Studies of Sulfate Utilization by Algae. 4. Properties of a Cell-Free Sulfate-Reducing System from Chlorella

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Abstract. A cell-free system from Chlorella pyrenoidosa Chick (Emerson strain 3) which produces acid-volatile radioactivity from $^{35}$SO$_2^–$ is described. A high speed supernatant from cells broken in the French Press at pH 7.0 shows maximal activity when fortified with ATP, an ATP-generating system (creatine phosphate and creatine phosphokinase), TPN, a TPN-reducing system (glucose-6-phosphate and glucose-6-phosphate dehydrogenase) and MgCl$_2$. This system is quite labile and is not stable to dialysis. Addition of low concentrations of 2,3-dimercaptopropan-1-ol (BAL) to the buffers used for enzyme preparation stabilize the extracts and permit them to be dialyzed for 4 hours without loss of activity. If additional BAL is also added to the incubation mixtures it can replace TPNH as a reductant. DPNH also shows appreciable activity.

The system prepared with BAL-containing buffers shows maximal activity at pH 9.0. At this pH, the system requires only ATP, Mg$^{2+}$ and additional BAL and has high activity and stability compared with the other conditions tried. The optimum concentrations of these reactants has been determined and the kinetics of production of acid-volatile radioactivity are described. Nucleoside triphosphates other than ATP are not appreciably active in this system. In all cases, anaerobic conditions are required for maximal activity, the enzyme extracts are labile to heat, and no unequivocal requirement for thioctic acid can be demonstrated.

There is considerable evidence from nutritional and genetic studies that plants and most microorganisms can reduce sulfate to satisfy their entire sulfur requirements (5, 15). Cell-free sulfate reducing systems have been obtained from yeast (1, 6, 16, 18), Desulfovibrio (8), and Salmonella (4), and several intermediates and enzymes involved in the process have been identified. Evidence is also available concerning the products formed from sulfate in these and other organisms in vivo (5, 15).

Chlorella pyrenoidosa was selected for the present investigation because it combines the advantages of studying sulfate reduction in a green plant with the ease of manipulation of a microorganism. We have previously shown that the products formed from sulfate by intact Chlorella cells are: cysteine, glutathione, homocysteine, S-adenosyl methionine, and adenosine-3′-phosphate-5′-phosphosulfate (PAPS) (11, 13, 14). Some of these compounds were also identified subsequently by others (3, 17).

In preliminary work, we adopted an assay based on acid volatility of the products formed, since several of the inorganic species which might be produced reductively from sulfate yield gases on acidification, while sulfate does not. Thus sulfite (SO$_3^{2–}$) and thiosulfate (S$_2$O$_3^{2–}$) would be expected to yield sulfur dioxide (SO$_2$) on acidification, while sulfide (S$^–$) would yield hydrogen sulfide (H$_2$S). Using this assay, it was found that an active crude broken cell preparation from Chlorella could be further fractionated by centrifugation without loss of activity (12). (The nature of the acid volatile product is described in an accompanying paper.) This active high-speed supernatant fraction could be prepared in imidazole or phosphate buffers, but tris proved to yield more active extracts, although relatively high concentrations of this buffer had to be used when working in the range of pH 7.0. This system appears to be rather insensitive to ionic strength, however, since buffers in the range of 0.05 to 0.5 M yield active extracts if the buffer capacity is adequate. Having found the conditions for obtaining active extracts from Chlorella, we turned to defining the optimum conditions for activity and the minimum requirements for cofactors. This is the subject of the present paper.

Materials and Methods

Growth of Organism. Chlorella pyrenoidosa Chick (Emerson strain 3) was grown on the medium shown in table I (Dr. R. C. Fuller, personal communication) under aseptic conditions. The cul-
Table I. Medium For the Growth of Chlorella

<table>
<thead>
<tr>
<th>Substance</th>
<th>Conc</th>
<th>Volume added</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH₂PO₄</td>
<td>0.5</td>
<td>ml/L</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>KNO₃</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Ca(NO₃)₂</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Fe-versenol¹</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>Trace elements²</td>
<td></td>
<td>1.0</td>
</tr>
</tbody>
</table>

¹ To make Fe-versenol, dissolve 59 grams Versenol-120 in 500 ml water, and add 24.9 g FeSO₄.7H₂O. Dilute to 1 liter and aerate overnight. Filter and use filtrate.

