Most important, in the system reported here, we test for the effect of mutagen on a regulator protein, a repressor determining lysogeny of phage $\phi 105$ in *B. subtilis*. This protein is not required for phage growth but rather for control of certain functions, and viable mutants are easily found.

The other important advantage of the transfection assay is its insensitivity towards any cyto-toxicity of the agent to be tested, since the chemical can be removed from the reaction mixture after the incubation period.

A limited number of chemical covering 9 major groups of chemicals showing positive results are indicative of the sensitivity and suitability of this test system. Experiments are in progress using a number of other chemicals to further confirm the usefulness of this test system.

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Cytogenetic evidence for hemizygosity at the thymidine kinase locus in P388 mouse lymphoma cells

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Summary. A detailed cytogenetic investigation was carried out on P388 mouse lymphoma cells. The cells have a mean chromosome number of 36.86 with a mode and median of 37 chromosomes. G-banding analysis of 12 spreads revealed a total of 15 marker chromosomes with chromosome 11, the determinant of thymidine kinase, being present only in single copy per cell. It is therefore concluded that the P388 cell line is hemizygous at the thymidine kinase locus. Thymidine kinase activities were assayed in P388 cells and two other malignant cell lines, clone 707 Friend mouse leukaemia cells and L5178Y mouse lymphoma cells. No clear relationship was observed between enzyme activity and gene dosage.

Resistance in cultured mammalian cells to thymidine analogues, e.g., bromodeoxyuridine (BrdU) or iododeoxyuridine (IdU) is brought about by deficiency of the enzyme, thymidine kinase. As thymidine kinase is autosomally linked in mammals, mutations are required in both copies of the relevant chromosome in order for BrdU (and IdU) resistance to occur. It therefore follows that the spontaneous frequency of cells resistant to either BrdU or (IdU) is usually very low e.g. 10^{-10} in mouse L cells[2] and 10^{-9} in hamster V79 cells[3].

Two exceptions to the observation and expectation of low spontaneous frequencies of cell lines resistant to halogenated thymidine analogues are clone 707 Friend mouse leukemia cells and P388 mouse lymphoma cells. Both cell lines have high spontaneous mutation frequencies to thymidine kinase deficiency: 5×10^{-5} in clone 707 Friend cells[4] and

Table 1. Results of G-banding analysis of P388 cells

Chromosome	% of cells containing specific chromosome	Mean No. of copies per cell	Chromosome	% of cells containing specific chromosome	Mean No. of copies per cell
1	100	1.0	M 1	100.0	1.0
2	100	1.92	M 2	100.0	1.0
3	100	2.0	M 3	100.0	1.08
4	100	1.42	M 4	75.0	0.92
5	100	1.83	M 5	41.67	0.42
6	100	1.08	M 6	91.67	0.92
7	100	2.0	M 7	100.0	1.0
8	100	1.0	M 8	83.33	1.42
9	100	1.33	M 9	25.0	0.25
10	100	1.17	M 10	8.33	0.08
11	100	1.0	M 11	8.33	0.08
12	100	1.08	M 12	8.33	0.08
13	100	1.33	M 13	8.33	0.08
14	100	1.08	M 14	16.67	0.25
15	100	1.67	M 15	8.33	0.08
16	100	1.17			
17	100	1.42			
18	100	1.75			
19	100	1.17			
X	0	0			
Y	0	0			

3×10^{-4} in P388 cells[5], suggesting that they may be heterozygous or hemizygous at the thymidine kinase locus. Chromosome 11 is the determinant of thymidine kinase in the mouse[6]. Cytogenetic analysis revealed that on average 2 normal copies of chromosome 11 are present per cell in the Friend cell clone[7] suggesting that these cells are probably heterozygous rather than hemizygous for the thymidine kinase gene. It has also been reported that deficiency of thymidine kinase leads to increased mutagen sensitivity in both cell lines[8-11].

In this communication we report on a detailed cytogenetic investigation of P388 cells involving conventional chromosome staining, and G-banding. The results indicate that P388 cells are hemizygous at the thymidine kinase locus. An attempt is also made to relate cellular thymidine kinase activities to gene dosage.

Methods. Three cell lines were used in the present study. P388 mouse lymphoma cells were originally described by Fox and Gilbert[12], clone 707 Friend mouse leukemia cells by Scher et al.[13]. and L5178Y mouse lymphoma cells by Fischer[14]. The cells were cultured in Ham's SF12 medium (Flow Laboratories) supplemented with MEM non-essential amino acids and 16% horse serum. The accumulation of metaphase spreads and G-banding was carried out as previously described[7]. Thymidine kinase activities were also determined by a previously described method[4].

