An electrosurgical technique for the production of localized tissue ablations in the early chick embryo

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SUMMARY

The passage of low-frequency alternating current was found superior to other methods considered for the production of small, discrete, electrolytic ablations in young chick embryos.

Active electrodes of tungsten metal less than 5 μm in diameter were prepared by controlled electrolytic corrosion. These gave reproducible, discrete foci of destruction of the required size, with currents less than 2 mA. The identification of destroyed tissue areas was immediately apparent under the operating microscope and confirmed histologically. Preliminary studies on bilateral extirpation of the ultimobranchial primordia show the absence of the ultimobranchial bodies 6 days after destruction of the primordia at 96 h of incubation.

INTRODUCTION

An electrosurgical technique has been developed for the selective ablation in the embryo of the primordia of one, or both, of the endocrine glands which are known, in the adult, to be concerned with calcium homeostasis - the parathyroids and the ultimobranchial body.

Previous experimental studies have shown that in the sheep (Scothorne, 1964), in the chick (McFarlane, 1965) and in the guinea-pig (Graham & Scothorne, 1971) the parathyroid glands in culture are capable of secretory activity at a very early stage in embryonic life. Stoeckel & Porte (1969) have put forward e.m. evidence of the secretory capacity of the ultimobranchial body of the 11-day chick embryo.

Little is known, however, of the respective roles of parathyroids and ultimobranchial bodies in calcium homeostasis in the intact embryo and the application of one of the classical methods of endocrinology in the adult - ablation - has awaited the development of a sufficiently refined technique which satisfies the following requirements:

(i) Ablation, in different embryos, of the primordia of one, two, three, or four parathyroid glands, destroying enough of the pouch endoderm to preclude regeneration, but with minimal damage to the immediately adjacent thymic primordia.

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(ii) Unilateral and bilateral ablation of the ultimobranchial primordium, without damage to the closely associated parathyroid IV.

(iii) Absence of damage to the closely adjacent aortic arch arteries.

To satisfy these requirements, the volume of tissue destroyed must be very small and, ideally, no effect should be produced on tissues outside the focal lesion.

After appraisal of a variety of possible methods (see Discussion) a method of using low-frequency alternating current was adopted and developed.

**MATERIALS AND METHODS**

*The apparatus*

Alternating current was obtained at 50 c/s from the mains supply and fed through a screening transformer which stepped the voltage down from 250 to 50 V. A variable output voltage range of between 0 and 50 V was obtained subsequently from a variable transformer. The output current was monitored by reading the voltage across a fixed series resistance of 1 kΩ with a millivoltmeter. The circuit was completed by the electrodes and the egg.

![Circuit diagram of low-frequency a.c. electrosurgical unit.](image)

The current was applied to the embryo by means of appropriate electrodes. The active electrode was a fine needle of tungsten metal. A length of tungsten wire, 30 s.w.g., was straightened by stretching the wire whilst it was being heated in a coal-gas flame. This was then cut into 4 cm lengths and each length was mounted in a 21 g hypodermic needle. The terminal 0.5 mm of wire was then reduced to a diameter of less than 10 μm by a process of controlled electrolytic corrosion, modified after the method of Hubel (1957). The tip of the wire tapered...
to a point of less than 2 μm, and the electrode was then insulated, by dipping with a surface-coating resin (Araldite Epoxy Resin for Surface Coating, PZ 985 + HZ 985).

The dispersive electrode consisted of a silver wire coated with silver chloride immersed in a bath of chick Ringer’s solution, the composition of which was given by New (1966). The whole bath of Ringer’s solution acted as the dispersive electrode.

The embryos

The parathyroids and ultimobranchial body begin to differentiate from the pharyngeal endoderm on the fifth day of incubation. Schrier & Hamilton (1952) confirmed experimentally the work of Verdun (1898), who described the origin of the chick parathyroids from the ventral parts of the 3rd and 4th pouches.

The ultimobranchial body was described by Rabl (1907) as arising in the duck from the caudal end of the primitive pharynx, as part of a ‘caudal pharyngeal complex’, in common initially with the 4th and 5th pouches. This view was confirmed for the chick by Dudley (1942) and by Nagy & Swartz (1966). It might appear best to attempt destruction of these primordia on or after the fifth day of incubation. Unfortunately, by this time the developing operculum has partly covered the third and fourth external pharyngeal grooves. The embryo must therefore be operated on during the fourth day of incubation.
The embryos used were $F_2$ hybrids (RIR-WL x WL). Eggs were incubated for 3 days in a Westernette incubator at 99.5 ± 0.5 °F and thereafter in a Hearson static incubator maintained at 101.5 ± 0.5 °F. At 84 h of incubation the eggs were candled and opened by the usual method (Harkmark & Graham, 1951).

