Deoxycytidylate Deaminase

PROPERTIES OF THE ENZYME FROM CULTURED KIDNEY CELLS OF BABY HAMSTER

By HILARY A. ROLTON* and HAMISH M. KEIR

Department of Biochemistry, University of Aberdeen,
Marischal College, Aberdeen AB9 1AS, U.K.

(Received 18 February 1974)

dCMP deaminase was partially purified from BHK-21/C13 cells grown in culture. The molecular weight of the enzyme was estimated by gel filtration and gradient centrifugation to be 130000 and 115000 respectively. The enzyme had a pH optimum of 8.4. Its activity versus substrate concentration curve was sigmoid, the substrate concentration at half-maximal velocity being 4.4 mM. dCTP activated the deaminase maximally at 40 μ M, gave a hyperbolic curve for activity versus dCMP concentration and a K_m value for dCMP of 0.91 mM. dCTP activation required the presence of Mg^{2+} or Mn^{2+} ions. dTTP inhibited the deaminase maximally at 15 μ M; the inhibition required the presence of Mg^{2+} or Mn^{2+} ions. The enzyme was very heat-labile but could be markedly stabilized by dCTP at 0.125 M and ethylene glycol at 20% (v/v).

dCMP deaminase (deoxycytidylate aminohydrolase, EC 3.5.4.12) has been partially purified and characterized from monkey liver (Scarano *et al.*, 1962), donkey spleen (Geraci *et al.*, 1967; Rossi *et al.*, 1967; Scarano *et al.*, 1967a,b) and chick embryo (Maley & Maley, 1964, 1968a,b). The activity of the enzyme is high in rapidly dividing cells but low in non-dividing cells (Maley & Maley, 1960). Moreover, dCMP deaminase activity fluctuates during the growth cycle of HeLa cells (Gelbard *et al.*, 1969), a rise occurring in late S phase and a decline in the ensuing G2 phase. These observations suggest that dCMP deaminase has a role to play in the provision of at least one precursor for DNA synthesis, dTTP [see Grav (1967) for a review of the relevant biosynthetic pathways].

The enzyme can utilize dCMP, 5-methyl-dCMP and 5-hydroxymethyl-dCMP as substrates and has neither metal ion nor coenzyme requirements. However, it is activated by dCTP and inhibited by dTTP but only in the presence of a bivalent metal cation such as Mg^{2+} (Scarano *et al.*, 1967a). Both dCTP and dTTP serve as allosteric effectors binding to the deaminase at a site distinct from the substrate-binding site (Scarano *et al.*, 1967b). Rossi *et al.* (1967) suggested that the enzyme exerts a regulatory function in the maintenance of nucleotide pool sizes appropriate to the physiological requirements of the cell. For example, both dCTP and dTTP are required simultaneously for DNA synthesis although most often in different amounts; if the amount of one drops below a critical value, DNA synthesis

stops and is restored only after that concentration is restored.

Since dTTP is an allosteric inhibitor of dCMP deaminase, Rossi *et al.* (1967) postulated that it can exert a negative feedback control on the enzyme to decrease the amount of dCMP being converted into dUMP and hence into dTTP. Should the concentration of dTTP become in excess of requirements, dCMP can then be converted into dCTP by the appropriate kinases. Conversely, if there is a surfeit of dCTP, dCMP deaminase is activated and channels dCMP into the production of dTTP. Thus the amounts of dCTP and dTTP are balanced by supply and demand.

In this paper, we describe the partial purification of dCMP deaminase from BHK-21/C13 cells. The work was part of a project comparing the properties of the enzyme with those of dCMP deaminase activity induced after infection with the virus of herpes simplex (Rolton, 1972; Rolton *et al.*, 1972).

Experimental

Materials

Non-radioactive deoxyribonucleotides were purchased from P-L Biochemicals Inc., Milwaukee, Wis., U.S.A.; [5- ^3H]dCMP (NH_4^+ salt) from The Radiochemical Centre, Amersham, Bucks., U.K.; 2-mercaptoethanol, (NH_4) $_2$ SO $_4$ (grade I) and NADH from Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey KT2 7BH, U.K.; lactate dehydrogenase (pig heart), catalase (beef liver), cytochrome *c* (horse heart) from Boehringer Corp.

