SMITH describes as a *non sequitur*, and therefore fallacious, my conclusion that (to quote SMITH) 'environmental stimuli affected control rats and LiCl-treated rats differently'[1]: it might well have been had I in fact drawn that conclusion. What I actually said was that 'lithium chloride... acted to suppress selectively activity which was stimulus controlled'[3]. My statement was carefully chosen to conform with the logic of the experiment and the rationale can be illustrated most directly by the use of Venn diagrams (Figure 1).

Of course, the form of analysis applied in Figure 1 to the behavioural effects of LiCl does not apply if LiCl either fails to affect vertical activity or fails to do so differentially as compared to measures of horizontal activity. SMITH made no test of the latter possibility, but he did confirm that LiCl reduced rearing significantly when compared with NaCl-treated control rats, albeit only in the first 5 min of the test period. However, he failed to confirm that vertical movements fall into the class of behaviours which are stimulus controlled. The explanation for this may lie in the methods used to record rearing activity. SMITH employed a fixed criterion of rearing height (9 cm above the floor of the apparatus) and a hand-operated recorder. In our studies we recorded vertical movements in an analogue form on a moving pen recorder, defining a rear as a 'peak on the activity record which was preceded by a rise of at least 1 cm from the previous low point and followed by a drop of at least 1 cm to the next low point'[2]. This system, which does not depend on a fixed criterion of rearing height arbitrarily imposed by the experimenter (Figure 2), was chosen because we had found, in previous work using other drugs[9], that it enabled quite subtle drug effects to be detected in a way not possible by simple observational techniques. In later studies[6] we further refined our recording method to provide greater sensitivity, by using the integrated area under the analogue curve as our index of vertical activity.

Using our recording technique we were able to establish the stimulus-dependence of rearing behaviour, confirming the finding of previous workers[10].

Many of the behavioural effects of lithium may be very subtle[11] and subject to the influence of factors still to be elucidated: the apparently contradictory findings which are so characteristic of studies involving lithium[12] may well stem from differences in behavioural recording techniques. It has, I think, to be remembered that failure to replicate an experimental finding can have many causes, all of which need to be carefully explored before firm conclusions may be drawn. It is a common error to confuse a failure to replicate an experimental finding with the refutation of an hypothesis based upon that finding, and SMITH's data, whilst interesting in themselves, do not warrant his conclusion about the falsity of our hypothesis concerning the mode of action of lithium salts in modifying behaviour.

[9] A. KEENAN and F. N. JOHNSON, Experientia *28*, 428 (1972).
[10] H. C. HOLLAND, B. D. GUPTA and E. WELDON, Activ. nerv. Super. *8*, 140 (1966).
[11] F. N. JOHNSON, Experientia *32*, 212 (1976).
[12] F. N. JOHNSON, in *Lithium Research and Therapy* (Ed. F. N. JOHNSON; Academic Press, London 1975), p. 315.

PRO EXPERIMENTIS

A Method for the Detection of Chemotaxis in Mammalian Tissue Cells

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Summary. A new method is described of culture of mammalian tissue cells beneath a solid medium, permitting the assessment of growth-promoting, growth-inhibiting and chemotactic substances.

Chemotaxis in mammalian leucocytes has been greatly clarified since the introduction of the BOYDEN chamber[2], but the equally important problem of detecting chemotaxis in tissue cells such as fibroblasts and smooth muscle cells has not yet been solved.

One approach would be to culture cells in a solid medium, in which diffusion gradients can be established; the medium must however lack a fibrillar structure, because cultured cells tend to grow along fibres. Accordingly the use of culture media containing agar was investigated.

Commercial preparations of agar were found to have varying properties; the lowest concentration to produce a solid (not sloppy) medium at room temperature was achieved by melting 17 ml of 3% agar (Oxoid) and adding 83 ml of culture medium when it had cooled to approx. 54°C. The medium used was Dulbecco and Vogt's modification of minimal Eagle's medium containing 10% foetal calf serum (Flow Laboratories, Scotland). The mixture was made under sterile conditions and introduced into 60 mm. Falcon plastic tissue culture dishes (4 ml in each).

Mouse fibroblasts of the 3T6 line were concentrated in suspension in fluid medium following trypsinization and a drop of suspension was inoculated on to the surface of the solid medium at room temperature. The dishes were incubated at 37°C in sealed Filde's jars, after gassing with 20% O_2, 5% CO_2 and 75% N_2 in order to buffer the bicarbonate-containing medium.

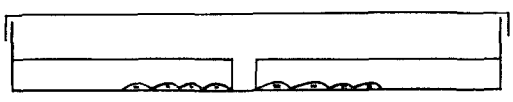

Fig. 1. Diagram of culture system.

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[2] S. V. BOYDEN, J. exp. Med. *115*, 453 (1962).

