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# Contractile Behaviour of the Human Pyelo-Ureteral Musculature

## II. Repetitive Electrical Stimulation Effects

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**Summary.** In normal proximal and distal pyeloureteral human strips and in pathological reflux or obstructive segments the influence of repetitive electrical stimulation at frequencies between 0.01 and 200 Hz on their contractile behaviour was studied. Between 0.01 and 1 Hz baseline tone and maximum amplitude of contraction were dependent on stimulation frequencies, but some strips showed an irregular response pattern. At frequencies between 1 and 200 Hz half of the strips showed a typical "on" and "off" contraction (at the beginning and end of the stimulus period respectively) separated by a plateau. We consider the "off" reaction to be a response to an ionic displacement at the end of the stimulus. Spontaneous activity was mostly observed in Tyrode solution and in calix and pyelum. Transmural nerve stimulation did not change the contraction patterns.

**Key words:** Repetitive electrical stimulation, Spontaneous activity, Normal and pathological ureteric strips, Similarities heart/pyeloureteral muscle.

## Introduction

In a previous paper (Part I of this communication) we showed that ureteric and cardiac muscle have many contractile similarities: an after rest activation, a long refractory period, and a frequency/force relationship. Since the heart muscle is known to adapt its contractile force to the frequency of repetitive stimulations, it seems logical to study the reaction of the pyeloureteral system to repetitive electrical stimuli. By changing the parameters of the stimuli we hoped to compare the influence of transmural nerve stimulation and direct muscle stimulation. Only human normal and pathological strips were used in order to exclude species variation.

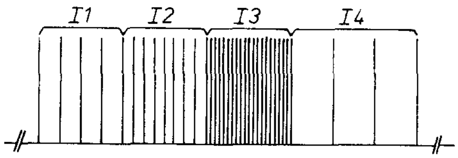


**Fig. 1.** Schemes of electrical stimulation

## Methods and Material

Normal and pathological upper urinary tract segments, removed because of kidney or bladder malignancy, urinary obstruction or reflux, were investigated. 4 strips from the same segment were tested simultaneously. After dissecting connective adventitial tissue, the segments were cut as longitudinal, transverse, helical or closed ring strips of about 15 mm length and 2.5 mm width. During preparation they were continuously immersed in warmed and oxygenated Hepes or Krebs-Tyrode solution. Then the segments were mounted in 10 ml thermostable warmed jacketed organ baths, filled with a solution of following millimolar composition:

a) Hepes solution: NaCl 131.5; $CaCl_2$ 2.5; $MgCl_2$ 1.2; KCl 5.9; Hepes 11.5; glucose 11.5.

b) Krebs-Tyrode: NaCl 136.9; $CaCl_2$ 2.5; $MgCl_2$ 0.5; KCl 5.9; $NaHCO_3$ 11.9; $NaH_2PO_4$ 0.3 and glucose 11.5.

The solutions were aerated with 99.6% $O_2$/0.005% $CO_2$. A pH of 7.35 was obtained by adding molar NaOH or HCl. The strip was attached to a fixed hook in the organ bath, while its other end was connected to a home made isometric force transducer. The characteristics of the transducer have been published elsewhere. During an adaptation period of at least 45 min the strips were equilibrated under an initial tension of 1 g [1]. Spontaneous activity was noted.

Electrical field stimulation was delivered by means of rectangular shaped pulses via 2 platinum wires located 8 mm apart at either site of the tissue. Pulse trains between 0.01 and 1 Hz had a duration of 200 ms duration; between 1 and 200 Hz (high frequency stimulation) the pulse trains had a 0.5 to 800 ms duration. All pulses were supramaximal (Fig. 1).

