polarization: one from the part on which the electrode had been placed, the other one from the corresponding part of the symmetrical nerve.

The emulsions obtained from these pieces of brain or nerve tissue were centrifuged; the centrifugate was placed in a water bath at 37° for one hour to destroy the acetylcholine. After this, the activity of cholinesterase was determined either by means of the gasometric method (in a Warburg apparatus) or by means of biological assay of acetylcholine not hydrolysed after a definite period (tested on the dorsal muscle of the leech and on the m. rectus abdominis of the frog). In all experiments, the action of the emulsion prepared from the polarized (cathodic and anodic) piece of tissue was compared to that of the emulsion prepared from the control piece.

More than seventy experiments were thus performed; in 90 per cent of the experiments the activity of cholinesterase was definitely affected by polarization: acetylcholine is destroyed by emulsion of nervous tissue more slowly when the tissue has been subjected to the action of the cathode than when it has been subjected to the action of the anode. This influence of polarization upon the activity of cholinesterase was equally manifested in the cerebral cortex and in the nerve fibres.

The above data are illustrated in Figs. 1 and 2. These figures demonstrate the kinetics of the enzymic hydrolysis of acetylcholine produced by cathodic, anodic and control emulsions of frog nerve, as determined by means of the gasometric method.

CHANGES IN THE ACTIVITY OF CHOLINESTERASE IN NERVOUS TISSUE
The figures denote the volume of carbon dioxide (c.mm.) liberated in 60 min. from solutions of bicarbonate due to the breakdown of acetylcholine.

No. of exp.	'Cathodic' emulsion	Control emulsion	No. of exp.	'Anodic' emulsion	Control emulsion
1	9	34	7	61	30
2	10	32	8	68·5	30
3	12	43	9	70	41·5
4	12	44	10	73	39·5
5	13	44	11	58·5	38·5
6	12·5	43	12	62·5	39·5

The fact that the cathode of constant current lowers the activity of cholinesterase, while the anode produces the opposite effect, that is, increases the activity of this enzyme, may be explained on the basis of the changes that take place in the distribution of ions in the nerve under the influence of polarization.

It is possible that the comparative increase of univalent cations in the region of the cathode lowers the activity of cholinesterase, while the comparative increase of bivalent cations in the anodal region raises it.

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Action of the Pigmentary Hormone of a Stick Insect, *Dixippus morosus*, on Vertebrate Melanophores

THE pigmentary hormone of *Dixippus*, the regulator of colour change in this animal, activates the melanophores of frogs (*Rana temporaria* and *R. esculenta*) and of the axolotl (*Ambystoma mexicanum*) by causing expansion of them. This effect can be obtained by injecting blood of *Dixippus* or Ringer's solution containing crushed tissues of *Dixippus* under the skin of these amphibians, and also by plunging fragments of frog's or axolotl's skin in Ringer's solution containing blood of *Dixippus*.

The expansion of melanophores begins after about 5–30 min. and sometimes longer, according to the species used, the individual characters of the specimen and the temperature, and lasts about twenty-four hours. Control injections and experiments show that the effect is of a specific hormonal character. The pigmentary hormone of *Dixippus* is not identical with the vertebrate melanophore hormone.

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Electrocardiogram of the Embryo at the Beginning of the Contractile Function of the Heart and of Explants Cultivated *in vitro*

WE have resumed the experiments of Olivo and Posteli[1] on the chick embryo heart on myocardial fragments cultivated *in vitro*, with the principal purpose of ascertaining whether the normal structural microscopic evolution of the myocardius during ontogenesis is accompanied by characteristic alterations of its electrical activity, and investigating the fundamental phenomena giving rise to the electrocardiogram of the adult heart.

The material and methods selected have proved particularly convenient for the analytical investigation of these questions. The electrodes were of the unpolarizable type, silver-chloridized silver: contact was established through Ringer's solution, by means of capillary glass tubing of 10–30 μ diameter moved by a micromanipulator.

