almost empty the tube connecting it to the 2-l. vessel is clipped off near the joint at the 5-l. reservoir and the latter is replaced by a fresh reservoir.

The apparatus has been used successfully to grow the strictly anaerobic *Selenomonas ruminantium*, a strictly anaerobic rumen lipolytic bacterium, and the facultatively anaerobic rumen strain of *Streptococcus bovis*. Although some failures have been caused by breakage of glass parts or contamination, cultures of the anaerobic bacteria have been kept going for more than a month and there seems no reason why longer runs should not be possible if necessary. The pumps have never failed during long runs and have had no parts renewed in two years' experiments. The rubber tubing is generally renewed between runs and all joints of rubber to glass tubing are taped and wire-bound.

The selenomonad and streptococcal cultures have been carried out under glucose-limiting conditions. Fig. 2 shows a run with the lipolytic bacterium growing on glycerol in which a nitrogenous (or other so far unidentified) factor was limiting, with glycerol in excess. The basal medium used was like medium 14 of Hobson and Mann[2]. The cell concentration is given in optical density units which were shown to be proportional to cell dry weight. The maximum growth in batch cultures was not attained when medium flow was started. The low value at 164 h was caused by failure of the carbon dioxide flow during the night. The culture continued to flow into the reservoir, but stirring by the pump *G* alone was not adequate. Under normal carbon dioxide flow mixing in the culture vessel is complete in 1 sec. This organism retained a uniform turbidity, but during long runs with the selenomonad granular growth occasionally occurred. However, this reverted to normal growth in a day or two. The buffering capacity of the medium is sufficient to keep the *p*H at an optimum level (about 6·2 usually).

Continuous culture is thus a feasible method of obtaining large amounts of cells of rumen bacteria for enzyme and other preparations as well as for metabolic studies.

P. N. Hobson
W. Smith

Rowett Research Institute,
Bucksburn, Aberdeen.

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CYTOLOGY

Duration and Rate of Mitosis after a Single Application of Methylcholanthrene

Several authors[1-4] have commented on the significant increase of the mitotic count (called 'mitotic activity' by many authors) shortly after application of methylcholanthrene and other carcinogens to the epidermis. The increased mitotic count has, as a rule, been interpreted as a sign of augmented growth, that is, an increased mitotic rate. The mitotic rate, defined as the number of cells that complete their mitoses per unit time, is related to the mitotic count and the mitotic duration as shown in the equation[5]: (mitotic rate) × (mitotic duration) = mitotic count.

Table 1. Changes in Epidermal Mitotic Count, Mitotic Duration, and Mitotic Rate after a Single Application of 20-methylcholanthrene

Time (weeks)	Mitotic count (%)	Mitotic duration (h)	Mitotic rate (%/h)
1	0·51 ± 0·13	1·57	0·33
2	0·30 ± 0·10	1·42	0·21
3	0·28 ± 0·12	1·20	0·23
4	0·19 ± 0·05	0·87	0·22
5	0·41 ± 0·05	1·44	0·29
7	0·21 ± 0·05	0·88	0·24
8	0·41 ± 0·14	1·39	0·29
9	0·34 ± 0·06	1·24	0·27
10	0·41 ± 0·12	1·25	0·33
11	0·24 ± 0·01	0·70	0·34

The mitotic counts represent the means of groups of four mice. Two thousand cells were counted in each specimen. The mitotic duration and rate represent the means of observations on 8 mice, 4 killed before and 4 after a delay of 4 h after injection of colcemid.

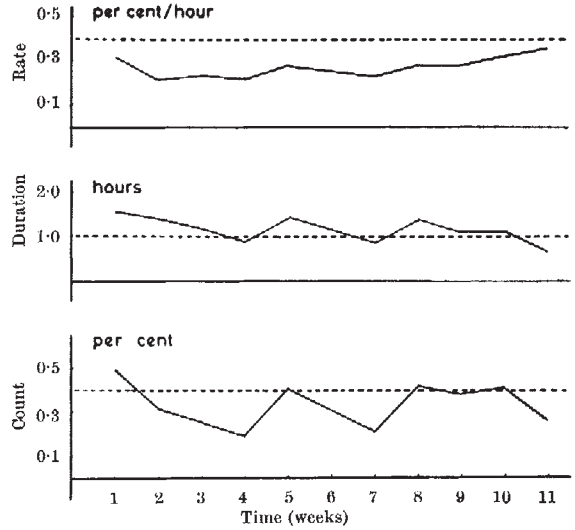

Fig. 1. Variations in mitotic rate, mitotic duration, and mitotic count after a single application of 20-methylcholanthrene to the epidermis

It can readily be seen that a high mitotic count can be the result of prolonged mitotic duration as well as of increased mitotic rate. The importance of variation in mitotic duration has sporadically been mentioned[6] but has not been thoroughly emphasized until recently. Evensen[7,8] has demonstrated that great variations occur in the mitotic duration during the first hours and days after application of a carcinogen to the epidermis.

To examine this phenomenon further, groups of eight hairless mice were painted with 0·03 ml. of a 1 per cent solution of methylcholanthrene in benzene, and the mitotic count, the mitotic duration, and the mitotic rate were determined weekly during a period of 11 weeks. The mitotic duration was evaluated by the colcemid method[7]. The results are shown in Fig. 1 and Table 1.

The initial increase and the subsequent fluctuations of the mitotic count are obviously a reflexion of the corresponding variations of the mitotic duration. The mitotic rate, however, decreases during the first two weeks to about half the normal value. Thereafter it displays a gradual and approximately linear increase.

The profound effect of a single application of methylcholanthrene, observed also by other authors, is evident. The present results do not, however, indicate whether these variations are causally related to the carcinogenic effect of this compound.

Kjell Elgjo

Institutt for Generell og Eksperimentell Patologi,
Universitetet i Oslo, Rikshospitalet,
Oslo.

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Fluorescence Microscopy and Autoradiography of Colchicine-induced Micronucleated Cells

Colchicine inhibits mitosis in plant and animal cells[1]. When colchicine-treated tissue-cultured cells are stained with acridine orange fluorochrome and viewed by fluorescence microscopy, an impressive result of its activity is seen as a morphological disorganization of the nuclei[2] and subsequent formation of micronucleated cells. X-irradiation causes a similar effect[3]. The origin of micronuclei may be accounted for either by fragmentation of the inter-