² One liter of trace elements solution contains: H₃BO₃ 1.43 g; MnSO₄·H₂O, 1.05 g; ZnCl₂, 50 mg; CuSO₄·5H₂O, 40 mg; H₂MoO₄·H₂O, 10 mg.

tubes were shaken in a water bath at 26° over “daylight” fluorescent tubes providing 800 ft-c at the lower surface, and were continuously bubbled with 5% CO₂ in air during growth.

Preparation of Extracts. Four types of extracts were employed and were prepared and maintained at 4° unless stored, as described below, for subsequent use.

A) “7” extracts. A 1 liter culture late in the log phase of growth was harvested by centrifugation at 1060 x g for 10 minutes yielding about 4 ml of packed cells. After resuspension of the pellet in 0.5 M tris-HCl (pH 7.0), the cells were sedimented at 1860 x g for 10 minutes and were resuspended in 20 ml of the same buffer. This suspension was then passed through a chilled French Pressure Cell (Amino) at 9 tons of pressure and was then centrifuged for 10 minutes at 1860 x g. The supernatant fluid was then centrifuged at 86,000 x g for 1 hour in a Spinco Model L2 preparative ultracentrifuge and the supernatant fluid constituted the undialyzed enzyme preparation. For dialysis, this preparation was used to fill tubing previously cleaned by boiling in distilled water 3 times, followed each time by a distilled water rinse, boiling once in a dilute solution of disodium ethylenediaminetetraacetic acid and rinsing in distilled water, and finally boiling and rinsing exhaustively in distilled water. Dialysis was carried out against 2000 ml of 0.5 M tris-HCl (pH 7.0) for 4 hours with 1 change of buffer. The contents of the dialysis tubing constituted the dialyzed extract.

B) The same procedures were employed as in A, but 2,3-dimercaptopropan-1-ol (BAL) at a concentration of 1.5 mM was included in all buffers.

C) “7-9” extracts. The same procedure was employed as in A, but the initial buffer used was 50 mM tris-HCl (pH 7.0) containing 1.5 mM BAL. The high speed supernatant fluid so prepared was then dialyzed against 50 mM tris-HCl (pH 9.0) containing 1.5 mM BAL, for 4 hours (with 1 change of buffer) as described in A above. The contents of the dialysis tubing constituted the enzyme preparation.

D) “9-9” extracts. The same procedure was employed as in C, but the initial buffer was 0.1 M tris-HCl (pH 9.0) containing 1.5 mM BAL, which was also used, subsequently, for dialysis.

If not used immediately, extracts prepared according to methods B, C, and D were frozen and stored at -20° in a series of small tubes to avoid repeated freezing and thawing of the same extract which progressively reduced the activity. Extracts prepared according to procedure A were always used immediately since they deteriorated rapidly even when frozen. Whole cells could be kept frozen for some time and still yield active extracts if they were first washed and resuspended in the initial buffer to be used for breakage in procedures B, C, and D. Again, these cell suspensions were dispensed into small tubes to avoid more than 1 freeze-thaw cycle for any suspension.

Incubation of Extracts. All experiments were carried out in Thunberg tubes evacuated twice, filled with nitrogen to remove and exclude atmospheric oxygen and finally left under a vacuum. Failure to do this resulted in a loss of half or more of the activity. The tubes were incubated at 30° in a constant temperature room for 2 hours unless otherwise indicated. At the end of the incubation period, 1.0 ml of the reaction mixture together with 100 μmoles of sodium thiosulfate as carrier (omission of carrier gave low recoveries of acid volatile radioactivity from radioactive thiosulfate or sulfite in control experiments) was placed in the middle concentric well of a Conway diffusion dish with a separated drop of acid (1.0 ml of 2 N H₂SO₄ containing 0.1% (v/v) octanol). The center well of the dish received 1.0 ml of 2 N NaOH containing 0.1% (v/v) octanol and the outermost concentric ring seal was the H₂SO₄-octanol solution. After the dish was covered, it was carefully rotated to mix the acid and the sample, and was then allowed to stand overnight. An aliquot (usually 0.1 ml) of the NaOH was then dried on a glass planchet with the aid of an infra-red lamp and was counted in a Nuclear Chicago Model D-47 automatic planchet counter operating in the proportional range.

