The synthesis of deoxyribose by the chick embryo

The conclusion has been drawn from tracer experiments *in vivo* that the rat can transform pyrimidine ribosides (or ribotides) to pyrimidine deoxyribosides (or deoxyribotides) without rupture of the glycosidic linkage[1-4]. This communication demonstrates the existence of a similar reaction in the chick embryo and presents evidence that the transformation takes place at the nucleotide level.

Experiments *in vitro* were carried out with minced 5-day old chick embryos[5]. On incubation with uniformly labelled $^{14}C_9$-cytidine, it was found that both the ribonucleic acid (RNA) and the deoxyribonucleic acid (DNA) of the cells rapidly became labelled. By isolation and degradation of the pyrimidine ribosides from RNA and the pyrimidine deoxyribosides from DNA[4], it was found that the isotope ratio, pyrimidine/sugar, was the same in the isolated compounds as in the administered precursor. This is similar to the earlier findings in different rat organs[2-4], and demonstrates the intact conversion of ribose bound to pyrimidine to deoxyribose bound to pyrimidine.

Next, the influence of the presence of non-labelled deoxycytidine and deoxycytidine-5'-phosphate on the labelling of RNA and DNA from $^{14}C_9$-cytidine was determined. In these experiments the amounts of isotope in compounds of the acid-soluble fraction (cytidine, cytidine-5'-phosphate, deoxycytidine and deoxycytidine-5'-phosphate) were also determined by a combination of ion-exchange and paper chromatography[6]. The presence of the deoxyribosidic compounds clearly did not appreciably influence the incorporation of cytidine into RNA, while relatively small but definite dilution effects were observed in the case of the DNA pyrimidines (Table I).

TABLE I

	Counts/min/μmole					
Expt No.:	*1*	*2*	*3*	*4*	*5*	*6*
RNA:						
Cytidine	340	1200	415	1380	340	1020
Uridine	250	610	280	520	210	580
DNA:						
Deoxycytidine	120	470	62	420	58	330
Thymidine		115		31		22

In each experiment 1 g of minced chick embryos was incubated in 2 ml of Tyrode solution containing 0.3 μmole $^{14}C_9$-cytidine (82,000 counts/min/μmole) at 37° for either 45 min (Expts. 1, 3 and 5) or 3 h (Expts. 2, 4 and 6).

Additions: Expts. 1 and 2: none
Expts. 3 and 4: 1.5 μmoles deoxycytidine
Expts. 5 and 6: 1.5 μmoles deoxycytidine-5'-phosphate.

In the acid-soluble fraction, a quite rapid accumulation of $^{14}C_9$-cytidine-5'-phosphate and a decrease of cytidine was found in all cases. In Expts. 3–6 (pools of non-labelled deoxyribosidic compounds present during incubation), a definite accumulation of $^{14}C_9$-deoxycytidine with time could be demonstrated. In the absence of the deoxyriboside pool (Expts. 1 and 2) much smaller amounts of this compound were formed. Scarcely significant amounts of $^{14}C_9$-deoxycytidine-5'-phosphate were formed in all six experiments.

The very small dilution effects observed tend to exclude free deoxycytidine as an intermediate in DNA formation from $^{14}C_9$-cytidine. It is very unlikely that the results were influenced to any large extent by a permeability barrier for the added deoxycytidine, especially in view of the observed incorporation of isotope into the deoxycytidine pools in Expts 3–6.

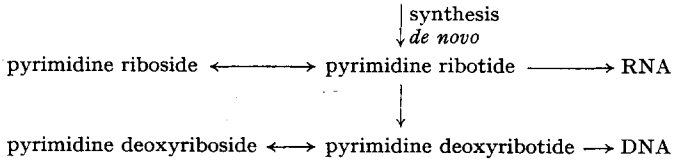
The well known impermeability of cells for mononucleotides[3], however, must be considered in the interpretation of Expts. 5 and 6. After 3 h almost all added deoxycytidine-5'-phosphate had disappeared and had to a large extent been transformed to deoxycytidine. It seems likely that only the dephosphorylated compound entered the cells and the experiments therefore do not exclude the intermediate formation of deoxycytidine-5'-phosphates during DNA formation from cytidine.

Positive evidence for the formation of a deoxyribotide from a ribotide has been obtained in preliminary experiments with homogenates and "high-speed supernatants" of homogenates from chick embryos. When 2-^{14}C-uridine-5'-phosphate was incubated with such preparations,

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