Embryos were operated upon at 95-97 h of incubation. The egg was half immersed in the bath of Ringer's solution which constituted the dispersive electrode (Fig. 2). Under a dissecting microscope the embryonic membranes were ruptured with glass needles and the embryo exposed.

The sterilized active electrode was then applied to the selected target, using a Singer-Barer low-power micromanipulator. For accurate positioning of the electrode, easily visible external landmarks are needed. The pouches themselves and their closing membranes are not always clearly visible. As a guide, a composite drawing (Fig. 3) was made, based on a wax construction of the caudal pharyngeal region and on indian ink injections of the aortic arch vessels. These vessels, easily visible in the living embryo, were then used as landmarks.

With the electrode in position, a low-frequency current of less than 2 mA was passed. The voltage drop across the fixed resistance, indicating the current flowing in the circuit, varied from egg to egg. The energy produced (energy = $I^2R = IV$) was kept constant from one operation to the next by varying the time interval for which current passed ($IV \times$ time).

The following ablations were attempted:

(i) Unilateral ablation of parathyroid 3 and 4, lesions being made at the ventral ends of pouches 3 and 4 of the right side, which is usually uppermost and therefore more accessible.
(ii) Bilateral ablation of parathyroids. This involved turning the embryo, to expose its left side, after making the right-sided ablations.

(iii) Unilateral ablation of the ultimobranchial body.

(iv) Bilateral ablation of ultimobranchial body. Turning the embryo was not necessary, as it was found possible to insert the electrode through the right fourth pharyngeal cleft and involve both the right and left body at one insertion.

Fig. 4. A, Ultimobranchial region in normal 96 h chicks; p3, third pharyngeal pouch; p4, fourth pharyngeal pouch; u.b., ultimobranchial body; 4, fourth aortic arch; 6, sixth aortic arch. ×120. B, Bilateral ultimobranchialectomy; complete absence of both bodies 4 h after operation. 3, third aortic arch; 4, fourth aortic arch; p3, third pharyngeal pouch. ×120.
RESULTS

Many experimental trials have established the following characteristics of the method:

(i) The destructive lesion is electrolytic in nature, as evidenced by the continued corrosion of the electrode when current is passed.

(ii) The active electrode does not adhere to the tissues at the site of the lesion, indicating that both chemical coagulation and liquefaction occurs.

(iii) The size of lesion produced is proportional to the energy supplied at the active electrode. Predictable and, therefore, repeatable lesions can be produced by appropriate adjustment of the energy at the electrode.

(iv) Operative mortality and morbidity were high during the preliminary experiments designed to assess the capabilities of the apparatus. Once the technique had been perfected the rate of survival improved. Out of an experimental batch of 12 eggs, two or three embryos die at operation as a result of

For unilateral ablation of the primordium of parathyroid 3 less than 0.2 J was required; for bilateral ablation of the ultimobranchial bodies about 0.5 J.

Fig. 5. A, Unilateral parathyroidectomy; destruction of ventral portions of both pouch 3 and 4 on the right side. Four hours after operation. p3, Position of third pouch, tissue destruction is complete. Liquefaction has occurred; p4, position of fourth pouch, damage severe. Necrotic tissue and debris can be seen; u.b., intact ultimobranchial body; 4 and 6, appropriate arch vessels. ×240. B, Unilateral ultimobronchialcystectomy, 6 h after operation. Right ultimobranchial body destroyed. u.b., Necrotic tissue and blood cells in position of ultimobranchial body; p4, fourth pharyngeal pouch, medial aspect is involved in lesion; 4 and 6, appropriate arch vessels. ×240.
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misplaced lesions or involvement of an arch vessel. Of the survivors, one further embryo may die in the first hour. Such post-operative deaths are normally caused by slow haemorrhage following minor damage to a vessel at operation. In primary experiments, where survival has been allowed for 6 days following operation, there is an occasional death in the first 24 h. Those embryos which survive this period normally survive until fixed.

(v) Histological appearances of some embryos allowed to survive for 3–6 h after operation are shown in Figs. 4 and 5.

(vi) Despite the apparently extensive destruction of tissue seen in sections 3 h after bilateral ablation of ultimobranchial bodies a few embryos have survived for 6 days after operation, and serial sections have shown the complete absence of the ultimobranchial body, without associated damage to other pharyngeal derivatives.

