A Time Study of the Incorporation of Radiophosphorus into the Nucleic Acids and Other Compounds of a Transplanted Mouse Mammary Carcinoma*

Cyrus P. Barnum, Robert A. Huseby,† and Halvor Vermund

(Departments of Physiological Chemistry, Physiology, and Radiology of the Medical School, University of Minnesota, Minneapolis, Minn.)

A previous study (2) with P³² and with normal mouse liver tissue indicated the marked metabolic heterogeneity of the pentosenucleic acid (PNA) isolated from various cellular fractions. It was hoped that by extending such a study to tumor tissue we could derive further information concerning possible metabolic interrelationships between the PNA of various fractions and also between PNA and desoxypentosenucleic acid (DNA). Such information should help us to better understand the growth process.

MATERIALS AND METHODS

Supply of tissue.—Such a study required the periodic supply of a uniform tumor tissue over a period of about 1 year. For this purpose a spontaneous mammary carcinoma that arose in an AZF mouse was carried by successive transplantation in AZF or A₂ZbF₁ mice. The transplantation was done by subcutaneous inoculation of a tumor cell suspension to the backs of the recipient animals. After several transplant generations the tumor appeared to have attained a uniform rate of growth which it maintained for 32 generations. The rate of growth was such that tumors of 1-2 gm. could be removed 13-16 days after inoculation.

The radioactive tracer.—At this period, 2 weeks after inoculation, groups of mice containing three or four animals per group were given injections intraperitoneally of a solution containing P³² as orthophosphate. The amount of P³² used ranged from 0.5 to 2 μC/gm body weight.

Fractionation of tissue.—At time intervals ranging from 15 minutes to 16 hours after injection of P³² the mice were sacrificed, and the tumors were homogenized and fractionated as previously described (1, 8, 14). The samples obtained were the nuclear fraction (N), the large granule or mitochondria! fraction (L), the microsomes (M), the ultrasedimentable fraction (U), and the nonsedimentable supernatant fluid (S).

In Table 1 are presented the results of the chemical analyses on the whole homogenate of this tumor line and on the separated cellular fractions. It may be seen that the total DNA and the total PNA are very similar in amount and that the bulk of the PNA is found associated with the particulate fractions (primarily M and U) derived from the cytoplasm. The value for S-PNA is probably somewhat low because of the difficulties encountered in quantitatively precipitating this fraction in several experiments. Consequently, two experiments were carried out in which the cytoplasmic extract (after centrifuging the homogenate to remove nuclei) was spun directly at 100,000 X g for 1 hour, and all the cytoplasmic particulates...
were sedimented together. The sediment and supernate were treated with cold 5 per cent TCA and then with hot TCA to remove the PNA. On the basis of orcinol-HCl tests the PNA P of the combined particulates averaged 493 μg/gm tissue, Table 2 are recorded the specific activities of the inorganic phosphate and of the various nucleic acid fractions. In seven experiments, performed at various time intervals, a large granule or “mitochondrial” fraction was also isolated. In six of

Table 1

<table>
<thead>
<tr>
<th>ACID-SOLUBLE</th>
<th>INORGANIC</th>
<th>DNA</th>
<th>PNA</th>
<th>LIPID</th>
<th>PROTEIN RESIDUE</th>
<th>PROTEIN RESIDUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRACTION*</td>
<td>µg P/gm wet weight of tissue</td>
<td>µg P/gm wet weight of tissue</td>
<td>µg P/gm wet weight of tissue</td>
<td>mg N/gm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>[570-650]</td>
<td>[510-540]</td>
<td>[584-628]</td>
<td>[785-828]</td>
<td>[301-328]</td>
<td>[107-113]</td>
</tr>
<tr>
<td>N (isolated)</td>
<td>[790-1130]</td>
<td>[550-910]</td>
<td>[284-318]</td>
<td>[101-118]</td>
<td>[12.5-15.5]</td>
<td></td>
</tr>
<tr>
<td>N (corrected for yield)*</td>
<td>127 (3)</td>
<td>59 (35)</td>
<td>27 (20)</td>
<td>6 (53)</td>
<td>3.1 (5)</td>
<td></td>
</tr>
<tr>
<td>N (corrected for yield)†</td>
<td>834</td>
<td>75</td>
<td>35</td>
<td>8</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>[35-75]</td>
<td>[59.147]</td>
<td>[8-7]</td>
<td>0.8-1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>253 (33)</td>
<td>89 (33)</td>
<td>6 (53)</td>
<td>0.5 (1.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>188 (35)</td>
<td>11 (33)</td>
<td>3 (53)</td>
<td>0.75 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>361 (5)</td>
<td>91 (21)</td>
<td>11 (33)</td>
<td>5 (0.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2N, L, M, U, S</td>
<td>061</td>
<td>345</td>
<td>30</td>
<td>18.6</td>
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<table>
<thead>
<tr>
<th>TIME AFTER P32 INJECTION (HOURS)</th>
<th>SPECIFIC ACTIVITY (BCC)* OF THE PHOSPHORUS OF VARIOUS NUCLEIC ACID FRACTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>7,470 (5)†</td>
</tr>
<tr>
<td>0.5</td>
<td>[4,000-9,500] † [5.1-10.1]</td>
</tr>
<tr>
<td>1</td>
<td>9,640 (4)</td>
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<tr>
<td>2</td>
<td>[8,000-11,700] [54-110]</td>
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<tr>
<td>4</td>
<td>6,940 (4)</td>
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<td>8</td>
<td>[6,100-8,000] [200-235]</td>
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<tr>
<td>16</td>
<td>4,130 (4)</td>
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<td></td>
<td>[5,200-4,700] [512-575]</td>
</tr>
<tr>
<td></td>
<td>4,290 (4)</td>
</tr>
<tr>
<td></td>
<td>[5,300-6,400] [200-418]</td>
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<tr>
<td></td>
<td>2,980 (4)</td>
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<tr>
<td></td>
<td>[6,100-5,300] [200-418]</td>
</tr>
<tr>
<td></td>
<td>2,670 (4)</td>
</tr>
<tr>
<td></td>
<td>[5,200-4,700] [512-575]</td>
</tr>
</tbody>
</table>

