Only 17 amino acids are spotted out from the haemolymph of starved nymphs. These amino acids appear in higher concentration than the control nymphs, suggesting that under normal conditions they are actively utilized in protein synthesis or in other metamorphic or metabolic processes, while during the starvation they are stored in the haemolymph as there is total or partial blockage of protein synthesis, metabolism and metamorphosis[8].

| Amino acids | Concentration* | | |
|---|---|---|---|
| | Control nymphs | Centrifuged nymphs | Starved nymphs |
| Ornithine | + | +++ | +++ |
| Histidine | ++ | + | +++ |
| Lysine | ++ | + | +++ |
| Aspartic acid | + | + | + |
| Serine | ++ | + | +++ |
| Arginine | + | ++ | +++ |
| Glycine | + | +++ | +++ |
| Threonine | + | +++ | ++ |
| Alanine | + | + | ++ |
| Proline | +++ | ++ | +++ |
| Tyrosine | + | + | ++ |
| Tryptophan | ++ | + | ++ |
| Valine | + | + | +++ |
| Isoleucine | + | + | ++ |
| Leucine | − | + | ++ |
| Phenylalanine | + | + | + |
| Glutamic acid | − | +++ | ++ |
| Methionine | − | + | − |
| α-Amino-*n*-butyric acid | − | + | − |
| Cystine | − | + | − |

*Concentration represented by the following: − negative; + low; ++ moderate; +++ high.

THAKARE and TEMBHARE[13] observed sudden release of neurosecretory material in haemolymph from the cerebral neurosecretory cells and the corpora cardiaca during stress, and its accumulation in the neurosecretory cells during starvation in the last instar nymph of *Orthetrum chrysis*. In the present study, an appearance of additional amino acids during stress and an accumulation of several amino acids in starvation have been tentatively observed. Thus there appears to be some functional correlation between the release of cerebral neurosecretory material and the metabolism of amino acids.

*Summary.* The free amino acids were determined by thin layer chromatography in the haemolymph of the last instar nymph of the dragonfly, *Orthetrum chrysis* (Selys) during normal, centrifugation and starvation periods and a functional correlationship between the cerebral neurosecretory material and amino acid metabolism has been suggested.

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## PRO EXPERIMENTIS

### A Cell Culture Substrate Obtained from Heat-Fused Collagen

The use of reconstituted rat tail collagen as a substrate for tissue explants or for certain types of cells is now a common practice. Recently, coverslips coated with dried collagen were accidentally included with glassware sterilized in a hot-air oven at 160 °C. The resulting clear film appeared to offer some advantages as a multipurpose substrate and further investigations were undertaken.

*Materials and methods.* Collagen was prepared by the BORNSTEIN modification[1] of the original method of EHRMANN and GEY[2], except that the entire process, including dialysis for 24 or 48 h, was carried through without interruption and aliquots of collagen solution were stored in sterile refrigerated containers. Coverslips placed inside petri dishes were coated with a thin film of liquid applied with a glass rod, and immediately dried for 24 h in a 37 °C oven[3]. The petri dishes were then wrapped in foil and placed in a hot-air sterilizing oven at 160 °C for 2 h. After cooling, they were stored at room temperature until required.

A drop of medium, dispersed with a glass rod to form a moist film, was placed on the surface of the substrate before explants were placed in position. The latter were left for several hours with minimal medium in order to attach them firmly.

When specific concentrations of dissociated cells were required, flat-ground glass rings were placed on the fused collagen films and standardized cell suspensions were seeded into them. To ensure that no liquid or cells escaped, the outer rim of each ring was usually sealed to the coated coverslip with hot wax but, with adequate care in handling, leakage could usually be avoided even without waxing. For electron microscopy, thicker layers of substrate, easily removed later from the coverslip, were obtained by placing small (10 mm diam.) glass rings on glass coverslips and filling the wells to varying depths with liquid collagen. This required extended drying time at 37 °C. The subsequent dry-heat sterilizing was carried out as usual with the rings in place. Glutaraldehyde and osmium fixation was carried out before the rings were removed.

*Results and discussion.* Heat fusion of dried collagen produced a transparent, even layer of unknown chemical composition, in which no evidence of structure has so far been demonstrated. A negative result was obtained with PAS staining. The substrate was produced entirely by dry heat.

Phase-contrast photography of cells through a film of fused substrate produced pictures of excellent clarity. Time-lapse cinematography indicated that the substrate provided an adequate locomotory surface for embryonic

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chick heart and adult mouse muscle fibroblasts, embryonic chick retinal epithelial cells and $SZ_3B16$ mouse melanoma[4] cells. The film could be cut into patterns with a scalpel, or stripped away with a silicone rubber wedge of desired width, to provide clean edges demarcating junctions between the prepared substrate and the underlying glass coverslip.

