The cells were washed 3 times with cold 0.9% NaCl solution, and 20 g of the moist cell paste were suspended in 40 ml of cold deionized water and disrupted by sonicating the suspension at 10 kc for 15 minutes. The cell debris was then removed by centrifugation at 9000 x g for 20 minutes. The extract contained about 2 mg of protein per ml and could be kept for several weeks in the cold with very little loss of enzymic activity. It was stable to freezing and thawing and could be dialyzed overnight against dilute buffer, pH 6.5, with little loss of activity.

The incubations were carried out in 3 ml cuvettes in a Beckman model DU spectrophotometer in which the cuvette chamber was kept at 37° C. The incubations contained between 0.001 and 0.02 ml of cell-free extract, 2 μmole of HAA or other o-aminophenols, 250 μmole of sodium acetate buffer, pH 5.3, and water to a total volume of 3 ml. A control cuvette, containing boiled cell-free extract, was used to correct for the slight nonenzymic oxidation of the aminophenols. The reactions could be followed by observing the increase in optical density in the 430 to 455 mμ region, characteristic of the phenozone nucleus. Under the conditions described, 0.01 ml of cell-free extract catalyzed the formation of between 0.04 and 0.06 μmole of phenozone in 10 minutes. The yellow product formed from HAA could be purified by extraction from the acidified incubation mixture into ether. It was tentatively identified as 2-aminophenoxazin-3-one-1,5-dicarboxylic acid (Fig. 2), by comparison with the synthetic compound prepared according to the procedure of Butenandt et al. (7). As shown in Fig. 3, the spectrum of the partially purified, enzymically formed material in methanol, and identical with that of the chemically synthesized compound. It also had similar spectral characteristics in methanol, and behaved in exactly the same way as the synthetic material when chromatographed on Whatman No. 1 paper in three solvent systems (propanol-water, 5:1; butanol-propanol-water, 1:3:1; and butanol-ethanol-water, 5:2:10). Incubation of 3-hydroxyphenylalanine resulted in the formation of a yellow compound with spectral characteristics similar to those reported by Butenandt et al. (10).

Further studies on the purification and properties of this enzyme have been carried out by Dr. T. Jacobs, Merck, Sharp and Dohme Research Laboratories, New Jersey, for supplying us with the 4-methyl-HAA methyl ester, and Dr. S. Udenfriend, Laboratory of Clinical Biochemistry, National Heart Institute, National Institutes of Health, for his advice and suggestions.

Acknowledgment.—The authors wish to thank Dr. H. Brockmann, University of Göttingen, West Germany, for supplying the 2-nitro-3-hydroxy-4-methylbenzoic acid used in the synthesis of 4-methyl-HAA, Dr. A. Robertson and Dr. B. Witkop, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland, for their help in synthesizing the latter compound, Dr. T. Jacobs, Merck, Sharp and Dohme, and Dr. S. Udenfriend, Laboratory of Clinical Biochemistry, National Heart Institute, National Institutes of Health, for his advice and suggestions.

REFERENCES

1. BUTENANDT, A., WEIDEL, W., AND BIEKER, E., Naturwiss., 28, 63 (1940).

Net Synthesis of Ribonucleic Acid with a Microbial Enzyme Requiring Deoxyribonucleic Acid and Four Ribonucleoside Triphosphates

SAMUEL B. WEISS AND TOKUMAS NAKAMOTO

From the Argonne Cancer Research Hospital* and the Department of Biochemistry, University of Chicago, Chicago 37, Illinois

(Received for publication, December 30, 1960)

Recent reports from this laboratory have described an enzyme system from rat liver which catalyzes the incorporation of ribonucleotides into the framework of ribonucleic acid (1, 2). This incorporation was shown to require the participation of all four ribonucleoside triphosphates. Inactivation of the enzyme system with small quantities of deoxyribonuclease pointed to a possible dependency of the reaction on intact deoxyribonucleic acid. Indeed, partial reactivation of the DNase-treated mammalian preparations could be achieved by the addition of heated or unheated purified rat liver DNA.1 This communication describes some of the properties of a similar partially purified enzyme from Micrococcus lysoedecticus which carries out the net incorporation of ribonucleotides into a polyribonucleotide fraction.

* Operated by The University of Chicago for the United States Atomic Energy Commission

1 S. B. Weiss, unpublished results.
A 2% suspension of M. lysodeikticus, in 0.02 M phosphate buffer, pH 7.5, was incubated with crystalline lysozyme for 45 minutes at 30°. The viscous, lysed preparation incorporated labeled nucleotides into an acid-insoluble fraction. This incorporation was stimulated 4-fold when all four ribonucleoside triphosphates were also present in the incubation medium. Exposure of the lysed bacterial preparation to sonic treatment or to the action of DNase, resulted in a disappearance of this stimulatory effect, as well as in a marked decrease in the viscosity of the extract.

