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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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

the sympathetic chain was isolated and placed in an in vitro test system[7]. The splenic supply to the ganglion was stimulated at a site proximal to the suture and the response elicited by supramaximal stimulation was recorded from the ganglion itself or from the sympathetic chain distal to the ganglion, using a technique similar to that described by PASCOE[8].

Recording of the compound action potential obtained in normal unoperated preparations confirmed the findings of OBRADOR and ODORIZ[9] that some preganglionic fibers synapse in the ganglion while other fibers pass directly through. In approximately 60% of the successfully reinnervated ganglia (Table), the pattern of the electrical response recorded was similar both to normal control ganglia and to ganglia where the sympathetic chain between L_3 and L_4 was cut and resutured (Figure 1).

Lumbar ganglia of the cat have been shown to have relatively few NA containing fibers under normal conditions[6], while the splenic nerve is known to be composed of nonmyelinated sympathetic fibers[10], containing a high concentration of NA[11]. There is also, however, a low concentration of acetylcholine in the splenic nerve[12]. Preliminary histochemical examinations of the in vitro stimulated ganglia using the Falck-Hillarp fluorescence technique[13,14] demonstrated numerous NA containing axons bridging over the site of anastomosis and an increased number of NA axons in the pre- and postganglionic trunks (distal sympathetic chain) of the splenic reinnervated ganglia. Furthermore, the proportion of fluorescent fibers in the preganglionic trunk as compared to the postganglionic trunk was increased in the splenic reinnervated ganglia.

The reinnervated ganglia could be classified into two groups on the basis of their electrical response. The first group consists of those ganglia showing a 'normal'

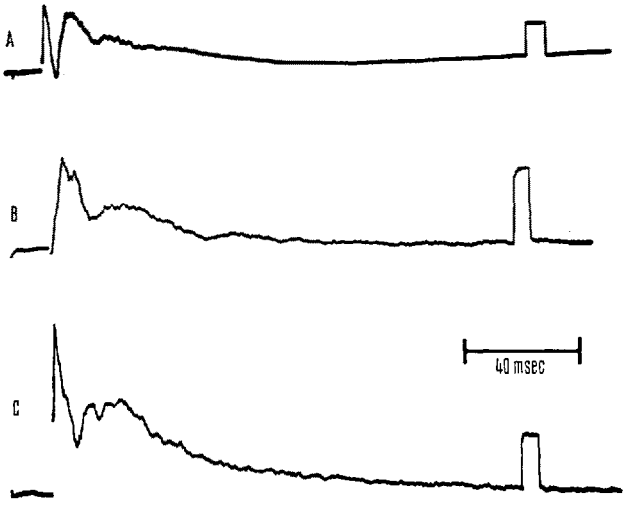

Fig. 1. Compound action potential of ganglia activated by orthodromic stimulation. A) Splenic reinnervated ganglion; B) self-reinnervated ganglion; and C) control. Calibration: A) 500 μV; B) 200 μV; C) 500 μV.

Summary of splenic-lumbar ganglion anastomoses

	No. of anastomoses	Functional anastomoses (%)
Total	28	
Not functional	7	
Group I (cholinergic)	13	62.0
Group II (monoaminergic)	5	23.8
Not defined (?)	3	14.2

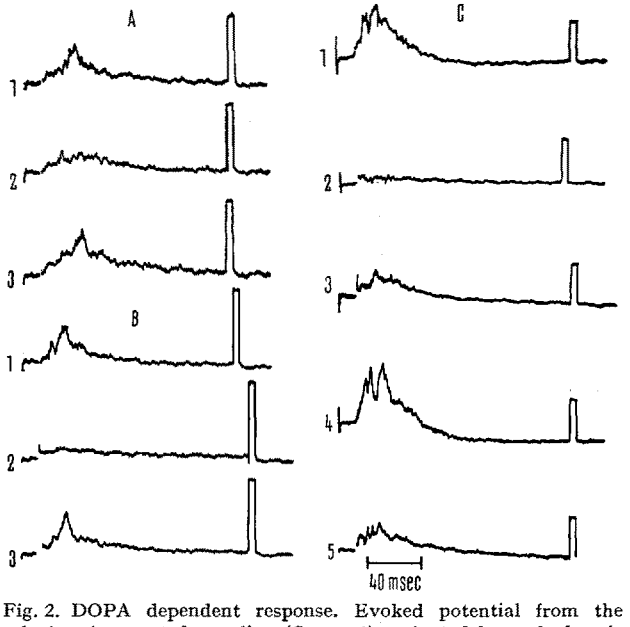

Fig. 2. DOPA dependent response. Evoked potential from the splenic reinnervated ganglion (Group 2) activated by orthodromic stimulation of the splenic nerve. 2 ganglia were used. A–B) Represents 1 ganglion and C) 1 second ganglion.
A-1. Control response, 2 h after isolation. 2. Following 60 min repetitive stimulation and successive 70 min rest. 3. 30 min after the addition of 25 ng/ml DOPA to the bath.
B-1. Control response, 7 h after isolation, twice restored by DOPA (Aa–Ac). 2. Following 30 min repetitive stimulation and successive 30 min rest. 3. 10 min after the addition of 125 ng/ml DOPA to the bath.
C-1. Control response, 7 h after isolation, twice restored by DOPA. 2. Following 60 min repetitive stimulation and successive 80 min. rest. 3. 3 h after the addition of 125 ng/ml DOPA to the bath. 4. 15 min after the addition of 125 ng/ml DOPA and 0.05 m*M* pp. 5. 10 min after the addition of hexamethonium bromide 70 μg/ml. Calibration: 200 μV.

