Phosphatase Activity in Homogenates from Normal and Poliovirus Infected Tissue Cultures

I. Characterization of the Normal Activity

By E. Frank Deic * and Louis P. Gerhardt

Department of Microbiology
College of Medicine, University of Utah **

A study was made on the total ATP-ase, alkaline phosphatase, and glucose-6-phosphatase activity in homogenates prepared from normal monkey kidney cells in tissue culture. A characterization of such activity under standardized assay conditions revealed that in comparable amounts of cell homogenate ATP-ase was 8—10 times as active as alkaline phosphatase and glucose-6-phosphatase. Substrate hydrolysis was brought about by the activity of each enzyme at a rate which was constant with respect to time. The amount of substrate hydrolyzed, in each case, was directly proportional to the concentration of homogenate used in the assay system. Within certain limits this was also true with respect to substrate concentration. However, with increasing amounts of substrate and a constant amount of cell homogenate a point was reached where the activity of each enzyme became independent of initial substrate concentration. Under one set of assay conditions the total ATP-ase in cell homogenates was shown to be optimally active at approximately $pg$ 7 while under another the enzyme showed a nearly directly proportional increase in activity per unit time between $pg$ 4 and $pg$ 10. Using standardized assay conditions such ATP-ase activity was markedly enhanced over that found in similar systems lacking added calcium and magnesium ion. No further stimulation of the activity of this enzyme, or of alkaline phosphatase and glucose-6-phosphatase, resulted from their interaction with 2,4-dinitrophenol. The substrate hydrolysis produced by the activity of each enzyme in cell homogenate preparations resulted from irreversible reactions. Such homogenates were incapable of carrying out an exchange reaction of radioactive orthophosphate with the phosphorous from any of the substrates used in this study.

Phosphatases such as ATP-ase, alkaline phosphatase, and glucose-6-phosphatase are found in almost all tissues which have been studied. Their exact role in metabolic processes, however, is still not well understood. Some evidence exists that they are associated to a certain extent with degenerative changes occurring in certain cell organelles. In addition, they are thought to have some function in inorganic phosphate conservation by cells in order to supply the requirements for this substance in many metabolic reactions.

Since poliovirus infection induces extensive degeneration (cytopathogenicity) in tissue cultured monkey kidney cells, and since this infection is known to markedly alter the phosphorous metabolism of mammalian cells, the study was undertaken to determine whether the activity of specific cellular phosphatases is also altered as a result of such infection.

One of the primary disadvantages to the study of ATP-ase, alkaline phosphatase and glucose-6-phosphatase, is their lack of specificity. With the use of impure preparations from cells (i.e. homogenates, particulates), therefore, it is necessary to carefully control and standardize the assay conditions with the understanding that a composite activity is being...
recorded. Having this necessity in mind, the initial part of this work was concerned with the characterization of the activity of these phosphatases under a variety of environmental conditions.

Materials and methods

Cells were obtained from the kidneys of cynomolgus monkeys. A 1:200 (vol/vol) suspension was prepared by the method of Youngner using the modifications recommended by Rapaport. The growth medium consisted of Melnick's tissue culture fluid containing 5% inactivated (56 °C/30 min.) calf serum. The cell suspension was plated in approximately 250 ml amounts into 5 l Povitsky flasks and incubation was carried out at 37 °C. After about 4 days a fluid change was usually made to enhance the cell yield. After incubation for a total period of 7 days the cells were utilized in experimental work. The yield from 14–22 flasks provided material for each experiment.

Suspensions of such cells were prepared by washing each monolayer with approximately 100 ml of phosphate buffered saline (P.B.S.) followed by treatment with 100 ml of 0.125% trypsin (Difco 1:250) in P.B.S. at 37 °C until all cells were removed from the glass surface. Such fluids were then drained into 250 ml centrifuge bottles. Each flask was washed with 50 ml P.B.S. to collect residual cells and these washings were pooled with the trypsinized cells. The bottles were centrifuged at 600 R.P.M. in an International SB-1 centrifuge for 3 minutes. After removing the supernatant fluids the cells were washed once with Melnick's fluid and resuspended in 200 ml of the same medium at 37 °C in a 500 ml baffled flask containing a magnetic bar. Low speed agitation of this material for about 2 hours at constant temperature was carried out on an isolated magnetic stirrer in order to bring the metabolic activity of such cells to optimal levels. During this period the pH of the medium dropped rapidly unless periodic additions of 2.8% NaHCO₃ were made in order to maintain it at approximately 7.4.