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**Table 1.** Spontaneous active strips: percentage of tested strips

| | Calyx | | Pyelum | | Prox ureter | | Mid ureter | | Dist ureter | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | n | % | n | % | n | % | n | % | n | % | n |
| Normal | 35 | 20 | 19 | 27 | 16 | 19 | 9 | 35 | 11 | 36 | 16 | 137 |
| Obstr. | 17 | 6 | 20 | 94 | 0 | 7 | 9 | 23 | 7 | 29 | 15 | 159 |
| Refl. | – | 0 | 30 | 10 | 0 | 12 | 0 | 16 | 21 | 38 | 14 | 76 |
| Inf. | 0 | 2 | 13 | 16 | 0 | 8 | 0 | 7 | – | 0 | 6 | 33 |
| Total | 29 | 28 | 20 | 147 | 7 | 46 | 6 | 81 | 14 | 103 | 15 | 405 |

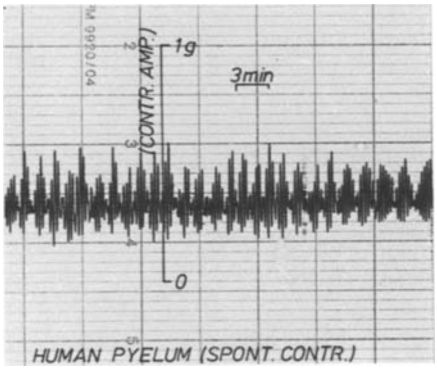


**Fig. 2.** Burst and irregular spontaneous activity

Tension changes were recorded, stored and processed by the same computerized equipment and techniques as described in the previous publication (Part I).

## Results

### *1. Spontaneous Activity*

All the muscle strips were checked for spontaneous contractions. Contractions were interpreted as spontaneous if they occurred under normal circumstances, i.e. bath solution containing 2.5 mM $CaCl_2$, t = 37 °C, without addition of drugs and in the absence of electrical stimulation. Out of 405 tested strips, 59 showed spontaneous contractions (= 15%). A distribution of active strips, given as a percentage of the tested strips based on origin and pathology, is given in Table 1. Calyces, pyelum and distal ureteric segments were more frequently active than proximal and mid ureteric specimens. Spontaneous activity may be regular, irregular, a regular burst of contractions, or totally irregular. In Tyrode solution 37% of the strips were spontaneously active, against only 6% in Hepes solution. Fig. 2 shows an example of a spontaneously active pyelum strip.

### *2. Influence of Frequency of Pulse Trains (Between 1 and 0.01 Hz)*

Chains of pulse trains of variable pulse intervals (Fig. 1) were applied on 17 ureteric segments. No statistically significant differences between several segments were found. Two types of reactions were observed.

In some experiments (Fig. 3) all stimuli of the same frequency were followed by a contraction of nearly the same amplitude. The latter decreases with increasing frequency, while the baseline progressively increased by incomplete relaxations between successive stimulations, so that the difference between maximum amplitude and baseline decreased (Fig. 4). Note that in Fig. 3 the return to low frequency stimulation provoked much larger amplitudes than were obtained for the same frequency in the beginning of the train pulses, and that the amplitudes of the first recovery contractions progressively increased. On the contrary, note that at a pulse interval of 4 and 2 s the amplitudes progressively diminished, before reaching a plateau value.

