TABLE IV

EFFECT OF THE ORDER OF ADDING REAGENTS ON THE DPNH INHIBITION OF THE NITRITE TEST

(Values found as % of NO_2 added)

Order of addition of reagents	*1. NO_2 2. Sulphanilamide 3. Naphthyl reagent*	*1. NO_2 2. DPNH 3. Sulphanilamide 4. Naphthyl reagent*	*1. NO_2 2. Sulphanilamide 3. DPNH 4. Naphthyl reagent*	*1. NO_2 2. Naphthyl reagent 3. DPNH 4. Sulphanilamide*
Expt. 1	100	34	100	60
Expt. 2	100	32	100	60

Assay mixture: 20 mμmoles NO_2 and 0.1 μmole DPNH; NO_2 determined as in Table I.

The mode of the DPNH interaction with nitrite was studied further by varying the order in which the reagents were added to the reaction mixture. The results are shown in Table IV.

It is clear that when sulphanilamide reacted first with NO_2^- to form the diazonium salt the subsequent addition of DPNH did not interfere with the azodye formation. Thus it appears that DPNH and TPNH compete with NO_2^- for the same site on the diazo compound. It is of interest that neither DPN nor TPN inhibit the diazotization process.

To overcome this serious interference with the nitrite reductase assay we propose the following modification in which residual DPNH and TPNH are removed by a barium acetate-alcohol treatment. At the end of the incubation period add 0.1 ml *M* Ba acetate and 2.5 ml 95% v/v ethanol, in the cold, to the reaction mixture, agitate well and centrifuge at 0° C. Nitrite is determined in the supernatant, in the usual way. The results obtained by this method are reproducible and devoid of interference.

ANTONIA MEDINA*
D. J. D. NICHOLAS

Agricultural Research Council Unit of Plant Nutrition (Micronutrients), Long Ashton Research Station, University of Bristol (England)

1 A. NASON AND H. J. EVANS, *J. Biol. Chem.*, 202 (1953) 655.
2 D. J. D. NICHOLAS, A. NASON AND W. D. MCELROY, *J. Biol. Chem.*, 207 (1954) 341.
3 D. J. D. NICHOLAS AND H. M. STEVENS, *Nature*, 176 (1955) 1066.
4 A. NASON, R. G. ABRAHAM AND B. C. AVERBACH, *Biochim. Biophys. Acta*, 15 (1954) 159.
5 F. D. SNELL AND C. T. SNELL, *Colorimetric Methods of Analysis*, Vol. I, Inorganic, D. Van Nostrand Co., Inc., New York, 1936, p. 644.
6 H. J. EVANS AND A. NASON, *Plant Physiol.*, 28 (1953) 233.
7 H. J. EVANS AND C. MCAULIFFE, in W. D. MCELROY AND B. GLASS, *Inorganic Nitrogen Metabolism*, Johns Hopkins Press, Baltimore, 1956, p. 189.

Received November 20th, 1956

* On leave from the Instituto de Edafologia y Fisiologia Vegetal, Madrid (Spain).

Correction of rotatory dispersion characteristics of adenosine triphosphate and related compounds

In a recent publication[1] the influence of pH on the value of λ_c and K_m of adenine, adenosine, AMP, ADP and ATP was presented in table-form. Because of a constant arithmetic error in the calculations, all values of slope (K_m), standard deviation of slope and molecular rotation are too large by a factor of 2. The intercept (λ_c) and the standard deviation of the intercept are not affected. It should be noted that this error in no way affects the ideas or interpretations presented in the publication.

A corrected table is given below.

TABLE I

INFLUENCE OF pH ON λ_c AND K_m OF ADENINE AND DERIVATIVES

Approx. pH*		*Adenine*	*Adenosine*	*AMP*	*ADP*	*ATP*
2.9	λ_c(A)	2520 ± 81	2385 ± 43	2580 ± 45	2223 ± 16	2069 ± 55
	$K_m \times 10^{-8}$	6.29 ± 0.64	−13.34 ± 0.15	−9.87 ± 0.16	−8.29 ± 0.07	−6.58 ± 0.08
	Mol. Rot.**	182	−764	−801	−631	−526
5.5	λ_c(A)		2365 ± 38	2584 ± 37	2324 ± 8	2328 ± 18
	$K_m \times 10^{-8}$		−15.72 ± 0.05	−10.18 ± 0.24	−8.88 ± 0.06	−7.58 ± 0.04
	Mol. Rot.		−901	−827	−780	−794
7.1	λ_c(A)	2432 ± 103	2353 ± 4	2500 ± 68	2346 ± 52	2332 ± 63
	$K_m \times 10^{-8}$	24.27 ± 1.21	−16.85 ± 0.14	−11.64 ± 0.22	−9.06 ± 0.11	−7.97 ± 0.31
	Mol. Rot.	745	−942	−899	−835	−866
10.2	λ_c(A)			2507 ± 23	2364 ± 17	2369 ± 54
	$K_m \times 10^{-8}$			−11.78 ± 0.08	−8.79 ± 0.12	−8.27 ± 0.13
	Mol. Rot.			−913	−832	−873
2.9 and returned to 7.1	λ_c(A)				2303 ± 10	2300 ± 21
	$K_m \times 10^{-8}$				−9.33 ± 0.08	−8.31 ± 0.05
	Mol. Rot.				−831	−857

* The pH of each solution was within ± 0.2 of the indicated value. All experiments were done in a medium of $M/15$ phosphate buffer at 23 ± 1° C.

** The molecular rotation values are calculated at 3200 A, which is the nearest wavelength available to the value of λ_c.

B. H. LEVEDAHL
Department of Zoology, University of California, Los Angeles, Calif. (U.S.A.) T. W. JAMES

[1] B. H. LEVEDAHL AND T. W. JAMES, *Biochim. Biophys. Acta*, 21 (1956) 298.

Received December 6th, 1956

Preliminary Notes

Photosynthetic activity of fragments of *Spirogyra* chloroplasts

II. Measurements with the mass spectrometer

In a previous preliminary note[1] experiments on light-induced carbon dioxide uptake and oxygen liberation by fragments of *Spirogyra* chloroplasts were reported. In the meantime, this investigation was extended with mass spectrometer measurements. This apparatus enables simultaneous recording of both gases and, thus, reliable determination of assimilatory quotients. The results of the latter study will be preliminarily reported here.

In contrast with the findings of ARNON *et al.*[3,4], who worked with spinach chloroplasts, nearly full-rate photosynthetic carbon dioxide fixation and oxygen evolution were observed with chloroplast fragments without addition of any enzymes or cofactors. This discrepancy is probably due to structural differences between the two types of chloroplasts. A more detailed discussion about this matter is in preparation.

The chloroplast fragments were prepared as described earlier[1]. They were suspended in a phosphate buffer, pH 7.2, previously flushed for 15 min with nitrogen containing 4.2% oxygen and 0.5 or 1.0% carbon dioxide. The suspension was transferred into a pre-cooled cuvette, which was adapted to the mass spectrometer. The cuvette was then quickly mounted in the operating