FUKAI, R., S. BALLESTRA, AND C. N. MURRAY. 1973. Intercalibration of methods for measuring fission products in seawater samples, p. 3–27. *In* Radioactive contamination of the marine environment. IAEA.

HARLEY, N. I., I. FISENNE, C. D. ONG, AND J. H. HARLEY. 1965. Fission yield and fission product decay. U.S. AEC Health Safety Lab. Fallout Program Quart. Summary Rep. HASL-264, p. 251–260.

KUPFERMAN, S. L. 1971. Cesium 137 in the North Atlantic measured by selective absorption in situ. J. Mar. Res. **29**: 11–18.

NOSHKIN, V. E., AND V. T. BOWEN. 1973. Concentrations and distributions of long-lived fallout radionuclides in open ocean sediments, p. 671–686. *In* Radioactive contamination of the marine environment. IAEA.

ROETHER, W. 1971. Flushing of the Gerard-Ewing large-volume water sampler. J. Geophys. Res. **76**: 5910–5918.

SCHUERT, E. A. 1971. Sr 90 and Cs 137 in deep ocean water, p. 69. *In* A. H. Seymour [ed.], Radioactivity in the marine environment. Natl. Acad. Sci.

WONG, K. M., V. E. NOSHKIN, AND V. T. BOWEN. 1970. Radiochemical procedures for the analysis of strontium, antimony, rare earths, caesium and plutonium in sea-water samples, p. 119–127. *In* Reference methods for marine radioactivity studies. Tech. Rep. Ser. 118. IAEA.

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The extraction and measurement of adenosine triphosphate from marine sediments[1]

Abstract—A technique has been developed, using boiling sodium bicarbonate buffer, to extract adenosine triphosphate (ATP) from marine sediments and has been tested on a variety of sediments, including those with high organic content, clay, and carbonate. Recovery of ATP, as measured by the addition of bacteria of known ATP content to sediment, varied from 64–100%. The technique also was as effective as the conventional Tris buffer for extraction of ATP from both pure cultures of bacteria grown in broth and natural seawater samples.

Because of their small size and adsorptive characteristics, microorganisms in natural waters, soil, and sediments are not separated readily from the material in or on which they grow. Since culture techniques are selective and underestimate the number of cells present, the procedure of Holm-Hansen and Booth (1966) for extraction and measurement of ATP from marine waters, as a means of measuring biomass in situ, has been of great interest to microbial ecologists. Holm-Hansen and Booth (1966) found a ratio of cellular carbon to ATP of about 250 : 1 for a wide variety of microorganisms. Techniques have been developed for the extraction of ATP from freshwater sediments (Lee et al. 1971*a*,*b*), soils (Doxtader 1969), and freshwaters. Karl and LaRock (1974) adapted the sulfuric acid extraction method, used for freshwater sediments and some terrestrial soil systems, to noncalcareous marine sediments and sands, while Ernst and Goerke (1974) adapted the Tris buffer technique to marine sediments.

Our study was undertaken to develop an ATP extraction procedure that could be used for all types of marine sediments and that would be simple, rapid, and reliable.

Clay, calcareous, and mangrove-litter sediments and pure cultures were examined by the following ATP extraction methods.

Boiling Tris—A mixture containing 4 ml of Tris (0.02 M, pH 7.8) + 1 ml of sediment sample was boiled for 1–3 min and diluted. The final volume was dependent on the concentration of ATP extracted (Holm-Hansen and Booth 1966).

H_2SO_4—Sediment samples of 1 ml were added to 0.6 N H_2SO_4 (1, 2, or 4 ml) on ice and mixed with a vortex stirrer. The extract was adjusted to pH 7.8, processed

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through a cation exchange resin, and diluted to the desired volume (1 : 30) by the method of Lee et al. (1971*a*).

Octanol-butanol—Sediment samples were extracted with octanol-butanol (Sparrow and Doxtader 1973).

$HClO_4$—Cold $HClO_4$ was used to extract sediments (Chapman et al. 1971).

$NaHCO_3$—Boiling 0.1 M $NaHCO_3$ (4, 8, 12, 16, 20, 24, or 32 ml) adjusted to pH 8.5 was added to 1-ml samples being mixed with a vortex stirrer. During a 30-s extraction period the sample was mixed two more times and then centrifuged in the cold at 19,000 × *g* for 10 min. The supernatant was diluted with 0.1 M Tris buffer, pH 7.8, to a final $NaHCO_3$: Tris ratio of 2.3. With the sediments thus buffered further adjustments of the pH of individual samples was unnecessary.

