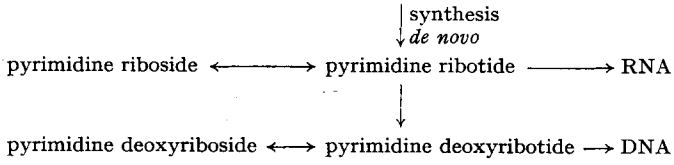
a compound was formed which on a Dowex-2-formate chromatogram moved together with uridine-5'-phosphate and had the ability to replace vitamin B_{12} during the growth of *L. leichmannii* 313[7]*. On paper chromatography, radioactivity was found in the deoxyuridine-5'-phosphate area. After dephosphorylation with phosphatase the radioactivity was found in the deoxyuridine area. These results tentatively identify the compound formed as deoxyuridine-5'-phosphate.

No such compound was formed when 2-^{14}C-uridine was used as substrate instead of 2-^{14}C-uridine-5'-phosphate.

It is believed that all these experiments are best explained by a reaction sequence of the following type:



In this scheme the ribose ⟶ deoxyribose transformation thus takes place at the nucleotide level, while ribosides and deoxyribosides enter the reaction sequence *via* side reactions, *e.g.* of the kinase type.

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The inhibition by glutamine of glutamyl transferase formation in cultures of human cells

The specific activity of glutamyl transferase[1] in cultures of human cells can vary strikingly with the growth medium. This is a preliminary account of the effects of L-glutamic acid (GA) and of L-glutamine (GM).

In our experiments replicate cultures of HeLa (cervical carcinoma) cells were grown in 1-l Blake bottles in EAGLE's medium[2] supplemented with 2 m*M* GM, 20 m*M* GA or both. The average generation time at 37° was about 30 h. At intervals during growth, cultures were drained and washed with ice-cold Earle's saline. The cells were scraped from the surface of the glass into ice-cold 0.85% NaCl with a rubber policeman and collected by centrifugation at 1500 × *g*. The cell suspensions (1–3 ml) in sealed plastic tubes were floated in water in the chamber of a 10 KC Raytheon Sonic Oscillator and were treated for 15–30 min. The extracts were centrifuged at 25,000 × *g* for 30 min. More than 90% of the protein remained in the supernatant fluids, which were used for enzyme assays. Growth was expressed as the total amount of protein formed by the cultures.

Glutamyl transferase effects the reaction:

$$\text{L-glutamine} + NH_2OH \rightleftharpoons \gamma\text{-glutamylhydroxamate} + NH_3$$

The reaction requires catalytic amounts of ADP, phosphate or arsenate[1], and Mn^{++}. Our assay mixtures contained in 1.0 ml: imidazole buffer, pH 7.4, 50 μmoles; L-glutamine, 40 μmoles; neutralized $NH_2OH \cdot HCl$, 100 μmoles; $MnCl_2$, 5 μmoles; ADP, 0.1 μmole; neutralized K_3AsO_4, 25 μmoles; protein, 0.5–2.0 mg. The reaction was stopped after 60 min at 37° by the addition of 1.0 ml of 10% $FeCl_3 \cdot 6H_2O$ dissolved in a mixture of 0.7*N* HCl and 0.2*N* trichloracetic acid. In such mixtures, 1.0 μmole of synthetic glutamylhydroxamate had an extinction at 540 mμ of 0.425 in a 1 cm cell. The transferase activity of our preparations required the joint presence of

ADP, arsenate and Mn^{++}. The reaction rate was constant for at least 90 min. Hydroxamic acid formation in the range 0–0.6 μmole was proportional to protein concentration. One unit of enzyme activity was the formation of 1 μmole glutamylhydroxamate/h.

Curve I shows that during an 8-fold increase in protein in 20 mM GA the specific activity rose to a value 13 times greater than the initial value. The cells could then be propagated indefinitely in 20 mM GA and the specific activity (0.410) remained about 15-fold greater than that (0.027) for cells grown in 2 mM GM. In contrast, enzyme formation was almost completely suppressed in medium containing 20 mM GA + 2 mM GM, in which the maximum increase in specific activity was 1.5-fold.

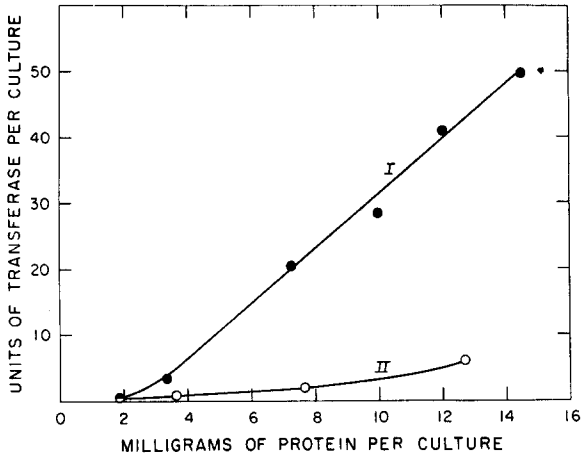

Fig. 1. Formation of glutamyl transferase during growth of the HeLa cells in glutamic acid (see text). I – 20 mM glutamic acid; II – 20 mM glutamic acid + 2.0 mM glutamine.

The heightened transferase specific activity of cells grown in 20 mM GA decreased when the cells were returned to media containing GM. In medium containing 2 mM GM + 20 mM GA the decrease in specific activity was proportional to growth and the total number of activity units remained constant through a 10-fold increase in protein. In medium containing only 2 mM GM the rate of decrease was twice as great. In neither case, however, was there evidence of a rapid inactivation of the enzyme by GM *per se*.

In the absence of GM and in concentrations of GA less than 10 mM, HeLa cells previously grown in GM fail to multiply[3]. However, cells that have multiplied 10-fold in 20 mM GA can be propagated for at least three doublings at a constant rate in medium containing 1 mM GA. At this concentration, 1 mM NH_4Cl enhances the growth rate (to be published). We are now determining if GA and NH_4^+ indeed fill the synthetic roles of GM under these conditions.

There is evidence that the transferase enzyme protein also catalyzes the synthesis of glutamine[4]:

$$\text{glutamic acid} + NH_3 + ATP \overset{Mg}{\rightleftharpoons} \text{glutamine} + ADP + P \text{ (synthase reaction)}$$

Such an identity would accord, on the one hand, with the ability of cells to satisfy their GM requirement and grow in GA, and on the other, with the rise in transferase activity that accompanies this growth. Synthase specific activity is about 1/10 that of transferase in our crude extracts. Significant increases in synthase activity do occur during growth in GA but the relationship between the two activities has yet to be defined.

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