Specific activity* of nucleic acid fractions.—In these experiments the PNA isolated showed a specific activity that was within the range observed for the PNA from the other particulate fractions of the cytoplasm. This will be noted from Table 2 that the differences in the specific activities of the PNA isolated from the M and U particulate fractions of the cytoplasm are not such as to indicate that these nucleic acids are metabolically distinct. This is particularly evident in the values recorded at the 15- and 30-minute time intervals when the differences might be expected to be most marked. In this tumor tissue, therefore, while that of the supernate averaged 104. This gives a ratio of particulate to supernatant PNA in the cytoplasm of 4:75, a figure that will be made use of later.

Specific activity* of nucleic acid fractions.—In this paper specific activity is expressed as the Biological Concentration Coefficient (BCC) as suggested by Schultman and Falkenheim (12). It is defined as follows:

\[ \text{BCC} = \frac{\text{counts/min/gm P}}{\text{counts/min administered/gm body weight}} \times 100 \]

The relative specific activities reported in a previous paper (2) may be converted to BCC by multiplying them by 101.
it appears reasonable to visualize all the PNA of the cytoplasmic particulates (P-PNA) as being metabolically homogeneous. In sharp contrast to such homogeneity in tumor are some recent unpublished results obtained with normal liver tissue in which the PNA isolated from the U fraction 15 minutes after administration of P\(^{32}\) had 4–5 times the specific activity of the M-PNA.

**TABLE 3**

<table>
<thead>
<tr>
<th>TIME (HOURS)</th>
<th>LIPID</th>
<th>PROTEIN RESIDUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>M and U</strong></td>
<td><strong>S</strong></td>
</tr>
<tr>
<td>0.25</td>
<td>17 (5)</td>
<td>99 (5)</td>
</tr>
<tr>
<td>0.5</td>
<td>54 (6)</td>
<td>187 (5)</td>
</tr>
<tr>
<td>1</td>
<td>150 (6)</td>
<td>284 (5)</td>
</tr>
<tr>
<td>2</td>
<td>377 (6)</td>
<td>688 (4)</td>
</tr>
<tr>
<td>3</td>
<td>607 (6)</td>
<td>771 (5)</td>
</tr>
<tr>
<td>4</td>
<td>850 (7)</td>
<td>935 (4)</td>
</tr>
<tr>
<td>8</td>
<td>1,220 (8)</td>
<td>1,350 (4)</td>
</tr>
<tr>
<td>16</td>
<td>1,470 (8)</td>
<td>1,520 (5)</td>
</tr>
</tbody>
</table>
* See footnote 4.
† Values are means of number of observations shown in parentheses. Ranges are shown in brackets below each mean.

It will be observed that the nuclear PNA shows by far the most rapid increase in specific activity of all the nucleic acid fractions isolated. This is similar to previous observations for normal liver tissue (2). Initially, the S-PNA has a specific activity much greater than that of the particulate PNA, but the latter rapidly approaches it.