Immediately after dilution all samples were either processed or stored at –20°C.

Pure cultures of marine culture *1309* (a marine *Vibrio* sp. isolated by W. S. Sottile) and *Enterobacter aerogenes* were grown in 100 ml of artificial seawater (Lyman and Fleming 1940), pH 7.4, and supplemented with 0.1% peptone, 0.1% yeast extract (ASWYE) in 250-ml Ryan flasks; optical density was measured with a Klett-Summerson photoelectric colorimeter (clinical model) with a No. 42 blue filter. Cells were counted and measured in a Petroff-Hauser bacterial counting chamber.

Enterobacter aerogenes grown in ASWYE medium was used to examine the ATP extraction efficiency from different materials. Recovery of ATP was estimated by adding *E. aerogenes* of known ATP content to sediment whose ATP content had been previously determined; recovery was calculated by the following formula:

$$\% \text{ recovery} = \frac{(\textit{E. aerogenes} \text{ ATP} + \text{sediment ATP}) - \text{sediment ATP}}{\textit{E. aerogenes} \text{ ATP}} \times 100.$$

ATP measurement—All glassware was washed in H_2SO_4 and rinsed in glass-distilled water. Tris buffer (Sigma Chem. Co.) at 0.02 or 0.1 M, pH 7.8, was used for standard preparation and sample dilution; 0.1 M Tris made pH adjustment unnecessary. Frozen dry firefly extracts (Sigma Chem. Co.) were diluted in buffer and held overnight at 5°C to reduce background activity. Stock reagent solutions were centrifuged at 19,000 × *g* for 10 min and stored on ice during use to minimize heat-induced kinetic variations in the bioluminescent reaction.

ATP standards containing 0.005, 0.025, 0.05, and 0.1 $\mu g\ ml^{-1}$ were prepared by dilution from a stock solution which contained 100 $\mu g\ ml^{-1}$ in a mixture of Tris buffer and $NaHCO_3$ like that used for sample extractions and stored at –20°C. Reaction mixtures contained 0.2 ml of sample added to 0.2 ml of buffered firefly extract in a scintillation vial; light emission was measured with an ATP photometer. Assays of blanks and standards were necessary because slopes of standard curves varied with the batch of firefly tail extract.

For the other extraction procedures, standard ATP curves were made using the same diluent as for sediment samples.

When we attempted to extract ATP from sediments by the various techniques listed above, we found that Tris buffer, octanol-butanol, and cold $HClO_4$ extractions gave poor recoveries (<65%). The sulfuric acid extraction yielded the highest ATP concentrations. However, because H_2SO_4 extraction resulted in the production of H_2S and soluble sesqui-oxides, it was necessary to pass the extract through a cation exchange resin (Lee et al. 1971*a*). This was a time consuming additional step which lost about 25% of the ATP in the sample. Furthermore, extraction efficiency varied greatly from sample to sample.

Extraction with boiling $NaHCO_4$ gave as much ATP as H_2SO_4 and sometimes significantly more. There was little sample-to-sample variability, and manipulation was minimal. We initially used this technique with Sapelo Island, Georgia, salt marsh sediments and later tested a variety of sediment types.

Table 1. Comparison of the volume of extractant and dilution on the levels of ATP extracted from clay sediment (Sapelo Island).

I			II		
$NaHCO_3$ (ml)	Final sediment dilution*	μgATP/ml	$NaHCO_3$ (ml)	Final sediment dilution*	μgATP/ml
4	1:30	0.3276 ± 0.0279	12	1:30	0.7878 ± 0.0831
	1:60	0.2865 ± 0.0187		1:100	1.5810 ± 0.2102
8	1:30	0.8939 ± 0.0567	16	1:40	1.0307 ± 0.0797
	1:60	0.7221 ± 0.0861		1:100	0.7248 ± 0.2144
12	1:30	0.6519 ± 0.0811	20	1:50	1.2128 ± 0.0346
	1:60	0.5760 ± 0.0411		1:100	2.3677 ± 0.2176
16	1:30	0.8642 ± 0.0941	24	1:60	1.2765 ± 0.0799
	1:60	0.7973 ± 0.0859		1:100	0.8657 ± 0.0946
32	1:80	0.4963 ± 0.0484			

*Dilution carried out with 0.1 M $NaHCO_3$, 0.1 M Tris buffer (pH 7.8) to approximate a final ratio of $NaHCO_3$:Tris of 2:3.