Incubation of all tissue explants so far tested (embryonic chick heart, foetal human connective tissue and adult human skin) appeared to be at least as successful on the fused substrate as on dried collagen. The mean outgrowth (based on 2 measurements at right angles) from 10 chick heart explants incubated for 24 h on fused collagen was probably significantly greater than that from 10 explants incubated on standard dried collagen film ($t = 2.9126$, d.f. 14; $0.02 > p > 0.01$).

Cells of permanent in vitro lines of mouse melanoma[4], human melanoma[5] and Ehrlich Lettré mouse ascites carcinoma[6] all grew successfully on fused collagen. However, no significant difference in mitotic index (based on 10 counts of 1000 cells) could be found among $SZ_3B16$ mouse melanoma cells seeded at a uniform density on fused collagen, dried collagen and glass.

Routine fixation of cells on fused collagen with formol saline and subsequent staining with Mallory's aqueous haematoxylin were entirely successful. So far E. M. sectioning has been limited to the substrate alone but control of the depth of film and the facility with which the latter can be removed from the coverslip for subsequent processing suggest that it will be perhaps even more convenient to handle than standard dried collagen[3]. The effects of heat treatment on microcrystalline collagen[7] have not yet been studied. If this also produces a satisfactory substrate, ethylene oxide sterilization and the subsequent period of delay before use[7] could be eliminated.

*Summary.* Coverslips coated with rat-tail collagen dried at 37 °C were placed in a hot-air sterilizing oven at 160 °C for 2 h. The resulting transparent sterile film was found to be a useful multipurpose substrate for cell culture and for subsequent histological sectioning.

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## A Simple Stimulus Isolation Unit Using a Complementary Metal Oxide Semiconductor Switch

Most neurophysiological experiments involve both the electrical stimulation of an excitable tissue (by passing an electrical current between 2 points) and the recording of the response (by measuring the voltage difference between 2 other points). If the current flows between a source and ground, the electrical recording is distorted, usually for several msec after the end of the stimulus[1]. This 'stimulus artifact' is greatly reduced or supressed by circulating the current through a path that is independent of the ground used for recording. There are at least 4 types of device stimulus isolation unit (SIU) that perform this function: The output of the secondary windings of a transformer ('inductorium'), the high-speed relay[2], the radiofrequency SIU[3,4] and the opto-isolator SIU[4–6]. The transformer output is necessarily AC. With high-speed relays it is difficult to obtain pulses of short duration (below 1 msec) without 'chatter'. The radiofrequency SIU is essentially a high frequency transformer whose output is rectified and filtered to produce DC outputs[3,4] and the opto-isolator SIU use a light beam to control the flow of current driven by a battery[4–6]. The last two SIU are multi-component instruments that could be replaced by just one battery, to drive the current, and one switch, to turn on and off the current. Here I am presenting a new type of SIU, with most of the features of the older instruments, whose construction is very simple and whose cost (excluding batteries) is ~ US $7.00. It consists of a very fast dual complementary metal oxide semiconductor (CMOS) switch that turns on or off the current flow between

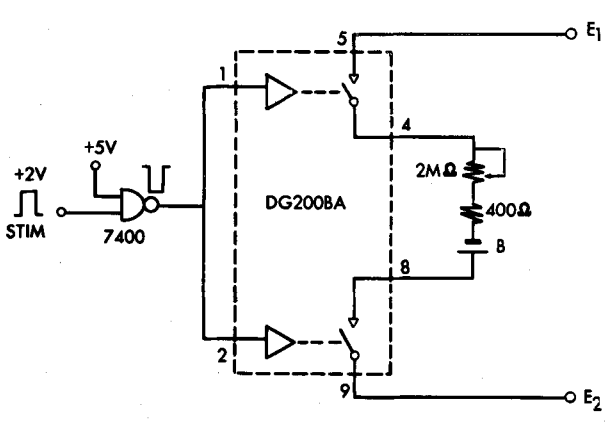


Fig. 1. Diagram of a CMOS SIU. The stimulating current flows between electrodes $E_1$ and $E_2$ when both switches are closed. The duration of the current pulse is determined by the duration of the positive square pulse (STIM), and the magnitude, by a 2 $M\Omega$ variable resistor. The numbers correspond to the pin numbers of Siliconix DG 200BA. B represents two 9.8 V mercury batteries, Mallory TR137.

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