Results and discussion. Chromosome numbers were counted in 50 metaphase spreads. The numbers per spread varied from 33 to 38. The mean chromosome number was 36.86 with a mode and a median of 37 chromosomes. The discrepancy between these figures and the 40 telocentric chromosomes found in normal mouse cells is partially offset when chromosome arm numbers are taken into consideration. Due to the presence of, on average, 1.06 metacentric chromosomes and 1.000 sub-metacentrics per spread the mean number of chromosome arms per spread was found to be 38.92 which is still however, significantly below that of normal mouse.

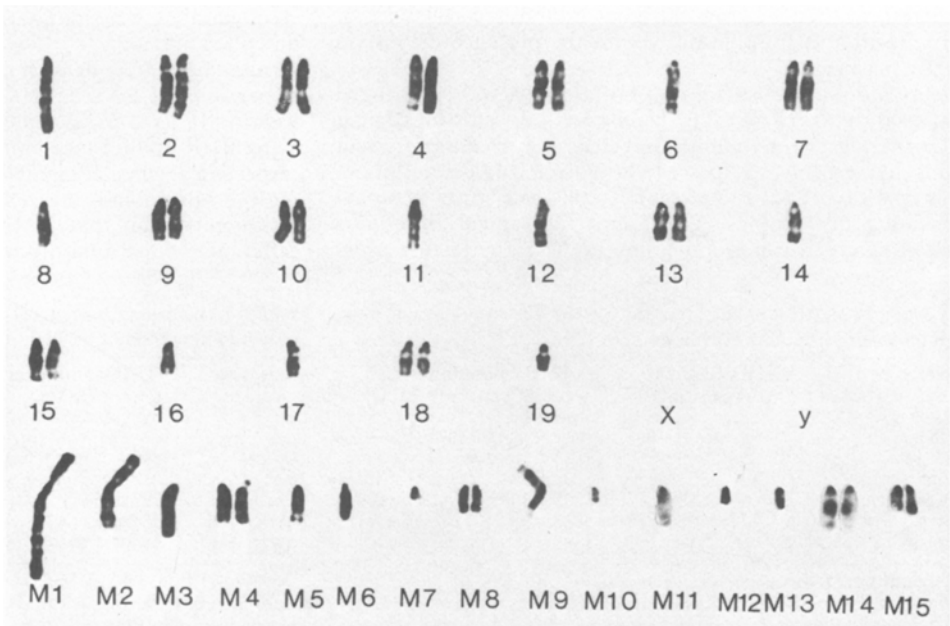
A more detailed analysis of the chromosome content of P388 cells was carried out by preparing G-banded karyotypes of 12 metaphase spreads. The figure is a composite karyotype in which the majority of chromosomes are taken from a single spread, however, where necessary chromosomes, from other spreads have been added to make up 2 copies of each chromosome. A number of marker chromosomes were observed. M1 is a sub-metacentric chromosome which appears to have resulted from a centric fusion between chromosome 1 and the Y chromosome. M1 was observed in all 12 spreads as were M2, and M7. M2 is an almost metacentric chromosome involving a centric fusion between chromosomes 13 and 14. M3-M15 are chromosomes of unclear origin, though M3 appears to be derived from the X chromosome.

Chromosome 11, the determinant of thymidine kinase in the mouse[6] was present in single copy in all 12 spreads examined. Although the exact origin of the marker chromosomes is unclear, careful examination of their banding patterns indicates that none of them are derived from chromosome 11.

In order to establish whether or not a relationship exists between thymidine kinase activities and dosage of the thymidine kinase gene, the activity of P388 cells was compared to 2 other suspension culture cell lines, namely clone 707 Friend mouse leukemia cells and L5178Y mouse lymphoma cells. All 3 cell lines are derived from the same strain of mice, DBA/2 and have similar growth rates. As stated earlier both P388 cells and clone 707 Friend cells have unusually high spontaneous mutation rates to thymidine kinase deficiency and are considered to be either heterozygous or hemizygous at this locus (i.e. $TK^{+/-}$). L5178Y cells have a low spontaneous mutation rate at the thymidine kinase locus of 5×10^{-11} $cell^{-1}$ $generation^{-1}$ (Clive et al.[15]) and are considered to be homozygous ($TK^{+/+}$). The thymidine kinase activities of the 3 cell lines are displayed in table 2. If thymidine kinase activity was directly related to gene dosage it would be expected that the respective ratios of thymidine kinase activities in the

Table 2. Thymidine kinase activities

Cells	pmoles dTMP min^{-1} mg^{-1} protein	% P388	% 707	% L5178Y
P388	9.784 ± 2.452	100	18.369	14.091
707	53.261 ± 2.24	544.368	100	76.709
L5178Y	69.432 ± 1.201	709.648	130.361	100



G-banded composite karyotype of P388 mouse lymphoma cells.