The amplifier[2] has direct coupling with symmetrical negative reaction and double entry in two channels, permitting, therefore, unipolar and bipolar derivation. The sensitivity is 10 microvolt and the maximum

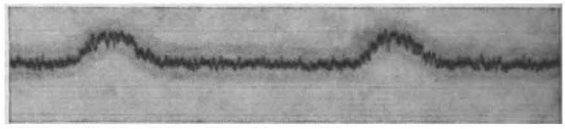

FIG. 1. ELECTROCARDIOGRAM OF A CHICK EMBRYO OF 25-H. INCUBATION, 9 SOMITES, *in vitro* FROM 40 MIN. (1 DIV. = 10^{-5} VOLT)

amplification $1{\cdot}5 \times 10^6$: deviation is practically nil. Registration is by means of a cathode ray oscillograph, photographic objective, sensitive paper and kymograph.

Young embryos. It has been shown[3] that the heart in the chick embryo begins to pulsate normally during the differentiation of the ninth pair of somites. Until the stage of 10–11 somites, the myocardius is free from myofibrillæ. Later on myocardial fibrillæ appear, first smooth then striated and in gradually increasing quantities.

The electrocardiograms of 26 embryos in the stages of 9–18 somites were recorded. In two embryos of 9 and one of 10 somites, we have obtained records consisting of a simple, slow, nearly sinusoidal half-wave. One perceives the absence of quick waves (Fig. 1). Thus we have so improved on the limit of 15 somites attained by Hoff, Kramer and others[4]. In two embryos at the stage of 7 and 8 somites respectively, the hearts of which had not begun to beat, it was possible to take a first record about an hour after the heart had begun to pulsate *in vitro*.

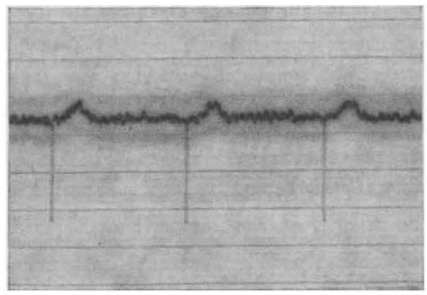

FIG. 2. ELECTROCARDIOGRAM OF AN ATRIAL FRAGMENT OF A CHICK EMBRYO OF 15-DAYS INCUBATION, *in vitro* FROM 12 DAYS (1 DIV. = 2×10^{-5} VOLT)

Quick impulses and myocardial fibrillæ. The whole embryos or the isolated cardiac primordia survive well *in vitro* and their histological differentiation progresses[5]. Taking the electrocardiogram of cardiac primordia at different stages of development, or repeatedly on the same primordium at an interval of 6–36 h., we have found that at the stage of 11–12 somites, with the appearance of the first myofibrillæ, one begins to perceive a very small quick wave which precedes the slow one. At 14–15 somites the quick wave is already stronger marked, until one obtains successively a complete electrocardiogram like that of the normal adult heart with the quick group *QRS* and the slow wave *T* (Fig. 2).

The explants cultivated *in vitro* for many days show increasing undifferentiation until the myofibrillæ totally disappear[6]. In those conditions their electrical records go back to the characters shown by the youngest primordia.

Electric potential of one fibre. We have recorded the electric potential of isolated fibres, spontaneously dissociated during cultivation *in vitro*. Such fibres are generally histologically undifferentiated. Their electric waves are slow, and quick impulses are absent.

Conduction velocity. Recording the electric potential of two points of the preparation, each in respect of the liquid in which it is immersed (unipolar derivation), and then the potential deriving from the difference of the two tensions (bipolar derivation), and as a check that obtained with the two electrodes inserted parallel, we have shown that there is a phase difference between the quick impulses at the two points. This phase difference is of the order of a hundredth of a second at distances between the two points of the order of one tenth of a millimetre.

Potentials recorded. In the very early stages (9–10 somites) the maximum potential is of the order of $50–100 \times 10^{-6}$ volt; but in all other cases, for whole embryos, myocardial fragments, and also single fibrillæ, the maximum potential recorded is of the order of $0{\cdot}5–1{\cdot}5 \times 10^{-3}$ volt.

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