There are three major sources of error in the extraction of ATP from sediments: all of the ATP may not be extracted; some of it may be degraded during extraction; the extract itself may contain factors that interfere with the luciferin-luciferase assay system. The quantity of ATP extracted from sediment was affected significantly by the volume of boiling $NaHCO_3$ solution (extractant) used and by the subsequent dilution with Tris buffer-$NaHCO_3$ (Tables 1 and 2). Generally, 16–20 ml of extractant added to about 1 ml of sediment yielded the most ATP. The effect of dilution varied; it was not related simply to the total dilution of the sample (Table 1). All combinations of extractant and dilution gave significantly different results above 0.025 confidence level, although there was considerable overlap in the total dilution of the sediment. However, sample-to-sample variation at fixed extraction and dilution volumes was ±10%. For our standard protocol we chose conditions that gave us generally the highest ATP concentration: 16 ml of extractant diluted to 40 ml with Tris buffer-$NaHCO_3$, to yield a $NaHCO_3$: Tris ratio of 2 : 3. We have tried to maximize the absolute concentration of ATP extracted, but we can never know if we have extracted all of the ATP from a sample. However, the recovery of ATP from a known mass of added bacteria is a good indicator of recovery efficiency.

ATP recoveries with respect to extraction and dilution volumes are presented in Table 3. By changing the extractant volumes, we obtained values consistently above 64%. Variations in the concentrations of ATP are probably caused by relatively large but infrequently encountered organisms in the sediment.

The extraction period also affected recovery. A 30-s period was superior to 3 min for both clay and calcareous sediments (Ta-

Table 2. Comparison of the volume of extractant and dilution on the levels of ATP extracted from calcareous sediment.

ml $NaHCO_3$	Final sediment dilution*	μgATP/ml	Standard Error
4	1:30	0.140	0.0104
	1:60	0.145	0.0119
8	1:30	0.224	0.0088
	1:60	0.343	0.0441
12	1:30	0.300	0.0051
	1:60	0.290	0.0047

*Dilution carried out with 0.1 M $NaHCO_3$ 0.1 M Tris buffer (pH 7.8) to approximate a final ratio of $NaHCO_3$:Tris of 2:3.

Table 3. Extraction of wet sediment and recovery of bacterial ATP (*Enterobacter aerogenes*) with 0.1 M $NaHCO_3$.

Sediment	Vol. orig. extract to final solution: 4:30 ATP of sediment µg/ml	4:30 ATP Recovery %	16:40 ATP of sediment µg/ml	16:40 ATP Recovery %
Estuarine (Sapelo)	0.28 ± 0.03	31	0.86 ± 0.09	114
Gulf of Mexico	0.12 ± 0.019	10	0.55 ± 0.12	64
Everglades	0.12 ± 0.018	82	0.75 ± 0.16	115
Everglades-litter	1.08 ± 0.23	24	3.3 ± 0.31	133
Florida Keys	n.d.		0.21 ± 0.018	91
Fresh water lake	n.d.		0.057 ±0.028	20
Fresh water river	n.d.		0.061 ± 0.004	65

ble 4). Re-extraction for 30 s did not increase significantly the amount of ATP. However, we found that boiling for up to 10 min with pure cultures did not decrease the amount of ATP extracted.

After extraction, centrifugation, and dilution, samples were placed on ice until assay or immediately frozen. During 2 h on ice there was no detectable loss of ATP. Some degradation of ATP may take place during extraction but recoveries were consistently ±10%, even though the time processing began varied from sample to sample over a range of about 10 min. The potential interference of the extract to the luciferin-luciferase assay is accounted for by the dilution factor used in the recovery experiments.

Table 4. Effect of time of extraction and dilution on the effectiveness of sodium bicarbonate to extract ATP from calcareous sediments (Everglades).