### Specific activity of phospholipid and "protein residue" fractions.

Table 3 shows the specific activities of the phospholipid P and the "protein residue" P. Since the phospholipid P of the M and U fractions showed no significant differences, these are reported together. Similarly, the phospholipid P of the "mitochondrial" fraction obtained in seven experiments showed no differences from that of the M and U fractions. On the other hand, the S-phospholipid P shows a significantly higher specific activity at early time intervals, but these values are approached by the particulate phospholipid values within 16 hours. The nuclear phospholipid has shown a specific activity of approximately 85 per cent of that of the cytoplasmic particulates.

The "protein residue" P shows an extremely rapid rise in specific activity, and no significant differences have been observed between any of the cytoplasmic fractions studied. Again, however, the nuclear "protein residue" P has shown a specific activity of approximately 85 per cent of that of the other fractions.

Chart 1 illustrates the time course of the specific activities of several of these fractions and demonstrates that by 16 hours they have very nearly reached equilibrium.

### DISCUSSION

The specific activity of the "protein residue" P shows an extremely rapid increase, comparable to the few observations previously made on this fraction from liver (2). An inspection of Chart 1 indicates that the curve, though rising more rapidly than that for the N-PNA, goes through its maximum at a value well below that for N-PNA. This seems to imply a heterogeneity of the P in this fraction (10)\(^6\) with one part incorporating P\(^{32}\) very rapidly and a second part more slowly. Even the slower fraction, however, has come to equilibrium by 16 hours.

**Chart 1.** Specific activity-time curves for inorganic P, nuclear PNA (N-PNA), "protein residue," particulate phospholipid (P-PL), and particulate PNA (P-PNA).

BCC refers to the specific activity. See footnote 4.

The particulate phospholipid incorporates P\(^{32}\) much less rapidly than the same fraction in liver (9). However, in tumor tissue the supernatant phospholipid shows a higher specific activity than the particulate phospholipid, whereas in liver no differences were observed.

### Analysis of the specific activity-time curves for nucleic acid fractions.

Our particular interest has been focused on the incorporation of P\(^{32}\) into the

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\(^6\) Kennedy (10), in an oral report, presented evidence for the isolation, from a protein residue fraction, of serine and threonine phosphates with specific activities several times that for the total P of this fraction.
various nucleic acids of the cells. One of our objectives was to find a tissue in which growth was sufficiently reproducible and constant over the experimental period that we could derive some useful information by following the time-course of the specific activity of the DNA and of the various PNA fractions.

One important simplifying assumption which is basic to all the calculations that follow was that even this rapidly proliferating tumor tissue could, as a first approximation, be visualized as a tissue in a steady state. This assumption was made since it seemed probable, on preliminary inspection of the curves, that the rates at which most of the constituents were equilibrating with their precursors greatly overshadowed the rate of growth. In view of the results reported in this paper the assumption of a steady state appears to have been a valid approximation.

Interrelationships between PNA fractions.—On the basis of previous observations (2) on mouse liver tissue a tentative hypothesis was suggested which proposed that some unknown intermediate, \( x \), which equilibrates very rapidly with inorganic P, \( i \), is the immediate precursor of the phosphorus of nuclear PNA, \( a \), and also of the cytoplasmic supernatant PNA, \( b \), which in turn is the precursor of the particulate PNA, \( c \), of the cytoplasm. Such a scheme can be pictured as follows:

\[
\text{Inorg. P} \rightarrow x \rightarrow N-PNA \quad \text{or} \quad \text{S-PNA} \Rightarrow \text{P-PNA} \quad \frac{1}{b} \frac{1}{a} \frac{1}{c} \cdot (1)
\]

The justification for considering the particulate PNA as the end of the reaction chain—thus permitting the formulation of equation (4) below—is that at early time intervals no P fraction that we have isolated from this tissue has shown a lower specific activity than the P-PNA. Since the major P fractions have been analyzed, it seems improbable that P-PNA could be the immediate precursor of any other appreciable P pool, which by a reversible reaction might affect its specific activity-time curve.