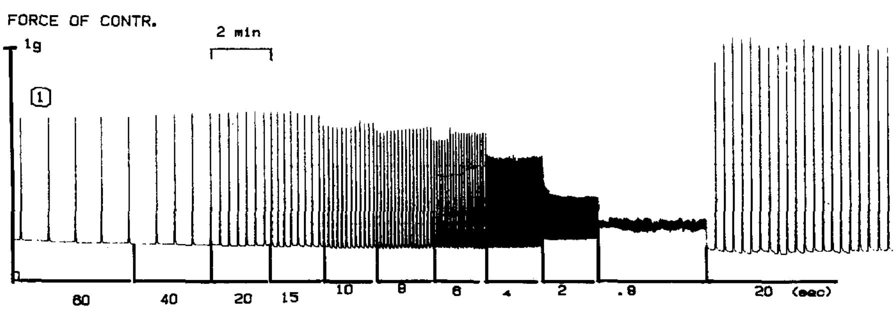


**Fig. 3.** Example of response of a normal distal transverse ureter strip to pulse trains of decreasing interval

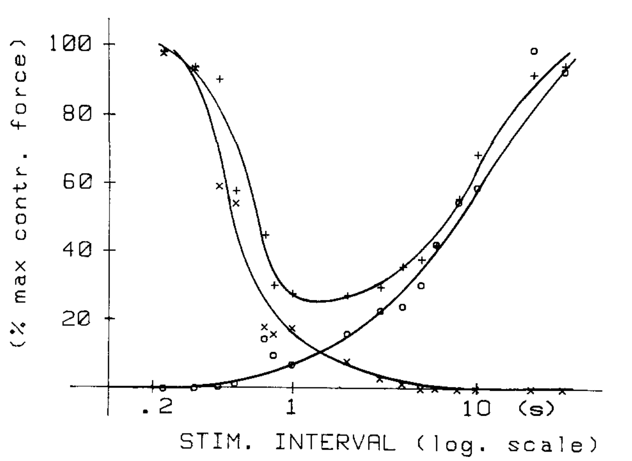


**Fig. 4.** Contraction force versus pulse interval of trains of stimuli. + = contraction amplitude; x = base line; o = contraction amplitude minus base line

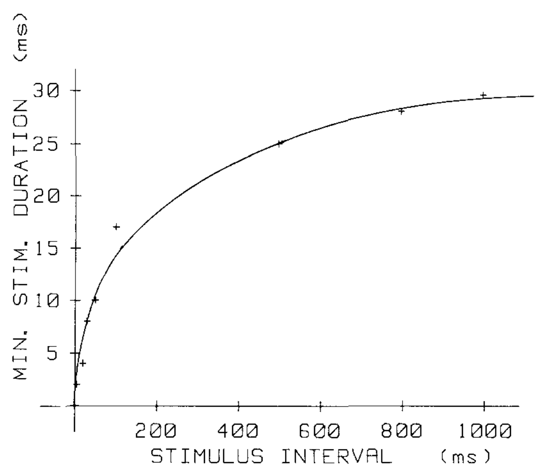


**Fig. 6.** Minimum pulse duration versus pulse interval

In other experiments an irregular reaction was observed: contraction amplitudes were of alternating size, totally irregular, and some pulses remained without a visible response (Fig. 5); in this group the baseline tone never increased.

### *3. High Frequency Stimulation (1 to 200 Hz)*

If high frequency trains (1–200 Hz) with a pulse duration of 0.5 to 800 ms were applied, the minimum pulse duration necessary to provoke a response was a function of the stimulus interval (Fig. 6). A single pulse below chronaxie value never provoked a response. The minimum train duration (2–12 s) necessary to obtain a reaction depended on both pulse duration and on frequency; only "on" responses (see below) were observed under these threshold circumstances.

In 14 out of 21 ureteral segments stimulated with high frequency pulse trains between 3 and 10 s duration, a three-phasic response was observed (Fig. 7).

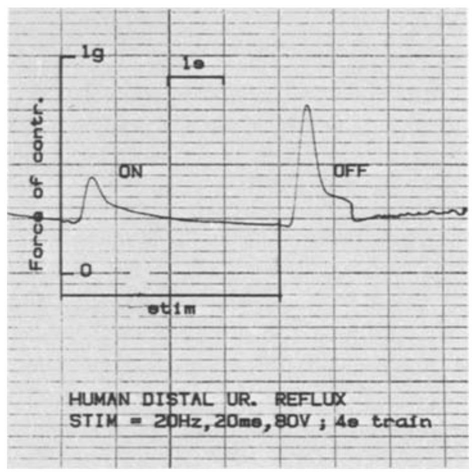


**Fig. 7.** On-off contractions in a human distal reflux ureteric strip

a. An initial "on" peak started at a mean latency period of 0.42 ± 0.02 s ($n = 80$) (X ± SEM) after the onset of the stimulus train. The longer the preceeding resting period, the higher the amplitude of the peak (Fig. 8). The time to peak force averaged 2.08 ± 0.02 s ($n = 80$) and was independent of stimulus parameters.

