A comparison of the rate of formation of desoxyribonucleic acid phosphorus (DNA P) with that of cell production in the liver and intestinal epithelium of the rat showed that, in both tissues, the amount of newly formed DNA P was about twice that generally assumed to be required for the formation of new cells by mitosis (1). Since the rate of mitosis is very slow in liver and very fast in the intestinal epithelium, it seemed indicated to repeat the measurements of DNA P formation in an organ exhibiting a moderately high mitotic activity, the alveolar tissue of the lung. The rate of formation of DNA P was then compared to the mitotic rate of this tissue (2).

Methods

In a first series of experiments, sixteen male Sherman albino rats, ranging in weight from 191 to 319 gm. (average 272 gm.), were given a single subcutaneous injection of 300 μc. of $^{32}$P in the form of sodium phosphate (pH 5.5). The animals were maintained on Purina fox chow and tap water. Groups of four rats were sacrificed at intervals of 3, 6, 12, and 24 hours after the $^{32}$P injection.

To reduce the blood cell content of the lung, the animals were exsanguinated via the abdominal aorta under ether anesthesia. The lungs were then removed and the intrapulmonary bronchi and adjacent bronchial lymph nodes excised with scissors. The trimmed lungs of the four rats of each group were pooled and forced through a plastic squeezer. 3 gm. of tissue pulp were ground in ice-cold 10 per cent trichloroacetic acid in a Potter-Elvehjem type homogenizer.

The phosphorus fractions were isolated by a slightly modified Schmidt-Thannhauser procedure (3). The specific activities of the inorganic, whole acid-soluble, and DNA P fractions were determined as described by Stevens et al. (1). The amount of DNA P newly formed during various time intervals after $^{32}$P administration was calculated by comparing the specific activity of the DNA P with that of either the whole acid-soluble phosphorus or inorganic phosphorus fractions (see below).

* Fellow of the Damon Runyon Memorial Fund for Cancer Research, Inc.
A second series of experiments was run in the same manner except for the following details: the animals weighed from 210 to 262 gm. (average 240 gm.), there were six animals per group, the dose of P32 was reduced to 150 μc. per animal, and only the most peripheral part of each pulmonary lobe was used, thus completely excluding bronchial and lymphatic tissue.

Results

Specific Activity of Phosphorus Fractions—In both experiments the specific activity of the whole acid-soluble phosphorus fraction, as well as

<table>
<thead>
<tr>
<th>Table I</th>
<th>Specific Activity* of Acid-Soluble and DNA P Fractions of Rat Alveolar Lung Tissue</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Experiment I</td>
</tr>
<tr>
<td></td>
<td>Whole acid-soluble P</td>
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<tr>
<td></td>
<td>End value†</td>
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<tr>
<td>hrs.</td>
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<tr>
<td>3</td>
<td>10.71</td>
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<tr>
<td>6</td>
<td>7.93</td>
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<td>12</td>
<td>4.22</td>
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<tr>
<td>24</td>
<td>4.20</td>
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</table>

* Expressed as percentage of the injected dose per microgram of phosphorus × 10⁻⁶, the figure for the injected dose being adjusted for 200 gm. of body weight.

† Specific activity at time of sacrifice.

‡ Average specific activity between time of injection and time of sacrifice, calculated as the mean of experimental and interpolated figures for each 3 hour interval during that time.

that of its inorganic component, showed a maximal value at 3 hours after P32 injection and then gradually decreased with time (Table I). By 12 hours, the specific activity of the whole acid-soluble fraction was similar to that of the inorganic phosphorus, a fact indicating that the main acid-soluble phosphorus compounds were then equally labeled. In contrast, the specific activity of the DNA P showed a progressive increase with time.

Rate of Formation of DNA P—The percentage of DNA P formed during

1 The explanation of the lower values obtained for the various phosphorus fractions in the second experiment is not known. Perhaps, the peripheral portions of the pulmonary lobes used possess a more sluggish circulation; hence, the entry of phosphorus would be slower than in the more central portions used in the first experiment. It will be shown (Table II), however, that the rate of formation of DNA P was the same in both experiments.
a given period was calculated as the percentage ratio of the specific activity of DNA P at the time of sacrifice to the average specific activity of the precursor from the time of injection to that of sacrifice (4). Such a method requires the assumption that no labeled DNA P was lost during this interval.

Free nucleotides are presumably the immediate precursors of nucleic acids (5-7). In fact, eight mononucleotides have been recently isolated from the acid-soluble phosphorus fraction of rat liver, at 12 hours after P32 administration, and, since seven of these nucleotides possessed an activity similar to that of the whole fraction, it was concluded that they were in rapid equilibrium with other acid-soluble phosphorus compounds. Therefore, the specific activities of the whole acid-soluble phosphorus fraction may be considered to be representative of those of the immediate precursors of DNA. The data calculated on this basis were first expressed as per cent of DNA P formed during each time interval, and, from these figures, the values for the daily formation of DNA P were obtained (Table II). Thus, the average values for the amount of DNA P formed per day were 10.82 and 10.50, respectively, in the two experiments. These two results are in good agreement.

Since many authors have considered the specific activities of the inorganic phosphorus as those of the precursor of DNA P (4), calculations of the rate of DNA P formation based on this assumption were also carried out. By using the data of the first experiment, the percentages of DNA P formed per day were found to be 7.80, 8.48, 7.56, and 8.15 on the 3, 6, 12, and 24 hour data, respectively, or 8.00 per cent on the average. Since the phosphorus present in the immediate precursors of DNA is necessarily

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R. Daoust and A. Cantero, unpublished.
DESOXYRIBONUCLEIC ACID
derived from the inorganic phosphorus, it will be of lower specific activity
than the latter at early intervals after injection of $P^{32}$. Therefore, the fig-
ure of 8.00 per cent for DNA P formation per day calculated from inorganic
phosphorus must be lower than the true value.

