physical agitation of the solutions in the presence of air causes some change in the secondary structure of the molecules resulting in higher radiation resistance.

Isotope Research Division,
Wantage Research Laboratory (A.E.R.E.),
Wantage, Berkshire (Great Britain)

B. A. BRIDGES*

1 B. A. BRIDGES, *Nature*, 188 (1960) 415.
2 B. A. BRIDGES, *J. Gen. Microbiol.*, 26 (1961) 467.
3 B. A. BRIDGES, *Radiation Res.*, 16 (1962) 232.
4 D. CAVALLINI, B. MONDOVI, B. GIOVANELLA AND C. DE MARCO, *Science*, 131 (1960) 1441.
5 F. H. WHITE AND C. B. ANFINSEN, in R. BENESCH *et al.*, *Sulfur in Proteins*, Academic Press, New York, 1959, p. 279.
6 B. HOLMES, *Nature*, 165 (1950) 266.
7 L. BRIGHENTI AND A. FALASCHI, *Biochim. Biophys. Acta*, 59 (1962) 376.
8 C. B. ANFINSEN, R. R. REDFIELD, W. L. CHOATE, J. PAGE AND W. R. CARROL, *J. Biol. Chem.*, 207 (1954) 201.
9 M. BOCCACCI AND M. QUINTILIANI, *Scient. Rep. Ist. Super. Sanita*, in the press.
10 A. B. ROBINS AND J. A. V. BUTLER, *Radiation Res.*, 16 (1962) 7.
11 M. EBERT AND T. ALPER, *Nature*, 173 (1954) 987.
12 C. S. BACHOFER AND M. A. POTTINGER, *Science*, 119 (1954) 378.
13 R. J. SHALEK, J. D. HUNTER AND C. E. SMITH, *Radiation Res.*, 16 (1962) 607.
14 F. HUTCHINSON, *Radiation Res.*, 14 (1961) 721.

Received September 7th, 1962

* Present address: Medical Research Council Radiobiological Research Unit, Harwell, Berkshire (Great Britain).

SC 11025

Mechanism of D-glutamyltransferase repression in mammalian cells

This investigation was undertaken to obtain information about the mechanism involved in an adaptive enzyme system in cultured mammalian cells. Repression of D-glutamyltransferase ,which is almost certainly identical with glutamine synthetase (L-glutamate : ammonia ligase (ADP), EC 6.3.1.2)[1] by glutamine was described by DEMARS[2] in human cervical carcinoma cells, strain HeLa[3]. We have observed a similar phenomenon in mouse subcutaneous (fibroblasts) strain L[4]. The cells were grown in WAYMOUTH's medium[5], modified as described below, to which 5% dialysed calf serum was added. They were harvested by treatment with trypsin and extracts were prepared by repeated freezing and thawing in 0.8% NaCl, followed by centrifugation at 12 000 × *g* for 45 min. D-glutamyltransferase activity in the supernatant was determined by measuring the capacity to form γ-glutamylhydroxamic acid[6] in conditions slightly modified from the original to obtain greater sensitivity[7].

When L cells were propagated in a glutamine-free medium containing 20 mM glutamic acid an 8-fold increase in glutamyltransferase activity occurred within 48 h. On the addition of glutamine at a final concentration of 2.4 mM D-glutamyltransferase activity rapidly decreased and reached the initial level within 16–24 h (Fig. 1). The questions posed by this observation are: Is active protein synthesis involved in

Fig. 1. The time course of induction and repression of D-glutamyltransferase in strain L cells.

the increase of enzyme activity and is a specific inactivation mechanism responsible for its rapid disappearance when glutamine is added to the medium?

In order to obtain information about the first point attempts were made to obtain derepression of the enzyme, as already described, in media to which had been added *p*-fluorophenylalanine, 8-azaguanine or 5'-fluorodeoxyuridine. As shown in Table I the enzyme increased in control cultures and also in cultures treated with 5'-fluorodeoxyuridine at concentrations adequate to inhibit DNA synthesis. On the other hand no extra enzyme activity was found in the presence of *p*-fluorophenylalanine or 8-azaguanine at concentration adequate to inhibit protein and RNA synthesis. Arylesterase (EC 3.1.1.2), which appears to behave constitutively in these cells, was assayed simultaneously and failed to show the same degree of fluctuation. These observations suggest that the increase in D-glutamyltransferase activity when glutamine is withdrawn from the medium involves *de novo* synthesis of the enzyme.

