now reported the results of experiments designed to examine (1) the effects of hyperbaric oxygen on these enteric bacteria; (2) the influence that nutrition might have on hyperoxia; and (3) the interaction of hyperbaric oxygen and antimicrobial drugs. Sulfisoxazole, the selected antimetabolite, is considered to resemble other sulphur drugs in interfering with *p*-aminobenzoic acid (PABA) metabolism. Some years ago Gottlieb's group found that the antitubercular agent *p*-aminosalicylic acid (PAS) exerted a synergistic effect with oxygen on the growth inhibition of *Mycobacterium tuberculosis*.

The use of surface, nutrient agar cultures demonstrated that hyperbaric oxygen tensions (>1·87 atm absolute of O_2 for 24 h) were bactericidal for all strains of *V. comma* examined and only bacteriostatic for *Salmonella* and *Shigella* species. Parallel experiments on a more nutritious medium, brain heart infusion, did not produce growth inhibition. The mechanism of this protection against hyperoxia has not been elucidated, but the nature of the carbohydrate source in the medium is at least contributory. Hyperoxia and its relief were not the consequences of elevated pressures, or the effects of oxygen on medium constituents or on *p*H phenomena. A synergistic action existed between oxygen and sulfisoxazole on the inhibition of vibrios even under conditions where hyperoxia *per se* was not apparent. The oxygen–sulfisoxazole synergism also could be induced by intermittent exposures to oxygen.

At present, the picture is of two widely diverse bacteria, *Vibrio* and *Mycobacterium*, responding similarly to oxygen in the presence of PABA antagonists. Although hyperbaric oxygen almost certainly acts in a detrimental way at other metabolic sites, the significance of Gottlieb and Pakman's findings is in the area of chemotherapy. If the biochemistry of the PAS/sulfisoxazole–oxygen synergism can be unravelled, it could stimulate a new approach to the design of agents that can synergize with PABA antagonists; success here might supplant the need of elaborate equipment now required for hyperbaric oxygen treatments of certain diseases.

Nitrogen Fixation

from a Correspondent

CURRENT views on many aspects of biological nitrogen fixation were summarized at the discussion meeting of the Royal Society held on June 6 and attended by more than 100 people.

The first speaker at the morning session, P. W. Wilson (Wisconsin), considered the early studies which have formed the basis for modern research in this field. J. Chatt (Sussex) reviewed recent developments in the chemistry of nitrogen fixation and discussed some inorganic models which may show a similarity to the nitrogen-fixing enzyme complex, nitrogenase. The biochemistry of the fixation process was reviewed by R. H. Burris (Wisconsin), who commented on the requirements for nitrogen fixation by bacterial cell-free extracts. These are ATP and a source of electrons (generally provided in the form of $Na_2S_2O_4$). The nitrogenase complex can be separated into two fractions neither of which fix N_2 alone but which do so when recombined. One fraction has a molecular weight of 100,000–135,000 and contains about 16 iron atoms and 1–2 molybdenum atoms per molecule, while the other has a molecular weight near to 40,000–50,000 and contains 3 iron atoms per molecule. A variety of substrates including N_2O, C_2H_2, NaN_3, HCN, CH_3NC and certain of their analogues are also reduced by the nitrogenase. Dr Burris considered that there may be multiple binding sites on the nitrogenase complex.

In the afternoon session, which was more biologically orientated, P. Fay (London) and R. M. Cox (Bristol) considered aspects of fixation by blue-green algae and concluded that in these organisms photosynthesis supplies the necessary energy, that direct photoreduction of nitrogen does not occur, and that electrons generated during respiration supply the necessary reducing power. W. D. P. Stewart (London) discussed the contribution of free-living nitrogen-fixing micro-organisms as providers of combined nitrogen and gave examples of several habitats including soils, lakes and marine environments where these organisms may be important. He emphasized the usefulness of the acetylene reduction technique for studying nitrogen fixation in the field.

The role of symbiotic associations of non-leguminous plants as contributors of combined nitrogen was discussed by W. S. Silver (Indiana). The endophytes of root nodules of non-legumes such as *Alnus* and *Myrica* have not yet been isolated with certainty. The requirements for nitrogen reduction by extracts of non-legume root nodules are similar to those of other nitrogen-fixing systems. F. J. Bergersen (Canberra) reviewed recent studies of nitrogen fixation by legume root nodules. It is now known that the bacteroids are the nitrogen-fixing sites and that they fix nitrogen under anaerobic conditions although the necessary energy and reducing power are generated aerobically. P. S. Nutman (Rothamsted) in the final paper of the session discussed the genetics of the symbiosis in legumes.

Antibiotics and RNA Polymerase

from our Medical Biochemistry Correspondent

THE claim that the antibiotic streptovaricin is unique in its mode of action (*Biochem. Biophys. Res. Commun.*, **30**, 379; 1968 and *Nature*, **218**, 11; 1968) has already been challenged. The bacterial DNA-dependent RNA polymerase reaction is thought to proceed in three stages; the formation of a DNA–enzyme complex, the initiation of RNA formation—a step which seems to involve nucleoside triphosphates—and then the polymerization of nucleoside monophosphates to form RNA. Streptovaricin was the first substance shown to inhibit the second stage of the reaction, the formation of the initiation complex. It had no effect on the RNA polymerase from Ehrlich ascites tumour cells, which suggested that the initiation of RNA synthesis differs in bacterial and mammalian systems.

Hartmann, Honikel, Knüsel and Nüesch (*Biochim. Biophys. Acta*, **145**, 843; 1967) reported last year that rifamycin and some of its chemically modified derivatives (antibiotics which are particularly active against Gram-positive micro-organisms) were very good inhibitors of the DNA-dependent RNA polymerase from *E. coli*. Because they did not inhibit the DNA-directed DNA polymerase from *E. coli* the inhibition probably did not involve reaction of the antibiotic with DNA, in contrast to the inhibition caused by actinomycin.

Sippel and Hartmann (*Biochim. Biophys. Acta*,