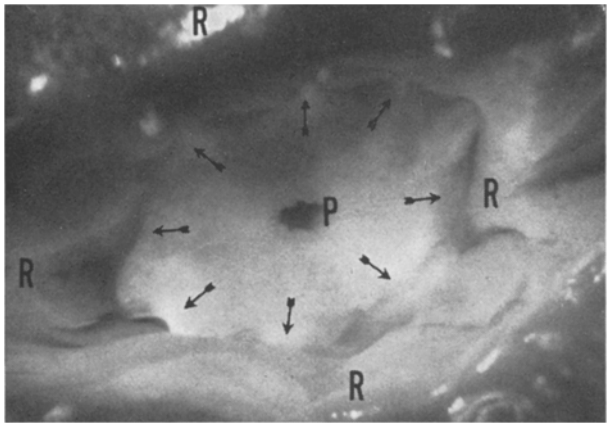
The multifocal origin of contraction waves as seen in the pig, but not in the dog, supports anatomical evidence which indicates that a number of pacemaker sites occur in multicaliceal and not in unicaliceal systems[6]. The observation that contractions which reached the pelviureteric region failed to 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.

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

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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

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with an inner diameter of about 35 mm can be chosen instead. See Figure 1.) The yolk sinks to the bottom while the blastoderm rotates upwards. Enough medium is poured out so that the yolk is about 1 mm below the surface. If the yolk is more than 3 mm below the surface, typical malformations are formed, probably owing to the lack of oxygen. The embryo grows more slowly, the brain becomes narrow, and the somites become wedgeshaped. In a stereomicroscope, the stage is determined according to HAMBURGER and HAMILTON[4] by oblique illumination of the blastoderm. The beaker is sealed with PVC-foil and a rubber band to avoid evaporation. The incubation then takes place at 37.5 ± 0.5 °C in an ordinary incubator. If the treatment is interrupted, the medium is gently sucked off, the yolk is rinsed with saline, and fresh medium is added. For treatment of not more than 48 h, the procedure is made partly sterile. Treatment over a longer period (4 days or more) makes full asepsis necessary, and the beakers are shaken at regular intervals so that the embryo has full access to the nutrient.

Results. Of 50 eggs preincubated for about 24 h (stage 3–6) and then cultivated for 24 h in vitro, as described in 'methods', 48 embryos developed normally. Experiments were also performed in sucking off the medium after 3–4 h incubation, rinsing the yolk twice in saline, and thereafter continuing the incubation for 20–24 h. Of 17 embryos treated thus, 16 developed normally. 87 blastoderms were incubated from 0 h. Of these, 95% developed normally up to 72 h, 53% reached 96 h, and in a few cases (14%) the embryos survived up to 5 days (Figure 2).

We have found that a 45% dilution of the albumen causes an abnormally large intake of liquid from the albumen, resulting in the vitelline membrane bursting in a circular line a few mm outside the sinus terminalis. This almost completely separates the yolk from the embryo and its vitelline blood vessels, which are surrounded by the incubation medium. This occurs after about 4 days incubation. From the onset of this separation, the embryo does not survive more than 30 h because of a disturbed circulation.

Discussion. The simplest way of culturing the embryo outside the eggshell consists of pouring the entire egg contents into a suitable container and providing this with a nonpermeable foil to prevent evaporation[5–15]. SCHMIDT[16], in an experiment in which he substituted part of the albumen with saline, proved the albumen to be necessary for starting the development of the embryo. In a medium consisting of $^2/_3$–$^3/_4$ albumen, the embryos (explanted at 1–2 days preincubation) developed for 4–6 days. The growth-promoting effect was still preserved in albumen diluted 20-fold. He also proved that, if the eggs were preincubated for 2 or 3 h before transferring them to a medium without albumen, the embryo developed normally although very slowly. Omitting the preincubation, the development would not start.

For our purpose, a medium composed of $^2/_3$–$^3/_4$ albumen proved too dense. The yolk floats up to the surface and exposes the blastoderm to a surface evaporation and an unfavourable distribution of the tested chemicals; thus it is necessary to shake the beakers every hour. A medium containing 45% of albumen has a density equivalent to that of the yolk, which maintains its natural spherical shape. A high saline content of the medium also makes it possible to use substances that must be warmed to be dissolved. The large percentage of albumen also makes aseptic conditions less important. The ease with which the stage can be determined, the continuous observations of the developing embryo, and the possibility of treatment at short intervals, together with the high reproducibility, makes the method very useful for studying the differentiation of the early chick embryo.

The modified Pannett-Compton saline, without buffer

NaCl	12.11 g
KCl	1.55 g
$CaCl_2 \times 2\,H_2O$	1.02 g
$MgCl_2 \times 2\,H_2O$	1.27 g
Aq. dest.	100 ml

This solution is autoclaved. For use, take 4 ml and add 96 ml aq. dest.

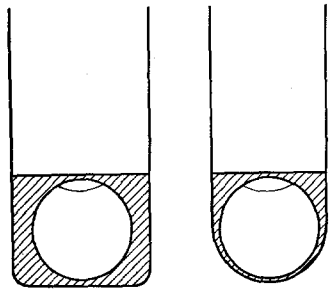

Fig. 1. The beaker and test tube with the yolk as described in the text.

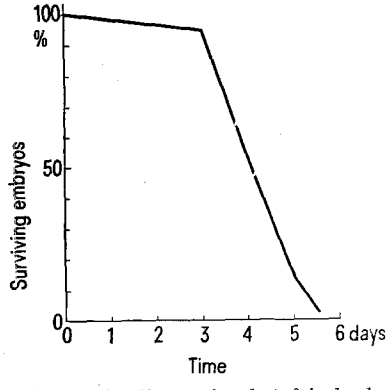

Fig. 2. Survival curve for 87 eggs incubated in beakers containing 45% albumen and 55% saline.

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