

Short Communications and Preliminary Notes

Stability of deoxypentosenucleic acid phosphorus (DNA-P) in growing tissue*

Since the LEBLOND school^{1,2} reported experiments pointing to the replacement of every one preexistent DNA molecule with two newly synthesised ones to result in the doubling of DNA per one mitotic division, data supporting this view have rapidly been accumulated along several different lines of approach^{3,4,5}. But this concept is acutely in contradiction with the results obtained with microorganisms^{6,7}, which show the conservation of DNA molecules during rapid proliferation, and even throughout many generations, of bacterial cells. Experiments using rat livers^{8,9} have also failed to prove the labilisation of DNA molecules in the course of cell proliferation. With growing young rats, it has also been shown by FUJISAWA AND SIBATANI⁶ that a nearly two-fold increase of liver DNA may not be attended by any extensive release of preexistent DNA-P. This last experiment, however, involved the data suggesting an eventual presence of DNA-P turnover to some extent.

Experiments of the same type with minor modifications are therefore being repeated to ascertain whether there is any measurable turnover of DNA-P in dividing and non-dividing liver cells of rats, and will be detailed in a later communication. The calculation of the fraction of DNA retained during the experimental period, as attempted by FUJISAWA AND SIBATANI⁶, was marred by an oversimplification which however is by no means unfamiliar to this sort of tracer studies^{10,11}. We have now developed a theory of estimating the turnover rate of nucleic acid P in growing systems, which may be summarised as follows:

In a growing system, A , the turnover rate, T , of nucleic acid P of a given type (NAP) is defined by $T = \frac{1}{Q} \frac{dD}{dt}$, where $Q(t)$ is the total amount of NAP in A and dD the amount of NAP released during the time interval dt . Assuming the homogeneity of the ³²P-labelled NAP pool of A , it is seen

TABLE I
THE TURNOVER RATE OF DNA-P AND PNA-P IN THE LIVERS OF YOUNG RATS

Expt. No.	Number of animals*	Period of expt. t (day)	Quotient of DNA increase $Q(t)/Q(0)$	Turnover rate T (day ⁻¹)	
				DNA-P	PNA-A
F-S ⁶	4 ~ 2	10	1.8	0.027	0.10
1-AB	2 ~ 1	7	1.7	—0.041	0.057
1-AC	2 ~ 1	7	1.2	—0.009	0.004
2-AB	4 ~ 2	10	2.4	0.034	0.27
2-AC**	4 ~ 4	10	1.0	0.041	0.38
2-AD	4 ~ 1	14	2.1	0.011	0.17
3-AB	3 ~ 1	7	1.9	—0.041	0.12
3-AC	3 ~ 1	16	2.6	—0.015	0.15
3-BC	1 ~ 1	9	1.3	0.011	0.13
Weighted mean***				0.012	0.18

* Numbers of animals of the two groups, yielding initial and final values respectively and corresponding to the alphabetic signs in col. 1, are given in the order mentioned.

** Supplied daily with restricted amounts of diet so as to keep the body weight unchanged.

*** T values of each line were multiplied with the sum of 2 figures in column 2 and averaged within individual experiments (Expt. 1, 2, etc.) to yield the primary weighted means. These were then multiplied with the actual total number of animals in respective experiments and finally averaged to give the values listed.

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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 equation¹² 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 system³, 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 SIBATANI⁶ 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 (pentose nucleic 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^{3,4,5}. 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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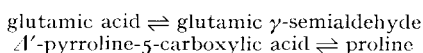
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The interconversion of glutamic acid and proline*

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



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

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