Homogenization of the cells was carried out after initially washing them 3 times in 0.85% NaCl. This step was done as rapidly and gently as possible to minimize leakage of cellular components into the extracellular environment. After the final wash the pellet was suspended in a small amount of physiological sodium chloride whose volume was determined by the volume of homogenate required for a given experiment. This highly concentrated cell suspension was distributed into Wasserman tubes (<2.5 ml/tube) which were then tightly sealed with rubber stoppers. These tubes were subjected as rapidly as possible to five cycles of freezing and thawing with a dry ice-ethanol mixture. Microscopic examination revealed that a minimum of 3 cycles was required before intact cells could no longer be observed. The contents of all tubes were pooled and stored at 4 °C until used.

The total ATP-ase, alkaline phosphatase, or glucose-6-phosphatase activity in such cell homogenate preparations was assayed in one or more of the following buffer systems:

1. Basic Carbonate (B.C.) — Sodium carbonate-sodium bicarbonate — 5.0·10⁻³ M (pH 9.2), sodium chloride — 5.8·10⁻² M, potassium chloride — 2.7·10⁻³ M, magnesium chloride — 9.9·10⁻⁴ M; and calcium chloride — 9.0·10⁻⁴ M.


3. Histidine. L-Histidine — 0.20 M (pH 7.2), and potassium chloride — 0.15 M. In certain experiments this buffer also contained magnesium chloride — 0.05 M and calcium chloride — 0.05 M.

4. Ethanolamine. Ethanolamine — 0.10 M (pH 9.5), and magnesium acetate — 0.005 M.

5. Maleic Acid. Maleic acid — 0.10 M (pH 6.9).

ATP-ase activity was assayed with the substrate di-sodium adenosine 5'-triphosphate (Sigma) — 5.0·10⁻³ M in the B.C., B.C.G.G., and Histidine buffer systems at a pH of 7.2. Alkaline phosphatase was assayed with the substrate sodium beta glycerophosphate (Merck) — 2.0·10⁻² M in the B.C. (pH 9.2). B.C.G.G. (pH 9.2), and Ethanolamine (pH 9.5) buffer systems. Glucose-6-phosphatase activity was assayed with the substrate di-sodium glucose-6-phosphate (Sigma) — 7.5·10⁻³ M in the B.C., B.C.G.G., and Maleic Acid buffer systems at a pH of 6.9. In most cases the pH of the substrate-buffer media was adjusted with 0.1 N NaOH or 0.1 N HCl to the required value. Such solutions were stored at 4 °C in stoppered flasks until used. Any variations from these standardized assay systems will be noted in the pertinent areas of the section on results.

To carry out each assay 9.0 ml of the appropriate substrate-buffer were placed in a 15 ml screw-cap tube. Duplicate solutions were usually set up on each preparation to verify reproducibility. All tubes were then placed in a 37 °C water bath. After temperature equilibrium, 1.0 ml of pooled uniform homogenate was added to each tube followed by thorough mixing.

As substrate hydrolysis proceeded under these conditions, periodic samples of 1.0 ml were removed from the homogeneous contents of each tube. These were placed in 4.0 ml of saturated ammonium sulfate in 0.20 M acetate buffer at pH 4.0 to prevent further enzymatic activity. The samples were then placed at 4 °C in tightly rubber stopped Wasserman tubes for several days to precipitate the protein in the solution.

Assay intervals of less than 10 hours were generally used to avoid excessive inactivation of the enzymes being studied. At the termination of the experiment the
The pH of each reaction mixture was usually checked. An alteration in pH greater than ±0.2 units was not encountered in most cases.