* Present address: Beatson Institute for Cancer Research, 132 Hill Street, Glasgow G3 6UD, U.K.

(London) Ltd., London W5 2TZ, U.K.; DEAE-cellulose (Whatman DE-23) from Reeve Angel Scientific Ltd., London SE1 6BD, U.K.; Sephadex G-25 and G-200 from Pharmacia (G.B.) Ltd., London W5 5SS, U.K.; calf serum and BHK-21/C13 cells from Flow Laboratories, Irvine, Ayrshire, U.K. and from Bio-Cult Laboratories Ltd., Paisley, U.K., and tryptose-phosphate broth from Difco Laboratories, East Molesey, Surrey, U.K. Sartorius collodion bags were purchased from V.A. Howe and Co. Ltd., London S.W.6, U.K. DNA polymerase [fraction VII from *Escherichia coli* strain W3110 (Richardson *et al.*, 1964)] was a kind gift from Dr. R. S. Hayward, Department of Molecular Biology, University of Edinburgh.

All reagent chemicals were A.R. grade.

Methods

Cell culture. Baby hamster kidney cells (BHK-21/C13 cells; Macpherson & Stoker, 1962) were grown in monolayer culture at 37°C in 2.24-litre (80oz) cylindrical roller-bottles (House & Wildy, 1965) by using the Glasgow modification of Eagle's medium containing 10% (v/v) tryptose-phosphate broth and 10% (v/v) calf serum. They were checked regularly for contamination with mycoplasmas by the method of Fogh & Fogh (1964).

Cell harvesting and disruption. For each routine experiment, cells in ten roller-bottles were grown to confluence (total cell number approx. 2.5×10^9) over a period of 3 days, harvested from the glass by treatment with 0.5mM-EDTA in phosphate-buffered saline (Dulbecco & Vogt, 1954) and collected by low-speed centrifugation at 4°C. They were washed once in iso-osmotic buffer [0.32M-sucrose-10mM-potassium phosphate buffer (adjusted to pH 8.0 with 10mM-KH₂PO₄)-5mM-2-mercaptoethanol] and collected again by centrifugation. They were resuspended in a hypo-osmotic buffer [10mM-potassium phosphate (pH 8.0)-2mM-2-mercaptoethanol] and allowed to swell for 10min at 4°C. The swollen cells were disrupted in a glass homogenizer tube with two strokes of a Teflon pestle (clearance 0.075mm) by using a Tri-R Stir-R homogenizer operating at 1100 rev./min. Immediately after homogenization, ethylene glycol was added to a final concentration of 20% (v/v); without this addition, dCMP deaminase is labile at 4°C (see Sonoda & Endo, 1969). The homogenate was centrifuged in a MSE Mistral 2L centrifuge (10min; 4°C; 800g_{av.}) and the sediment of nuclei and cell debris discarded. The supernatant fluid was centrifuged in the MSE Superspeed 65 ultracentrifuge (60min; 4°C; 100000g_{av.}) in the 8×25ml fixed-angle rotor.

Assay of dCMP deaminase. The enzyme was assayed by a method developed from that described by Maley & Maley (1964). The assay mixture (0.2ml)

contained: [5-³H]dCMP (0.4μmol; 5μCi/μmol); dCTP (8nmol); MgCl₂ (0.8μmol); Tris-HCl buffer, pH 8.0 (20μmol with respect to Tris), and enzyme solution as indicated in the Results section. Incubation was for 30min at 37°C and the reaction was stopped by rapid cooling to 0°C by shaking the tubes in an ice bath. Portions (5μl) were taken and applied to strips of Whatman no. 1 chromatography paper together with 5μl samples (10μg) each of non-radioactive dUMP and dCMP which served as markers. The paper strips were submitted to electrophoresis in a Shandon electrophoresis tank (Kohn model U77 SAE-3225) by using 0.05M-sodium citrate buffer, pH 3.5 (prepared by addition of 0.05M-trisodium citrate to 0.05M-citric acid). A constant voltage of 300V was applied for 1.5h from a Vokam d.c. power-supply unit. After electrophoresis, the papers were dried and the dCMP and dUMP spots located by examination in u.v. light; under these experimental conditions, the dCMP remained at the origin whereas the dUMP moved 5cm towards the anode. The spots were cut out, placed in glass vials (scintillation grade) containing 10ml of a toluene-based liquid-scintillation fluid (Hayton *et al.*, 1973) and 0.5ml of Hyamine hydroxide in 1M-methanol. The vials were incubated for 30min at 56°C, then cooled and measured for radioactivity in the Intertechnique model SL-30 liquid-scintillation spectrometer.