DISCUSSION

1. Advantages of low-frequency alternating current lesions

Various properties of electrical currents have been utilized for the production of destructive lesions. Tissue destruction may be brought about by:

(i) Thermal coagulation – the heating effect produced by the passage of electrical currents is utilized. Direct current, low-frequency alternating current and high-frequency alternating current may all be used in the production of heat and thus cause protein denaturation.

(ii) Electrolysis – direct current or low-frequency alternating current may be used to produce destruction resulting from the chemical action of the ions liberated.

(iii) High-frequency superficial dehydration and cutting – these effects depend upon the heat developed by the current in passing from the active electrode into the tissues or upon the ohmic heat developed by the current in passing through the tissues.

The methods of desiccation and electrocoagulation were used to hypophysectomize 33 h chick embryos by Hilleman (1942), who stated that the heat precipitation of the cell contents by thermal coagulation can be carried out at any temperature from 65 to 100 °C. During the electrocoagulation the temperature of the active electrode itself does not rise; only the tissue surrounding the free end suffers a rise in temperature and becomes coagulated. The method has a number of advantages: it is quick; there is no release of bubbles of chlorine or hydrogen which may cause ruptures, pressure distortions or emboli; and tissues do not stick to the electrode, which aids in withdrawal.

However, Watkins (1965) investigated heat gains in the brain during the production of electrocoagulative lesions and found that tissue destruction occurred up to 2 mm from an electrode heated to 60 °C for 30 s; temperatures of 40 °C+ were still obtained 6 mm from the active electrode.

Experiment showed that such heating effects applied to the pharyngeal
targets, considered in this work, produce effects on the aortic arches resulting in direct and irreversible damage to the heart.

Direct current involves electrolysis in the tissues, and this method was also refined to the microscale of experimental embryology by Hilleman (1942). The results he obtained using this method to hypophysectomize young chick embryos were inconsistent. Several difficulties presented: with anodal lesions protein coagulation occurs, and the coagulum often adheres to the electrode hindering its withdrawal; also polarization effects and resistance variations, owing to the deposition of decomposition products upon the electrodes, made current control very difficult and were the main cause of irregularity.

Low-frequency alternating current appears to have overcome the drawback of both d.c. and high-frequency a.c. At mains frequency, i.e. 50 c/s, the reaction is electrolytic and therefore the destruction is chemical in nature. However, with the change of polarity every cycle, neither polarization effects nor resistance variations are encountered. Furthermore, both coagulation and liquefaction occur at the active electrode and there are no difficulties in removing the electrode after the passage of current. The heating effect of such a reaction is minimal, since the amount of heat energy developed per unit time depends on the intensity of the current and the ohmic resistance impeding its flow.

2. Nature of the reaction

Although Faraday's Law applies for any individual experimental system (i.e. each egg and electrode set up), the parameters governing this law vary from one egg to another. For example, the resistance of each system is individual to that system and is dependent upon the resistance of the egg shell and the electrodes. Therefore to ensure that each lesion is repeatable the energy produced must be maintained constant from one egg to the next. The amount of energy required for any proposed lesion is first determined empirically. Once established, current is supplied at a fixed voltage and the time period varied so that the energy supplied is the same for any given lesion. The value of the fixed voltage is such that the current does not exceed 2 mA, for above this the foci produced may no longer be discrete or reproducible (Mullan et al. 1965).

3. Conclusion

Unlike the method of Hilleman in 1942, which gave rather unpredictable results, this study has demonstrated that, once the nature of the reaction is fully understood, good repeatable lesions can be obtained. The major drawbacks of unipolar anodal or cathodal lesions have been overcome, although bubbles of hydrogen and chlorine are still released and occasionally obscure the operative field.

The examination and identification of the destroyed or damaged tissue areas presented no difficulties. The tissue immediately in the electrode path was com-
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completely disaggregated and to a large extent liquefied. The tissue slightly removed from this immediate area also showed degenerative changes.

The apparatus and technique is capable of producing localized discrete foci of destruction. The shape of such foci is dependent on the shape of the electrode used and their size upon the time during which power is supplied. The basic restriction of the method is one of electrode application. The unilateral lesions and bilateral ultimobranchiallectomy are straightforward, but bilateral parathyroidectomy, with the complications of turning the embryo and the number of electrode applications required, is hazardous and for all practical purposes not yet as refined as the other three ablations.

There are probably many further microsurgical applications for the method where access of the electrode is not denied by morphological limitations.

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REFERENCES


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