For kinetic experiments, a number of identical incubation mixtures were constituted from a single enzyme extract in individual Thunberg tubes, each of which was opened and assayed at the indicated time after the beginning of the experiment.

For the determination of pH optima, double strength extracts were prepared by resuspending the cells in one-half the usual volume of buffer used for breakage. The buffer used in all cases was 0.05 M tris-HCl (pH 7.0). When BAL was omitted each aliquot of the high speed supernatant fluid was diluted with an equal volume of 0.5 M tris-HCl or MES-HCl of the appropriate pH. When BAL was included in the buffer used for breakage (at a conc of 1.5 mM) the high speed
supernatant fluid was dialyzed against the same buffer in the manner described above before aliquots of it were diluted with an equal volume of 0.5 M tris-HCl or MES-HCl of the appropriate pH. The final pH was checked, after mixing, in all cases. By diluting many aliquots of the same double strength extract, an entire pH curve could be measured with a single preparation.

Reagents. ATP (disodium salt), TPN, TPNH, DPN, DPNH, thioic acid, creatine-P, glucose-6-P, creatine phosphokinase, and glucose-6-P dehydrogenase were purchased from the Sigma Chemical Company. The glucose-6-P dehydrogenase solution was dialyzed against 0.1 M tris-HCl (pH 7.0) overnight at 4° before being frozen for further use. When high ATP concentrations were to be used (as in the ATP optimum experiments) 10 µmoles of ATP were dissolved per 0.1 ml of 0.1 M tris before being added to the reaction mixtures. Nucleoside triphosphates other than ATP were purchased from the Schwartz Chemical Company (UTP and CTP), Cal Biochemicals (dATP and TTP), and Pabst (GTP). Two-(N-morpholino)ethanesulfonic acid (MES) and 2,3-dimercaptopropan-1-ol (Versenol-120) was purchased from the Fisher Scientific Company.

Results and Discussion

Extracts Prepared at pH 7.0. Table II shows the results obtained with a typical extract prepared by method A (pH 7.0). The undialyzed extract shows requirements for ATP and Mg2⁺. The small stimulation observed in the absence of ATP, but in the presence of an ATP generating system (creatine phosphate and creatine phosphokinase) indicates that there is probably some endogenous ADP. The addition of TPNH or TPN and a reducing system (glucose-6-P and glucose-6-P dehy-

Table II. Reduction of Sulfate at pH 7: Cofactor Requirements and Lability to Dialysis in the Absence of BAL

<table>
<thead>
<tr>
<th>Complete system</th>
<th>Acid-volatile radioactivity of Undialyzed extract CPM/2 ml</th>
<th>Dialyzed extract CPM/2 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>-TPN</td>
<td>66,680</td>
</tr>
<tr>
<td></td>
<td>-TPN, glucose-6-P</td>
<td>23,780</td>
</tr>
<tr>
<td></td>
<td>and glucose-6-P dehydrogenase</td>
<td>15,060</td>
</tr>
<tr>
<td></td>
<td>-TPN, glucose-6-P</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>and glucose-6-P dehydrogenase;</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+TPNH (1.56 µmoles)</td>
<td>35,800</td>
</tr>
<tr>
<td></td>
<td>-ATP</td>
<td>3500</td>
</tr>
<tr>
<td></td>
<td>-ATP, creatine-P</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>and creatine phosphokinase</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>-MgCl₂</td>
<td>2680</td>
</tr>
<tr>
<td></td>
<td>-thioic acid</td>
<td>67,040</td>
</tr>
</tbody>
</table>
|                 | Chlorella extract heated at 70° for 3 min               | 0                        | 0

Fig. 1. Kinetics of formation of acid-volatile radioactivity from 35SO₂⁻ by an undialyzed Chlorella extract at pH 7 lacking BAL. Conditions are the same as in Table II, except incubation times other than 2 hours were employed by using several identical incubation mixtures in separate Thunberg tubes.
hydrogenase) is quite stimulatory and implicates TPNH as a reductant. The results also indicate that a small amount of endogenous TPN is probably present since the added reducing system, in the absence of added TPN results in some increase in activity. Thiocytic acid appears to have little effect in this system, although the effect of this compound is quite variable, occasionally it is stimulatory or inhibitory, to some extent, but it has not been possible to show an absolute requirement. The heat lability of the extract is consistent with a requirement for enzymes contained in the *Chlorella* extract.

