cannot be excluded. Sometimes, very thin (0.4 μm diameter) beaded arborizations of the nerve fibres were seen (Figure 2c), resembling, for instance the endings in the depressor tibiae muscle of the locust, stained with methylene blue by AUBER[5].

Better resolution was obtained by applying Timm's intensification method after cobalt-staining[6]. This revealed two different types of nerve-endings: a compact type in muscles M197 and M198 (Figure 2e) and a more dispersed one (Figures 2c and d) in M202 and M203 (nomenclature after[7]). Electrophysiological and ultrastructural investigations have shown that the abdominal intersegmental muscles of locusts each receive nerve endings from several axons[8–10]. However, with cobalt-staining multiple innervation could not be demonstrated as yet, although in some cases, two fibres could be seen ramifying side by side. An interesting feature is the innervation of some muscles in two distinct fields which are similar in different individuals (Figure 2b). Whether this finding has also physiological implications concerning, for instance innervation by two functionally different nerve fibres, remains to be investigated.

[5] J. AUBER, Z. Zellforsch. *51*, 705 (1960).
[6] N. M. TYRER and E. M. BELL, Brain Res. *73*, 151 (1974).
[7] F. O. ALBRECHT, *The Anatomy of the Migratory Locust* (Athlone Press, University of London 1953).
[8] N. M. TYRER, J. exp. Biol. *55*, 305 (1971).
[9] N. M. TYRER, J. exp. Biol. *55*, 315 (1971).
[10] G. W. LEWIS, P. L. MILLER and P. S. MILLS, J. exp. Biol. *59*, 149 (1973).

The Origin and Propagation of Upper Urinary Tract Contraction Waves. A New in vitro Methodology

J. A. GOSLING[7] and C. E. CONSTANTINOU[8]

Department of Surgery, Division of Urology, Stanford University School of Medicine, Stanford (California 94305, USA), and Department of Anatomy, University of Manchester, Manchester (England), 28 July 1975.

Summary. A new in vitro experimental method which has enabled direct visualization of the upper urinary tract with minimal damage to the wall of the region is described. Evidence is presented which supports the concept of an autonomous pacemaker system located proximal to the pelviureteric junction.

The present paper describes an experimental method which enables direct visualization of the region in which contraction waves originate in the upper urinary tract, namely, that part proximal to the pelviureteric junction. New data are provided about the origin and progagation characteristics of contraction waves in unicaliceal and multicaliceal systems demonstrating their species differences in pacemaker activity.

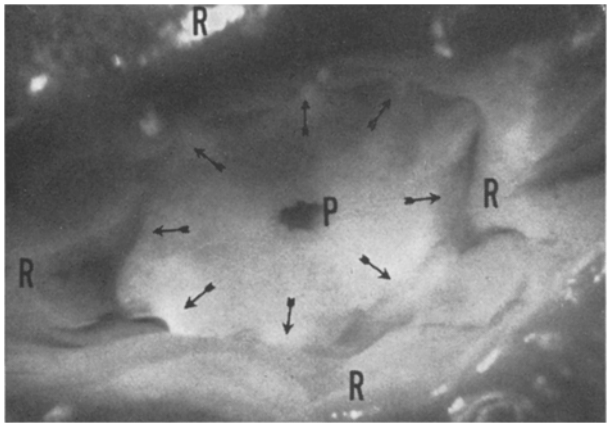
Materials and methods. The kidneys and ureters were removed in toto and the cortex was incised along its lateral border in a plane midway between the anterior and posterior surfaces from upper to lower poles, through the cortex into the outer medulla. In the dog, the medulla formed a single ridge projecting into the lumen of the ureter and the plane of the incision divided this ridge longitudinally. In the pig, each minor calix lying in the plane of the incision was identified by blunt dissection of renal medullary tissue. The interior was then partially exposed by an incision minimizing interference with the renal attachments of the minor calyces. Specimens were transferred into a tissue bath containing Krebs solution. The anterior and posterior surfaces of each kidney were retracted along the line of the original incision to allow visualization as far distally as the pelviureteric junction (Figure). The ureter was placed so that ureteric contraction waves could be easily visualized. Observations and filming were begun after a tissue stabilization.

Results and discussion. Regular spontaneous contraction waves were readily observed crossing the exposed inner surface of the upper urinary tract as soon as the preparations were examined. These rhythmical events continued throughout the period of observation (up to 3 h). Whilst the exact sites of origin and the rates and form of propagation of each wave varied according to the species, every contraction began proximal to, and always moved initially towards, the pelviureteric junction. The fact that spontaneous activity can be directly observed is evidence against those who believe that contraction waves are initiated solely by the stretching forces[1]. In the present study, distention forces were reduced since the renal pelvis was converted into an open system thereby decreasing the resting tension on the renal attachments. Thus, the present findings support the concept of spontaneously active pacemaker cells responsible for the initiation of contraction waves[2–4]. It is argued that the active pacemaker site is located at the pelviureteric junction[5]. The present observations, however, clearly indicate that in both unicaliceal and multicaliceal systems, contractions always develop proximal to the pelviureteric junction and propagate towards this region.

In the dog, regular contraction waves occurred at a rate of 13–17/min, and were seen to arise on the wall of the renal pelvis a few mm distal to the septa which attached the pelvis to the renal parenchyma (Figure). Each wave moved distally along a concentric front and approached the pelviureteric junction at 1–2 cm/sec but waves failed to propagate along the ureter. In the pig, contraction waves usually originated from circumscribed regions in the junctional area between one minor calix and the renal pelvis. Although one such region acted as the source for several contractions, this activity frequently changed to a similar region in another part of the system. Contraction waves were observed 5–9/min and each moved from its origin across the adjacent part of the renal pelvis towards the pelviureteric junction forming a crescentic wave front (1–2.5 cm/sec). At the pelviureteric junction, however, the wave frequently continued across the pelvis in a direction away from the ureter moving towards that pole of the kidney opposite to the one in which the contraction had originated. As in the dog, peristaltic waves are not observed along the pig ureter.

