splenic Nerve Homografts

Synaptic transmission in sympathetic ganglia is mediated by acetylcholine[1]. The adrenergic structures in sympathetic ganglia might participate by inhibiting this cholinergic transmission[2]. Several findings supporting such a role[3] have led to the theory of an adrenergic modulating system in ganglia[4]. Additional support for this theory is the presence of noradrenaline (NA) containing nerve terminals in sympathetic ganglia; these have been postulated to be derived from interneurons[5] or to represent adrenergic collaterals[6]. This morphological arrangement precludes the possibility of selectively stimulating these fibers outside the ganglion.

The intraganglionic effects of the adrenergic axons could be analyzed more directly by surgically providing a sympathetic ganglion with a direct input of NA containing fibers. By cutting the sympathetic chain of the cat between the lumbar$_3$ (L_3) and lumbar$_4$ (L_4) ganglion and suturing the splenic nerve to the proximal stump (L_4) we have surgically produced such a sympathetic ganglion. This report describes the electrophysiological results obtained by directly stimulating the noradrenaline containing fibers.

One year after the surgery the L_4 ganglion together with its splenic nerve attachment and distal segment of