Final sediment dilution	µgATP/ml	Standard Error
30-s Extraction		
1:10	0.096	0.0046
1:20	0.148	0.0081
1:30	0.184	0.0097
1:50	0.238	0.0100
30-s Reextraction		
1:10	0.007	0.0010
1:20	0.013	0.0004
1:30	0.009	0.0011
3-min Extraction		
1:10	0.069	0.0006
1:20	0.071	0.0245
1:30	0.072	0.0014
1:50	0.042	0.0125

Extraction procedure: 4 ml 0.1 M $NaHCO_3$ + 1 ml sample. Dilution carried out with 0.02M Tris, pH 7.8.

When sediments with bacterial cells added are used to determine the percentage recovery to reduce variability, it is important that the ratio of sediment ATP to added cell ATP be about 1 : 1 (data not shown). This procedure also serves as a further check for extract effects on the luciferin-luciferase reaction.

Since the population composition of natural samples is not likely to be known, we decided to standardize ATP concentrations for biomass estimates in terms of a pure culture. We selected *E. aerogenes* because it has a long, stable, stationary phase over which the ATP content remains relatively constant and had been used by Lee et al. (1971*b*) in studies of ATP extraction techniques. Optical density (OD), ATP, cell numbers, and cell volumes were measured over the growth cycle (Table 5). During the stationary phase OD was constant while cell numbers decreased. Cell volumes, es-

Table 5. Determination of optical density, ATP, cell numbers, and cell volumes over time for *Enterobacter aerogenes*.

Time (h)	O D (Klett Units)	nos. x10^8/ml	Average cell vol. (μm^3)	µgATP/ml	C:ATP
0	25	-	-	-	-
1	33	1.42	1.912	0.299	100:1
2	47	3.30	1.912	0.655	106:1
3	72	7.12	1.913	1.336	110:1
4.5	108	31.20	0.327	1.090	103:1
6	128	20.20	0.270	1.088	55:1
7	129	11.60	0.238	0.682	45:1
10.5	128	10.12	0.238	0.624	42:1

timated mathematically, decreased throughout the growth cycle; ATP concentration was maximal in midlogarithmic phase growth. We also examined an isolate from marine sediment, *1309* (Table 6). The carbon content of the cells was calculated from numbers and average volumes, assuming that 10% of cell weight was carbon and that the specific gravity was 1.1. The absolute relationships of C : ATP for *E. aerogenes* and marine culture *1309* were variable, although values for both were maximal in midlogarithmic growth; ratios decreased from 110 : 1 to 42 : 1 for *E. aerogenes* and 222 : 1 to 87 : 1 for marine isolate *1309* in the stationary phase.

The carbon content of nonviable cells produced between the time numbers were maximum in the logarithmic phase and the stationary phase was calculated by measuring the difference in total numbers for the two times. The weight of carbon in the non-

Table 6. Determination of optical density, ATP, cell numbers, and cell volumes over time for marine culture *1309*.

Time (h)	O D (Klett Units)	nos. x 10^8 /ml	Average cell vol. (μm^3)	µgATP/ml	C:ATP
0	18	1.12	3.470	0.386	97:1
1	22.5	1.00	2.592	1.340	21:1
2	46	2.72	2.961	2.800	26:1
3	61.5	5.45	2.416	2.780	56:1
4	97.5	11.73	1.870	1.730	132:1
5	118	18.60	1.307	1.960	196:1
6	133	12.60	1.541	0.880	222:1
10.5	160	7.58	1.494	0.630	191:1
14	169	6.24	1.315	0.910	100:1
25	160	5.80	1.280	0.870	87:1

Table 7. Effects of nonviable cell carbon on C : ATP ratios of viable cells.

	Non viable cells (x 10^8)	average size (μm^3)	µgC/ml (non viable)	Viable & non viable carbon (µg/ml)	C:ATP
1309 (cf. 5 h and 25 h)	12.80	1.293	182.05	253.85	292:1
E. aerogenes (cf. 4.5 h and 10.5 h)	21.08	0.283	55.19	81.69	132:1

viable cells was then calculated (Table 7). Total carbon as measured before would overestimate the carbon in viable cells by 55.19 μg ml^{-1} for *E. aerogenes* and 182.05 μg ml^{-1} for marine isolate *1309*. By addition of nonviable to viable cell carbon, carbon : ATP ratios become 132 : 1 and 292 : 1.

