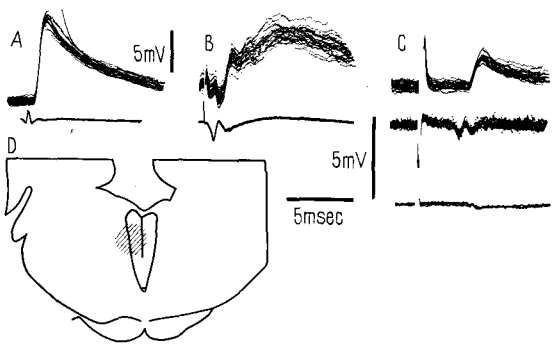
A Descending Pathway with Monosynaptic Action on Flexor Motoneurones

Alpha motoneurones of the lumbosacral region in the cat receive monosynaptic EPSPs from descending pathways[1,2]. Extensor motoneurones receive this action from the ipsilateral lateral vestibular (Deiters') nucleus[2]. The pathway to flexor motoneurones also descends from supraspinal centres but does not originate in Deiters' nucleus[2]. The aim of the present experiments has been to find the origin of the pathway from which monosynaptic EPSPs can be evoked in flexor motoneurones.

Cats anaesthetized with chloralose and paralysed with Flaxedil were used for intracellular recording from motoneurones. Stereotaxic stimulation was made in the brain stem after vermal cerebellectomy with a thin tungsten electrode (insulated but for the tip) against an indifferent electrode in the neck muscles. At the lower thoracic level, the dorsal columns were removed for about two segments and the spinal cord hemisectioned contralateral to the side of motoneuronal recording. The ventral roots were left intact. Microelectrodes filled with $2M$ K-citrate and a resistance of 3–5 MΩ were used. Recordings were made from 25 flexor motoneurones in which a monosynaptic EPSP was evoked on stimulation of ventrolateral funicles in the thoracic region (Figure, B). The cells were identified by antidromic stimulation of the axon at a peripheral level. A monosynaptic EPSP can be evoked in flexor motoneurones from a dorsomedial region in the upper medulla and lower pons. Figure, C shows that stimulation of this region produces a descending volley conducted at about 120msec (middle trace in C) and an EPSP in a flexor motoneurone after a segmental latency of 0.6 msec, which shows that the linkage is monosynaptic. The amplitude of the EPSP from the brain stem corresponds to the amplitude of the early EPSP evoked by maximal stimulation of the ipsilateral ventrolateral funicles (Figure, B) and is about 25% of the amplitude of the maximal homonymous Ia EPSP (Figure, A; note different voltage calibration in A and B). Careful exploration in a transverse plane revealed that the effect is evoked at very low threshold (< 0.05 mA) from the hatched region in the drawing. The region extends for several millimetres in a rostrocaudal direction, but there was a large increase in threshold when the stimulating electrode was moved in the rostral direction to the midpontine level.



The upper traces in A–C are intracellular recordings from a posterior biceps-semitendinosus (PBSt) motoneurone. The lower traces in A and B and the middle trace in C are recorded from the L7 dorsal root entry zone. Lowermost trace in C is recorded after withdrawal of the microelectrode to a just extracellular position. Voltage calibration refers to microelectrode recordings only. A shows the maximal monosynaptic Ia EPSP from the PBSt nerve. B shows the effect of supramaximal stimulation of the ipsilateral ventrolateral funicles in the lower thoracic region. In C the brain stem is stimulated in the hatched area in the drawing (D), which represents a transverse section of the brain stem at a level 6.5 mm rostral to obex.

Fibres from different centres descend in the region from which these low threshold effects are evoked[3], and it is not possible to disclose the origin of the axons with monosynaptic effect on flexor motoneurones only from the stimulation experiments. However, there is evidence that the axons do not originate from the medial vestibular nucleus because descending monosynaptic EPSPs can be evoked in flexor motoneurones from the thoracic region several weeks after complete destruction of the ipsilateral medial vestibular nucleus[4]. Our stimulation experiments suggest that the axons do not come from centres rostral to the lower pons. It is tentatively suggested that the pathway with monosynaptic connection to flexor motoneurones is reticulospinal.

Zusammenfassung. Erregung eines dorsomedialen Stammhirngebietes verursacht ein monosynaptisches EPSP in Motoneuronen der Beugemuskeln.

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Action of Cortisone on Human Fibroblasts in vitro

Human embryonic cells can be maintained for several months in vitro with retention of some of their in vivo properties[1,2]. These cells are probably derived from mesenchymal cells. Therefore, since cortisone is one of the factors controlling the metaoblism of mesenchymal tissue in vivo[3], it seems important to study the behaviour of these cells when they are exposed to a medium containing this hormone.

