that *^{32}P present in NAP at a given time* ($t = 0$) is lost in accordance with an exponential function with the specific rate constant of T. But the total amount of ^{32}P in NAP of A, to be observed at the time $t > 0$, is further contributed by ^{32}P coming from the precursor pool which may still be retaining its label. To estimate the magnitude of this secondary incorporation, BRANSON's integral equation[12] may be employed in the present case that is quite favourable in this respect (*cf.* ref.[13]). Postulating a simple exponential decrease in the specific activity of acid-soluble P, which should be in equilibrium with direct precursors of NAP in our system[3], and an "autocatalytic" synthesis of NAP, the BRANSON's equation was solved to yield a compound exponential equation relating initial and final values of $Q(t)$, $Q^*(t)$, and $c(t)$, to be obtained experimentally, to T (as a constant), where Q^* denotes the total activity of NA-^{32}P in A and c the specific activity of total acid-soluble (or inorganic) P. In this equation, T can take negative as well as positive values, the point $T = 0$ showing no peculiarity in the $Q^*(t)$-T curve, although a negative T value is of no biological significance.

T values obtained up to the present time, from our recent experiments, along with the ones recalculated from FUJISAWA AND SIBATANI[6] are compiled in Table I. Although the individual T values are subject to considerable fluctuation from experiment to experiment, this may be due to errors introduced in various ways. It is now almost certain that T of DNA-P is not increased to any significant extent by the augmentation of $Q(t)/Q(0)$. Moreover, the weighted mean of T for DNA-P is very close to nil while that for PNA-P (pentosenucleic acid P) has a positive value of a reasonable magnitude.

It may thus be inferred that in growing young rats there is little if any turnover of DNA-P, whether or not the synthesis of new DNA molecules is actively going on. This conclusion is at variance with the one supported by the LEBLOND group[1,2] and some other workers[2,4,5]. But the data of the Canadian authors for rat livers at least would be subject to appreciable inaccuracy, since the SCHMIDT-THANNHAUSER fractionation of DNA as employed by them may well have caused a contamination of non-DNA ^{32}P-compounds, as will be shown elsewhere on the basis of our recent findings with preparations of highly polymerised DNA obtained from livers of ^{32}P-injected adult rats (AMANO, KIHARA AND SIBATANI, to be published). It follows that the specific activities of DNA-P in rat livers as submitted by the LEBLOND school were in all likelihood too high to represent the true rate of formation of new DNA molecules.

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The interconversion of glutamic acid and proline*

Several lines of evidence indicate that in animal tissues glutamic acid can be converted to proline and that proline is oxidized to glutamic acid (*cf.*[1]). It has also been shown in growth experiments with microorganisms that glutamic acid can serve as a precursor of proline with the intermediate formation of glutamic-γ-semialdehyde (*cf.*[2]). The experimental procedures used in these investigations are not very suitable for a study of the enzymic mechanisms involved but do suggest the following series of reactions

glutamic acid $\rightleftharpoons$ glutamic γ-semialdehyde
Δ^1-pyrroline-5-carboxylic acid $\rightleftharpoons$ proline

In our laboratory, results bearing on the enzymic mechanisms of the above reactions have been

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obtained with whole cells of a mutant strain of *E. coli* and with extracts of liver mitochondria. Washed resting cells of *E. coli* (55-1) accumulate Δ'-pyrroline-5-carboxylic acid (glutamic γ-semialdehyde) with glutamic acid as the substrate. (Δ'-pyrroline-5-carboxylic acid was determined by reaction with *o*-amino benzaldehyde[3] and by assay with another mutant strain of *E. coli* (55-25)[4].) The formation of glutamic γ-semialdehyde in these cells is an aerobic process and is markedly stimulated by the addition of any of the following compounds; lactate, pyruvate, glucose and formate, pyruvate being most effective. An additional stimulation in the presence of pyruvate is obtained upon addition of adenosine monophosphate (AMP) and to a lesser extent with adenosine diphosphate (ADP) (Table I).

TABLE I

The incubation mixture (2.0 ml) contained 0.3 ml of a 30 % suspension of cells, 30 μM of potassium glutamate, 50 μM of potassium pyruvate, 10 μM of AMP and 100 μM of either tris(hydroxymethyl)aminomethane or potassium phosphate buffer (pH 7.6). (Δ'-pyrroline-5-carboxylic acid (glutamic γ-semialdehyde) was assayed by reacting with *o*-amino benzaldehyde[3] and determining the absorption at 430 mμ.)

Components	D_{430}
Cells alone	0.105
Cells + glutamate	0.460
Cells + glutamate + pyruvate	1.350
Cells + glutamate + pyruvate + AMP	1.800
Complete systems — oxygen	0

TABLE II

The complete system (2.0 ml) contained 0.3 ml of the enzyme solution obtained by subjecting a sucrose suspension of mitochondria of rat liver to sonic oscillation, 0.9 μM of Δ'-pyrroline-5-carboxylic acid, 200 γ of DPN, 100 μM of tris-(hydroxymethyl)aminomethane buffer (pH 7.6) and 50 μM of either potassium phosphate or sodium arsenate. The disappearance of Δ'-pyrroline-5-carboxylic acid was determined as the difference between an enzymic and non-enzymic incubation mixture.

Components	D_{430}
Complete system	0.500
Complete system — phosphate	0
Complete system — DPN	0
Complete system — phosphate + arsenate	0.460

Efforts to obtain a cell-free preparation of the enzyme system from *E. coli* have up to now not been successful. However, extracts of rat liver mitochondria catalyze the oxidation of synthetic Δ'-pyrroline-5-carboxylic acid. This oxidation requires diphosphopyridine nucleotide (DPN) and inorganic phosphate or arsenate (Table II).

Paper chromatography of the products of the oxidation of Δ'-pyrroline-5-carboxylic acid shows the presence of glutamic acid roughly proportional to the disappearance of Δ'-pyrroline-5-carboxylic acid.

These mitochondrial extracts also catalyze the formation from proline of a compound which reacts with *o*-amino benzaldehyde. The reaction proceeds anaerobically but it has not been possible up to now to increase the activity by addition of soluble components. Addition of DPN and phosphate results in the disappearance of the enzymically formed compound. Using an enzyme obtained from *Neurospora crassa* YURA AND VOGEL[5] have observed a pyridine nucleotide dependent reduction of synthetic Δ'-pyrroline-5-carboxylic acid to proline. Purification of the enzyme systems will aid in the study of the detailed mechanisms of the metabolic interconversions of glutamic acid and proline.

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