[1] J. LAPIDES, J. Urol. *59*, 501 (1948).
[2] J. A. GOSLING and J. S. DIXON, Br. J. Urol. *44*, 550 (1972).
[3] K. GOLENHOFEN and J. HANNAPPEL, Pflügers Arch. ges. Physiol. *341*, 257 (1973).
[4] C. E. CONSTANTINOU, Am. J. Physiol. *226*, 1413 (1974).
[5] R. M. WEISS, M. L. WAGNER and B. F. HOFFMAN, Invest. Urol. *5*, 42 (1967).

The multifocal origin of contraction waves as seen in the pig, but not in the dog, supports anatomical evidence which indicates that a number of pacemaker sites occur in multicaliceal and not in unicaliceal systems[6]. The observation that contractions which reached the pelviureteric region failed to propagate support in vivo data where the number of pelvic contractions successfully propagated along the ureter was directly related to the urine flow rate[4]. Thus at higher urine flow rate, more ureteric contractions were propagated even though renal pelvic activity remained constant. Collectively, these findings indicate a urine flow dependent mechanism in the region of the pelviureteric junction which determines whether a renal pelvic contraction will result in a propagated ureteric peristaltic wave. Since this mechanism appears to depend upon urine flow, it may be that the stretching forces exerted upon the pelviureteric junction by accumulating fluid lowers the threshold of the region. The arrival of a renal pelvic contraction can now result in a propagated contraction through the pelviureteric junction and along the ureter. Clearly, in the present experimental design, the renal pelvis has been opened and bolus formation is unlikely to occur. Thus, on the basis of the above hypothesis it is not surprizing that regular renal pelvic contraction waves failed to propagate as ureteric peristalsis.



The renal parenchyma (R) has been incised and reflected to expose the inner surface of the renal end of the upper urinary tract in the dog. Contraction waves were first identified in the regions marked by the arrows and moved as a concentric wave front towards the pelviureteric junction (P). The preparation was quiescent at the time of this illustration, in which the shape of the pelviureteric junction is clearly evident.

In conclusion, the present study has provided some preliminary observations which are of importance in an understanding genesis of peristaltic activity in the unicaliceal and multicaliceal systems. Whilst the method does have the limitations imposed upon in vitro techniques, it is hoped that its application in future investigations should provide valuable information on this important subject.

[6] J. A. Gosling and J. S. Dixon, Invest. Urol. *11*, 418 (1974).

[7] Department of Anatomy, University of Manchester, Manchester, England.

[8] Supported in part by United States Public Health Service Training Grant No. 5 TO1 AM05513.

A Simple Method for Cultivating the Early Chick Embryo in vitro[1]

K. Palén and L. Thörneby

Zoophysiological Institute, Helgonavägen 3, S-223 62 Lund (Sweden), 3 July 1975.

Summary. A method for cultivating the early chick embryo in an artificial medium has been developed that permits the determination of the stage at the onset of the treatment and the continuous observation of the embryogenesis.

Previous investigations at this institute[2] into the influence of chemicals on the embryogenesis of the chick employed two different methods for applying the chemicals: the New-method[3] and the window-method, the latter based on injection of the substance directly under the blastoderm. The New-method is, for our purpose, too slow and laborious. The blastoderm is exposed to a strong mechanical stress, and the ring is a limiting factor for the outgrowth of the area opaca. Injection of the substance under the blastoderm is more rapid, but produces an uneven material, owing to the difficulty of controlling the exact position of the injection and therefore of the concentrations of the chemicals. Malformations sometimes occur which cannot be explained in any other way than as a consequence of the mechanical stress the embryo is exposed to by an injection, no matter how carefully executed. Also the difficulty in examining the stage by this method is an obvious disadvantage when it is desirable to begin the treatment at a fixed stage. To eliminate the various difficulties inherent in the New-method and the window-method, we developed a method wherein we incubated the yolk in an artificial medium. Moreover, we needed a method that permitted 1. the determination of the stage at the onset of the treatment; 2. the continuous observation of the embryogenesis for any greater length of time; and 3. administration as well as removal of the tested chemicals at a well-defined stage.

Material and methods. All experiments were made on freshly laid White Leghorn eggs. Experiments starting with non-incubated eggs were prepared at room temperature. If later stages were required, the eggs were incubated at 37.5 ± 0.5 °C and the following treatment performed at 37.5 °C.

Dense and thin albumen was carefully homogenized, avoiding any foam formation, with a knife-homogenizator until the albumen flowed thinly. We used a modified Pannet-Compton's saline (Table) in which we dissolved any chemicals we wished to test. The egg was cleaned with 70% alcohol and the shell broken with forceps. The albumen was gently removed and the chalazae were cut off with a pair of scissors. The yolk was gently poured into an ordinary 100–150 ml beaker, containing about 80 ml of the medium. (To lessen the amount used, a test tube

[1] Kungliga Fysiografiska Sällskapet, Lund, supported this work.

[2] H. Emanuelsson and K. Palén, Wilhelm Roux' Arch. EntwMech. Org. *177*, 1 (1975).

[3] D. A. T. New, J. Embryol. exp. Morph. *3*, 326 (1955).