the acid at different temperatures, also show some similarity with data obtained after hydrolysis in 1 *N* HCl at 60 °C for varying periods of time. In fact the result in the present study with 12 *N* HCl at 20 °C is intermediate between that of 5 *N* HCl at 26 °C and 1 *N* HCl at 60 °C. Therefore, the present author feels that the process of breakdown of purine in materials hydrolyzed in 12 *N* HCl at 20 °C starts quickly, and even more quickly in 12 *N* HCl at 35 °C, just like after 1 *N* HCl at 60 °C; but whereas in the latter case further degradation of the DNA complex and loss of apurinic acid are brought about by heat, in the former case with 12 *N* HCl these are caused by the optimum normality of the acid and temperature.

Résumé. On a étudié l'effet des conditions d'hydrolyse de tissus ammaliens par l'acide chlorhydrique concentré sur la coloration de Feulgen en lumière UV.

M. K. DUTT

Department of Zoology, University of Delhi, Delhi – 110007 (India), 13 February 1975.

A Rapid and Simple Method for the Detection of Mycoplasma and Other Intracellular Contaminants

There are many published methods for the detection of mycoplasmas in cultured eukaryotic cells (for reviews see[1,2] and references within), most of which are complex, time consuming and often inaccurate giving false-negative results. Recently a method has been published[3] that involves detection of cytoplasmic DNA-containing cell contaminants by staining their DNA with either Hoechst 33258 or 4′-6-diamidino-2-phenylindole and viewing by fluorescence microscopy. This method, as are several others, is based on the fact that mycoplasmas and some DNA animal viruses replicate in the cytoplasm. However, one drawback of this procedure is that cells must be grown on coverslips. In the method I describe, a small culture vessel (e.g. a 6 oz medical flat) routinely used for sub-culture is adequate for the assay. An answer can be obtained within 2 h, thus making it feasible to check for the presence of mycoplasma in sister cultures before each experiment, as suggested by LEVINE[2].

The method I describe is a simpler and more sensitive double-label modification of the one described by SCHNEIDER et al.[4] and makes use of the fact that mycoplasmas synthesize the enzyme pyrimidine phosphorylase[5] which both hydrolyses uridine (and thymidine) to uracil (and thymine), and can 'salvage' pyrimidines to nucleosides by the reverse reaction; this means that exogenous uracil can be incorporated into RNA in mycoplasma-infected cells but not in uninfected cells[5,6] where this enzyme is in the main absent (but see ref.[2]).

Methods. A 50 times concentrated mixture of ^{3}H-uridine and ^{14}C-uracil was added to a rapidly growing, subconfluent bottle of cells to final levels of 1 μCi/ml and 0.1 μCi/ml respectively, without changing the medium. This bottle was allowed to incorporate label for 1 h, after which time the medium was poured off and the attached cells washed twice with a balanced salt solution (e.g. Earle's, Hank's, phosphate buffered saline). 5 ml of 0.1% sodium dodecyl sulphate (SDS; w/v) was then added and, after leaving for 5 min to allow complete lysis, the viscous solution was poured into 10 ml of 20% trichloroacetic acid (TCA; w/v) at 0 °C. A further 5 ml of 0.1% SDS was used to rinse the culture vessel, and this was also added to the 20% TCA. The mixture was left for 20 min at 0 °C before filtration onto a Whatman GF/A or GF/C filter; this filter was washed sequentially with 20 ml of ice-cold 10% TCA, 10 ml of ethanol-ether (1:1 by vol.) and 10 ml of ether. Finally the filter was dried in an oven at 100 °C for 5 min, added to a vial containing PPO-POPOP toluene scintillant and counted in a Packard liquid scintillation spectrometer; the settings were such that ^{3}H and ^{14}C could be differentiated e.g. ^{3}H: window 50–400, gain 90%; ^{14}C: window 350–1000, gain 10%. With these settings ^{3}H is counted at 47% relative efficiency of counts, and ^{14}C at 35%; spillover from ^{14}C channel into ^{3}H channel is 15% of the corrected ^{14}C cpm, while there is no spillover of ^{3}H cpm into the ^{14}C channel. Background values were obtained by stopping the incorporation at time zero (i.e. immediately after addition of label), and processing exactly as above.

Total cellular DNA was isolated essentially as described by MARMUR[7].

A comparison of ^{3}H-uridine and ^{14}C-uracil incorporation in healthy, mycoplasma-infected, and kanamycin-treated infected mouse L cells

Condition of cells	Corrected TCA-insoluble radioactivity (cpm)	
	^{3}H-uridine	^{14}C-uracil
Healthy cells	81,540	7
Infected cells	3,050	5,285
Kanamycin (200 μg/ml) treatment for 7 days	7,488	1,522

Results and discussion. The importance of using radioactively-labelled nucleic acid precursor compounds in a wide variety of types of experiments with tissue culture cells over the last 20 years or so is obvious. If cell lines are contaminated with mycoplasma, then the host's metabolism is radically altered [1–6]. For example, mycoplasmas synthesize pyrimidine phosphorylase, an enzyme that hydrolyses pyrimidine nucleosides to ribose and the free base. An important consequence of this is that the use of thymidine or uridine either to label cellular nucleic acids, or in the case of thymidine, to synchronize cells, becomes impractical, since these coupounds are degraded by the mycoplasma contaminants. The presence of pyrimidine phosphorylase, however, can be exploited in a convenient assay for the presence of mycoplasma.