Assay of proteins used in calibration of Sephadex G-200 columns. The catalase, DNA polymerase I and lactate dehydrogenase used as marker enzymes during gel filtration on columns of Sephadex G-200 were assayed by standard techniques, catalase as described by Chance & Maehly (1955), DNA polymerase I as described for their 'assay A' by Richardson *et al.* (1964) and lactate dehydrogenase as described by Kornberg (1955). Cytochrome *c* in the column eluates was monitored by measurement of extinction at 412nm.

Purification of dCMP deaminase. The enzyme was precipitated from the 100000g supernatant fluid by stepwise addition of solid (NH₄)₂SO₄ at 0°C (Dawson *et al.*, 1969), the deaminase activity being recovered in the fraction precipitating between 40% and 70% (w/v) saturation with respect to (NH₄)₂SO₄. The precipitate was dissolved in buffer A [10mM-potassium phosphate buffer (pH 8.0)-20% (w/v) ethylene glycol-5mM-2-mercaptoethanol], and the solution passed through a column (1.6cm×30cm; 60ml bed vol.) of Sephadex G-25, previously equilibrated against buffer A, at a flow rate of 25ml/h. The void volume (20ml) was collected and loaded on a column (1.7cm×15cm; 34ml bed vol.) of DEAE-cellulose, previously equilibrated with buffer A. Almost all of the loaded protein was retained by the DEAE-cellulose. The flow rate was 10-15ml/h and elution was continued at this rate by using a linearly

increasing gradient (200ml) of KCl rising to 0.5 M-KCl. dCMP deaminase was eluted within an ionic-strength range 0.05–0.15 in a volume of about 30ml, immediately before the main peak of eluted protein. The column fractions containing high enzyme activity were pooled and concentrated to 6ml by using a Sartorius collodion bag mounted in a suction vessel containing buffer A, and stored at -70°C .

Under the standard assay conditions, the activity of the deaminase was linear with time of incubation up to 60min and with protein concentration up to 200 μg /assay. The enzyme was therefore assayed as a routine well within these limits. One unit of activity is taken as 1 μmol of dCMP converted into dUMP in 30min at 37°C .

Determination of the molecular weight. The molecular weight of the dCMP deaminase was determined by gel filtration through Sephadex G-200 (Andrews, 1965) and centrifugation on sucrose density gradients (Martin & Ames, 1961).

The column (1.4cm $\times$ 90cm; 140ml bed vol.) of Sephadex G-200 was calibrated by using four standard proteins (see above) which were loaded on the column together with the purified sample (420 μg of protein) of dCMP deaminase. Fractions (1ml) were collected from the column and assayed for the different proteins.

Sucrose density gradients [4.6ml; 5–20% (w/v) sucrose in buffer A] were prepared in 5ml polypropylene centrifuge tubes and 0.1ml samples containing dCMP deaminase (210 μg of protein) and lactate dehydrogenase (25 μg of protein) were layered on top of each gradient. Gradients were centrifuged for 14h at 39000rev./min at 4°C in the SW50 swing-out rotor (3 $\times$ 5ml) in the Beckman L2 ultracentrifuge. The gradients were fractionated by piercing the base of each tube and collecting samples drop-wise (6 drops/fraction; 34 fractions/gradient). Enzyme activities were assayed in each fraction.

In these experiments, the molecular weights of catalase, lactate dehydrogenase, DNA polymerase and cytochrome *c* were taken respectively as 245000 (Sund *et al.*, 1967), 140000 (Goldberg, 1972), 109000 (Englund *et al.*, 1968) and 12400 (Andrews, 1965).

Miscellaneous. DEAE-cellulose was prepared for column chromatography by the washing and equilibration cycles recommended by the manufacturers (Whatman Technical Bulletin IE2). Sephadex G-25 and G-200 were prepared for use by the methods recommended by Pharmacia ('Sephadex Gel Filtration in Theory and Practice'). Protein was determined by the method of Miller (1959).