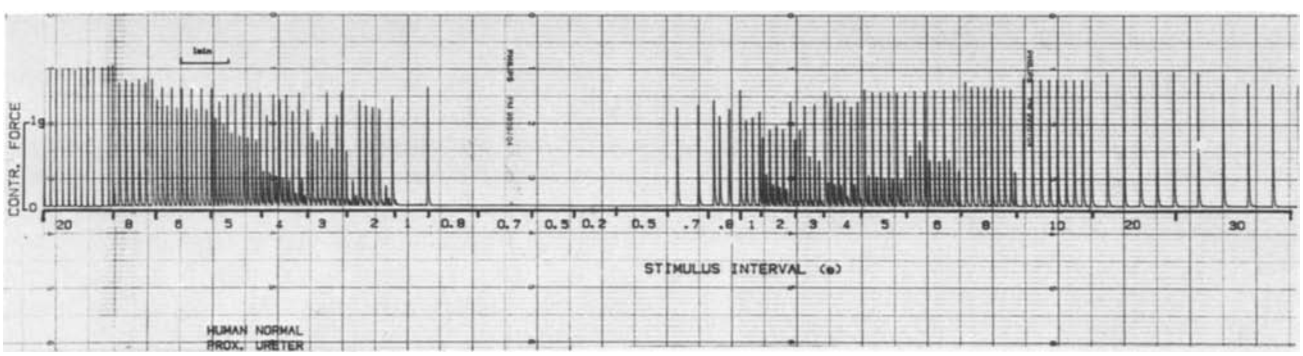


**Fig. 5.** Example of irregular response of a normal proximal ureter to pulse trains of decreasing and increasing intervals

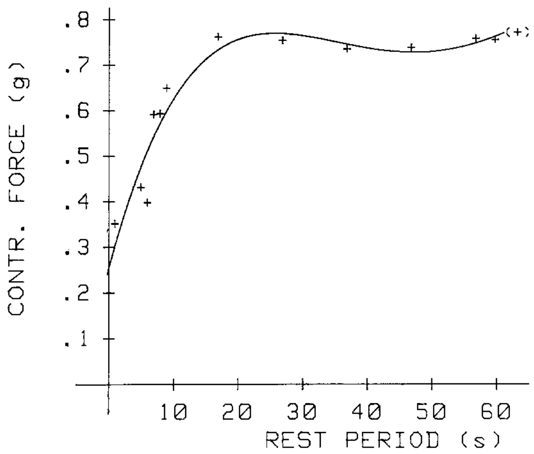


**Fig. 8.** Contraction force of the "on" contraction as a function of the preceding rest period

b. In 85% of the tests the "on" peak was followed by a plateau of lower amplitude which persisted throughout the train course. The test plateau amplitude was directly correlated to the stimulus frequency and duration.

c. The "off" contraction occurred after the stimulus train was interrupted. It started after $1.09 \pm 0.03$ ($n = 67$) ($X \pm SEM$) seconds beyond the last pulse of the train and reached its maximal amplitude $2.99 \pm 0.02$ ($n = 67$) s later (= rise time). The amplitude of this after-contraction but not its time to peak was related to the train duration, frequency and pulse duration, the rise time was not significantly different from that of the "on" contraction. The amplitude of the "off" contraction was sometimes 3 times that of the "on" contraction.

The "on-off" contraction was influenced by the direction of the stimulus current: reversal of polarity increased the "on" contraction in 50% and decreased it in 33% of the cases, while the "off" contraction increased in 85% of stimuli.

We measured the pH of the bathing solution after high frequency stimulation: at the anode the pH was $6.97 \pm 0.12$ ($X \pm SEM$) and at the cathode $7.76 \pm 0.10$ ($X \pm SEM$) which was a highly significant difference ($n = 8$, $t = 4.90$, $p < 0.001$).