DISCUSSION

The DNA content of each one of the two cells arising from mitosis is
equal to that of the mother cell. Accordingly, it is generally assumed that
mitosis is associated with the addition of an amount of new DNA equal to
that of original DNA and, therefore, the rate of cell production should equal
the rate of formation of DNA (and also of DNA P) (8).

In the case of the alveolar tissue of the lung, the rate of cell production
was estimated by the colchicine method in rats weighing 256 gm. on the
average. The number of cells formed daily by mitosis in this tissue was
found to be 3.57 per cent of the cells present (2). When corrected for a lag
phase of 45 minutes between the injection of colchicine and its effect on
the cells of this tissue, the daily cell formation amounted to 4.08 per cent.
Since bronchi and bronchial lymph nodes were excluded from these estima-
tions of the mitotic rate, as well as from the measurements of the rate of
DNA P formation here presented, it is presumed that both the histological
and the radiochemical estimations were performed on similar material. If
the daily rate of cell production, that is, 4.08 per cent, is compared with
that of DNA P formation, that is, 8.00 per cent (with inorganic phosphorus
as precursor) or 10.66 per cent (with the whole acid-soluble phosphorus as
precursor), it is apparent that the rate of cell formation in the lung is not
equal to but is approximately half that of DNA P synthesis. The same
conclusion had previously been reached with rat liver and intestine (1).
Thus, in the three organs, twice as much newly formed DNA P appears
as is necessary to account for the amount of DNA P generally assumed
to be required for the production of new cells.

The presence of this apparent excess of new DNA P suggests that a re-
newal of preexisting DNA P must also occur. The possibility that the
renewal of DNA P takes place continuously in non-dividing cells may be
ruled out since (a) radioautographic studies demonstrated that only cells
undergoing mitosis incorporated phosphorus into the chromosomal DNA
(9), and (b) a direct relationship between the rates of DNA P formation
and mitosis was suggested in the past (4, 10–12) and demonstrated in the
present series in liver, intestine, and lung. The dependence of the DNA P
formation rate on mitosis in these three organs precludes that a significant
amount of DNA P appears in the cell in the absence of mitosis. Thus,
renewal of DNA P must also occur during the mitotic process.

Since only half the new DNA P is accounted for by the synthesis of the
additional amount of DNA P which appears at mitosis, the other half must have been introduced for the renewal of an equal amount of preexisting DNA P. In other words, all the DNA P in cells arising from mitosis must be new. Presumably, all this new DNA P is formed during the duplication stage of the mitotic cycle (8).

The complete substitution of old by new DNA P at mitosis may well indicate that the duplication of a DNA molecule does not take place by addition of 1 similar molecule, but rather consists of the replacement of each preexisting molecule by 2 new ones.  

SUMMARY

The specific activities of the inorganic, whole acid-soluble, and DNA P fractions of the lung were estimated in rats sacrificed at 3, 6, 12, and 24 hours after administration of radiophosphorus in two series of experiments.

Considering the specific activity values of the whole acid-soluble phosphorus as representing those of the DNA P precursors, the daily formation of DNA P in the lung was calculated to be 10.66 per cent of the total DNA P present, while a figure of 8.00 per cent was obtained by using the values of the inorganic phosphorus as those of the precursors.

Since a value of 4.08 per cent was obtained for the daily cell formation in the same tissue, it appears that the amount of DNA P newly formed per day in the lung is about twice that expected from a simple duplication of DNA in the cells undergoing mitosis during the same time interval.

These results confirm previous conclusions derived from similar investigations on liver and intestine and thus further support the view that, during the duplication stage of mitosis, the preexisting DNA P is catabolized and replaced by twice its amount of new DNA P, both daughter cells receiving exclusively newly formed DNA P.

This work was supported by grants of the National Cancer Institute of Canada.

Addendum—Since the publication of our results on the rate of formation of DNA P in rat liver and intestine (1), Barnum, Huseby, and Vermund (14) calculated the rate of DNA P formation in transplanted mouse mammary carcinoma and confirmed our finding that DNA P is formed at a rate which is twice that of cell formation.

These authors criticized our method of calculating the results obtained at the early intervals after P²³ injection (3 and 6 hours). Admittedly, the averages used for these periods are less accurate than those used for the first 12 hours or longer periods. Thus, in the case of the lung, the most accurate data are those computed

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The breakdown of the preexisting DNA at the duplication stage of mitosis may be related to the appearance, in the nucleus, of substances absorbing ultraviolet light, which are Feulgen-negative, in an amount equal to that of the original DNA (13).
for the 0 to 24 hour period. In the case of the liver, the data for the 0 to 72 hour period and, in the case of the intestinal mucosa, those for the 0 to 12 hour interval are the most precise. In the three tissues, the rates of DNA P formation computed over these intervals were almost exactly twice the rate of cell formation, a conclusion in agreement with that reached in the present and previous paper (1).

It should be added that some of the "correction factors" suggested by Barnum et al. were based on results obtained with liver tissue and, therefore, may be helpful in evaluating our data on liver at the early intervals. However, it is doubtful whether a factor calculated for transplanted mouse mammary carcinoma should be used to correct our data on the intestinal mucosa. In any case, the figures obtained at the late intervals would not be significantly altered by these "correction factors."

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