Results

Purification of dCMP deaminase

A purification of 50-fold was achieved as a routine, but occasionally, by selecting only peak enzyme fractions from the DEAE-cellulose column, a purification of 100-fold was attained (Table 1). The overall recovery at best was about 32%. These purified preparations from DEAE-cellulose were used for enzyme kinetic studies and were stable at -70°C for at least 2 months in the presence of ethylene glycol at 20% (v/v). In the absence of ethylene glycol the enzyme was labile and activity was lost rapidly at 4°C and above.

The purified enzyme fraction was free from phosphatase activity, which might have interfered with the assay by converting dCMP into deoxycytidine and dUMP into deoxyuridine. This was confirmed by checking the distribution of radioactivity on the paper strips after electrophoresis of samples from enzyme assays. Marker bands of cytosine, deoxycytidine, uracil and deoxyuridine did not contain radioactivity above background values. Other experiments (H. A. Rolton & H. M. Keir, unpublished work) indicated that there was no phosphatase activity that would affect deoxyribonucleoside 5'-triphosphates included in the dCMP deaminase assay.

Molecular weight of dCMP deaminase

After elution from the Sephadex G-200 column, the standard proteins and the dCMP deaminase were assayed. A semi-logarithmic plot of molecular weight

Table 1. *Partial purification of dCMP deaminase*

Step	Total protein (mg)	Activity (total units)	Yield (%)	Specificity activity (units/mg of protein)	Purification (fold)
Cell homogenate	392.7	43.2	100	0.11	1
Low-speed supernatant	186.3	33.6	78	0.18	1.6
100000g supernatant	117.6	29.4	68	0.25	2.3
(NH ₄) ₂ SO ₄ fractionation followed by Sephadex G-25 gel filtration	38.2	17.2	40	0.45	4.1
DEAE-cellulose	2.5	13.8	32	5.52	50.2
DEAE-cellulose (peak fractions)	—	—	—	11.10	100.9

against elution volume for the proteins was constructed. The molecular weight of dCMP deaminase was estimated from this linear plot to be 130000 ± 10000 .

After sucrose-density-gradient centrifugation and assay of dCMP deaminase and lactate dehydrogenase in the fractions obtained, the molecular weight of the deaminase was estimated to be 115000 ± 7000 .

pH optimum

Deaminase displayed optimum activity at pH 8.6 under the standard conditions of assay. Activities at pH 7.0, 8.2 and 9.0 were 60, 98 and 96% respectively of that at pH 8.6.

At all pH values studied, the enzyme showed neither inhibition nor activation by salt (KCl) over the range 0–0.3M.

Activation of dCMP deaminase by dCTP

At low substrate concentrations, the deaminase was activated by dCTP. The activation curve at 2mM-dCMP showed that the optimum concentration of dCTP was about $40 \mu\text{M}$ (Fig. 1). When assayed with $40 \mu\text{M}$ -dCTP (i.e. standard assay conditions), the enzyme activity response was hyperbolic with respect to dCMP concentration (Fig. 2a) as determined in several experiments, and gave a K_m value for dCMP of 0.91mM as measured by the method of Lineweaver & Burk (1934) (Fig. 2b).

Effects of Mg^{2+} ions and EDTA

All preparations of the enzyme assayed in the presence of $40 \mu\text{M}$ -dCTP gave maximum deaminase

Fig. 1. dCMP deaminase activity as a function of dCTP concentration

dCTP was added to the standard assay as indicated. Each assay contained $38 \mu\text{g}$ of protein from the enzyme preparation.

activity without addition of Mg^{2+} ions. However, if 1mM-EDTA was present in the assay mixture the activity was decreased by about 90%, but it was restored to the maximum value by inclusion of Mg^{2+} in the range 1–5mM in the presence of 1mM-EDTA. Mn^{2+} ions gave a response similar to that shown by Mg^{2+} . It seems justifiable to conclude that the presence of a bivalent metal cation is necessary for the activation effect elicited by dCTP, and that there is an amount of bivalent cation in the assay mixture sufficient to promote the activation, perhaps bound to macromolecular material in the purified enzyme fraction.

Fig. 2. dCMP deaminase activity as a function of substrate concentration with dCTP present

dCTP was present in all assays at $40 \mu\text{M}$. Each assay contained $25 \mu\text{g}$ of protein from the enzyme preparation. (a) Activity versus dCMP concentration; ● and ○ were two different enzyme preparations. (b) Double-reciprocal plot (Lineweaver & Burk, 1934) of the data given in (a) by symbols ○.