### *4. Nerve Stimulation*

Pulses at a frequency of 10 Hz and a duration of 0.5 ms are considered as specific nerve stimulators. In 69 strips such pulses were superimposed through a separate stimulator on the muscle stimulation (1 pulse per 40 s, 200 ms, 80 V). No alteration of contractile force or shape, contraction amplitude or frequency of spontaneous contractions was observed.

## Discussion

We are surprised that only one seventh of the examined specimens showed spontaneous activity. Although most spontaneous activity arose from calyces, pyelum and distal ureter, it was less regular and constant than in guinea pigs, where we always found spontaneous regular contractions at a frequency decreasing from calyx to pyeloureteral junction [7]. Longrigg [5] found only immediate and regular rhythmical contractions in human minor calyx preparations. In contrast to Malin et al. [6], we found spontaneous activity in circular segments, especially from the calyces, pelvis and distal ureteric segments. An explanation for the low incidence of spontaneous activity may be that we mostly used Hepes solutions instead of Krebs Tyrode; in the latter spontaneous activity was observed more frequently. Deane [3] noted that Ringer and low temperature decrease spontaneous activity. It is interesting to note that we observed more often irregular spontaneous activity in specimens in bad condition (either dilated or which had suffered from prolonged ischemia). In this situation cell anoxia may be accompanied by increased $Ca^{++}$ entry, provoking the spontaneous contractility; the widespread use of $Ca^{++}$ entry blockers in myocardial lesions rely on an analogous mechanism. We found that proximal and mid ureteric specimens have little spontaneous activity, in agreement with other authors. We cannot explain why some ureters follow the stimulation frequency (Fig. 3) and others do not (Fig. 5). The same is true for the heart muscle. Irregular activity is most likely due to Wenckebach periods Weiss [8]: the latency period of the response to successive stimuli increases while its amplitude decreases until a stimulus falls in the absolute refractory period following the preceding contraction, so that no response can be triggered. In the ureters which follow the electrical stimuli a changing stimulus frequency may be followed by either progressively increasing or progressively decreasing amplitudes of the contractions: such phenomena also exist in the heart, where they are termed as a positive or negative staircase.

The "on" reaction of a high frequency stimulation is totally different from a single shock response. Its latency period is about double and its rise time about 1.5 times larger. The higher the frequency and the shorter the duration of pulses, the more pulses are required to provoke a reaction: a threshold level of depolarisation must be obtained.

The "off" reaction observed at high frequency stimulation has already been described by Weiss [9]; they could not find any explanation. Firstly we observed that only 2 out of 3 strips showed the phenomenon; secondly that it is largely influenced by reversal of stimulus polarity, and finally that its amplitude can be very large as compared to the "on" reaction, even if the stimulus is applied after a rest period. Therefore we believe that the "off" reaction is due to a new chemical stimulus, provoked by a polarization phenomenon within the bath fluid and/or by the tissue itself: during stimulation ions of opposite polarity accumulate around the cathode or anode of the stimulus electrodes, thus provoking a capacity field which discharges when the electrical pulses are interrupted; the larger and stronger the electrical field, the stronger the ionic displacements and thus the stronger the chemical stimulus occurring at the withdrawal of the electrical stimulus. Analogous reactions may occur in the tissue itself. It therefore should be considered as an artefact. Furthermore there is in fact no physiological correlation with such high frequency stimulation.

It is generally accepted that myogenic ureteral activity is modulated by nervous influences [2]. It is therefore interesting to know in what manner stimulation of intramural nerves influences this activity. Transmural stimulation is obtained by electrical square wave pulses of 10 Hz and 0.5 to 1 ms duration [3]. In contrast to Weiss et al. [9] who found an increase of the contraction force, we could not find any difference between trains with or without superimposed nerve stimulation. The increased activity observed by Weiss suggests a noradrenergic activity and is in accordance to NOR dosages used by del Tacca [4]; in our experiments either the environmental conditions may have been insufficient or the strips too small to obtain sufficient NOR release. However our results agree with those of Longrigg [5] who found that in man intrinsic nerve stimulation was without effect in areas distal to the minor calyces. Again species differences may play a role.

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