Table 1. Relation between Body Temperature and Changes of Posture after Chlorpromazine

	n	Time after injection of chlorpromazine (min.)						
		0	3	6	15	90	180	390
Body temperature (° C.)*	40	37·3 ± 0·5	37·2 ± 0·5	36·5 ± 0·4	34·9 ± 0·4	31·7 ± 0·4	29·2 ± 0·3	31·3 ± 0·3
Placing reactions†	43	0·0	0·0	13·8	89·4	78·5	84·7	90·4
Righting reflexes†	43	0·0	42·8	73·3	57·4	52·6	43·5	37·8

* Mean ± *S.E.*
† Percentage of animals in the group where they are absent.

induced mainly by the disturbance of placing reactions. The reversible exclusion of these reactions is mainly responsible for the exposure of a wider surface of the animal's body to a lower temperature of the environment at the time when the chemical component of thermoregulation decreases.

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Release of Acetylcholine in Rat Diaphragm

Several attempts have been made in the past to correlate the amount of acetylcholine released during activity at nerve endings with the amount needed to produce activation of the effector cell[1–3]. It is clearly of crucial importance for the acetylcholine hypothesis that there should be quantitative agreement between these amounts. Until recently, a very substantial discrepancy has remained, supporting the suggestion that acetylcholine is merely a by-product of transmission. One source of difficulty in making such correlations has been the paucity of results concerning the release of acetylcholine and the excitation produced by its application in the same preparation.

Intracellular recording of end-plate activity in muscle, and the ionophoretic application of acetylcholine in controlled amounts from micro-pipettes, have made possible a detailed analysis of end-plate potentials under conditions closely approaching those which obtain during normal transmission at the neuro-muscular junction. End-plate potentials have been produced by such methods in the isolated rat diaphragm with as little as $1{\cdot}5 \times 10^{-17}$ mole of acetylcholine[4]. We have now found that acetylcholine is released by nerve endings in the rat diaphragm, during stimulation of the phrenic nerve, in amounts which are of the same order of magnitude.

The isolated phrenic nerve diaphragm preparation was stimulated in a 'Perspex' chamber at room temperature (19–23° C.). The muscle was bathed in 5·0 ml. of Ringer–Locke solution continually agitated by oxygen bubbles, and the rate and duration of nerve stimulation were kept to a minimum consistent with the sensitivity of the preparation used for assay (the leech dorsal muscle, the heart of *Mya areneria* or the rat duodenum). To prevent destruction of acetylcholine, we either added physostigmine sulphate to the solution (10^{-5}), or administered di*iso*propyl fluorophosphonate to the rat (1 mgm./100 gm. body-weight). For control, active solutions were treated with strong alkali whenever possible.

It is difficult to be certain in this type of experiment that one is exciting all the nerve endings. The phrenic nerve was always stimulated with shocks two or three times greater than maximal, and the action potential of the nerve trunk was recorded close to its entrance into the muscle throughout the period of stimulation. This gave us a useful index of failure of excitation or conduction in the nerve, but no positive information about the activation of the nerve endings.

During periods of stimulation at a rate not greater than 5/sec. and lasting not more than 5 min., the amounts of acetylcholine collected from hemi-diaphragms varied between $0{\cdot}4$ and $2{\cdot}5 \times 10^{-13}$ mole per impulse. Fourteen estimates gave a mean value of $1{\cdot}1 \times 10^{-13}$ mole. Since there are about 10,000 muscle fibres in the hemi-diaphragm[5], the mean release per ending was $1{\cdot}1 \times 10^{-17}$ mole.

It is clear that in the rat diaphragm there is overlap between the least amount of acetylcholine needed to produce appreciable excitation at an end-plate ($1{\cdot}5 \times 10^{-17}$ mole) and the greatest amount released at nerve endings during stimulation ($2{\cdot}5 \times 10^{-17}$ mole). The latter should be more significant than the mean value, since the principal sources of error, imperfect activation of nerve endings and hydrolysis of acetylcholine, would both lead to an underestimate.

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Effect of Supernatant Potassium-Level on Cardiac Activity in Quantitative Tissue Culture

Inadequate functional 'survival' of hearts cultured in supernatant containing serum with a high potassium-level from lysed red cells led us to investigate the effects of supernatant potassium-levels on the electrical activity of 1,222 hearts in quantitative tissue culture. The criterion for 'adequate survival' is an ability to produce potentials of at least 0·02 mV. during a minimum of three periods of observation per day (8 a.m., 1 p.m. and 5 p.m.). The apparatus, etc., is described in previous papers[1]. The supernatants are 0·25 per cent solutions of human serum protein in balanced salt solutions—however, they differ in potassium-level. The 'adequate survival' time is given to the nearest day using the smallest figure in cases of doubt.

Fig. 1*a* relates time and percentage adequate 'survival' for three separate series of 6-day chick embryo whole hearts, all from the same breeder.