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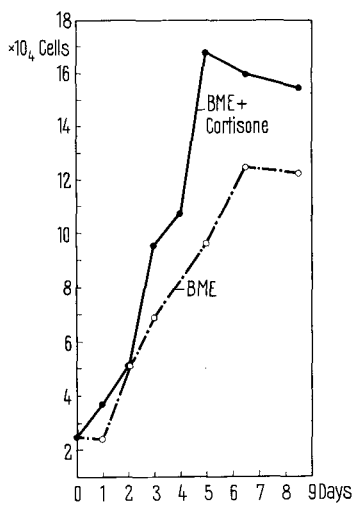
Materials and methods. Two cell strains originating from human embryonic lung were used. One strain, S41, was obtained from Dr. LEONARD HAYFLICK, and the other strain, referred to here as HAB, was started by ourselves. Lung tissue from a 4-month-old embryo was divided into 2 pieces which were fragmented and minced separately with paired forceps and placed into 2 Petri dishes. One dish contained medium supplied with 2.5 µg/ml cortisone. The fragmented tissues, after being aspirated several times with nutrient medium, were placed in culture flasks covered with aluminium foil and incubated in a 37 °C humidified atmosphere with 5% CO_2. Tissue culture methods and the nutrient medium[4] and trypsin used were the same as described by HAYFLICK and MOORHEAD[2]. Cortisone acetate (Vitamix Corp., USA) was added to prewarmed medium in both a final concentration of 2.5 µg/ml and of 50 µg/ml.

Results. At the 21st passage, strain S41 was split into two series: one was cultivated further in the original medium (BME), the other in this medium containing cortisone at a concentration of 2.5 µg/ml. The series carried in BME died at the 48th passage; the series supplemented with cortisone died at the 65th passage. A culture was removed from its cortisone-containing medium at the 38th passage and replated in BME without cortisone. It died at the 50th passage.

Both series of strain HAB which were carried in two different media were assayed at the 11th passage for plating efficiency: Petri dishes were seeded with 100 cells each from the cortisone series, and other dishes with an equal number of untreated cells. 10 days later the colonies which formed were stained and counted, a group of at least 7 cells being considered as a colony. An average of 17 colonies per dish were formed in the untreated series and 27 in the cortisone treated series (cortisone 2.5 µg/ml). At the 25th passage cultures were made from the 2 series with an initial inoculum of $2.5 \cdot 10^4$ cells/ml. The cells from 4 cultures of each series were counted daily for 9 days. Cells were trypsinized and stained with trypane blue for counting. Results plotted on growth curves representing the average count (Figure) showed a generation time of 24 h for the cells maintained in BME and 20 h for the cells maintained in BME with cortisone (2.5 µg/ml).



Growth curve of strain HEL. For explanation see text.

This strain was also carried in BME supplemented with 50 µg/ml of cortisone. No stimulating action was observed with this concentration of the hormone; nevertheless, there was no growth inhibition.

The HAB cells cultivated in BME died at the 61st passage. The culture originating from the same tissue, which was grown in the medium supplemented with 2.5 µg/ml of cortisone, died at the 76th passage.

Discussion. In our experiments, human embryonic strains which can be maintained for long periods in different tissue culture media[1,2] could be carried for longer periods with the addition of cortisone. The lifespan of the two cell strains maintained in medium containing the hormone at a concentration of 2.5 µg/ml increased respectively by 35 and 25%. This effect seems to disappear when the hormone is withdrawn, since strain S41 grown in cortisone medium lasted as long as the strain in the controls when replated in BME. TODARO and GREEN[5] could also increase the lifespan of human embryonic strains with the addition of albumin to the medium, and GILLETTE et al. found that cortisone delayed the degenerative changes of organized skin in vitro[6]. The hormone also had a stimulatory effect on cellular growth of strain HAB, which was evidenced by the shortening of the generation time and the increase in the plating efficiency. This effect was previously demonstrated by CASTOR[7] in his work with human fibroblasts. ARPELS et al.[8] also found a sustaining effect of hydrocortisone on different established cell lines.

The strains we used also seem to have a specific capacity to metabolize cortisone, since they could be carried without any toxic effect at a concentration (50 µg/ml) which is inhibitory to other cell systems[9–11].

It seems possible that the improvement of tissue culture media will further delay the appearance of the degenerative stage observed in tissues of different origins during their life in vitro.

Résumé. Deux sources de fibroblastes originaires de poumon d'embryon humain ont été cultivées en milieu de culture contenant 2,5 µg/ml de cortisone. L'hormone a prolongé la vie des souches et stimulé la division cellulaire.

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