Although boiling Tris buffer, H_2SO_4, and $NaHCO_3$ solutions extracted equivalent amounts of ATP from pure cultures of bacteria, for marine sediments, boiling 0.1 M $NaHCO_3$ was substantially more effective than Tris buffer, simpler to use, and less variable in recovery than H_2SO_4. The H_2SO_4 procedure required ion-exchange treatment before ATP assay and could not be used with calcareous sediments because of the carbonate buffering.

Unlike Ernst and Goerke (1974), we found that the Tris buffer extraction yielded very low recoveries. They used a 10-s incubation at 100°C for their extraction and this may account for their success with the technique. However, they did not measure the ATP in the supernatant but adsorbed it on a mixture of sediment and calcium carbonate and then measured ATP by adding this mixture to luciferin-luciferase. Pamatmat and Skjoldal (1974) also used Tris buffer for ATP extraction from sediments, boiling for 1 to 1.5 min and then centrifuging. They measured ATP in the centrifugate but did not report data on recovery or the effects of different dilutions.

Recovery of ATP from sediment by the $NaHCO_3$ procedure was maximal in 30-s extraction; this period was chosen for our standard procedure, although extraction for 1 min did not reduce the ATP recovered. The decrease in sediment ATP with longer boiling times is curious. Holm-Hansen and Booth (1966) showed that ATP can be extracted from water by boiling for 5 min in Tris buffer, and we found that boiling in $NaHCO_3$ for up to 10 min did not reduce ATP recovered from pure cultures.

In our initial work we used 4 ml of extractant. However, subsequent experiments showed that maximum ATP concentrations were extracted with 16–20 ml of 0.1 M $NaHCO_3$; we chose the former volume for later work because of ease of handling, particularly in vortex mixing.

Lee et al. (1971*a*) found that the quantity of ATP measured was related to the total dilution of the sample; they suggested that there were interfering materials in the extract whose effects could be diluted out (*see Table 1*). We found similar effects with dilution of our extracts, but whatever the effect on the ATP or luciferin-luciferase, recovery was not a simple function of the total dilution of the sediment. Interaction between the extractant and different properties of the individual sediments (e.g. pH, sediment type, structure, etc.) must affect recovery. The working system we chose for the Sapelo Island clay sediments comprised 16 ml of 0.1 M $NaHCO_3$ extractant subsequently diluted to 40 ml with 0.1 M Tris buffer (pH 7.8). This eliminated making a final pH adjustment before analysis. Because recovery of ATP appears to be a function of sediment type, ATP concentra-

tion, volume of extractant, and final dilution, we suggest that preliminary experiments be conducted to determine the optimum procedure for each sediment.

Various procedures have been used to examine the conditions affecting recovery of ATP from sediments. Ausmus (1973) reported about 100% recovery from soils; the ATP was added directly to predried soil. Karl and LaRock (1974) reported recoveries of 45–100% with an EDTA-treated acid extraction procedure. Addition of known amounts of ATP to an acid/EDTA mixture in the presence of glass beads was used to determine residual interference by cations and the recovery of ATP. Lee et al. (1971*a*,*b*) got a 63% recovery of ATP using sulfuric acid extraction and treatment with a cation exchange resin. Recovery was determined by addition of known quantities of ATP to raw and presterilized soils.

We felt that addition of ATP as living bacteria to natural substrates before extraction more closely approximated natural conditions, although we recognize that not all organisms may be extracted with equal efficiency. Some sample-to-sample variability in sediment replicates is no doubt due to the inhomogeneity of the suspended organisms in distribution and in size. We suggest that the ratio of bacterial ATP added : sediment ATP should also be determined for each sediment type, since we cannot predict what interactions occur.