Since the steady state is assumed in scheme 1, then \( v_1 \) (the rate at which P in compound \( x \) is incorporated into compound \( a \)) is equal to \( v_2 \) (the rate at which P returns from \( a \) to \( x \)). Similarly, \( v_3 = v_4 \) and \( v_5 = v_6 \). As previously described (2), one can define the time changes in the specific activities of compounds \( a \), \( b \), and \( c \) by means of the following equations:

\[
\frac{dA}{dt} = \frac{v_1}{a} (X - A) = k (X - A) \quad (2)
\]

\[
\frac{dB}{dt} = \frac{v_3}{b} (X - B) - \frac{v_5}{b} (B - C)
\]

\[
= l (X - B) - n (B - C) \quad (3)
\]

\[
\frac{dC}{dt} = \frac{v_5}{c} (B - C) = m (B - C) \quad (4)
\]

In these equations \( a, b, \) and \( c \) refer to the \( \mu g \) PNA P/gm wet tissue to be found in nuclei, cytoplasmic supernate, and cytoplasmatic particulates, respectively, while \( X, A, B, \) and \( C \) refer to the specific activities of the compounds \( x, a, b, \) and \( c \), respectively. The constants \( k, l, m, \) and \( n \) define that fraction of a given compound replaced each hour. Thus, \( k \) is that fraction of the P present in \( a \) which traverses the path from \( x \) to \( a \) each hour.

Metabolic reactions involving DNA.—In attempting to analyze the data with respect to DNA we have made the assumption that the incorporation of \( P^{32} \) into DNA occurs only in cells that are preparing to undergo division, i.e., that in most of the cells at any given time the DNA is metabolically inert as far as its P is concerned. This assumption rests on the observation of Osgood et al. (11) that in three nondividing tissues (skeletal muscle, cartilage, and brain cortex) of an adult patient treated with \( P^{32} \) for 1 year the DNA showed no incorporation of \( P^{32} \); on our observation (2) that the DNA of adult mouse liver cells incorporates extremely little \( P^{32} \); and on the observation of Brues et al. (5) that when \( P^{32} \) is incorporated into liver during regeneration it remains in the DNA for a long time, whereas it gradually leaves the other P compounds of the liver.

In a cell that is preparing to undergo division and is synthesizing an equivalent amount of new DNA, one could visualize several possibilities: (a) the pre-existing DNA of the mother cell might remain metabolically inert, and therefore only half of the DNA of the daughter cells would become labeled; (b) the pre-existing DNA might go completely into the pool of its precursors from which a double amount of DNA, all labeled, would emerge; or (c) the pre-existing DNA might enter into reversible reactions with its labeled environment at, or at about, the same time that an equivalent amount of new DNA was being synthesized from the precursor pool. Regardless of which of these three possibilities is correct, the rate of incorporation of \( P^{32} \) into DNA will be a function of the rate of synthesis of DNA. Furthermore, since we have found no significant differences in the content of DNA/gm of tissue between small and large tumors, one can also conclude that the incorporation of \( P^{32} \) is a function of tumor growth.
We may visualize a general reaction scheme as follows:

\[ \text{Inorg. } P \rightarrow x \rightarrow \text{DNA} \quad \text{or} \quad \text{Inorg. } P \rightarrow x \rightarrow \frac{1}{2} d \quad (5) \]

where \( v_r \) is the rate at which \( P \) goes from a precursor, \( x \), to DNA, \( d \), and \( v_b \) is the rate of a possible reverse reaction. This system is not in a steady state, and we know that \( v_r > v_b \). If possibility (a) above is correct, then \( v_b = 0 \); if possibility (b) is correct, then \( v_b = v_r/2 \); and if possibility (c) is correct, then \( 0 < v_b < v_r/2 \).

The quantity \( (v_r - v_b)/d \) represents the fractional increase of DNA per unit time and will be referred to as the growth constant, \( g \). If one assumes that the number of cells in which DNA is being synthesized is proportional to the number of cells present (or to the total DNA present) at any given time, and that this proportionality remains constant over the experimental period of 16 hours, then one can write that \( d = d_0 e^{gt} \) where \( d_0 \) is the amount of DNA at time zero, \( d \) the amount of DNA at any specified future time, \( g \) the growth constant, and \( t \) the time in hours. Using this assumption of the constant percentage increase in DNA over the experimental period, one can derive the following equation defining the time change in the specific activity of DNA:

\[ \frac{dD}{dt} = \frac{v_r}{d} (X - D) = f(X - D) = (g + h)(X - D) \quad (6) \]

where

\[ f = \frac{v_r}{d}; \quad g = \frac{v_r - v_b}{d}; \quad h = \frac{v_b}{d}; \quad \text{and} \quad 0 \leq h \leq g. \]

It is surprising at first to find the form of the equation identical to that for reversible, steady-state systems, but this is a direct result of the assumption that the rate at which DNA is formed in the tissue is proportional to the amount there.