After 4 days, most of the cells remained on the surface of the solid medium as spherical clusters of about 20–50 cells, but some were found to have migrated through the medium, appearing as flattened individuals on the floor of the dish. In subsequent experiments, therefore, the cell suspension was inoculated directly on to the floor of the dish, after cutting a central 5 mm well through the whole thickness of the medium. After incubation for 14 days these cells had proliferated centrifugally beneath the gel to produce a circular monolayer about 1 cm in diameter clearly visible to the naked eye (Figure 1). Subcultures into fluid medium showed that the cells survived in these conditions up to 6 weeks after inoculation, without any further addition of medium. This long survival suggests applications as a transport medium.

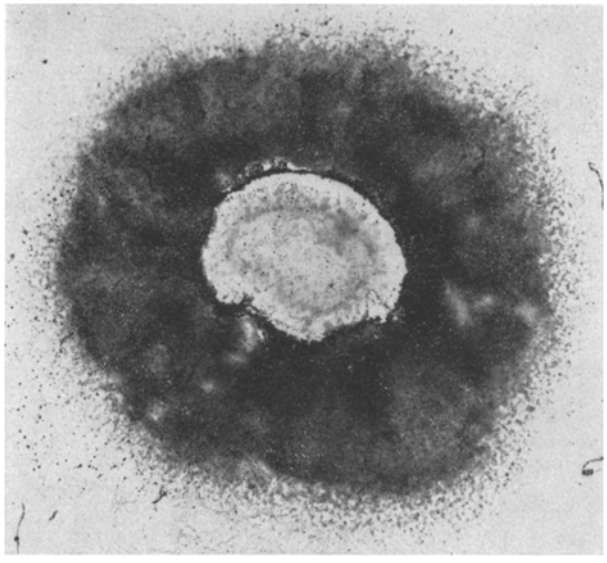

Fig. 2. Pattern of distribution of 3T6 fibroblasts after 14 days incubation. Haematoxylin and eosin. ×7.

The experiment was then repeated using smooth muscle cells, cultured in vitro from explants of pig aortic media. These cells did not survive, so the experiments were again repeated using, instead of agar, 3% agarose (initially agarose F.3083, later Indubiose A.45, L'Industrie Biologique Francaise, S.A.). This change was suggested by CUTLER's use of agarose-containing media[3], in a very similar system, for the detection of neutrophil chemotaxis. In agarose medium, the smooth muscle cells flattened on the floor of the dish and survived for up to 3 weeks beneath the medium close to the central well; the rate of cell proliferation was much less than that of the 3T6 fibroblasts, but for the present purpose that is of no consequence.

Experiments are now in progress in which materials are introduced into shallow wells in the periphery of the medium. These experiments suggest that the distribution pattern of the cells can be modified by this means; and therefore that the technique is suitable for the detection of both chemotaxis and growth-promoting substances (GOLD, TAYLOR, LEVENE and MITCHINSON, unpublished results).

An experiment can be concluded at any convenient time by fixing the adherent cells in situ by the addition of fixatives such as formol saline and then tipping the solid disc of medium out of the plate. The cells can then be stained by various routine methods (e.g. haematoxylin and eosin) but, because xylene dissolves the plastic, araldite should be used as the mountant. In this way a permanent record of the growth pattern is obtained (Figure 2). We believe the technique has broad applications.

[3] J. E. CUTLER, Proc. Soc. exp. Biol. Med. *147*, 471 (1974).

Blood Plasma Investigations by Resonance Raman Spectroscopy: Detection of Carotenoid Pigments

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Summary. Using Raman spectroscopy, we demonstrate that low levels of β-carotene, lycopene, and xanthophyll give rise to resonance enhanced bands in blood plasma. These results explain the significance of previously unidentified spectral maxima which have been related to the state of health of the blood donor.

In 1974 LARSSON and HELLGREN[1] reported that both the Raman and fluorescence spectra of human blood plasma change markedly depending on the state of health of the individual. The laser Raman spectrum of blood plasma exhibits three scattering maxima superimposed on a fluorescence background. We now report that these previously unidentified bands[1] arise from the carotenoids in blood plasma, and thus report the direct detection of carotenoids in blood plasma by resonance Raman spectroscopy.

Carotenoid pigments are intensely colored, and through the coupling of electronic and vibrational modes give rise to a resonance enhanced spectrum which can be detected at 10^{-7} M[2,3]. Previous work has shown that carotenoids are carried by plasma proteins at levels of ca. 10^{-6} M[4]. Thus, we believed that direct observation of carotenoids by the resonance Raman technique should be possible. Repetitive measurements on human blood plasma showed the three maxima at 1517, 1157 and 1005 cm^{-1} (Figure a, Ar^+ laser at 514.5 nm). These frequencies and relative

Resonance Raman spectra (cm^{-1}) in chloroform

	β-Carotene			Lycopene		Xanthophylls	
	Lit.[2,3]	Authentic sample[a]	Fraction I	Lit.[2,3]	Fraction II	Authentic sample[b]	Fraction III
ν_2	1527	1523	1523	1516	1516	1525	1525
ν_1	1158	1157	1157	1156	1155	1158	1157
ν_4	1006	1006	1006	1004	1004	1007	1005