All samples removed during the assay interval were analyzed for ortho-phosphate phosphorous by the method of Lowry and Lopez 17, using a Coleman Jr. spectrophotometer (Model 6 A). Preliminary work showed that non-enzymatic hydrolysis of the respective substrates did not occur at 37 °C over a period much longer than that used for the assay interval in each experiment.

An assumption was made in this study that the concentration of each enzyme was directly proportional to the amount of homogenate used as a source of the enzyme. Therefore, the total nitrogen present per unit volume was taken as a means to measure the relative amounts of enzyme in different preparations. After the preparation of cell homogenates homogeneous samples were removed and analyzed for total nitrogen content by the micro-Kjeldahl method described by Steyermark 18, using a single piece steam distillation unit.

Results

The Effect of the Buffer System on Enzyme Activity. Cell homogenates were prepared and assayed for total ATP-ase, alkaline phosphatase, and glucose-6-phosphatase according to the standard procedures given above.

The results obtained using the B.C. buffer system are plotted in Figs. 1, 2 and 3. It can be seen that the activity of each enzyme was such that substrate hydrolysis proceeded at a rate which was constant with respect to time. ATP-ase activity tended to level off as the substrate became limiting in concentration. These data also indicate that substantial amounts of orthophosphate were present in each assay system at the beginning of the reaction period. Presumably this was due to intracellular phosphorous released during the homogenization procedure.

The data from this experiment also indicated that the addition of glucose and L-glutamine to the B.C. buffer system had no effect on phosphatase activity under the assay conditions. Therefore, their use was discontinued in further work.

When substrate hydrolysis versus time was plotted as a measure of ATP-ase, alkaline phosphatase, and glucose-6-phosphatase activity in the presence of the Histidine, Ethanolamine, and Maleic Acid buffer systems, respectively, it was again shown that such activities were directly proportional to time. However, in all cases the velocity was significantly enhanced over that observed when the B.C. buffer system was used. The quantitative relationships of these differences can be noted from the data which follow.

The Effect of Homogenate Concentration on Enzyme Activity. Experiments were carried out to determine the ATP-ase, alkaline phosphatase, and glucose-6-phosphatase activity in varying concentrations of homogenate using the otherwise standardized conditions of assay. It can be seen from the results which are plotted in Figs. 4, 5, and 6 that the total activity of each enzyme, obtained with a variety of substrate concentrations in the standard B.C. buffer system, was directly proportional to the concentration of cell homogenate used as a source of the enzyme. Over the range of homogenate concentrations used, the enzymatic activity observed in each


The Effect of Substrate Concentration on Enzyme Activity. When experiments were carried out to determine the effect of varying substrate concentrations on the activity of the three phosphatases under
study the results plotted in Figs. 11, 12, and 13 were obtained. Under the conditions of each assay the amount of substrate hydrolyzed was again found to be directly proportional to time for a period of 4 to 6 hours or longer.

It can be seen from these figures that the total activity of each enzyme in a constant amount of cell homogenate was directly proportional to the initial concentration of substrate in the assay system. The presence of non-enzymatically produced orthophosphate at the beginning of each assay period was particularly marked in these experiments due to the rather high concentration of cell homogenate used as a source of each enzyme.

The relationship observed, however, between activity and initial substrate concentration was found in each case to hold only within a certain range of values. As the enzyme source was reduced in amount, and the substrate concentration was increased, a point was reached where the activity of each enzyme became independent of the initial substrate concentration. This is illustrated in Fig. 14 by data obtained for ATP-ase.

The Effect of $p_{II}$ on Enzyme Activity. Preliminary experiments indicated that an assessment of the effect of $p_{II}$ on these enzymes could only be made in the case of ATP-ase. The very low activities which were obtained with the other two enzymes when the $p_{II}$ of the assay system was varied were not significantly reproducible.