**Proposed interrelationships between DNA and the various PNA fractions.**—With equation (6) one can readily demonstrate that neither inorganic P nor any of the fractions of PNA P isolated can fulfill the role of \( x \). Thus, as an initial assumption to be tested, we have visualized the following:

\[ \text{DNA} \quad \text{or} \quad \text{S-PNA \rightarrow P-PNA} \quad (7) \]

In words, this assumption says that the phosphorus of the DNA, \( d \), of the nuclear PNA, \( a \), and of the cytoplasmic supernatant PNA, \( b \), is all derived from precursors that share a common metabolic box. It is possible that the phosphorus precursors of each of these nucleic acids are identical, though this is not necessary and does not seem very probable. If the precursors of \( a, b \), and \( d \) are so rapidly equilibrated among themselves that their specific activities are indistinguishable, then for our subsequent considerations \( x \) may be considered a single substance.

**Evaluation of proposed mechanism.**—The first step in testing the above hypothesis involves analysis of the postulated chain of reactions from \( x \) to \( b \) to \( c \) as shown in equation (7). Calculations on the cytoplasmic constituents have been carried through the 8-hour interval. From Chart 2 it may be seen that it was possible to draw a smooth curve through, or very close to, all the experimental points for \( C \), i.e., the points for the specific activity of the particulate PNA of the cytoplasm. In the case of the supernatant PNA, the experimental points for specific activity, \( B \), were less consistent, and a smooth curve, \( B_1 \), was drawn among the points as a first approximation. At this stage the areas under curves \( B \), and \( C \) (i.e., \( B_1 \) and \( C \)), respectively) were evaluated and introduced into the integrated form of equation (4):

\[ C = m (\beta_1 - \gamma) \quad (8) \]

By plotting \( C \) against \( (\beta_1 - \gamma) \), it was possible to draw the best straight line through the points. The slope of this line would represent the most probable value of \( m \), which was evaluated as 1.18. Using this value for \( m \), along with the fixed values for \( C \) and \( \gamma \), equation (8) can be used to re-evaluate \( \beta_1 \), this time calling it \( \beta_2 \). Finally, the values for \( \beta_2 \) at the various time intervals may be numerically differentiated, and a smooth curve drawn through these differentials to give \( B_2 \) as shown in Chart 2. It may be seen, then, that a curve \( B_2 \), varying only a little from the curve \( B_1 \) first drawn among the experimental points, will fit exactly into the role of precursor for the particulate PNA.

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The next step involves writing equations (6), (2), and (5) in their integrated forms.

\[ D = f(\xi - \delta) \]  
\[ A = k(\xi - \alpha) \]  
\[ B = l(\xi - \beta) - n(\beta - \gamma). \]

In these equations, \( \alpha, \beta, \delta, \delta, \) and \( \xi \) represent the integrals of \( A, B, D, \) and \( X, \) i.e., they represent the areas under the respective specific activity-time curves up to any given point. Since \( (\beta - \gamma) = C/m, \) as seen from equation (8), and since \( n/m = \)

\[ c/b = 4.75 \] as seen from equations (8) and (4), equation (11) may be written as

\[ B = l(\xi - \beta) - 4.75C. \]  
\[ \text{(11a)} \]

Finally, equation 11a may be rearranged to read

\[ B + 4.75C = \bar{B} = l(\xi - \beta). \]  
\[ \text{(11b)} \]

The hypothesis to be tested assumes that \( \xi \) is identical in equations (9), (10), and (11b). Thus, from equations (10) and (11b) we have

\[ \frac{\alpha - \beta}{A} - l^{-1} = \frac{\beta}{A} = -k^{-1}. \]  
\[ \text{(12a)} \]

Dividing through by \( A \) and rearranging we get

\[ \frac{\alpha - \beta}{A} = \frac{\beta}{A} = \frac{1}{A}. \]

Similarly, from equations (9) and (10) we get

\[ \frac{\alpha - \delta}{D} + \frac{k^{-1}}{D} = f^{-1}. \]  
\[ \text{(13)} \]

\[ 4.75 \] is the ratio of the particulate PNA in the cytoplasm to the supernatant PNA. See first paragraph under "Results."

If we now plot \( \bar{B}/A \) against \( (\alpha - \beta)/A \) and \( A/D \) against \( (\alpha - \delta)/D, \) as shown in Charts 3 and 4, we can evaluate \( k^{-1}, l^{-1} \) and \( f^{-1} \) from the proper slopes and intercepts, provided the points can reasonably be considered to fall on straight lines. Since \( k^{-1} \) must be the same for both plots, then the selection of best values for all three constants is influenced...
by all the experimental data, thus minimizing errors inherent in the data for any single time curve. The values for $l$, $k$, and $f$, as evaluated by this graphical method, are 0.38, 1.5, and 0.05, respectively. Using these constants we can calculate $\xi_1$ from equation (9), $\xi_2$ from equation (10), and $\xi_3$ from equation (11a) and compare them as is done in Table 4. In this calculation the actual

