are: 1. lack of a calcium source, normally obtained from the eggshell[8]; 2. the much lower carbon dioxide concentration as compared with the normal egg, where the CO_2 is known to reach a very high level towards the end of incubation[9]; and 3. the altered geometry of the chorio-allantoic membrane.

Cultured embryos were motorically responsive to tactile stimulation, and also displayed 'spontaneous' motility which was quite similar in character to that described in ovo[10]. Most of the oldest preparations in fact were in almost continuous movement, as is normal for that stage of development[11].

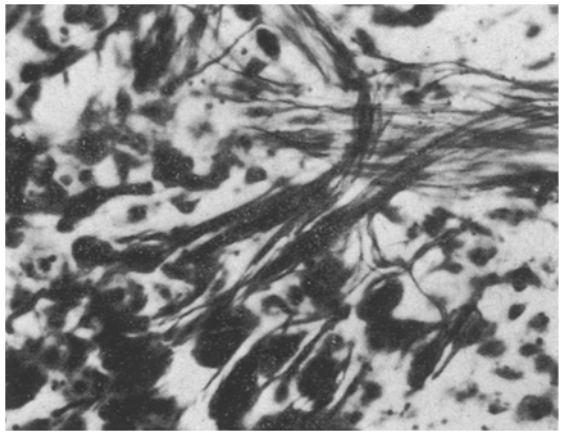

Fig. 2. An abundant, well-fasciculated, outgrowth of nerve fibres from a monolayer cluster of neurons and neuroblasts in various stages of development (lower left). Isolated at 6 days of incubation and cultured for 10 days on the chorio-allantoic membrane (background cells) in vitro. Bodian's protargol-silver staining method, magnified × 500.

The chorio-allantoic membrane was prepared for grafting of isolated neural tissues according to the standard procedure[2]. In most of the experiments to date, spinal cord segments of 1 or 2 somites in length were dissected out from donor embryos of about the same age as the host (4–7 days). The neural tissue was cleaned of adherring mesoderm and then placed onto the membrane. The grafts became quickly vascularized and usually survived for as long as did the host embryo. Explanted tissues were fixed in situ after varying periods of culture (up to 12 days) and then silver stained in toto using the Bodian method. Despite the absence of any readily recognizable histotypic organization in these flattened pieces of spinal cord, an extensive neuritic outgrowth was found to have taken place and nerve cells in various stages of differentiation could be identified (Figure 2).

Résumé. On décrit une technique pour obtenir la survie prolongée des embryons du poulet in vitro, et son emploie pour cultiver des tissus isolés du système nerveux centrale.

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Cell Cycle Determination of Phytohemagglutinin-Stimulated Lymphocytes from the Opossum, *Didelphis virginiana*

Due to a number of remarkable cytogenetic characteristics, the opossum (*Didelphis virginiana*) has been utilized recently to investigate chromosomal morphology, nucleic acid metabolism, and sex chromatin physiology[1–4]. The lymphocyte culture technique was employed exclusively in these investigations due to the ease of obtaining large numbers of dividing cells in a very short period of time. Since investigations of nucleic acid synthetic patterns require a knowledge of the cell cycle and its component parts, a generation time (PLM) curve was constructed for opossum lymphocytes in vitro.

Materials and methods. Lymphocytes obtained from either thoracic duct lymph or cardiac blood of 8 opossums (7 males and 1 female) were cultured according to modifications of the technique of MOORHEAD et al.[5] (see SCHNEIDER and RIEKE[2], for details). Following 36 h of incubation, the cultures were pulse-labeled for 30 min with 1 μC/ml of H^3-thymidine (spec. act. 6.05 C/m*M*, New England Nuclear). The cells were subsequently washed and replaced in fresh media containing 120 times excess non-radioactive thymidine. Cultures were harvested at intervals from 0–28 h. Slides were coated with liquid Kodak NTB 2 emulsion, exposed 1–3 days at 4 °C, developed and stained. Between 100 and 600 mitotic figures were scored at each interval.

Results and discussion. The Figure is the PLM curve derived from opossum lymphocytes in tissue culture. Labeled mitotic figures first appeared 1.5 h after administration of H^3-thymidine. The ascending limb of the first peak approached 100% labeled mitotic figures at 7 h. The descending limb, which was skewed somewhat to the right, dipped only slightly below 50% labeled mitoses before immediately giving rise to a second similar, but much lower, peak. A third peak appeared at approximately 22 h after introduction of isotope, followed by a general trailing off of the curve to 28 h. Both thoracic duct and blood lymphocytes produced the same basic curve configuration.