When constant amounts of substrate in the B.C. buffer system were standardized with respect to $p_{II}$, and the ATP-ase activity of a uniform concentration of cell homogenate was determined under these conditions, the results plotted in Fig. 15 were obtained. It can be seen that substrate hydrolysis proceeded per unit assay time in a manner which was nearly directly proportional to the $p_{II}$ of the assay system.

As in the previous experiments the total activity of this enzyme, in each case, was directly proportional to time. However, the rate of substrate hydrolysis was so rapid at higher $p_{II}$ that under these conditions this proportionality was maintained over an assay period of only two hours or less because limiting concentrations of substrate were reached in this time. Incubation of all substrate-buffer solutions at 37 °C, in the absence of cell homogenate, for periods in excess of 6 hours resulted in insignificant substrate hydrolysis.

A similar experiment carried out with the Histidine buffer system gave the results which are plotted in Fig. 16. These show that the enzyme was opti-
mally active at about pH 7. The reason for this striking variation in activity of ATP-ase in the two buffer systems, which was confirmed by additional work, is not clear but some speculation on the observation follows in the Discussion section.

The Effect of Certain Divalent Cations on Enzyme Activity. An experiment was carried out to assess the effect of calcium and magnesium ions in the B.C. buffer system on total ATP-ase activity. The results plotted in Fig. 17 were obtained. It can be seen that in the absence of these ions the activity of the enzyme was markedly reduced. Similar experiments where each of these ions was varied independently of the other over an increase in concentration of three and five times the standard one gave essentially identical results to those shown in Fig. 17.

Undoubtedly the cell homogenate used as a source of the enzyme contained significant amounts of Ca\(^{2+}\) and Mg\(^{2+}\). Presumably this ionic material contributed to that ATP-ase activity which was observed under conditions where neither of these ions was added to the buffer system.

The Effect of 2,4-Dinitrophenol (DNP) on Enzyme Activity. When DNP was incorporated into the standard B.C. buffer system in concentrations of 1 \(\cdot\) 10\(^{-6}\) M, 5 \(\cdot\) 10\(^{-6}\) M, 1 \(\cdot\) 10\(^{-5}\) M, 5 \(\cdot\) 10\(^{-5}\) M, 1 \(\cdot\) 10\(^{-4}\) M, 5 \(\cdot\) 10\(^{-4}\) M, 1 \(\cdot\) 10\(^{-3}\) M, and 5 \(\cdot\) 10\(^{-3}\) M and these solutions were then adjusted to the proper pH, after the addition of the respective substrates, it was found that in no case was an enhancement of ATP-ase, alkaline phosphatase, or glucose-6-phosphatase activity brought about in cell homogenates by the presence of this DNP, over that obtained in the corresponding assay system under standard conditions.

This is illustrated in Fig. 18 from data obtained on ATP-ase activity. Assay systems containing the other concentrations of DNP gave similar results to those produced with the substance at a concentration of 5 \(\cdot\) 10\(^{-5}\) M. Only at the highest concentration of DNP (i.e. 5 \(\cdot\) 10\(^{-3}\) M) was a partial inhibition of the enzyme encountered. Whether this inhibition was direct or resulted from other causes could not be determined.

The Reversibility of Enzyme Activity. Experiments were conducted to determine whether the ATP-ase, alkaline phosphatase, and glucose-6-phosphatase of cell homogenates were capable of orthophosphate esterification when incubated with their normal reaction products under otherwise standardized assay conditions.

The reversibility of ATP-ase activity was tested for by incubating cell homogenate in both the standard B.C. and Histidine buffer systems containing 5.0 \(\cdot\) 10\(^{-3}\) M Na\(_2\)HPO\(_4\) and the sodium salts of either 5.0 \(\cdot\) 10\(^{-3}\) M adenosine-5'-diphosphate or
**Table I. The Incorporation of Radioactive Orthophosphate (Pi₃²) into Phosphate Esters by Cell Homogenates.**

* Counts x 500 for total radioactivity of intact assay system (10 ml). Delay involved between experimental use of isotope and counts: 3 days. Homogenate concentration: 7.82 mg N/ml. Background radiation: 22 counts/min.

