

The broken line X-Y represents a dividing line in each polynucleotide chain, such that portion B always has a base sequence complementary to portion A and could fold back on itself to give the correct base pairing. Each chain as a whole is identical to the other and in consequence during cell division each individual deoxyribonucleic acid chain will exactly replicate itself. For the particular segment shown in the diagram the sequence will be invariably A-G-T-C-G-A-C-T. In general, the one-half of a parent deoxyribonucleic acid chain may have any arbitrary base sequence, but the sequence of the second half must be complementary to the first.

Unlike the Watson-Crick model this scheme does not require the existence of two different, though complementary, chains for each type of acid in a cell. It does however require that each deoxyribonucleic acid chain be composed of an even number of bases. The scheme would account for a double helical structure of the acid during cell division, but also permits of the existence of single deoxyribonucleic acid chains during resting periods. A similar duplex model consisting of identical deoxyribonucleic acid chains but with pairing between identical bases has been suggested by Donohue and Stent<sup>3</sup>. Their model does not however account for the X-ray diffraction results obtained with deoxyribonucleic acid from a variety of sources.

The scheme proposed would account for the existence of a plane of symmetry in living organisms, and might also explain the symmetrical division of chromosome material during spermatogenesis.

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<sup>1</sup> Watson, J. D., and Crick, F. H. C., *Nature*, **171**, 737, 964 (1953).

<sup>2</sup> Perutz, M. F., *Endeavour*, **17**, 190 (1958).

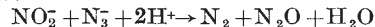
<sup>3</sup> Donohue, J., and Stent G. S., *Proc. U.S. Nat. Acad. Sci.*, **42**, 734 (1956).

### Measurement of Inhibition by Azide in Biochemical Assay Systems Involving Nitrite Estimation by Diazotization

VILLANUEVA<sup>1</sup> recently directed attention to the interference by azide in diazotization procedures used in assay systems. He suggested that the reported inhibition by azide of numerous enzyme systems in which nitrite production has been measured might require re-investigation.

We have independently reached this conclusion during a study of nitrate reduction by the aldehyde oxidase system of potato, and our method for dealing with this point may be of interest in connexion with the re-measurement of inhibition by azide in nitrate reductase and xanthine or aldehyde oxidase systems.

First attempts to test for azide inhibition of a nitrate reduction system showed that nitrite reacts immediately with azide when the acidified sulphanilamide reagent is added in the diazotization step. This reaction is probably that shown by Partington<sup>2</sup>:



Azide at concentrations normally used in inhibition tests ( $10^{-4}$ - $10^{-3}$  M) caused proportional losses of nitrite estimated in standard solutions, and almost total losses of nitrite produced enzymically in concentrations of the order of  $2-5 \times 10^{-5}$  M. The results given by Villanueva show that in his system containing azide at a final concentration of  $3.3 \times 10^{-3}$  M, where 92 per cent interference was observed, the concentration of nitrite was approximately  $1.2 \times 10^{-3}$  M.

The reaction between azide and nitrite can, however, be applied in the manometric estimation of the latter when produced in enzymic reactions. The effect of inhibition of azide is then determined by comparison of evolution of gas produced in the reaction between nitrite and azide in acid solution when azide is added at the end of the assay period or initially also, when present as an inhibitor. In practice, 0.5 ml. of  $10^{-2}$  M azide solution in one side-arm was added at the end of the assay period to the reaction mixture containing 3.5 ml. of 0.13 M phosphate buffer pH 6.0 in the main compartment and 0.4 ml. of 2N hydrochloric acid was added from a second side-arm to produce rapid gas evolution. The enzymic reaction was started by adding 0.1 ml. of M acetaldehyde solution by dislodging a glass cup hooked on the centre well.

The following points merit note. As nitrous oxide is appreciably soluble in water, the volume of gas measured is less than the theoretical volume. In our experiments, 2 μ moles nitrite yielded 82-86 μ litres of gas at 27°C. in an atmosphere of nitrogen instead of 89.6 μ litres. Manometric estimation of nitrite production using azide is nevertheless more sensitive than that using sulphamic acid proposed by Robinson<sup>3</sup>, as nearly twice as much gas is evolved. Sodium azide is unstable when acidified, and yields variable small amounts of gas, depending on initial concentration, which must be accounted for so far as possible in blank tests, since azide is in excess of the equivalent amount of nitrite. The activity of the uninhibited system should also be estimated independently by a colorimetric determination of nitrite in an aliquot from a parallel assay mixture incubated and shaken under conditions identical with those used in the manometric procedure.

Using the procedures outlined, we have shown that aldehyde oxidase of potato is inhibited 80-90 and 45 per cent by 1 to  $5 \times 10^{-3}$  and  $5 \times 10^{-4}$  M azide concentrations respectively when reduction of nitrate to nitrite is used to measure activity. The inhibition is probably immediate and is completely reversible, since dialysis for 3 hr. after incubation with azide permits normal rates of nitrite production in the usual assay system.

The details of this work will be published elsewhere.

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<sup>1</sup> Villanueva, J. R., *Nature*, **184**, 549 (1959).

<sup>2</sup> Partington, J. R., "A Text Book of Inorganic Chemistry", 5th edit., p. 562 (Macmillan, London, 1946).

<sup>3</sup> Robinson, J., *Arch. Biochem. Biophys.*, **52**, 148. (1954).