SCHNEIDER et al.[4] labelled separate cultures with either ^{3}H-uridine or ^{3}H-uracil, purified total cellular RNA from both, and determined their specific radioactivities. Thus the ratio of the specific radioactivities of the RNA labelled with ^{3}H-uridine to the RNA labelled with ^{3}H-uracil gave an indication of elevated levels of uridine phosphorylase and hence of mycoplasma contamination. This method has now both been simplified and made more sensitive by using uridine and uracil labelled with different radioisotopes in the same culture vessel, and also speeded up by labelling for much shorter periods. Using 2 radioisotopes obviates the necessity for determining the precise amounts of RNA present.

If cells are labelled for 1 h with a mixture of ^{3}H-uridine and ^{14}C-uracil to final concentrations of 1 μCi/ml and 0.1 μCi/ml respectively, the incorporation into RNA can be measured as described in Methods. The Table shows results from a typical experiment using mouse L cells. Isolation of total cellular DNA followed by analytical isopycnic CsCl gradient centrifugation (data not shown) confirmed the presence of mycoplasma DNA (density approx. 1.68 g/ml). Furthermore, treatment of mycoplasma-infected cells with kanamycin (200 μg/ml) for 7 days suppressed the infection but did not eliminate it; this suggests that incorporation of ^{14}C-uracil indicates the presence of some cell culture contamination.

Since the medium used (modified 199) contains uracil (2.68 μM) but nor uridine, its was necessary to check if the addition of cold uridine (to 3 μM and 30 μM) had any effect on either ^{3}H-uridine or ^{14}C-uracil incorporation. As expected, ^{3}H-uridine incorporation was decreased by addition of cold uridine, but ^{14}C-uracil incorporation was unaffected. Also using a meduim (e.g. F10) lacking uracil slightly increased ^{14}C-uracil incorporation in mycoplasma-infected cells.

So far this method has been successfully applied to the following cell types: to human lymphocyte suspension cultures; to monolayer cultures – human HEP, D98/AH2 and FLA cells, mouse L and 3T3 cells, kangaroo rat (*Dipodomys ordii*) cells, marsupial (*Sminthopsis crassicaudata*) cells and *Xenopus* kidney cells; and to several human-mouse somatic hybrid cells. Obviously this method will also detect other cell culture contaminants (e.g. viruses) if they produce enzymes that utilize uracil. Mycoplasmas, however, are the most common and troublesome cell culture contaminants.

Summary. A simple, rapid and sensitive double radioisotopic method is described for the detection of mycoplasma infection in tissue culture cell lines.

K. W. C. PEDEN[8]

MRC Mammalian Genome Unit, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT (Scotland), 21 April 1975.

1 P. P. LUDOVICI and N. B. HOLMGREN, *Methods in Cell Biology* Ed. D. M. PRESCOTT; Academic Press, Inc., New York 1973), vol. 6, p. 143.
2 E. M. LEVINE, *Methods in Cell Biology* (Ed. D. M. PRESCOTT; Academic Press, Inc., New York 1974), vol. 8, p. 229.
3 W. C. RUSSELL, C. NEWMAN and D. H. WILLIAMSON, Nature, Lond. *253*, 461 (1975).
4 E. L. SCHNEIDER, E. J. STANBRIDGE and C. J. EPSTEIN, Expl. Cell Res. *84*, 311 (1974).
5 E. M. LEVINE, Expl Cell Res. *74*, 99 (1972).
6 A. G. PEREZ, J. H. KIM, A. S. GELBARD and B. DJORDJEVIC, Expl. Cell Res. *70*, 301 (1972).
7 J. MARMUR, J. molec. Biol. *3*, 208 (1961).
8 The author thanks P. MOUNTS, V. VAN HEYNINGEN, P. R. MUSICH, J.M.R. HATFIELD and P. M. B. WALKER for discussions and criticism of the manuscript, and gratefully acknowledges receipt of a MRC Scholarship for Training in Research Methods.

PRAEMIA

Prize 'Biochemical Analysis'

The prize of DM 10000.– is donated from Boehringer, Mannheim, and is awarded every 2 years at the conference 'Biochemical Analytic' in Munich, Germany, for outstanding work in the field of biochemical instrumentation and analysis. The donation will take place during the 1976 conference between the 9th and 13th of April. One or several papers concerning one theme, either published or accepted for publication between October 1st 1973 and September 30th 1975, may be sent in triplicate before November 15th 1975 to: Prof. Dr. Ivar Trautschold, secretary of the prize 'Biochemical Analysis', Medizinische Hochschule Hannover, Karl-Wiechert-Allee 9, D–3000 Hannover-Kleefeld, Germany.

CONGRESSUS

Canada

International Symposium on Flammability and Fire Retardants

in Toronto, 6–7 May 1976

Papers should deal with flammability and fire retardancy of polyurethanes, plastics, textiles and fabrics, paints and coatings, testing procedures and marketing. Papers are now being solicited and tentative titles should be sent by October 15, 1975 to: Vijay Mohan Bhatnagar, Editor, Advances in Fire Retardands, 209 Dover Road, Cornwall, Ontario, Canada K6J 1T7.

Italy

International Symposium on Thrombosis and Urokinase

in Roma, 30 October–1 November 1975

The Symposium is organized by the Istituto Superiore di Sanità and the chairmen are: Prof. Sol Sherry of Philadelphia, USA, and Prof. R. Paoletti of Milano, Italy. Main topics: Physiopathology of thrombosis. Chemical, biochemical and pharmacological aspects of urokinase. Effects of urokinase on thrombosis. Clinical applications of urokinase.

Registration fee will be US Dollars 30.00. Information and registration by Prof. Rodolfo Paoletti, Via A. Del Sarto 21, I–20129 Milano, Italy.

Corrigendum

MARIAN DORR, HANNAH STEINBERG and M. SHAPERO: *Stimulation of Sexual Behaviour in Rats by a Benzodioxane Derivative*, Experientia *31*, 91 (1975). In paragraph 1, line 6, aminoethyl should read correctly **aminomethyl.**