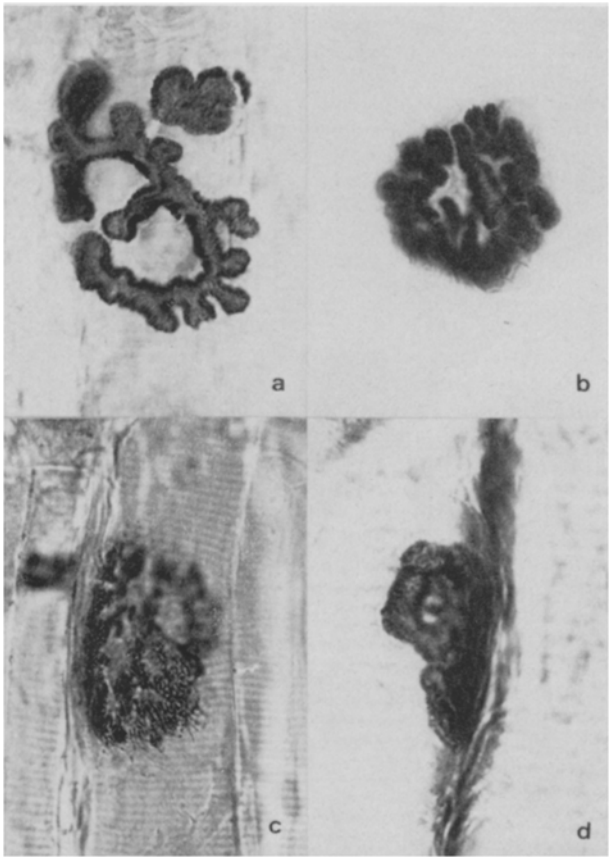
Small cholinesterase-positive sites of round shape are arranged in rows or groups and are the early representatives of the new endings. Later on the endings become more compact. But, even 11 weeks after transplantation, they still differ in shape from normal endplates. In longitudinal sections of the muscles, two or more zones of endplates could be distinguished: the band of degenerated ones and at least one additional and less distinct region with the new, innervated endings.



In spite of great difficulties due to large amounts of connective tissue, it was possible, in some cases, to isolate fragments of muscle fibres showing 1 old, degenerated, non-innervated endplate (Figure 2c), and at some distance from it, a new innervated motor ending (Figure 2d). The distance of the 2 endings obviously depends on the site at which the new nerve enters the muscle. If the nerve reaches the muscle near its tendon, the endings are situated excentrically on the muscle fibres.

The formation of new ectopic endplates indicates that in at least a high percentage of fibres, the ingrowing axons did not reach the original endings to reinnervate them. Probably the large time-lag between denervation and the formation of new neuromuscular contacts in free muscle transplantation without implantation of a nerve is one of the reasons for the de novo formation of motor endings. An additional factor might be the severe damage of the muscle.

Zusammenfassung. Ungefähr 7 Wochen nach Transplantation zeigte sich eine Reinnervation der Muskeln durch kollaterale Sprossung aus den darunterliegenden Interkostalnerven. Die Transplantate reagierten auf indirekte Stimulation. Histochemisch waren neugebildete motorische Endplatten nachweisbar. In einigen Fällen konnte an Isolationspräparaten an ein- und derselben Muskelfaser zusätzlich zur alten, degenerierten eine neugebildete motorische Endplatte beobachtet werden.

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Fig. 2. Photomicrographs of motor endplates in EDL muscles. a) Normal endplate in control muscle. b) Denervated endplate 7 days after transplantation. c) Denervated endplate 70 days after transplantation. d) New endplate 70 days after transplantation. Modified Koelle-technique, Magnification: ×500 (a–c); ×800 (d).

Effects of Incubation Temperature on the Kinetics of Cultured Lymphocytes from the Opossum, *Didelphis virginiana*

The common opossum (*Didelphis virginiana*) possesses a body temperature which is considerably lower than that of the majority of marsupials and eutherian mammals[1–3]. In ambient temperatures of 14–26 °C, the rectal temperatures of opossums ranged from 32–34.5 °C[1]. In in vivo experiments[4], the mean durations of the G_2 and S phases for cells from opossum stomachs were longer than those of eutherian mammals[5–7]. It was suggested that this was caused by the lower body temperature of opossums as the result of a Q_{10} phenomenon[4]. Day-old rats lacking thermoregulatory mechanisms demonstrated increases in G_2 and S phase durations at temperatures substantially below normal for those animals[8]. In contrast, a short S phase for chicken intestinal epithelia has been related to the higher body temperature (40.5 °C) of this species[6]. A number of investigators have demonstrated that incubation of in vitro cell systems at temperatures above or below the optimum range (generally 37–39 °C) resulted in prolonged durations of individual phases and/or the total cell cycle[9–12]. This study was undertaken to determine if incubation of opossum lymphocytes at 34 °C

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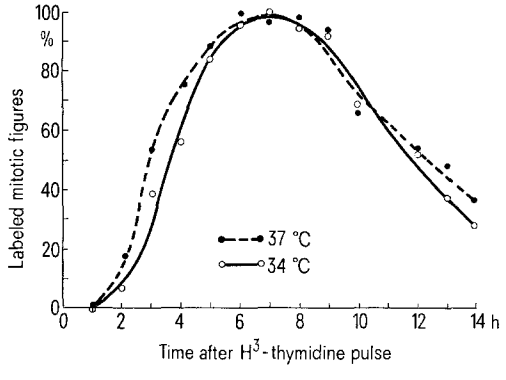
(mean body temperature of this animal) rather than at the conventionally employed 37 °C would alter the durations of the G_2 and S phases.