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response to electrical stimulation. The second group had the following characteristics: 1. The voltage of the response was lower than the first group; 2. the time course of the rise of the evoked potential was slower than that of controls; 3. the voltage of the response was unstable and was made up of a number of peaks, the pattern of which was irregular; and 4. the amplitude of the response was labile and showed marked fatigue to repetitive stimulation.

Ganglia were subsequently subjected to pharmacological studies to establish whether or not 2 groups of ganglia could also be differentiated on the basis of their synaptic pharmacology. Some of these ganglia had the 4 characteristics of the novel electrical response listed above. In the latter preparations, repetitive stimulation (0.3 msec, 20 Hz, supramaximal, 30–60 min) of the splenic nerve abolished the ganglionic and the postganglionic responses to electrical stimulation and no restoration occurred with prolonged rest (up to 3 h). Complete restoration was accomplished, however, within 30 min after the addition of 25 ng/ml DL-3,4-dihydroxyphenylalanine (DOPA) to the bath medium (Figure 2A). Following a second stimulation period and rest period, complete restoration was seen in less than 8 min after the addition of a higher concentration of DOPA (125 ng/ml) (Figure 2B). DOPA by itself was only capable of restoring the abolished ganglionic response 2 or 3 times. Ganglionic transmission was thereafter restored only by the combined addition of DOPA and pyridoxal phosphate (pp, 0.05 m*M*) to the bath (Figure 2C). This DOPA-dependent ganglionic transmission was blocked by the ganglionic blocker, hexamethonium bromide (70 µg/ml) (Figure 2C).

In one of these 5 splenic reinnervated ganglia, no response was detected 2 h after isolation (normal recovery period). The addition of pp and DOPA to the bath yielded a ganglionic response to electrical stimulation within 10 min.

As a control, normal L_4 ganglia and self-reinnervated L_4 ganglia (reinnervated with its own preganglionic fibers from the sympathetic chain) were subjected to the same treatment. Following stimulation and recovery the ganglionic response showed at most a slight decrease in amplitude. Addition of pp alone caused a slight increase in the amplitude of the response, while DOPA alone or in combination with pp caused a decrease. Likewise, the compound action potentials of an isolated normal splenic nerve, showed a decreased amplitude after the addition of DOPA to the bath.

The data presented here suggest that when the L_4 sympathetic ganglion is reinnervated with fibers from the splenic nerve, new excitatory synapses are formed. In about half of the cases, the reinnervated ganglion shows normal electrophysiological responses, probably due to the presence of cholinergic fibers. The novel electrophysiological response of the other reinnervated ganglia and the dependence of their synaptic activation on the presence of DOPA and pp suggests that these sympathetic neurons may now have 'adrenergic' preganglionic fibers. Until further studies are done, however, a cholinergic component cannot be ruled out nor can the hexamethonium sensitivity be explicitly interpreted as an action of the monoaminergic transmitter on a nicotinic receptor. The sympathetic homoneurograft may provide a unique preparation for the analysis of sympathetic physiology and for synaptic plasticity[15].

Zusammenfassung. Sympathische Ganglien, wenn mit Fasern des *N. splenicus* reinnerviert, bilden 2 pharmakologisch differente Synapsengruppen: normale, vom cholinergischen Typ und ca. 20% exitatorische vom «monoaminergischen» Typ, wenn sie von adrenergen Fasern reinnerviert wurden.

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Synchronous Growth of *Polytomella agilis*

The growth characteristics of the flagellate protozoan, *Polytomella agilis*, have been studied in batch culture[1]. Under these conditions, cell volume, protein and carbohydrate decline during the logarithmic phase of growth. Thus, in spite of the constant rate of cell multiplication the growth of the population is unbalanced. From these observations, it is difficult to come to any meaningful conclusions about the description or regulation of growth of single cells in the population. Therefore, cultures of this organism were synchronized by means of a repetitive temperature cycle, using the rationale of James[2].

Cells were grown in 500 ml Erlenmeyer flasks in a complex medium consisting of 0.2% (w/v) tryptone, 0.1% (w/v) yeast extract and 0.2% (w/v) sodium acetate. Cell counts were made periodically using a hemocytometer. It was found that a cycle of 22 h at 9 °C followed by 2 h at 25 °C resulted in the complete doubling of the population during the warm period. This is in contrast to the mean generation times of this organism in batch cultures grown at these temperatures, which are 32 and 4.7 h respectively.

Figure 1 illustrates the synchronous growth of a population maintained on this repetitive temperature cycle. In addition to the change in cell number, the change in the division index (the proportion of cells undergoing division at any time) from 0.2% during the cold period to between 0.8% and 1.5% of the population

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