The requirement for ATP and Mg$^{2+}$ is not unexpected since most sulfate-utilizing systems rely on 1 or 2 activation steps to form successively, adenosine-5'-phosphosulfate (APS) and adenosine-3'-phosphate-5'-phosphosulfate (PAPS) (2, 9, 10). The formation of PAPS from sulfate by this species of *Chlorella* has already been mentioned (11, 17). TPNH has also been implicated in sulfate-reducing systems from yeast (6, 18).

Extracts prepared in this way are quite labile and rapidly lose activity. Activity is lost almost completely on dialysis for 4 hours as shown in table II, where dialyzed and undialyzed samples of the same extract are compared.

The undialyzed extracts show a brief lag followed by essentially linear kinetics from about 1 hour to 12 hours but beyond this time the activity progressively diminishes (fig 1). While MES-HCl is a somewhat inferior buffer to tris-HCl as judged from the overlap region in figure 2, 2 pH optima are distinguishable, 1 in the region of pH 7.0, the other at pH 8.5 indicating that several enzymes are probably involved in the production of acid-volatile radioactivity whose interactions provide the double optima.

**Table III. Reduction of Sulfate at pH 7.0: Cofactor Requirements and Stability to Dialysis in the Presence of B.11.**

<table>
<thead>
<tr>
<th>Complete system</th>
<th>Enzyme undialyzed</th>
<th>Enzyme dialyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid-volatile radioactivity of CPM/2 ml</td>
<td>CPM/2 ml</td>
</tr>
<tr>
<td><strong>TPN</strong></td>
<td>102,300</td>
<td>175,900</td>
</tr>
<tr>
<td><strong>TPN, glucose-6-P and glucose-6-P dehydrogenase</strong></td>
<td>58,060</td>
<td>67,720</td>
</tr>
<tr>
<td><strong>TPN, glucose-6-P and glucose-6-P dehydrogenase; +TPNH (1.56 μmoles)</strong></td>
<td>2440</td>
<td>28,660</td>
</tr>
<tr>
<td><strong>ATP</strong></td>
<td>129,960</td>
<td>71,060</td>
</tr>
<tr>
<td><strong>ATP, creatine-P and creatine phosphokinase</strong></td>
<td>4480</td>
<td>880</td>
</tr>
<tr>
<td><strong>MgCl$_2$</strong></td>
<td>1060</td>
<td>160</td>
</tr>
<tr>
<td><strong>-thiocytic acid</strong></td>
<td>1800</td>
<td>2000</td>
</tr>
<tr>
<td><strong>Enzyme heated at 70° for 3 min</strong></td>
<td>67,480</td>
<td>99,580</td>
</tr>
</tbody>
</table>

It was subsequently found that the inclusion of low concentrations (1.5 mM) of BAL in the buffer used to prepare the extracts (method B) increased their activity and protected them from deterioration. Table III shows that the undialyzed extracts are.

**Fig. 2. pH optima for formation of acid-volatile radioactivity from $^{35}$SO$_4^{2-}$ by an undialyzed *Chlorella* extract lacking BAL.** Conditions are the same as in table II, except that the pH of the enzyme extracts was adjusted with either MES or tris buffer as described in Materials and Methods and additional buffer was not added to the incubation mixtures.
Table IV. Reducing Agents Active in System at pH 7.0

The complete system contained: ATP (10 μmoles), MgCl₂ (50 μmoles), thiocetic acid (0.49 μmole), Chlorella extract (method B) (1.0 ml), carrier-free ³⁵SO₄²⁻ (4 × 10⁶ cpm), creatine-P (10 μmoles), creatine phosphokinase (10 μg) and 0.5 M tris-HCl, pH 7.0 (0.1 ml) in a total volume of 2.0 ml.