Fig. 3. dCMP deaminase activity as a function of substrate concentration in the absence of dCTP

dCTP was omitted from the standard assay. Assays each contained 40 μ g of protein from one enzyme preparation (●) or 124 μ g from a second enzyme preparation (○).

To ensure maximum activation by dCTP 2mM- Mg^{2+} was included in all routine assays for dCMP deaminase.

Activity of dCMP deaminase in the absence of dCTP

When dCTP was omitted from the standard deaminase assay and the enzyme activity measured over a range of concentrations of dCMP, a sigmoid activity-substrate concentration curve was obtained (Fig. 3). The experiment was performed with two different batches of purified enzyme. These had different protein concentrations and one batch had been stored at $-70^{\circ}C$ for 2 months longer than the other. The substrate concentration at half the maximum velocity was 4.4 ± 0.4 mM for both enzyme preparations.

Comparison of Figs. 2 and 3 shows that the addition of dCTP to the assay mixture results in a shift of the curve at low substrate concentrations from right to left and a change in its shape from sigmoid to hyperbolic. Therefore dCTP increases the affinity of the enzyme for the substrate.

Inhibition of dCMP deaminase by dTTP

In the presence of Mg^{2+} (or Mn^{2+}), dTTP inhibits the activity of the enzyme. At dCMP concentrations of 3mM and 6mM (Fig. 4a) the inhibition exerted by dTTP was 95% or greater. Although the inhibition by dTTP could be demonstrated even without the addition of exogenous Mg^{2+} to the assay mixture, the effect was abolished by addition of EDTA just as was the activation effect exerted by dCTP. Mg^{2+} or Mn^{2+} are therefore required to elicit the activation by dCTP and the inhibition by dTTP.

Fig. 4. Inhibition of dCMP deaminase activity by dTTP

dCTP was omitted from the standard assay mixture and dTTP added as indicated. Each assay contained 83 μ g of protein from the enzyme preparation. (a) Deaminase activity versus dTTP concentration at 3mM-dCMP (●) and 6mM-dCMP (○). (b) Dixon plots [reciprocal of the reaction velocity in the presence of dTTP ($1/v_i$) versus the dTTP concentration (i) (Dixon, 1953)] of the data in (a) at 3mM-dCMP (●) and 6mM-dCMP (○).

The dTTP does not act as a classical competitive inhibitor. By plotting the reciprocal of the velocity of the reaction ($1/v_i$) against the inhibitor concentration (i) at two different substrate concentrations (Dixon, 1953), plots with a convex-upwards curve were obtained (Fig. 4b). This agrees with the view that the deaminase is an allosteric enzyme (Scarano *et al.*, 1967a,b) and does not behave according to classical Michaelis-Menten kinetics.

Fig. 5. Heat-inactivation of dCMP deaminase

The enzyme preparation (50 μ l; see the Experimental section) was incubated at 50°C for the times indicated, cooled to 4°C and the other components of the standard assay system added. Incubation was then continued at 37°C as in the standard assay. Each assay contained 40 μ g of protein from the enzyme preparation.

Heat-inactivation studies

Incubation of the deaminase in the absence of substrate, dCTP and Mg^{2+} at 50°C, before the standard assay at 37°C resulted in rapid loss of activity (Fig. 5). The rate of inactivation could be decreased by increasing the protein concentration or by including 0.125 mM-dCTP in the preliminary incubation. Further studies showed that preliminary incubation of the enzyme at 45°, or even 37°C, produced inactivation although less markedly than at 50°C.

dCMP deaminase activity in resting cells

The above experiments were all performed on preparations from cells grown just to confluence in monolayer culture. The cells could therefore be regarded as being in the final stage of the exponential phase of growth. Moreover the dCMP deaminase activity extracted from them was quite high. In an extension of these experiments, the serum concentration of the growth medium was decreased from 10% (v/v) to 0.5% (v/v) as described by Bürk (1970) after confluence of growth had been attained, in order to place the cells in a state of quiescence. Under these conditions the metabolic activities of the cell decline to basal values after 4 days and dCMP deaminase activity reflects this by being

barely detectable. The activities after 1, 2, 3 and 4 days in the 0.5% serum were respectively 62, 19, 6 and 0–1% of the value found for the enzyme in exponentially growing cells.