<table>
<thead>
<tr>
<th>TIME AFTER INJECTION (HOURS)</th>
<th>$\xi_1$</th>
<th>$\xi_2$</th>
<th>$\xi_3$</th>
<th>$\chi$</th>
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<td>102</td>
<td>112</td>
<td>720</td>
</tr>
<tr>
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<td>510</td>
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</tr>
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<td>1,720</td>
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<td>2,700</td>
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<td>10,900</td>
<td>11,700</td>
<td>3,200</td>
</tr>
<tr>
<td>5</td>
<td>21,000</td>
<td>21,200</td>
<td>21,300</td>
<td>2,000</td>
</tr>
<tr>
<td>16</td>
<td>55,400</td>
<td>56,400</td>
<td>56,400</td>
<td>1,640</td>
</tr>
</tbody>
</table>

* $\xi_1$, $\xi_2$, and $\xi_3$ calculated from equations 9, 10, and 11a respectively. Values of the constants, obtained from the graphical solution, were 1.5, 0.05, and 0.38 for $l$, $k$, and $f$, respectively. Values of $A$, $B$, $C$, and $D$ used were those experimentally observed, but values of the areas $\alpha$, $\beta$, and $\delta$ were obtained from the smoothed time curves of $A$, $B$, and $D$.

† $\chi$ is derived by numerical differentiation of the time curve of the average $\xi$ values.

...Experimental values for $A$, $B$, $C$, and $D$ at the various time points were used, but it was necessary to use the areas $\alpha$, $\beta$, and $\delta$ derived from the smoothed curves. Inspection of Table 4 shows that the $\xi$ values calculated from the three reaction pathways are in good agreement, indicating the essentially identical course of the specific activity-time curves for the precursors of DNA, nuclear RNA, and cytoplasmic supernatant RNA. Also, shown in Table 4, are values of $\chi$, the specific activity of these precursors at the various time intervals. These values of $\chi$ are obtained by graphical differentiation of a smooth curve drawn through the average $\xi$ values. The relationship of $\chi$ to several other fractions is shown graphically in Chart 5.

While these calculations indicate that the experimental data are compatible with the reaction scheme outlined in equation (7), they do not rule out the possibility that the data might be equally compatible with some other scheme. A currently widely accepted alternative scheme has been presented by Jeener and Szafarz (9), and they have marshalled the arguments supporting it. In this scheme it is proposed that nuclear RNA is a precursor of the nonsedimentable RNA of the cytoplasm. If the values of $B + 4.75C$ are plotted against $(\xi - \beta)$, it may be seen from equation (11b) that a straight line of slope 1 should result. Such a plot, using the average $\xi$ values derived above, gives an excellent straight line passing through the origin. Regardless of what the precursor of this cytoplasmic RNA ($B$) might be, if the area under its curve were substituted for $\xi$ in equation (11b) and a similar plot made, a straight line through the origin should result. However, when the values for $\alpha$, the area under the nuclear RNA curve, were substituted for $\xi$, the resulting plot described a curve for which the points were very far from falling on a straight line. Thus, at least in this tissue, the nuclear RNA isolated cannot be considered the precursor of the supernatant RNA of the cytoplasm.

Comparison of $l$ with the growth rate of the tumor.

Of all the constants that emerge from these calculations, $f$ has special interest because it is related to the growth constant, $g$, which can be approximated by independent means. Unfortunately, the tumor transplant line used in these studies was accidentally lost before several contemplated experiments could be undertaken. However, three independent evaluations of increase in tumor volume by caliper measurements had previously been done on this tumor for other experi-

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In Chart 5.—Specific activity-time curves for inorganic $P(D)$, nuclear RNA ($A$), DNA ($D$), and a postulated precursor ($X$). BCC refers to specific activity. See footnote 4.
12th and 17th days, in the second experiment 2.0 per cent between the 13th and 18th days, and in
the last experiment 2.6 per cent between the 9th and 12th days.

A growth rate of 5 per cent per hour, which was
the calculated value of \( f \), implies that the tumor
should triple its size each 24 hours, and this tumor
was clearly not growing that fast. From the crude
estimation of its actual growth rate, \( g \), it would ap-
pear that this might be one-half of \( f \). Referring
back to equation (6) it would appear clear then
that \( v_4/d \) or \( h \) does have a finite value and that the
best approximation currently possible is that it is
equal to \( g \). Thus, it is tentatively concluded that,
in a cell preparing to synthesize new DNA, the
pre-existing DNA does enter into metabolic reac-
tions with its environment and that this pre-
exisiting DNA goes completely (so far as its \( P \) is
concerned) into the metabolic pool of its pre-
cursor.

