Experimental. Methyl β-D-glucopyranoside (2.00 g) was dissolved in liquid nitrogen dioxide (1.90 g) in a two-necked flask fitted with an efficient water-cooled condenser carrying a calcium chloride tube. In one experiment a slow stream of oxygen, and in another a slow stream of nitrogen, was passed through the solution, which was maintained at 12° for 5 h. The product was then evaporated under reduced pressure (at 30°), dissolved in water and deionised (Dowex 3 (free base)). Paper chromatographic (solvents: ethyl acetate—acetic acid—water, 3:1:1 and butan-1-ol—ethanol—water, 10:3:5) and electrophoretic (buffer: 0.1 M hydrogen sulphite, pH 4.7 used at 50°) examination of the neutral products from the two experiments revealed similar patterns. Unchanged starting material was the main product together with the four possible mono-carbonyl glycosides and some unidentified products. Methyl β-D-glucopyranoside (10.00 g) in nitrogen dioxide (9.50 g) was kept at 12° for 17 h. The fractionation of the neutral components in the reaction mixture was carried out essentially as previously described (Ref. [4] and earlier papers) by chromatography on a carbon-Celite column and sub-fractionations by chromatography and electrophoresis on thick filter papers. The amounts of carbonyl glycosides isolated are given in the text. They were all shown by paper chromatography and electrophoresis to be indistinguishable from authentic samples. The 3-keto compound, methyl β-D-*ribo*-hexopyranosid-3-ulose, was obtained in the crystalline state, m.p. and mixed m.p. 129—130°.

The acids were recovered from the anion exchange resin as their ammonium salts (6.95 g); a brown, sticky, partially crystalline product. After carbon treatment the acids were converted to their potassium salts, and the relatively insoluble crystalline potassium hydrogen salt (2.37 g) of D-glucaric acid was isolated in the usual way. The product after removal of the cations from the light yellow salt was shown to be indistinguishable from an authentic sample of D-glucaric acid by paper chromatography and electrophoresis (buffer: 0.1 M acetate, pH 4). After one reprecipitation the salt was colourless and its IR-spectrum (KBr) was identical with that of an authentic sample.

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The Use of a Recording Beckman DB Spectrophotometer for the Assay of ATP with a Luciferin-Luciferase Reagent *

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The assay of ATP *** with the luciferin-luciferase reagents is usually carried out with a photofluorometer or a specially designed instrument.[1,2] Manufacturers of the reagents also describe methods with somewhat lower sensitivity utilizing the Beckman DU spectrophotometer. The use of this instrument does not, however, allow registration of the maximum values of the light emission. The procedure therefore

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*** The following abbreviations are used:
ATP, adenosine-5′-triphosphate
GTP, guanosine-5′-triphosphate
ITP, inosine-5′-triphosphate
UTP, uridine-5′-triphosphate.

necessitates reading after a definite time interval following the mixing of the reagents and the substrate. If a short time interval is chosen, the slope of the curve will make the result of the reading very sensitive to small variations in time. At longer time intervals the readings are smaller, thus giving rise to other errors, and when crude enzyme preparations are used, one also faces the problem of ATP generation in the presence of nucleoside di- and triphosphates.

We want to describe a technique circumventing these difficulties by measuring in a recording Beckman DB spectrophotometer the light emitted from the ATP assay mixture. The method is simple and rapid and allows the determination of ATP quantities down to 0.05 μg per ml with a 10 % variation between duplicate samples.

The spectrophotometer, which may be used in either single or double beam operation, is turned to the ON position[1] with both light sources off. The recorder scale expansion and base line are adjusted and the recorder left in the STAND BY position. A semimicro silica cell containing an appropriate volume of the sample to be tested is placed in the sample compartment. The luciferin-luciferase reagent † is prediluted with a solution of 0.02 M $MgSO_4$ in a 0.05 M potassium arsenate buffer pH 7.4 to give a total volume of the reaction mixture of one ml. The diluted enzyme solution is added by blowing it from a Pasteur pipette into the solution to be tested, while the recorder is simultaneously started and the cell house is closed as quickly as possible.

Tracings as shown in Fig. 1 were obtained with a chart speed of one inch per min. With appropriate amounts of ATP and enzyme, the ascending part of the galvanometer deflection curve could be registered. This indicated to us that the main part of the light emission took place while the instrument was measuring. The effect of UTP, GTP and ITP, respectively, when replacing ATP is also shown in Fig. 1.

By using the scale expansion of the recorder, measurements of smaller quantities of ATP were possible as shown in Fig. 2. 0.1 ml of enzyme solution was found sufficient for ATP concentrations up to 0.5 μg per ml. Maximum galvanometer deflection as well as readings taken after 15 and 60 sec were plotted and gave a linear correlation with the ATP concentration up to 0.4 μg per ml in all three instances.