<table>
<thead>
<tr>
<th>Complete system</th>
<th>CPM/2 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>+TPN (1.56 μmoles), glucose-6-P (10 μmoles) and glucose-6-P dehydrogenase (0.05 unit)</td>
<td>12,000</td>
</tr>
<tr>
<td>+TPNH (1.56 μmoles)</td>
<td>39,020</td>
</tr>
<tr>
<td>+DPNH (1.56 μmoles)</td>
<td>31,020</td>
</tr>
<tr>
<td>+glucose-6-P (10 μmoles) glucose-6-P dehydrogenase (0.05 unit) and BAL (3 μmoles)</td>
<td>21,980</td>
</tr>
<tr>
<td>+BAL (3 μmoles)</td>
<td>49,180</td>
</tr>
<tr>
<td>+BAL (3 μmoles), —ATP</td>
<td>71,600</td>
</tr>
<tr>
<td>+BAL (3 μmoles), —MgCl₂</td>
<td>1780</td>
</tr>
<tr>
<td>+BAL (3 μmoles), —thiocetic acid</td>
<td>78,760</td>
</tr>
<tr>
<td>+BAL (3 μmoles), —ATP, creatine-P and creatine phosphokinase</td>
<td>0</td>
</tr>
<tr>
<td>+BAL (3 μmoles), enzyme heated at 70°C for 3 min</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 3. pH optima for formation of acid-volatile radioactivity from ³³SO₄²⁻ by a dialyzed Chlorella extract containing 1.5 mM BAL. Each incubation mixture contained: ATP (10 μmoles), MgCl₂ (50 μmoles), thiocetic acid (0.49 μmole), carrier-free ³³SO₄²⁻ (4 × 10⁶ cpm), creatine-P (10 μmoles), creatine phosphokinase (10 μg), BAL (3 μmoles), and Chlorella extract brought to proper pH as described in Materials and Methods (1.0 ml), all in a total volume of 2.0 ml.

quite active and clearly require TPNH as a reductant. ATP is still required as is Mg²⁺. In this particular experiment, thiocetic acid was somewhat stimulatory. The system retained its lability to heat. Under BAL protection, the extracts could be dialyzed for 4 hours without loss of activity, indeed an increase in activity was frequently observed as though some inhibitory material was being removed. The general pattern of requirements remained essentially the same as did the heat lability of the extracts. The protective effect of BAL might be attributed to its maintenance of sulfhydryl groups of the enzyme extract in the reduced form or to its well-known properties as a chelator of heavy metals. Perhaps it acts in both capacities.

To our surprise, when the concentration of BAL was increased by adding it to the incubation mixtures already constituted with enzyme extracts containing BAL, this compound could replace TPNH as a reductant. Table IV demonstrates that BAL is even more effective when the TPN-reducing system is omitted. DPNH is somewhat less active than TPNH as a reductant but when BAL replaces these compounds, the system still requires ATP and Mg²⁺ and retains its lability to heat. Thiocetic acid is somewhat inhibitory in this experiment. The participation of BAL might reflect a thio-redoxin-like mechanism of electron transfer from TPNH as in deoxy-riboside biosynthesis (7).

Figure 3 shows the pH curve for activity of dialyzed extracts prepared in BAL-containing buffers where additional BAL is used as the reductant, rather than pyridine nucleotides. Under these conditions, MES-HCl and tris-HCl appear to be equally good buffers. There is the suggestion of a small shoulder in the region of pH 7 to 8, but the major activity is found at pH 9.0. These extracts em-
Table V. Minimal Requirements For Sulfate Reduction at pH 9.0

The complete system contained in a total volume of 2.35 ml: ATP (10 μmoles), MgCl₂ (50 μmoles), BAL (3.0 μmoles), 1.0 M tris-HCl (pH 9.0) (0.1 ml), carrier-free 35SO₄²⁻ (3.8 × 10⁶ cpm), and Chlorella extract, either method C (“7-9”) or method D (“9-9”) as indicated (1.0 ml).