Discussion

The dCMP deaminase partially purified from BHK-21/C13 cells has properties similar to those of the well-characterized corresponding enzymes from monkey liver (Scarano *et al.*, 1962), donkey spleen (Scarano *et al.*, 1964; Geraci *et al.*, 1967; Rossi *et al.*, 1967; Scarano *et al.*, 1967a,b) and chick embryo (Maley & Maley, 1964, 1965, 1968a,b). It was purified to the extent that it was free from contamination by factors that might interfere with the assay. For example, it contains no phosphatase that would affect the substrate (dCMP), the product (dUMP) or the effectors (dCTP, dTTP). Further, it seems reasonable to conclude from Figs. 2, 3 and 4 that the enzyme preparation does not contain amounts of endogenous deoxyribonucleotide that would significantly influence the activity measurements conducted in this study.

Soluble low-molecular-weight compounds would, of course, be expected to be removed during the fractionation with $(NH_4)_2SO_4$ and the ensuing gel filtration on Sephadex G-25. However, it is recognized that it is possible that such compounds might still contaminate the enzyme preparation if they were firmly bound either to macromolecules co-fractionating with the deaminase or to the deaminase itself. Thus it was not possible to demonstrate the requirement for a bivalent metal cation in the dCTP activation and dTTP inhibition effects unless EDTA was added to the assay; it therefore seems likely that bound bivalent metal cations were present.

The observations do not exclude the additional possibility that proteins (or other macromolecules) other than dCMP deaminase in the enzyme preparation could interact with the deaminase and thereby modify its activity. From that point of view it would be ideal to purify the enzyme to homogeneity, but this was not feasible owing to the relatively small amounts of starting material available and to the rather poor recovery of enzyme (about 30%; Table 1) after only three purification steps. Geraci *et al.* (1967) purified the dCMP deaminase of donkey spleen to 95% homogeneity but achieved only 5% recovery.

The molecular weight of the deaminase was 130000 ± 10000 as determined by gel filtration and 115000 ± 7000 as determined by centrifugation on sucrose density gradients. These results suggest that the enzyme has a molecular weight close to that of the dCMP deaminase of donkey spleen, which was found by Geraci *et al.* (1967) to be 122000 ± 10000 .

Other properties of the BHK-21/C13 cell deaminase were similar to those of the corresponding

enzymes from chick embryo (Maley & Maley, 1964) and donkey spleen (Geraci *et al.*, 1967; Scarano *et al.*, 1967a). It displayed conventional Michaelis-Menten kinetics in the presence of dCTP (Fig. 2) and in the absence of this activator a sigmoid curve was obtained for the plot of activity against substrate concentration (Fig. 3). The corresponding double-reciprocal plots (Lineweaver & Burk, 1934) gave straight lines when dCTP was present in the assay (Fig. 2) and curves (concave upwards) when it was omitted. Therefore, at the substrate concentrations used in these experiments, dCTP increased the affinity of the enzyme for the substrate. In contrast, dTTP was inhibitory and Dixon (1953) plots gave curves that were concave upwards (Fig. 4). A bivalent metal cation was necessary for both activation and inhibition effects.

The observations with dCTP and dTTP agree with those of Scarano *et al.* (1967a,b), who concluded that they act as allosteric effectors, binding at regulatory sites on the deaminase in complexes with Mg^{2+} .

dCMP deaminase is very heat-labile and one must suppose that the resistance to heat-inactivation afforded by the inclusion of dCTP and Mg^{2+} in the preparation during heating is attributable to the enzyme being stabilized by binding of the Mg^{2+} -dCTP complex.

The substantial decrease of deaminase activity in cells maintained in medium containing the low concentration of serum, is expected. For example, Maley & Maley (1960) showed that the enzyme is essentially absent from normal rat liver, but that it is measurable as early as 12h after partial hepatectomy and rises to a maximum value at about 48h. The activity then declines to the extremely low values found in normal rat liver. Further, Gelbard *et al.* (1969) showed that the deaminase activity of HeLa cells is up to fivefold higher in cells in late S phase than in cells early in the G1 phase.