The reading at the maximum galvanometer deflection will give the highest sensitivity and specificity.

With the enzyme preparation used, the generation of ATP did not seem of any significance except when small amounts of ATP should be determined in mixtures with large amounts of other nucleoside triphosphates. However, 10 μg of UTP would interfere seriously with the determination of 0.05 μg of ATP.

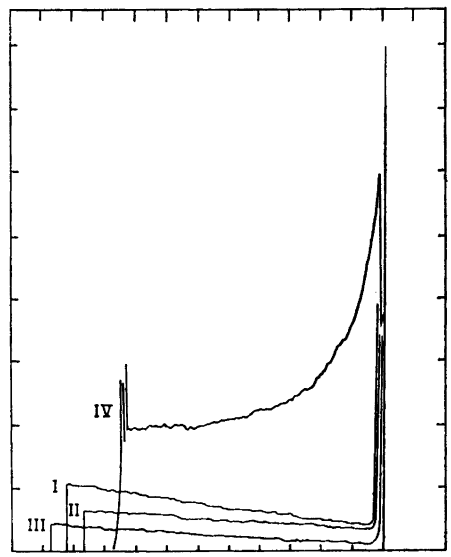

Fig. 1. Reaction of nucleoside triphosphates in the luciferin-luciferase system as recorded with the Beckman DB spectrophotometer. The tracings have been superimposed on the same graph directly from the recorder chart. The abscissa gives 1/4 inch distances corresponding to 15 sec. The ordinate gives the galvanometer deflection corresponding to the decadic units of the recorder chart. For procedure see the text. The curves I, II, III, and IV were obtained with test solutions containing 36 μg UTP, 35 μg GTP, 59 μg ITP, and 5 μg ATP, respectively.

† The luciferin-luciferase reagent was obtained from Worthington Biochemical Corporation, Freehold, N. J., U.S.A. (Designation FFX 6221) as a lyophilized water extract of firefly lanterns. Each vial is equivalent to 50 mg dry lanterns and when reconstituted with 5 ml of water is 0.05 M in potassium arsenate buffer and 0.02 M in $MgSO_4$, pH 7.4.

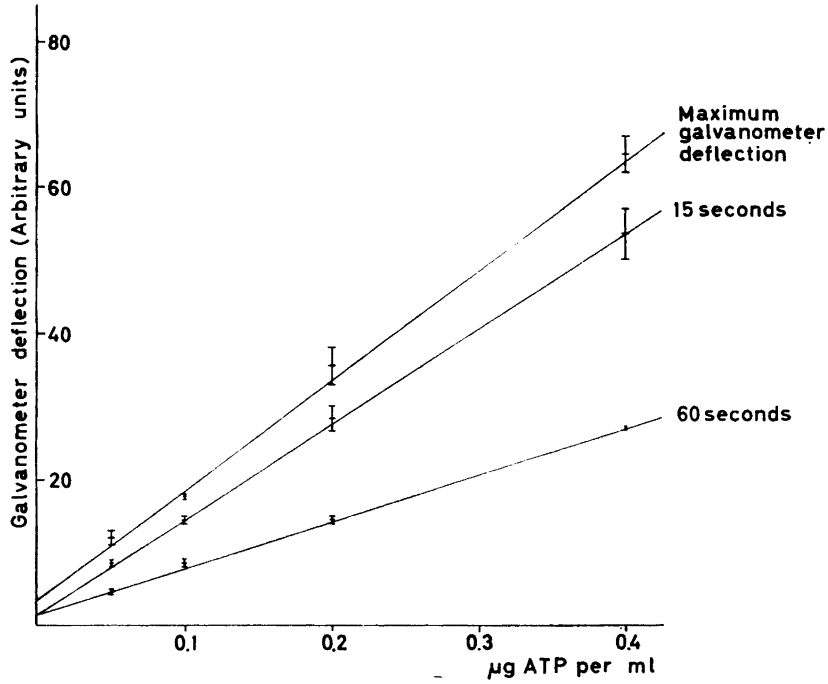

Fig. 2. Standard curves for the luciferin-luciferase assay of ATP. The readings are taken at maximum galvanometer deflection, and after 15 sec and 60 sec. For procedure see the text. Each point represents the mean of two determinations as indicated.

The maximum values in Fig. 2 were used for the determination of the Michaelis-Menton constant by the method of Lineweaver and Burke.[3] A K_m for ATP of 3.6×10^{-4} M was found. This is in good agreement with the values given by Green and McElroy.[4]

Thus the recording Beckman DB spectrophotometer is sensitive, accurate and reliable for the assay of ATP with the luciferin-luciferase system. If special precautions are taken (*e.g.* injection of the enzyme solution in the absence of light), even smaller amounts of ATP can probably be measured. The results reported here are, however, obtained under ordinary routine conditions.

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