The question of the appropriate C : ATP ratio to be used for calculating biomass has been the subject of several papers. Holm-Hansen and Booth (1966), Holm-Hansen (1969), and Ausmus (1973) have suggested that it is about 250 : 1. However, there is abundant evidence that this ratio varies with the growth phase for pure cultures of bacteria. Our results agree with those of Kao et al. (1973), that as cultures approach the stationary phase of growth ATP concentration decreases simultaneously with cell numbers. The C : ATP ratios varied greatly during growth of *E. aerogenes* and *1309*, as in work by Forrest (1965) and Lee et al. (1971*b*). Falsely high ratios would be inherent if conventional dry weight analysis were used, particularly during the stationary phase where nonviable cells and cell fragments are present. We have chosen to count cells microscopically and calculate the carbon content from the volumes. Table 7 shows that a total carbon measurement in stationary phase would produce an overestimate of 55.19 μg ml^{-1} for *E. aerogenes* and 182.05 μg ml^{-1} for culture *1309*. The C : ATP ratios would then become 132 : 1 and 292 : 1, closer to the 250 : 1 ratio (Holm-Hansen and Booth 1966). The 132 : 1 ratio for *E. aerogenes* is similar to those given by Harrison and Maitra (1969) and Strange et al. (1963); comparable data are not available for culture *1309*. Christian et al. (1975) used C : ATP ratios of 100 : 1 and 250 : 1 to estimate the possible range of living biomass.

The ATP technique provides a sensitive and rapid assay for the biomass of living organisms in natural materials. It serves well for comparative studies and for estimating, within limits, the biomass of organisms in situ. The $NaHCO_3$ extraction technique provides a simple and reliable procedure for determining ATP in both clay and calcareous sediments.

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References

Ausmus, B. S. 1973. The use of the ATP assay in terrestrial decomposition studies. Bull. Ecol. Res. Comm. (Stockholm) **17**: 223–243.

Chapman, A. G., L. Fall, and D. E. Atkinson. 1971. Adenylate energy charge in *Escherichia coli* during growth and starvation. J. Bacteriol. **108**: 1072–1086.

Christian, R. R., K. Bancroft, and W. J. Wiebe. 1975. Distribution of microbial adenosine triphosphate in salt marsh sediments at Sapelo Island, Georgia. Soil Sci. **119**: 89–97.

Doxtader, K. G. 1969. Estimation of microbial biomass in soil on the basis of adenosine

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triphosphate measurements. Bacteriol. Proc. **69:** 14.

ERNST, W., AND H. GOERKE. 1974. Adenosin-5'-triphosphat (ATP) in Sedimenten und Nematoden der nordostatlantischen Tiefsee. *Meteor* Forschungergeb. Reiche D Biol. **18:** 35–42.

FORREST, W. W. 1965. Adenosine triphosphate pool during the growth cycle of *Streptococcus faecalis*. J. Bacteriol. **90:** 1013–1016.

HARRISON, D. E. F., AND P. MAITRA. 1969. Control of respiration and metabolism in growing *Klebsiella aerogenes*. The role of adenine nucleotides. Biochem. J. **112:** 647–652.

HOLM-HANSEN, O. 1969. Determination of microbial biomass in ocean profiles. Limnol. Oceanogr. **14:** 740–747.

———, AND C. R. BOOTH. 1966. The measurement of adenosine triphosphate in the ocean and its ecological significance. Limnol. Oceanogr. **11:** 510–519.

KAO, I. C., S. U. CHIU, L. T. FAN, AND L. E. ERICKSON. 1973. ATP pools in pure and mixed cultures. J. Water Pollut. Control Fed. **45:** 926–931.

KARL, D. M., AND P. A. LAROCK. 1974. Extraction and measurement of adenosine triphosphate in sediments and sands. Bacteriol. Proc. **74:** 53.

LEE, C. C., R. R. HARRIS, J. D. H. WILLIAMS, D. E. ARMSTRONG, AND J. K. SYERS. 1971*a,b*. Adenosine triphosphate in lake sediments: 1. Determination. 2. Origin and significance. Soil Sci. Soc. Am. Proc. **35:** 82–86; 86–91.

LYMAN, J., AND R. H. FLEMING. 1940. Composition of sea water. J. Mar. Res. **3:** 134–146.

PAMATMAT, M. M., AND H. R. SKJOLDAL. 1974. Dehydrogenase activity and adenosine triphosphate concentration in marine sediments in Lindaspollene, Norway. Sarsia **56:** 1–12.

SPARROW, E. B., AND K. G. DOXTADER. 1973. Adenosine triphosphate (ATP) in grassland soil. Its relationship to microbial biomass and activity. IBP Tech. Rep. 224.

STRANGE, R. E., H. E. WADE, AND F. A. DARK. 1963. Effect of starvation on ATP concentration in *Aerobacter aerogenes*. Nature **199:** 55–57.

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