<table>
<thead>
<tr>
<th>Assay Time [hr]</th>
<th>Pi₃² Exchange with ATP [counts/min/ml Pi extract]</th>
<th>Pi₃² Exchange with Glycerophosphate [counts/min/ml Pi extract]</th>
<th>Pi₃² Exchange with Glucose-6-Phosphate [counts/min/ml Pi extract]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer: B.C.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>system #1</em></td>
<td><em>system #2</em></td>
<td><em>system #1</em></td>
</tr>
<tr>
<td>0</td>
<td>—</td>
<td>1261</td>
<td>981</td>
</tr>
<tr>
<td>1.5</td>
<td>1062</td>
<td>1123</td>
<td>1153</td>
</tr>
<tr>
<td>2.5</td>
<td>1153</td>
<td>1213</td>
<td>1020</td>
</tr>
<tr>
<td>4.0</td>
<td>805</td>
<td>1071</td>
<td>868</td>
</tr>
<tr>
<td>5.5</td>
<td>865</td>
<td>898</td>
<td>766</td>
</tr>
<tr>
<td>8.5</td>
<td>962</td>
<td>905</td>
<td>—</td>
</tr>
<tr>
<td>Buffer: Histidine (Mg²⁺)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1208</td>
<td>1045</td>
<td>834</td>
</tr>
<tr>
<td>1.5</td>
<td>1191</td>
<td>1008</td>
<td>962</td>
</tr>
<tr>
<td>2.5</td>
<td>1196</td>
<td>1202</td>
<td>970</td>
</tr>
<tr>
<td>4.0</td>
<td>874</td>
<td>1354</td>
<td>776</td>
</tr>
<tr>
<td>5.5</td>
<td>1032</td>
<td>951</td>
<td>898</td>
</tr>
<tr>
<td>8.5</td>
<td>1300</td>
<td>1237</td>
<td>—</td>
</tr>
</tbody>
</table>

1.0·10⁻² M adenosine-5'-monophosphate. Such reversibility of alkaline phosphatase activity was tested for in both the standard B.C. and Ethanolamine buffer systems containing 0.02 M Na₂HPO₄ and 0.02 M glycerol. Finally, the reversibility of glucose-6-phosphatase activity was tested for in both the standard B.C. and Maleic Acid buffer systems containing 7.5·10⁻³ M Na₂HPO₄ and 7.5·10⁻³ M glucose.

Such incubations over a period of 15.5 hours (with intermediate sampling intervals at 0, 1.5, 2.5, 4.0, 5.0, 6.0, 7.75, and 10.75 hours) brought about no significant orthophosphate esterification in any of the assay systems tested.

**The Enzymatic Exchange of Orthophosphate with Substrate Phosphorus.** Standardized substrate-buffer system were prepared for the assay of ATP-ase, alkaline phosphatase, and glucose-6-phosphatase. In addition, about one microcurie of radioactive orthophosphate (Pi₃²) (0.047 mg P/2.54 millicuries) plus 5 µg carrier orthophosphate phosphorus was added to each assay system. Following the addition of a uniform amount of cell homogenate, incubation was carried out under standardized conditions. Periodically, samples were removed over an 8.5 hour interval. The total orthophosphate was extracted from each sample by the procedure of Marsh. A 1.0 ml volume from each extract was distributed into a planchet and radioactive counts were made in a Nuclear-Chicago Model 182 A scaling unit with a Model DS 5 scintillation detector. Duplicate assay systems were set up in each case.

The results from this experiment are presented in Table I. It can be seen that although some variation in Pi₃² measurements existed between corresponding samples with respect to time, the data tended to indicate that little or no change in the concentration of the isotope was taking place in the orthophosphate fraction. Therefore, an exchange reaction apparently was not operative under any of the conditions used in the assay of ATP-ase, alkaline phosphatase, or glucose-6-phosphatase activity.