This same conclusion has been recently reached
by Stevens, Daoust, and Leblond (13) from studies on rat liver and intestine in which they
compared the "rate" of incorporation of P\( ^{32} \) into
DNA to evaluations of the rate of new cell forma-
tion in these tissues. They calculate the rate of
incorporation of P\( ^{32} \) on the assumption that the
precursor of DNA has a specific activity-time
course which can be represented by that for the
acid-soluble \( P \) of their tissues. Their values on
the rate of incorporation are expressed as DNA
specific activity at the time of sacrifice as a per-
centage of the "average specific activity" of the
acid-soluble \( P \) from the time of injection to that
of sacrifice. They do not explain how they calcu-
late the average specific activity of the acid-sol-
uble \( P \), but from their data one can calculate\(^4\) that
the value they use for the average activity from
0 to 3 hours is the observed value at 3 hours and
that the value they use for the average from 0 to 6
hours is the mean of the observed values at 3 and
6 hours. From our previous study (2) and from
unpublished data pertaining to acid-soluble \( P \) ob-
tained in the same study, we can calculate that
for mice injected intraperitoneally with P\( ^{32} \) the
observed specific activity of liver acid-soluble \( P \) at
3 hours is 73 per cent of the average activity over
the 3 hours. We assume that a somewhat com-
parable relation exists for the liver acid-soluble \( P \)
in their rats which were injected subcutaneously.
Furthermore, their calculation of the 6-hour aver-
age tacitly assumes that the average between 3
and 6 hours is equal to the value observed at 6
hours, rather than to some value intermediate be-
tween the 3- and 6-hour values as would be neces-
sary on this descending portion of their curve. We
have recalculated their data, assuming that the
3-hour value for acid-soluble \( P \) should be divided
by 0.73 to give the average over the first 3 hours,
and using, as a first approximation, the mean of
any two adjacent values as the average activity
throughout that time interval. On this basis we
find that their liver DNA at 24 hours has a specific
activity that is 1.11 per cent that of the average
for acid-soluble \( P \) instead of 1.36 per cent as they
report. This makes what they call the "rate of for-
mation of DNA" considerably less than twice the
rate of new cell formation which they give as
0.71 per cent per day. On the other hand, the ob-
servations reported in this paper indicate that the
postulated precursor of DNA in mammary tumor
tissue has a specific activity-time course well be-
low that for inorganic \( P \) (and also below that for
total acid-soluble \( P \)) throughout the first 8 or 12
hours. This implies that if Stevens et al. had been
able to calculate the average specific activity of
the actual precursor they would have obtained a
value for DNA formation greater than 1.11 per
cent per day and probably in the range of double
the rate they report for new cell formation—as
they claim their study demonstrates. It would ap-
pear that they reached their conclusion because of
a canceling of errors.

A similar recalculation of their data on intesti-
nal DNA leads to a value for DNA at 3 hours of
10.75 per cent that of the recalculated average ac-
tivity of acid-soluble \( P \). This may be expressed
as 86 per cent per day, whereas they report 117.6
per cent. Again, one is justified in assuming that
the actual immediate precursor of the \( P \) in intes-
trnal DNA has an average specific activity over
the first 3 hours that is substantially below the
average for acid-soluble \( P \) and would thus lead to
a calculation of DNA formed per day substantially
greater than 86 per cent. In mouse mammary tu-
more the average specific activity of the postulated
DNA precursor during the first 3 hours is only 50
per cent that of the acid-soluble \( P \) during the same
time. If this same correction factor should apply
to intestine, it would imply a daily rate of DNA
formation of 170 per cent, thus making it much
more than twice their calculated rate of new cells
formed per day of 52.7 per cent. Whether this dis-
crepancy—(a) results from the possibility that the
correction factor to be applied to intestine should
be much less than would apply in mammary car-
inoma, (b) indicates need for re-evaluation of the
52.7 per cent per day of cell increase which was
based on the piling up of cells in mitosis over a

\(^4\) A personal communication from Dr. Leblond confirms
that the calculation described here is equivalent to the one
used by them.
4-hour period after the administration of colchicine, or (c) implies that the thesis advanced by us as well as by Stevens, Daoust, and Leblond is invalid—must await the accumulation of additional experimental evidence.

