INCREASED ACTIVITY OF TRYPTOPHAN PEROXIDASE IN HeLa CELLS

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Substrate induction of enzymes has been demonstrated by Klein (1960, 1961) to occur in animal cells cultured as monolayers in vitro, presumably by the same mechanism that operates in bacteria and other microorganisms.

We have now carried out a parallel study on an established strain of HeLa cells cultured in monodisperse suspension on a rotatory water bath shaker. Air was used as the gas phase; enrichment of the air with CO_2 was found to be unnecessary. The medium used consisted of 20% calf serum and 80% Morgan's Mixture #199[1] to which this strain of HeLa cells has been adapted for over a year. Because the cell suspensions were monodisperse, we were able to use bacteriological methods exclusively to obtain successive generations of cells.

Monodisperse suspensions seemed to offer material inherently more homogeneous and tractable for the study of "induced enzyme synthesis" than did cells grown as monolayers. We have tested their suitability for such a study by following

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[1] From Microbiological Associates, Bethesda, Md.

the "induction" of tryptophan peroxidase (TPO) in such cell suspensions under various conditions.

There was no evidence of TPO synthesis in cells cultured in the medium alone. Raising the L-tryptophan concentration of the medium from 10^{-4} M to 10^{-2} M did not give rise to a measureable increase of TPO activity. Klein (1961) encountered a similar difficulty with the induction of arginase but was subsequently successful after adding RNA to the culture medium. The addition of RNA was made on the basis of an hypothesis of Speigelman et al. (1955).

We therefore tried the effect of RNA and L-tryptophan separately and together. Ten cultures of cells in basal medium, and ten cultures of cells in basal medium plus 250 µg/ml yeast RNA (Sigma Chemical Co.), were grown for 24 hours. The L-tryptophan concentration in five of the "basal" cultures and five of the "RNA" cultures was then raised from 10^{-4} M to 10^{-2} M. The cultures were then assayed from time to time for the enzyme according to a method essentially the same as that used by Knox (1955).

No activity was observed in any culture except those containing extra RNA and extra tryptophan. In these cultures, TPO activity increased as culture age increased. The activity was measured by the spectrophotometric determination of the amount of L-kynurenine formed during the aerobic incubation of homogenate with L-tryptophan (Knox, 1955) at 365 mµ.

Age of Culture	Activity (Knox units)
0 days	0.00
4 days	1.62
8 days	2.62
9 days	3.00
10 days	3.41

The monodisperse suspensions are thus perfectly suitable for the study of "induced enzyme synthesis".

We are currently attempting to determine whether this phenomenon is due to substrate induction of TPO synthesis or to activation of TPO protein already present in the cells. The curious effect of RNA on the system is also being investigated.

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