occurring relaxant in this species we feel that such a role should not be excluded solely on the results of experiments using very large concentrations of the drug.

J. A. Holgate
G. W. Cambridge

Department of Physiology,
School of Medicine, Leeds, 2.

[1] Hoyle, G., and Lowy, J., *J. Exp. Biol.*, **33**, 295 (1956).
[2] Twarog, B. M., *J. Cell. Comp. Physiol.*, **44**, 141 (1954).
[3] Cambridge, G. W., and Holgate, J. A., *J. Physiol.*, **130**, 22*P* (1955).
[4] Blaschko, H., Symposium on 5-Hydroxytryptamine (1957).

Recently, Evans and Schild[1] have examined the effects of electrical stimulation and drugs on vertebrate smooth muscle which had been depolarized by the external application of potassium ions. The conducted response to electrical stimulation was abolished under these conditions but the contraction in response to drugs, presumed to be due to local excitation of smooth muscle cells, was maintained. Experiments on invertebrate smooth muscle, the anterior retractor muscle of the byssus of *Mytilus edulis*, have given results which differ from these. This muscle can be depolarized by exposure to 0·56 *M* potassium chloride and this produces, under isometric conditions, a prompt development of tension which is maximal in about 10 sec., and relaxation occurs over a period of 30–40 sec. The muscle is then inexcitable by electrical stimulation (either d.c. which normally produces a tonic contraction or 2-m.sec. pulses 4/sec. which normally produce a phasic contraction), and it also fails to respond to acetylcholine in concentrations up to 2 μgm./ml. The course of depolarization of the muscle by potassium chloride was determined by Twarog[2], who found the maximum potential on depolarization was obtained 20–25 min. after the potassium chloride contraction, and in the experiments reported above the responses to electrical stimulation and drugs were examined within this period. Soaking in sea water restored the excitability to electrical stimulation and to drugs.

Owen[3] reported that lamellibranchs could be narcotized and caused to relax by exposure to propylene glycol monophenyl ether ('Propylene phenoxetol'), and preliminary experiments indicate that the anterior retractor muscle of the byssus may be rendered insensitive to electrical stimulation and to acetylcholine by soaking in a 0·25 per cent solution of this compound in sea water. Furthermore the contraction produced by 0·56 *M* potassium chloride is abolished under these conditions; the effect of phenoxetol is reversible on soaking the muscle in sea water.

A further agent which may be of value in differentiating the mode of action of drugs and electrical stimulation on the membrane is the toxin from *Gymnodinium veneficum*[4] which is presumed to render nerve and muscle, including the anterior retractor muscle of the byssus, inexcitable by increasing the permeability of the membrane to sodium ions. So far, experiments I have made with the impure toxin have not yielded clear-cut results, a marked change in threshold occurring rather than an absolute failure of response to stimulation.

Details of this investigation, including the effects of other ions on the response of the muscle to drugs, will be reported elsewhere.

I wish to thank the Director of the Marine Biological Association Laboratory for the facilities placed at my disposal at Plymouth, and to acknowledge a personal grant from the Browne Research Fund of the Royal Society which enabled me to undertake this work.

G. W. Cambridge

Department of Physiology,
University of Leeds.

[1] Evans, D. H. L., and Schild, H. O., *Nature*, **180**, 342 (1957).
[2] Twarog, B. M., *J. Cell. Comp. Physiol.*, **44**, 141 (1954).
[3] Owen, G., *Nature*, **175**, 434 (1955).
[4] Abbot, B. C., and Ballantine, D., *J. Mar. Biol. Assoc. U.K.*, **36**, 169 (1957).

ACTION OF HYDROCORTISONE ON RESPIRATION OF HUMAN CANCER CELLS IN TISSUE CULTURE

By HENRY GROSSFELD

Departments of Medicine and Orthopædic Surgery, Columbia University College of Physicians and Surgeons, and the Edward Daniels Faulkner Arthritis Clinic of the Presbyterian Hospital, New York

A PRIMARY human epidermoid cancer of the larynx, *H.Ep.*2(Toolan), was transplanted into X-irradiated and cortisone-treated rats[1], and, after two generations in rats, has been grown in tissue culture since September 1952[2]. The tissue culture cells when re-implanted into an irradiated, cortisone-treated rat form a typical epidermoid cancer. In tissue culture these cells, growing on the glass surface of *T*-60 flasks[3], form dense epithelial sheets very much like the original epidermoid cancer. Their rate of growth is relatively high, and their number doubles in about three days[2]. In the following experiments the culture medium consisted of 10 per cent of half-diluted embryo extract, 40 per cent horse serum, and 50 per cent Earle's balanced salt solution containing 0·22 per cent sodium bicarbonate and phenol red as *p*H indicator. Cultures of several flasks were thoroughly scraped from the glass surface, the cell suspensions pooled, and 5 ml. were put in each of four *T*-60 flasks. 0·5 ml. of the pooled cell suspension was used for enumeration of cell nuclei in the hæmocytometer[5], after trypsinization. Except for enumeration of cell nuclei, no trypsinization was used. Approximately equal distribution of cells in the four *T*-60 flasks was achieved by continuous mixing of the stock suspension with a pipette. 5 ml. of fresh medium were then added to each of the four flasks, and to two of them hydrocortisone in a final concentration of 0·62–1·25 γ per ml. Since the hydrocortisone stock solution contained 50 per cent ethanol, ethanol in the same dilution was added to the control flasks. Hydrocortisone was added to the cultures 3–4 times at intervals of about three days, and also to the Warburg vessels before they were attached to the manometers.

The medium of the four *T*-60 flasks was completely removed with a Pasteur pipette, and to each flask 3 ml. of fresh medium were added. The cells of the