The following cycle times may be derived from the curve: mean T_c (generation time) = 10.5 h, mean G_2 = 2.75 h and mean S = 9.5 h. T_m (mitotic time), calculated from the mitotic index, was approximately 30 min. It

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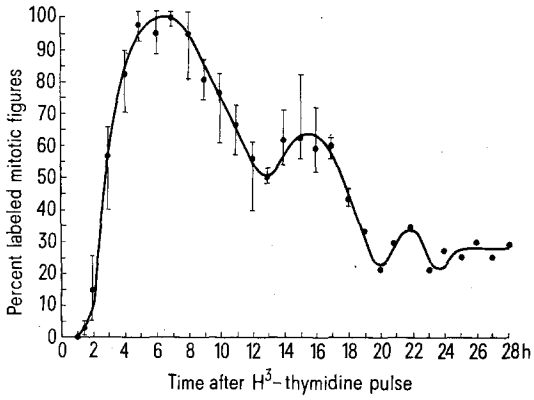
is readily apparent that the sum of G_2, S, and M (mitosis) is greater than the total generation time as determined from the PLM curve, G_1 not considered. The assumption that the G_1 phase may be lacking is supported by similar observations on Ehrlich ascites tumor cells[6,7], rabbit ameloblasts[8], and Chinese hamster lung cells[9]. Nevertheless, it is not expected that the remaining phases should exceed the calculated T_c.

The initial wave of the present curve is nearly identical to those reported for opossum stomach cells[10]. As a result, the calculations for G_2 and S of opossum lymphocytes in vitro can most likely be accepted with some degree of accuracy. On the other hand, the rapid appearance of subsequently damped out peaks suggests considerable variability in cycle times and phases of individual cells. It is suggested that this variation exists primarily in the length of G_1 of perhaps two or more functionally diverse populations of lymphocytes[11].



PLM curve for opossum lymphocytes in vitro. Each dot between 1 and 18 h represents the mean value for 2–5 animals; vertical lines represent the range of labeled mitoses observed at each interval.

Résumé. La courbe PLM (pourcentages indiqués de mitoses) pour les lymphocytes d'opossum in vitro a fait preuve d'un T_c (temps générateur) que n'atteignait pas la somme de G_2, S, et M (mitosis). Tandis que les calculations pour les phases de G_2 et S sont représentées avec exactitude sur la courbe, il y a des variations dans le G_1 parmi les population de lymphocytes au fonctionnement divers (au nombre de deux ou peut-être plus parmi celles-ci) qui sont responsables de ce désaccord apparent.

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Substructure of Paramyosin Filaments Prepared by Freeze-Substitution Technique

Certain mollusc muscles – the so-called 'catch muscles' – have thick filaments with very large dimensions. These filaments contain the unique protein, paramyosin[1], which forms the core of the filament, and myosin which is probably present at the surface[2,3]. Small angle X-ray-diffraction revealed that the thick filaments of the catch muscles had a 725 Å axial periodicity with a prominent 145 Å repeat[4,5]. Electron micrographs of isolated and negatively stained or shadowed filaments showed a surface net pattern from which Elliott and Lowy[6] proposed a model of a helicoidal structure of the paramyosin filaments. In longitudinal sections of the oyster adductor muscle, Elliott[7] observed thick filaments with a variety of band patterns which he interpreted in terms of a paramyosin filament consisting of a stack of longitudinal lamellae. A very clear substructure of paramyosin filaments can be observed in freeze-substituted preparations of catch muscles, as demonstrated in this study.

Material and methods. The muscle employed was the anterior byssus retractor muscle (ABRM) of *Mytilus edulis* which was frozen with isopentan, substituted with glycol at –50°C[8], embedded without any fixation in Araldite resin, sectioned and stained with uranyl acetate and lead citrate.

Results and discussion. Figure 1 shows a longitudinal section through a muscle fibre of the ABRM. The filaments present a complex and variable fine structure. Most obvious is a lattice-like pattern produced by two sets of bands being placed obliquely to the axis. At one side, consecutive bands (thickness 100 Å) are 720 Å apart and have an angle of about 15 degrees in the direction of the filament axis. On the other side, the bands (thickness 160 Å) show a 360 Å periodicity and an angle of 45–50 degrees. The dimensions of the periodic markings appear to be identical in both, the exact longitudinally sectioned filaments as well as in those cut obliquely. At a higher magnification each band can be seen to comprise series of short dark lines or spots, staggered to each other (Figure 2). Preliminary results obtained with the Markham technique suggest that the lines and spots are composed of such a geometrical pattern that each lattice point gives rise to a 725 Å periodicity.

Another typical pattern observed in the longitudinally sectioned filaments consists of a repeat of 5 dark cross lines, separated by a wide unstained gap. The length of one repeating unit is again around 725 Å. The cross striation can extend over a great part of the filament diameter (Figure 3), or it may occur only at the filament periphery, whereas the net-like pattern is present in the middle part (Figure 1). The various patterns observed

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