The active disappearance of the enzyme on addition of glutamine was in contrast to the observation made by DEMARS[2] in HeLa cells, where the enzyme was simply diluted out. We have confirmed DEMARS' observations and conclude that this mechanism is different in the two cell lines, and probably in the two species

TABLE I

THE EFFECT OF METABOLIC INHIBITORS ON THE INDUCTION OF GLUTAMYLTRANSFERASE IN STRAIN L

Duplicate cultures ($2 \cdot 10^6$ cells/ml) were set up in WAYMOUTH's medium (-glutamine) + 20 mM glutamine acid + dialysed calf serum. Inhibitors were added to the medium as indicated and the cells were assayed for enzyme activity after 48 h.

Additions to the medium	*Concentration (M)*	*Incubation time (h)*	*Glutamyl transferase units/million cells*	*Esterase Units/million cells*
None	—	0	0.014, 0.012	0.019
None	—	48	0.120, 0.122	0.021
8-Azaguanine	$2 \cdot 10^{-4}$	48	0.011, 0.014	0.009
p-Fluorophenylalanine	$2 \cdot 10^{-4}$	48	0.016, 0.018	—
5'-Fluorodeoxyuridine	$2 \cdot 10^{-6}$	48	0.093, 0.080	—

(human and mouse) from which the cells were derived. In order to investigate the nature of the phenomenon, crude extracts of mouse liver were prepared in 0.1 M phosphate buffer (pH 7.4). On incubation at 37° D-glutamyltransferase activity progressively disappeared from these extracts. The enzyme could, however, be stabilized to varying extents by substances related to the substrate (glutamic acid, glutamine), by maintenance under nitrogen and by $NADH_2$ (Table II). These observations suggest that inactivation of the mouse enzyme may occur as a result of oxidation and that it can be stabilized in the presence of its substrate.

TABLE II

THE EFFECT OF VARIOUS SUBSTANCES IN PROTECTING MOUSE LIVER D-GLUTAMYLTRANSFERASE FROM INACTIVATION BY INCUBATION AT 37° IN 0.15 M PHOSPHATE BUFFER (pH 7.2)

Substance	Concentration (mM)	% Protection*
D-Glutamic acid	30	47
L-Glutamine	30	25
$NADH_2$	2	56
N_2	—	82
L-Glutamic acid + N_2	30	186
L-Glutamine + N_2	30	123
L-Glutamic acid + $NADH_2$	30 2	172
L-Glutamine + $NADH_2$	30 2	53

* $\%\ \text{Protection} = \frac{\text{Activity of test} - \text{Activity of control}}{\text{Activity of initial} - \text{Activity of control}} \times 100$

Therefore it would appear that D-glutamyltransferase in the strain L cell is an unstable protein which tends to be spontaneously inactivated in the presence of oxygen and is stabilized by its substrate. Nevertheless the appearance of increased enzyme activity on releasing the cell from the repressive influence of glutamine appears to involve the synthesis of new protein and therefore represents a true example of enzyme induction.

This research was supported by grants from the Scottish Hospital Endowments Research Trust.

Biochemistry Dept.
University of Glasgow (Great Britain)

J. PAUL
P. F. FOTTRELL*

1 P. R. KRISHNASHWAMY, V. PAHILJANS AND A. MEISTER, *J. Biol. Chem.*, 235 (1960) pc 39.
2 R. DE MARS, *Biochim. Biophys. Acta*, 27 (1958) 435.
3 G. O. GEY, W. C. COFFMAN AND M. T. KUBICEK, *Cancer Research*, 12 (1952) 264.
4 K. E. SANFORD, W. R. EARLE AND G. D. LIKELY, *J. Natl. Cancer Inst.*, 9 (1948) 229.
5 C. WAYMOUTH, *J. Natl. Cancer Inst.*, 22 (1959) 1003.
6 P. K. STUMPF, W. D. LOOMIS AND C. MICHELSON, *Arch. Biochem.*, 30 (1951) 126.
7 P. F. FOTTRELL, Ph. D. thesis, University of Glasgow, 1962.

Received July 25th, 1962

* Beit Memorial Fellow.