Our present results established some of the fundamental properties of the deaminase from BHK-21/C13 cells and provide the basis for comparison of these properties with those of the corresponding enzyme from cells infected with the virus of herpes simplex (H. A. Rolton & H. M. Keir, unpublished work).

We thank the Medical Research Council for support. H. A. R. was in receipt of a scholarship from the Council. We are grateful to Miss Helen Milne, Mrs. Alison Blair and Mrs. Janet Elson for skilled technical assistance.

References

Andrews, P. (1965) *Biochem. J.* **96**, 595-606

- Bürk, R. (1970) *Exp. Cell Res.* **63**, 309-316
- Chance, B. & Maehly, A. C. (1955) *Methods Enzymol.* **2**, 764-775
- Dawson, R. M. C., Elliott, D. C., Elliott, W. H. & Jones, K. M. (eds.) (1969) *Data for Biochemical Research*, 2nd edn., p. 616, Clarendon Press, Oxford
- Dixon, M. (1953) *Biochem. J.* **55**, 170-171
- Dulbecco, R. & Vogt, M. (1954) *J. Exp. Med.* **99**, 167-199
- Englund, P. T., Deutscher, M. P., Jovin, T. M., Kelly, R. B., Cozzarelli, N. R. & Kornberg, A. (1968) *Cold Spring Harbor Symp. Quant. Biol.* **33**, 1-9
- Fogh, J. & Fogh, H. (1964) *Proc. Soc. Exp. Biol. Med.* **117**, 899-901
- Gelbard, A. S., Kim, J. H. & Perez, A. G. (1969) *Biochim. Biophys. Acta* **182**, 564-566
- Geraci, G., Rossi, M. & Scarano, E. (1967) *Biochemistry* **6**, 183-191
- Goldberg, E. (1972) *J. Biol. Chem.* **247**, 2044-2048
- Grav, H. J. (1967) *Methods Cancer Res.* **3**, 243-389
- Hayton, G. J., Pearson, C. K., Scaife, J. R. & Keir, H. M. (1973) *Biochem. J.* **131**, 499-508
- House, W. & Wildy, P. (1965) *Lab. Pract.* **14**, 594-595
- Kornberg, A. (1955) *Methods Enzymol.* **1**, 441-443
- Lineweaver, H. & Burk, D. (1934) *J. Amer. Chem. Soc.* **56**, 658-666
- Macpherson, I. A. & Stoker, M. G. P. (1962) *Virology* **16**, 147-151
- Maley, F. & Maley, G. F. (1960) *J. Biol. Chem.* **235**, 2968-2970
- Maley, F. & Maley, G. F. (1964) *J. Biol. Chem.* **239**, 1168-1176
- Maley, F. & Maley, G. F. (1965) *J. Biol. Chem.* **240**, PC3226-PC3227
- Maley, F. & Maley, G. F. (1968a) *J. Biol. Chem.* **243**, 4506-4512
- Maley, F. & Maley, G. F. (1968b) *J. Biol. Chem.* **243**, 4513-4516
- Martin, R. G. & Ames, B. N. (1961) *J. Biol. Chem.* **236**, 1372-1379
- Miller, G. L. (1959) *Anal. Chem.* **31**, 964
- Richardson, C. C., Schildkrant, C. L., Aposhian, H. V. & Kornberg, A. (1964) *J. Biol. Chem.* **239**, 222-232
- Rolton, H. A. (1972) Ph.D. Thesis, University of Aberdeen
- Rolton, H. A., Bates, J. & Keir, H. M. (1972) *Biochem. J.* **129**, 14p-15p
- Rossi, M., Geraci, G. & Scarano, E. (1967) *Biochemistry* **6**, 3640-3645
- Scarano, E., Bonaduce, L. & de Petrocellis, B. (1962) *J. Biol. Chem.* **237**, 3742-3751
- Scarano, E., Geraci, G. & Rossi, M. (1964) *Biochem. Biophys. Res. Commun.* **16**, 239-243
- Scarano, E., Geraci, G. & Rossi, M. (1967a) *Biochemistry* **6**, 192-201
- Scarano, E., Geraci, G. & Rossi, M. (1967b) *Biochemistry* **6**, 3645-3650
- Sonoda, S. & Endo, H. (1969) *Biochim. Biophys. Acta* **191**, 425-429
- Sund, H., Weber, K. & Mölbert, E. (1967) *Eur. J. Biochem.* **1**, 400-410