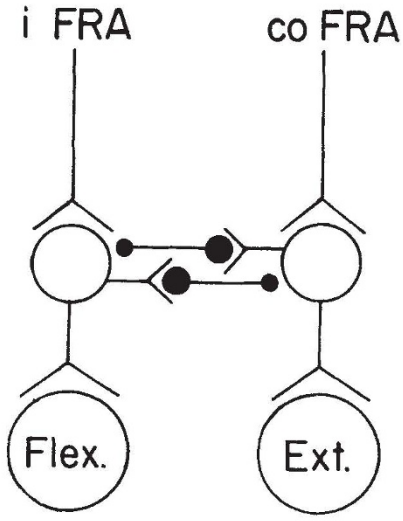

Fig. 3. Diagram showing the organization of the neuronal paths giving long-lasting reciprocal activation of flexor (Flex.) and extensor (Ext.) motoneurones. In this diagram an interneurone may represent an interneuronal pool. Excitatory interneurones are indicated by open circles, inhibitory interneurones by filled circles. Termination of inhibitory interneurones on cell bodies merely indicates inhibition and there is no commitment whether inhibition is postsynaptic on the cell bodies or presynaptic on the terminals of interneurones

tion of the inhibitory interaction found is indicated in Fig. 3.

The neuronal organization of Fig. 3 seems eminently suited to subserve alternate rhythmic activation of extensors and flexors and may be the neuronal correlate of Graham Brown's 'half centres'[3]. However, true reciprocal innervation, for example with extensor relaxation during flexor contraction, requires that opposing reflexes should not operate at the same time. With most reflexes this is achieved through postsynaptic inhibition of antagonist motoneurones, but reciprocal innervation through the neuronal paths now disclosed does not seem to depend on postsynaptic inhibition of motoneurones. Hence it is of interest that at the same time as reflex activation of motoneurones there is a primary afferent depolarization in Ia afferents so that during flexor contraction the Ia stretch reflex to extensors is not allowed to counteract the flexor movement.

E. Jankowska *
M. G. M. Jukes †
S. Lund
A. Lundberg

Department of Physiology,
University of Göteborg,
Göteborg, Sweden.

* International Brain Research Organization Fellow.
† Sir Henry Wellcome Travelling Fellow.

[1] Andén, N.-E., Lundberg, A., Rosengren, E., and Vyklický, L., *Experientia*, **19**, 654 (1963).
[2] Anden, N.-E., Jukes, M. G. M., Lundberg, A., and Vyklický, L., *Nature*, **202**, 1344 (1964).
[3] Brown, T. Graham, *Proc. Roy. Soc.*, B, **86**, 140 (1913).

Oxygen Requirements for Liver Cells *in vitro*

Over the past 50 years many workers have tried to maintain and multiply parenchymal liver cells in tissue culture without success. The following discussion indicates that inadequate oxygen supply may be the basis for this failure. The oxygen tension (pO_2) of pooled venous blood is about 40 mm Hg while that of arterial blood is about 95 mm Hg. Therefore the pO_2 to which liver cells are exposed *in vivo* should be somewhere between these values. Direct measurements of liver pO_2 in the dog gave a median value of 70 mm Hg[1]. Let us take 40 mm Hg as the lowest tension in which liver cells can survive.

Assume that we wish to grow a sq. cm of a monolayer of liver cells on glass over which there is a stationary layer of fluid. Our problem is to determine the maximum thickness of the fluid phase which can maintain a pO_2 of 40 mm at the cell surface. The equation will be:

$$b = k\,\frac{y_0 - y}{a}$$

where b is the distance in cm from the gas/fluid interface to the fluid/cell interface, k is the diffusion constant of Krough[2] for water at 37° C, y_0 is the O_2 pressure in atmospheres at the gas/fluid interface, y is the O_2 pressure at the fluid/cell interface and a is the rate of oxygen consumption by the cells in ml./min.

For water, $k = 3{\cdot}4 \times 10^{-5}$, $y_0 = 0{\cdot}21$, $y = 0{\cdot}05$ (40 mm Hg for the limit of acceptable oxygen tension converted to atmospheres). Liver cells use a great deal of oxygen, the value for rabbits being about $8{\cdot}3 \times 10^{-2}$ ml. O_2/gm wet wt./min[3]. Since liver contains about $1{\cdot}3 \times 10^8$ cells/g (ref. 4) the consumption/cell is $6{\cdot}4 \times 10^{-10}$ ml. O_2/min. From the number of cells/g we can compute that a liver cell is about 20 μ^3 and one sq. cm will therefore contain $2{\cdot}5 \times 10^5$ cells using $1{\cdot}6 \times 10^{-4}$ ml. O_2/min, the value of a. Substituting these values in the equation, we find that to maintain a monolayer of rabbit liver cells *in vitro* in air there should be a fluid overlay not greater than 0·34 mm thick. If oxygen were used, this distance could be increased to about 1·7 mm. These values are very similar to those calculated by Hill in 1928 for the depth of oxygen diffusion into muscle[5]. The tissue culture systems in which liver is usually cultured expose the cells to greater depths than these; it is impossible to control pH without doing so. These values probably represent maximum depths; it is likely that multiplication of liver cells will require an even greater pO_2.

Trowell[6] has made the most sophisticated attempts to supply adequate oxygen using liver cubes above, but wetted by, the medium. However, his tissues used only a fraction of the oxygen used *in vivo*. He stated that 20 explants, each approximately a 2-mm cube, used about 1·5 ml. of O_2 daily. This represents 7×10^{-3} ml. O_2/g/min. The *in vivo* O_2 consumption by the liver of 60-g rats is about $1{\cdot}7 \times 10^{-1}$ ml. O_2/g/min[3]. Hence these liver cells *in vitro* were obtaining only 4 per cent of their requirement and degenerated rapidly. It is therefore clear that liver cells cannot obtain the energy needed for maintenance and division in tissue culture from aerobic respiration. Those cells which do grow well in tissue culture have high anaerobic glycolysis; for example, cancer cells, foetal cells and fibroblasts. In contrast, liver has extremely low anaerobic glycolysis[7] and cannot obtain adequate energy in this way.

I thank Dr. Joseph Engelberg for assistance with the diffusion aspects of this work, which was aided by grants from the National Institutes of Health and the American Cancer Society.

K. M. Stevens

Department of Medicine,
University of Kentucky
College of Medicine,
Lexington.

[1] Derrick, J. R., and Russell, D., *Arch. Surg.*, **88**, 1059 (1964).
[2] Krough, A., *J. Physiol.*, **52**, 26 (1919).
[3] Brauer, R. W., *Physiol. Rev.*, **43**, 115 (1963).
[4] Allard, C., Mathieu, R., de Lamerande, G., and Cantero, A., *Cancer Res.*, **12**, 407 (1952).
[5] Hill, A. V., *Proc. Roy. Soc.*, B., **104**, 39 (1928).
[6] Trowell, O. A., *Exp. Cell Res.*, **16**, 118 (1959).
[7] Aisenberg, A. C., *The Glycolysis and Respiration of Tumors* (Academic Press, New York, 1961).