**Discussion**

The nature of the variations which were observed in the total activity of the three phosphatases in a standard amount of cell homogenate cannot be specified, primarily because of problems of specificity and the fact that the function these enzymes serve in cell metabolism is not well understood. However, as has already been mentioned, the ATP-ase activity is enhanced as a result of degenerative changes in the metabolic activity of many intact or disrupted cell preparations. Such changes usually occur spontaneously in "ageing" cell isolates and therefore

---


one would expect a fairly high activity from the enzyme under the experimental conditions used in this study.

On the other hand a very low alkaline phosphatase and glucose-6-phosphatase activity may be explained by the observations of a number of workers \(^6\) \(^7\) \(^21\) \(^22\) \(^10\) that in certain instances these enzymes are markedly depressed in activity in the presence of inorganic or esterified phosphate. However, in several instances \(^23\) \(^24\) where such an effect has been looked for in mammalian tissue culture material the concentration of inorganic phosphate in the medium has been shown to have little or no effect on the reaction unless relatively high concentrations were present.

It is also possible that the low activity of these enzymes resulted from the change of the highly differentiated cells in the intact kidney to the type which replicate in tissue culture. Some evidence for this idea has been presented by Bang and Niven \(^25\), Lieberman and Ove \(^26\), and Rossi et al. \(^27\). As Cox and MacLeod \(^28\) have shown, however, it is entirely possible that considerable variation may exist in such activity even when strains of tissue cultured cells derived initially from the same source are used. Certain of these were found to contain little or no phosphatase activity unless suitable activation was initiated \(^28\). Kovács et al. \(^29\) demonstrated such a low alkaline phosphatase in Rhesus kidney subcultures. The activity observed was linear with time.

The results obtained in the characterization of the activity of the three phosphatases with respect to substrate and homogenate concentration parallel those obtained by other workers using a variety of sources for such enzymes. Thus the ATP-ase activity of a digitonin extract of rat liver mitochondria was found by Cooper \(^30\) to be linear with respect to time.

Similar results were obtained by Bowen and Gershfeld \(^31\) using myosin B as a source of ATP-ase. Avi-Dor and Gonda \(^32\) showed, within the limits of 0.1 and 0.3 mg particulate nitrogen, that ATP was hydrolyzed in their assay system at a velocity directly proportional to the concentration of mitochondria (from tissues of A. aegypti) which were used.

It should be pointed out that under the experimental conditions of this study the ATP-ase activity brought about substrate hydrolysis in excess of that stoichiometrically predicted for the total removal of the terminal phosphate of ATP. It is likely that other enzymes were responsible for this result, notably myokinase and nucleoside monophosphate kinase, among other possibilities.

Studies on the activity of alkaline phosphatase from rat small intestine by Triantaphyllopoulos and Tuba \(^33\) revealed that the reaction under their conditions was directly proportional to time over an assay period of 1 hour. In addition, they found that so long as the concentration of the enzyme in homogenates did not exceed 80 units/l, a straight line was obtained by plotting activity against enzyme concentration. McCarthy and Hinselwood \(^34\) demonstrated that the activity of the enzyme in B. lactis aerogenes was directly proportional to the number of cells which were used in the assay system. Kinetic studies by Nitowsky and Herz \(^35\) on alkaline phosphatase from tissue cultured liver cell fractions showed the reaction to be zero order, with the activity linearly dependent on enzyme concentration over a 100-fold range. A similar reaction order was shown by Ahmed and King \(^36\) to exist for purified placental alkaline phosphatase. Its duration depended on the type of substrate as well as the buffer used in the assay system. A zero order reaction also existed for an alkaline phosphomonoesterase

30 C. Cooper, Biochim. biophysica Acta [Amsterdam] 30, 529 [1958].
31 W. J. Bowen and M. Gershfeld, Biochim. biophysica Acta [Amsterdam] 21, 315 [1957].
32 Y. Avi-Dor and O. Gonda, Biochem. J. 72, 8 [1959].
isolated by Kus and Blumenthal\textsuperscript{37} from Neurospora crassa.