3 cell lines namely P388, clone 707 Friend, and L5178Y would be 1:1:2. While the L5178Y cells have clearly the highest activity, P388 cells have considerably less activity than would been expected in the event of a direct relationship existing between enzyme activity and gene dosage. The ratios of enzyme activities between P388 and L5178Y cells are similar to those observed earlier by Fox and Anderson[16] It would therefore, appear that gene regulation plays a greater part in determining thymidine kinase activities than does gene dosage.

In conclusion, Anderson and Fox[5] in reporting on a high mutation requency in P388 cells to thymidine kinase deficiency, postulated that they may be heterozygous or hemizygous at the thymidine kinase locus. The latter possibility is clearly the case as the results of the cytogenetic part of the present investigation show that only one copy of chromosome 11, the determinant of thymidine kinase is present per cell.

1 The authors acknowledge a grant from the Department of Health and Social Services for Northern Ireland and the technical assistance of Mr D.J. Mooney and Mr H. Termonia. Author for reprint requests: P.G. McK.

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The serum-free growth of different cell types in buffalo milk plasma

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Summary. Buffalo milk plasma can replace the fetal calf serum required for the growth of various types of cells. However, the addition of serum is essential for the initial attachment of the cells.

Serum has been an essential component of the medium for growing almost all types of cells. In addition to nutrients, it also provides growth factors and hormones. Recently attempts have been made to formulate serum-free media using various combinations of hormones and growth factors[1-3]. Human milk has been shown to contain epidermal growth factor (EGF)[4], and it also stimulated the DNA synthesis and cell division in Balb/c 3T3 cells[5]. Bovine colustrum supported the serum-free proliferation of epithelial cells, but not of fibroblasts in long-term culture[6]. We, therefore, tried to grow different established cell lines of epithelial and fibroblastic type in the presence of buffalo milk plasma.

Materials and methods. Fresh buffalo milk was centrifuged at 8000 × g for 15 min followed by another centrifugation at 120,000 × g for 60 min. The clear supernatant, free of micellar casein, was passed through a 0.45-μm millipore filter. HeLa, BSC-1, Vero, BHK cell lines (from National Institute of Virology, Pune) and rat vaginal fibroblasts (RVF) were grown in a medium prepared by mixing equal volumes of Dulbecco's minimal essential medium (DME) and Ham's F-12 (F-12) medium containing fetal calf serum (FCS) or milk plasma (MP). For growing the cells in MP, the cells were initially plated in 4 ml of the medium containing 5% FCS in 6-cm Petri dishes (Sterilin, UK) and incubated in a humidified incubator at 37 °C with 5% CO_2. After the cells were attached, the medium was removed, the cells were washed twice with F-12 medium and incubated further in the medium containing MP. The cells were counted using a hemocytometer. The incorporation of ^{3}H-thymidine was monitored according to a previously described method[7].

Results. The effect of FCS or MP on the total cell count is shown in table 1. Vero, BHK and RVF cells did not grow as well in MP as in FCS-containing medium. In milk plasma, Vero, BHK and RVF cell numbers were 76%, 69% and 58%, respectively, when compared with those in fetal calf serum. On the other hand, the cell number of HeLa and BSC-1 was higher than that in FCS. There was no significant difference in the total protein content per cell when either

Table 1. Total cell-count after the growth of different cell types in fetal calf serum or milk plasma*

Cell type	Initial cell count ($\times 10^4$ cells/ml)	Total cell number ($\times 10^4$ cells/ml ± SEM) FCS	MP
HeLa	9	94 ± 2.3	115 ± 1.1
BSC-1	8	73 ± 0.9	81 ± 1.2
Vero	7	55 ± 1.0	42 ± 0.8
BHK	6	36 ± 2.6	25 ± 2.4
RVF	7	41 ± 1.7	24 ± 0.7

*Counting of the cells was done on the 4th day of culture and the results are average values from 4 Petri dishes in each group.

Table 2. Incorporation of ^{3}H-thymidine by different cell types grown in the presence of fetal calf serum or milk plasma*

Cell type	^{3}H-thymidine incorporation (cpm ± SEM) FCS	MP	Incorporation (%)**
HeLa	78475 ± 6865	125940 ± 6027	160
BSC-1	67533 ± 2621	69087 ± 1873	102
Vero	29125 ± 785	21255 ± 486	73
BHK	25491 ± 643	12831 ± 291	50
RVF	56392 ± 2783	30187 ± 5620	53

*On the 3rd day, 20 μCi of ^{3}H-thymidine was added and the cells were incubated further for 24 h. **Expressed as percentage of incorporation in FCS.