This conclusion, that the pre-existing DNA of a cell that is preparing to divide does incorporate $P^{32}$, is also consistent with the suggestion made by v. Euler and Hevesy (7) and with the data presented in a previous paper by the present authors (14). In our studies on mouse mammary carcinomas it was observed that a dose of $x$-radiation, sufficient to cause complete cessation of tumor growth and complete inhibition of new cells entering mitosis, inhibited the incorporation of $P^{32}$ into the DNA to only 50 per cent of that seen in the non-irradiated control tissue. It was proposed that $x$-radiation blocked the net synthesis of DNA but did not affect the “turnover” of the pre-existing DNA.

**SIGNIFICANCE OF CONSTANTS AND IMPLICATIONS CONCERNING STEADY STATE.**—The values for the various constants together with the amount of $P$ in each of the nucleic acid fractions, as taken from Table 1, enable us to calculate the rate at which $P$ is passing via the various pathways in equation (7). These rates are recorded in Table 5.

As may be seen in Table 5, the amount of nucleic acid $P$, in this case presumably intact PNA, that is going back and forth each hour between a nonsedimentable form in the supernate (b) and the particulate lipoprotein-nucleoprotein form (c) is 75 per cent of all the PNA $P$ in the cell. It shows also that the fractional turnover between S-PNA and P-PNA is nearly as great as that between precursor and N-PNA, and should make one cautious about concluding that a certain fraction is metabolically less active than some other merely because its specific activity is much lower. Table 5 also appears to justify the original approximation that this system could be treated as a steady state, since even the slowest reaction ($x \rightleftharpoons b$) has a turnover of 38 per cent/hour where the net increase due to growth is of the order of 2-2.6 per cent/hour.

**SUMMARY AND CONCLUSIONS**

Mice bearing a transplanted mammary carcinoma have been sacrificed at time intervals varying from 15 minutes to 16 hours after the intraperitoneal injection of $P^{32}$. The tumors have been fractionated by differential centrifugation to yield nuclei (N), microsomes (M), an ultraseedimentable particulate fraction (U), and a supernatant fluid (S). From each of these fractions the nucleic acids, phospholipids, and “protein residues” have been isolated and the specific activities of the $P$ of these constituents measured along with the specific activity of the inorganic $P$ derived from the whole homogenate.

Next to inorganic $P$, of those fractions studied the “protein residue” $P$ showed the most rapid initial rise in specific activity. However, the shape of its specific activity-time curve leads us to conclude that it is a heterogeneous fraction with respect to its $P$, part of which equilibrates very rapidly with inorganic $P$ and the rest more slowly. Nevertheless, it has all equilibrated by 16 hours. No significant differences were observed in the specific activities of this “protein residue” $P$ derived from any of the cytoplasmic fractions, but the “protein residue” $P$ from the nuclei consistently showed a slightly lower specific activity.

The phospholipid $P$ from the cytoplasmic particulate fractions was indistinguishable on the basis of specific activity. However, the supernatant phospholipid $P$ showed a significantly higher specific activity at early time intervals but its time curve was approached by the curve of the particulate phospholipid $P$ as both of them approached equilibrium with the inorganic $P$ at 16 hours. Again, the nuclear phospholipid $P$ consistently showed a slightly lower specific activity than that from the other fractions.

The pentosenucleic acids could be placed in three metabolically distinct fractions—the nuclear (N-PNA), the cytoplasmic supernatant (S-PNA), and the cytoplasmic particulate (P-PNA). The specific activity-time curves for these three fractions and for the DNA were consistent with the assumption that the $P$ of DNA, N-PNA, and S-PNA came from precursors whose specific activities had essentially identical time courses. This is interpreted to mean that the immediate precursors (perhaps a mixture of desoxypentose- and pentose-mononucleotides) were all in rapid equilibrium with some common $P$ donor (such as ATP). The data were also consistent with the assumption that S-PNA was the immediate precursor of P-PNA. On the
other hand, the data were not consistent with the prevalent assumption that N-PNA is the precursor of either fraction of cytoplasmic PNA.

Turnover constants, expressed in terms of that percentage of the P in a product which passes from precursor to product each hour, were calculated as 150 per cent for N-PNA, 38 per cent for S-PNA, and 118 per cent for P-PNA. In the case of DNA, the amount of P incorporated per hour was calculated as 5 per cent of that present, whereas the net increase of DNA was estimated to be 2 – 2.6 per cent/hour. This led to the tentative conclusion that, in a cell preparing to synthesize new DNA, the pre-existing DNA enters into metabolic reactions with its environment and that this pre-formed DNA goes completely (so far as its P is concerned) into the metabolic pool of its precursor.

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A Time Study of the Incorporation of Radiophosphorus into the Nucleic Acids and Other Compounds of a Transplanted Mouse Mammary Carcinoma

Cyrus P. Barnum, Robert A. Huseby and Halvor Vermund


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