Segal et al.\textsuperscript{38} and Segal and Washko\textsuperscript{39} determined that the glucose-6-phosphatase activity in whole rat liver homogenates, and in microsomal fractions, was directly proportional to assay time and, further, that a plot of activity with respect to enzyme concentration gave a straight line relationship. Koide and Oda\textsuperscript{3} demonstrated the presence of a very low glucose-6-phosphatase activity in human serum. Substrate hydrolysis by this enzyme proceeded in a manner which was directly proportional to time for a period of at least one hour.

Experimental work reported by others can be taken to support some of the additional findings. Optimal ATP-ase activity in the region of neutrality has been reported by Bronk and Kielley\textsuperscript{40}, Tonzetich and Kare\textsuperscript{41}, and Skow\textsuperscript{42}—among others—in preparations from different sources. However, Myers and Slater\textsuperscript{43} showed that the $p_H$-activity curves for the ATP-ase of rat liver mitochondria (or mitochondrial fragments) appeared to represent four superimposed optimum $p_H$ values which were located in the regions of $p_H$ 6.3, 7.4, 8.5, and 9.4. Under most conditions the ATP-ase activity was proportional to $p_H$ within these peak regions. Similarly, Avi-Dor and Gonda\textsuperscript{32} demonstrated increasing ATP-ase activity in their mitochondrial preparations over a $p_H$ range from 6.0 to 9.0, with direct proportionality existing between 6.5 and 8.5. Since this type of data has been interpreted in terms of a number of functional ATP-ases it might thus serve to explain the differences noted with regard to the effect of $p_H$ on ATP-ase activity in the two buffer systems. In the B.C. system a number of ATP-ases (and other phosphatases) might be functional, while in the Histidine system such activity would perhaps be confined primarily to the enzyme(s) which gives optimal effects in the region of $p_H$ 7.0.

Landau and Schlamowitz\textsuperscript{44} have pointed out that the method of preparation affects the behavior of certain phosphatases, and that a multiplicity of activity peaks does not necessarily establish the existence of several enzymes in the native tissue. This observation might be relevant to our conditions.

Metal ion activation of ATP-ase has been demonstrated by a large number of workers; for example, by the publications of Gamble and Lehninger\textsuperscript{45} and Myers and Slater\textsuperscript{46}. Bronk and Kielley\textsuperscript{47} have presented data which indicate that the optimum concentration of substrate used in the assay. It is quite likely that if we had considered this possibility, and also metal ion interaction, considerably advanced activity of ATP-ase in the kidney cell homogenates might have been obtained.

While 2,4-Dinitrophenol (DNP) is generally regarded as a "stimulator" of ATP-ase activity, evidence exists that in a number of situations this is not the case. For example, Klempere\textsuperscript{20} showed that a concentration of DNP of $5 \cdot 10^{-5}$ M inhibited oxidative phosphorylation of freshly isolated rat liver mitochondria almost 100% but had no effect on the ATP-ase activity of the particles. A similar effect with different systems was noted by Perry and Chappell\textsuperscript{47}, Cooper\textsuperscript{2} and Purvis\textsuperscript{48}, among others. Gilmour and Griffiths\textsuperscript{49} have observed an inhibition of ATP-ase activity brought about by DNP, and concluded that this inhibition was in some way dependent on the substrate concentration used in the assay system.

The absence of a substrate-P$_3$ exchange reaction under the experimental conditions of this study was not unexpected. "Aged" preparations were used, and Plaut\textsuperscript{50} has shown that the enzyme from pig liver and heart mitochondria catalyzing this reaction is unstable. It has also been reported by Eggleston\textsuperscript{51} that their preparations required certain amino acids.