<table>
<thead>
<tr>
<th>Complete system</th>
<th>&quot;9-9&quot; extract</th>
<th>&quot;7-9&quot; extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>465,088</td>
<td>1,053,975</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>423</td>
<td>0</td>
</tr>
<tr>
<td>BAL (3.0 μmoles)</td>
<td>634</td>
<td>376</td>
</tr>
<tr>
<td>Enzyme heated 3 min at 70°</td>
<td>4935</td>
<td>50,995</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>235</td>
</tr>
</tbody>
</table>

![Diagram](image1)

**Fig. 5.** Optimum concentrations of MgCl₂ for the formation of acid-volatile radioactivity from 35SO₄²⁻ by 2 types of Chlorella extract at pH 9. Conditions the same as in Table V, except that MgCl₂ concentration was varied.

![Diagram](image2)

**Fig. 4.** Optimum concentration of ATP for the formation of acid-volatile radioactivity from 35SO₄²⁻ by 2 types of Chlorella extract at pH 9. Conditions the same as in Table V, except that ATP concentration was varied.

Employing BAL at pH 9.0 were so much more active and stable than any obtained previously, that they were adopted for further study.

**Extracts Prepared at pH 9.0.** Two types of extracts were employed in these studies; the details of their preparation are given in methods C and D of Materials and Methods. Both contain BAL at a concentration of 1.5 mM but "7-9" extracts are prepared by breaking the cells at pH 7.0 and dialyzing them against pH 9.0 buffer, while "9-9" extracts are prepared by using pH 9.0 buffer for both steps. The 7-9 extracts are usually more active, perhaps because pH 9 buffer extracts some inhibitory materials from the cells; these latter extracts generally contain much more pigment than 7-9 extracts. As is evident, however, the 2 types of extract seem to be equivalent in their general properties, and are used interchangeably by us.

Table V shows the minimal requirements for formation of acid-volatile radioactivity by these extracts. ATP, Mg²⁺ and additional BAL are all essential and the system remains labile to heat. Figure 4 shows that both types of extracts yield optimal acid-volatile radioactivity at about 10 μmoles of ATP per 2.35 ml of reaction mixture, high
concentrations of ATP are markedly inhibitory. Mg²⁺ (fig 5) displays a broad optimum in both types of extract at 50 μmoles per 2.35 ml. Additional BAL, as a reductant, is active and relatively non-inhibitory over a wide range of concentrations (fig 6), with the best activity between approximately 3 and 10 to 12 μmoles per 2.35 ml. The region of rise in the BAL concentration curve is somewhat different from experiment to experiment and probably reflects the basal amount of BAL remaining in the initial enzyme extract, which is variable, since BAL is continually removed through air oxidation during the preparation of the enzyme extracts. Figure 7 shows that the kinetics of formation of acid-volatile radioactivity is essentially linear from time zero until about 8 hours in both types of extract. For this reason, 2 hours of incubation was adopted as a convenient time of assay in most experiments. The activity of the system was also proportional to the amount of enzyme added, over a considerable range, when concentrated enzyme extracts were employed.

Table VI shows that ATP is the preferred nucleoside triphosphate in this system, as in other systems involving sulfate activation (2).

Table VI. Nucleotide Specificity for the Production of Acid-Volatile Radioactivity

<table>
<thead>
<tr>
<th>Nucleotide added to complete system</th>
<th>Acid-volatile radioactivity (CPM/min 2.0 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No addition</td>
<td>580</td>
</tr>
<tr>
<td>ATP</td>
<td>389,020</td>
</tr>
<tr>
<td>Desoxy-ATP</td>
<td>3440</td>
</tr>
<tr>
<td>GTP</td>
<td>13,020</td>
</tr>
<tr>
<td>TTP</td>
<td>10,920</td>
</tr>
<tr>
<td>UTP</td>
<td>28,300</td>
</tr>
<tr>
<td>CTP</td>
<td>3560</td>
</tr>
</tbody>
</table>

Acknowledgment

The expert technical assistance of Miss Jeannette Lemieux is gratefully acknowledged.

Literature Cited


7. **Laurent, T., E. Moore, and P. Reichard.** 1964. Enzymatic synthesis of deoxyribonucleotides. IV. Isolation and characterization of thioredoxin, the hydrogen donor from *Escherichia coli* B. J. Biol. Chem. 239: 3436-44.