While this work was in progress, Stoevna (3) and Hurwitz et al. (4) presented evidence that extracts from Escherichia coli B catalyzed the incorporation of labeled ribonucleotides into RNA. This incorporation also required the presence of all four ribonucleoside triphosphates. Hurwitz et al. (4) further showed that their preparations were dependent on DNA for optimal activity.

The partial purificication of the M. lysodeikticus enzyme was effected by exposing the viscous lyzate to sonic oscillation in a Raytheon 10 kc sonic oscillator. The enzyme was precipitated by the addition of proteinate sulfate and the precipitate was extracted with 0.05 M phosphate, pH 7.5. This first extract was discarded. A second extraction was made with 0.20 M phosphate of the same pH, and this solution contained most of the enzyme. The pH of this extract was lowered to 5.4, and the precipitate which formed was collected and suspended in 0.02 M phosphate, pH 7.5. The enzyme was adsorbed onto calcium phosphate gel and removed again by washing the gel with 0.20 M phosphate of the same pH. Extracts so prepared had a ratio of absorbancy at 280 to 260 μm of about 1.4, and were used as the source of enzyme in the work reported here.

The partially purified bacterial preparation catalyzes the incorporation of radioisotope from CTP\(^{32}\), labeled in the ester phosphate only, into a fraction containing RNA (Table I). This incorporation shows nearly an absolute requirement for the presence of ATP, GTP, and UTP as well as DNA. The reaction is specific for the ribonucleoside triphosphates. Substitution by the corresponding diphosphates or the deoxyriboadenosyl triphosphate results in a marked reduction of isotope incorporated. Table I also illustrates that equivalent amounts of RNA cannot replace the DNA requirement. The system is completely dependent upon the addition of certain divalent metal ions: manganese and cobalt can activate the enzyme; magnesium is approximately one-third as effective. The product of the bacterial enzyme reaction resembles RNA in that it is (a) acid-insoluble, (b) non-dialyzable, (c) sensitive to RNase but not DNase, and (d) hydrolyzed by alkali. Alkaline hydrolysis yields 2'- and 3'-nucleoside monophosphates, of which all four mononucleotides are labeled.

### Table I

<table>
<thead>
<tr>
<th>Additions</th>
<th>Total counts incorporated</th>
<th>Total optical density units</th>
<th>Optical density units</th>
<th>Total F¹</th>
<th>ΔF¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete (zero time)</td>
<td>0</td>
<td>4.67</td>
<td>0.00</td>
<td>0.33</td>
<td>0.00</td>
</tr>
<tr>
<td>Omit ATP</td>
<td>1,800</td>
<td>4.98</td>
<td>+0.31</td>
<td>0.67</td>
<td>+0.06</td>
</tr>
<tr>
<td>Omit GTP</td>
<td>510</td>
<td>4.35</td>
<td>-0.42</td>
<td>0.64</td>
<td>-0.11</td>
</tr>
<tr>
<td>Complete: DNA sonicated</td>
<td>19,300</td>
<td>8.36</td>
<td>+3.09</td>
<td>0.94</td>
<td>+0.42</td>
</tr>
<tr>
<td>Complete (zero time)</td>
<td>34,400</td>
<td>11.59</td>
<td>+6.53</td>
<td>1.20</td>
<td>+0.73</td>
</tr>
</tbody>
</table>

* Total optical density measurements were made at λ\_max in a Zeiss spectrophotometer.

† Total phosphate were determined by the method of Fiske and SubbaRow (5).
material located in the acid-insoluble fraction is approximately doubled. Omission of one nucleotide (UTP) or DNA prevents a net increase. In another experiment, a similar inhibition was obtained when GTP alone was omitted. Exposure of DNA to sonic oscillation, before its addition to the reaction mixture, reduces by nearly 50% the amount of ribonucleotide incorporated. This evidence strongly suggests that net RNA synthesis was achieved.

REFERENCES
Net Synthesis of Ribonucleic Acid with a Microbial Enzyme Requiring Deoxyribonucleic Acid and Four Ribonucleoside Triphosphates
Samuel B. Weiss and Tokumasa Nakamoto


Access the most updated version of this article at http://www.jbc.org/content/236/3/PC18.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/236/3/PC18.citation.full.html#ref-list-1