Materials and methods. Cardiac blood lymphocytes of 5 male opossums were isolated and cultured with phytohemagglutinin (Difco) according to a previously described technique[13]. In each experiment, the total number of lymphocytes collected was divided equally (final dilution approximately 3×10^6 cells/ml of culture medium) and placed in incubators set at 34 °C and 37 °C. All conditions for the 2 sets of cultures were identical with the exception of temperature. This was monitored and recorded at regular intervals to the nearest 0.05 °C. After 36–42 h of incubation, both sets of cultures were pulse-labeled for 30 min with 1 μc/ml of H^3-thymidine (New England Nuclear, 6.7 c/m*M*) followed by a chase in 120 times excess nonradioactive thymidine. Colchicine (0.1 μg/ml) was added 1 h prior to culture harvest. Cultures from each temperature were sacrificed at intervals from 1–14 h; the slides were coated with liquid NTB 2 emulsion (Kodak), exposed 3 days, developed, fixed and stained. Percent labeled mitoses (PLM) curves were constructed for lymphocytes grown at each tempearture by scoring on radioautographs approximately 900 mitotic figures per interval.

Results. Analysis of approximately 400 temperature readings over a 33-dayperiod provided the following data. The mean incubation temperatures of the 2 sets of cultures were 34.27 ± 0.34 °C and 37.29 ± 0.34 °C. The mean difference between incubation temperatures was 3.0 ± 0.29 °C. The temperatures of the 2 incubators varied slightly in a manner similar to a diurnal cycle (attributed to cyclic variations in line current), but variations were simultaneous and in the same direction. Rectal and esophageal temperature measurements of anesthetized opossums used in this laboratory revealed a mean value of 33.0 °C.

The PLM curves for lymphocytes incubated at 34 °C and 37 °C are presented in the Figure. It may be observed that the average G_2 phase (the time required for the curve to reach 50% labeled mitoses) was considerably shorter for the cells incubated at 37 °C (2.8 h) than for those grown at 34 °C (3.6 h). In contrast, the average S phase (the time between 50% labeled mitoses on the ascending and descending limbs of the curve) was longer for the cells grown at 37 °C (9.4 h) than for those at 34 °C (8.4 h). The mean durations of the G_2 + S phases for the 2 sets of cultures were approximately equal.



PLM curves for opossum lymphocytes incubated at 34 °C (solid line) and at 37 °C (dashed line). The mean G_2 phase was shorter at 37 °C than at 34 °C, while the reverse was true for the S phase.

Discussion. The results for the G_2 phase observed in this investigation are in agreement with other reports available in the literature[9–12] and appear to support the suggestion that phase duration may be related to a Q_{10} phenomenon[4]. On the other hand, the results for the S phase are inconsistent with this hypothesis. Considerable evidence exists, however, to support the observation that different incubation temperatures can affect individual phases differently. For example, it has been stated that a temperature change could: a) result in an equal change in all phases; b) affect only 1 or 2 phases; or c) affect different phases to different degrees[12]. Highly variable mean G_2 durations (greater than 2 h difference) were observed in 2 sets of cultures of L-strain mouse cells, both with a generation time of 20 h[14]. This means that in the culture with the shorter G_2 phase, one or more of the other phases (G_1, S or M) was longer. The investigators believed that the variations were due to 'differering culture conditions'. Other investigators believe that the fact that each part of the cell cycle has a different temperature response curve 'indicates that there are different rate-limiting steps in the various parts of the cycle'[9]. It has been suggested that two types of variability operate in cell cultures, one between individual cells of a population, and the other related to changes in extracellular environment[15]. The curve for opossum lymphocytes incubated at 37 °C in this investigation, however, is nearly identical to that previously reported[16]. Therefore, although some variation among individual cells or cell populations may occur, incubation temperature also plays an important role in cell cycle phase duration[17].

Résumé. La courbe PLM (pourcentages indiqués de mitoses) pour les lymphocytes d'opossum à 34 °C (température normale de cet animal) a attesté une phase G_2 plus courte mais d'une phase S plus longue que chez ceux qui ont été incubés à la température conventionnelle de 37 °C. Les résultats obtenus sont discutés dans leurs rapports avec les réactions liées à des différences de température pour les deux phases du cycle cellulaire.

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[17] Supported by a grant from the American Cancer Society, Arizona Division, Incorporated. The authors thank Bonnie L. Jackson and David L. Grundy for technical assistance.
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