\textsuperscript{39} H. L. Segal and M. E. Washko, J. biol. Chemistry 234, 1937 [1959].
\textsuperscript{40} J. R. Bronk and W. W. Kielley, Biochim. biophysica Acta [Amsterdam] 29, 369 [1958].
\textsuperscript{41} J. Tonzetich and M. R. Kare, Arch. Biochem. Biophysics 86, 195 [1960].
\textsuperscript{42} J. C. Skow, Biochim. biophysica Acta [Amsterdam] 23, 394 [1957].
\textsuperscript{43} D. K. Myers and E. C. Slater, Biochem. J. 67, 588 [1957].
\textsuperscript{44} W. Landau and M. Schlamowitz, Arch. Biochem. Biophysics 95, 474 [1961].
\textsuperscript{46} J. R. Bronk and W. W. Kielley, Biochim. biophysica Acta [Amsterdam] 21, 440 [1957].
\textsuperscript{47} S. V. Perry and J. B. Chappell, Biochem. J. 65, 469 [1957].
\textsuperscript{48} J. L. Purvis, Exp. Cell Res. 16, 98 [1959].
\textsuperscript{49} D. Gilmour and M. Griffiths, Arch. Biochem. Biophysics 72, 302 [1957].
\textsuperscript{50} G. W. E. Plaut, Arch. Biochem. Biophysics 69, 320 [1957].
\textsuperscript{51} L. V. Eggleston, Biochem. J. 68, 673 [1958].
Phosphatase Activity in Homogenates from Normal and Poliovirus Infected Tissue Cultures

II. Variations Induced by Infection and Bicarbonate Poisoning*

By E. FRANK DEIG * and LOUIS P. GEBHARDT

Department of Microbiology
College of Medicine, University of Utah ** (Z. Naturforschg. 18b, 912–918 [1963]; eingegangen am 5. März 1963)

Concentrated suspensions of equal numbers of normal and poliovirus infected cells at 37°C were sampled periodically. Cell homogenates prepared from these samples were analyzed for total nitrogen content and for total ATP-ase, alkaline phosphatase, and glucose-6-phosphatase activity. The homogenates prepared from equal numbers of virus-infected cells showed a logarithmic decrease in nitrogen with respect to time over the period from four to twelve hours after infection. No additional loss in nitrogen occurred during the following twelve hours. Such nitrogen losses were associated with a decrease in the activity of the three enzymes. In the case of ATP-ase this decrease between the four and twelve hour period was linear with respect to time. The activity of this enzyme remained constant during the following twelve hours. The nitrogen content and activity of the three enzymes in homogenates prepared from normal cell suspensions did not increase over the sampling interval. The large amount of bicarbonate required to maintain the pH of the culture medium, at standard pHi, containing excess amounts of bicarbonate showed no metabolic activity. Such cells exhibited a marked reduction in total nitrogen and in the activity of the three enzymes over a sampling interval of twelve hours. When normal cells were placed in the culture medium, at standard pHi, containing such excessive concentrations of bicarbonate, and immediately infected with poliovirus, a delay in the synthesis of, and a marked reduction in final titer of the virus resulted from that found under control conditions.

A wealth of published experimental data exist at the present time concerning the cellular alterations of certain phosphorous-containing substances—notably the nucleic acids—during the course of virus infections. However, fairly little work has been done to assay the activity of specific enzymes concerned with phosphorous metabolism during this process. The present study was undertaken to determine the total ATP-ase, alkaline phosphatase, and glucose-6-phosphatase activities in homogenates prepared from normal and poliovirus infected cell suspensions over a 12 or 24 hour infection period.

* Aided by grants from the National Foundation and the National Institutes of Health, PHS, Grant 2-G-502.
** Senior author's present address: University of California, Naval Biological Laboratory, Building 841-C, Naval Supply Center, Oakland, California.
1 M. Green, Virology 9, 343 [1959].
3 M. Starelfi, Biochim. biophysica Acta [Amsterdam] 29, 43 [1958].
6 E. Volklin, L. Astrachan and J. L. Countryman, Virology 6, 545 [1958].
9 E. Kovacs, J. exp. Medicine 104, 589 [1956].
10 B. Thorell and E. Yamada, Biochim. biophysica Acta [Amsterdam] 31, 104 [1959].
12 E. Kovacs, G. Wagner and V. Sturte, Z. Naturforschg. 15b, 506 [1960].