Bedeutsam ist, dass bei der wasserlöslichen W-HS nur wenig Radikale nachgewiesen werden. Das geringe Teilchengewicht sowie der mögliche – und vielfach vermutete – Gehalt an aktiven Huminsäurevor- und -zwischenstufen in der wasserlöslichen Huminsäurefraktion liessen hier einen höheren Radikalgehalt erwarten. Offenbar handelt es sich bei diesen Teilchen jedoch um relativ stabile Aggregate, die die W-HS als selbständige Gruppe der Huminstoffe auszeichnen.

Summary. The electron spin resonance was measured with several humic acids. The preparations used here contain stable free radicals. Grey humic acids contain more radicals than brown humic acids.

H. KLEIST und D. MÜCKE[9]

Institut für Physiologische Chemie der Universität, Rostock (DDR), 20. September 1965.

[9] Herrn Dr. SEIFERT und Fräulein Dr. EBERT (Deutsche Akademie der Wissenschaften Berlin-Adlershof, Arbeitsgruppe: Physikalische Methoden der Analytischen Chemie, Labor für Hochfrequenz-Spektroskopie; Dir.: Prof. Dr. KRIEGSMANN) danken wir für die Unterstützung bei der Durchführung der Messungen.

Excited Molecular O_2 and Inactivation of Indolebutyric Acid (IBA)

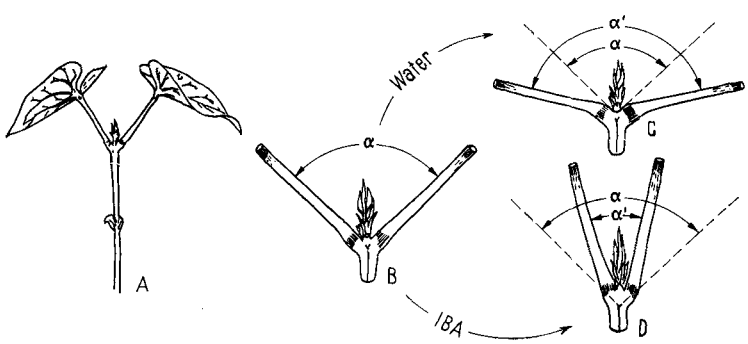
The existence of a metastable excited molecular oxygen in solution is of outstanding interest in several problems. Originally KAUTSKY[1], about 1935, had speculated on the existence of such a species in connection with dye (e.g. eosin) photosensitized oxidations[2], but the suggestion was lost for want of direct evidence. The present report confirms KAUTSKY's earlier speculations and places them on a firm footing.

The observation that a red-orange chemiluminescence is produced during the reaction of hydrogen peroxide and sodium hypochlorite in aqueous solution was reported by SELIGER[3]. KHAN and KASHA[4], extended the spectroscopic study of this chemiluminescence and, in view of the nature of the reaction and the bands observed, they tentatively assigned the red chemiluminescence bands as the 0.0 and 0.1 bands of the $^1\Sigma k^+ \rightarrow {}^3\Sigma k^-$ transition of the *molecular oxygen*. The study of the mechanism of the reaction by CAHILL and TAUBE[5], using isotopic labelling of the peroxide, also indicates that the O–O bond remains intact, reaffirming the possibility of excited *molecular oxygen* rather than *atomic oxygen* as a primary product. The results of the present investigation distinctly show that there is a direct *cause-and-effect* relationship between molecular oxygen and its capacity of inducing the oxidation of IBA.

In this endeavour, the author employed the 'bean test' devised by FERRI and CAMARGO[6] for the rapid assay of plant growth regulators. Y-shaped segments from 2- to 3-week-old plants of *Phaseolus vulgaris* L. var. *'Blue Lake'* enhance the angle between the petioles while reposing in water and decrease it while immersed in IBA solution (Figure). The behaviour is qualitatively the same whether the test objects are kept in light or in darkness and is not attributable to pH alterations or osmotic influences.

With the aid of this biological test, it was possible to evaluate the influence of excited molecular O_2 produced in aqueous solution by the interaction of sodium hypochlorite and hydrogen peroxide on IBA solutions. The mean values of a representative experiment are charted in the Table. It is apparent that, while the pieces reposing in water increased the average angle between the petioles, those resting in IBA solutions decreased it both in the light and in the dark. The two ingredients of the reaction mixture, viz. sodium hypochlorite and hydrogen peroxide (at pH 7.0), were unable to sensitize the destruction of IBA, as reflected in the response of the plant segments. But as the two constituents mingled together, the resulting red-orange chemiluminescence was instrumental in abolishing the IBA activity with unusual avidity and the test objects behaved as if they were in pure water. This highly reproducible experiment was repeated with other indole analogues, e.g. β-(indole-3)-*n*-propionic acid, with identical results.

[1] H. KAUTSKY, in *Luminescence, A General Discussion of the Faraday Society* (Gurney and Jackson, London 1938), p. 216.
[2] R. K. KAKKAR, Naturwissenschaften *52*, in press (1965).
[3] H. SELIGER, Anal. Biochem. *1*, 60 (1960).
[4] A. U. KHAN and M. KASHA, J. Chem. Phys. *39*, 2105 (1963).
[5] A. E. CAHILL and H. TAUBE, J. Am. chem. Soc. *74*, 2312 (1952).
[6] M. G. FERRI and L. S. V. CAMARGO, Ann. Acad. Brasil. Ci. *22*, 161 (1950) (in LINSER und KIERMAYER, *Methoden zur Bestimmung pflanzlicher Wuchsstoffe* (Springer, 1957), Figur 39).



A upper part of the 2-week-old bean plant showing the primary leaves; *B* Y-shaped piece prepared from the same; *C* increase of α in water; *D* decrease of α in presence of IBA.

Oxidation of IBA by excited molecular oxygen. IBA 50 mg/l, sodium hypochlorite 30% w/v, hydrogen peroxide 30% v/v (pH 7.0, temp. 20 °C ± 2). Each value determined with 15 plant segments

Angles	Light				Dark		
	Water	IBA + sodium hypochlorite	IBA + H_2O_2	IBA + sodium hypochlorite + H_2O_2	IBA + sodium hypochlorite	IBA + H_2O_2	IBA + sodium hypochlorite + H_2O_2
Initial	88.8	94.6	86.6	90.6	112.1	81.5	91.6
Final	141.3	66.4	56.4	139.9	86.1	58.3	131.8
Difference	+ 52.5 ± 6.1	− 28.2 ± 4.8	− 30.2 ± 5.9	+ 49.3 ± 5.3	− 26.0 ± 6.6	− 23.2 ± 3.1	+ 40.2 ± 5.4

On the basis of the above findings, the author is tempted to hazard the explanation that the H atoms, originating from the oxidation of the side-chains of IBA molecules, are transferred to an acceptor, the acceptor being the molecular O_2 itself. The author believes that in many biological peroxide systems, the chemiluminescence may be found as an indication of the breakdown mechanisms; and in addition, similar observations may be made in radiobiological and radiation chemical reactions. Application of these ideas deserve further investigations[7].

Zusammenfassung. Indolbuttersäure wird durch angeregten molekularen Sauerstoff in vitro inaktiviert. Die Bedeutung dieses Befundes liegt darin, dass dadurch ein möglicher Weg für die Inaktivierung pflanzlicher Wuchsstoffe in vivo aufgezeigt wird.

R. K. KAKKAR

Botany Department, The University, Allahabad (India), August 31, 1965.

[7] The author acknowledges with appreciation the generous gift of IBA and IPA from Messrs. Calbiochem AG, Lucerne (Switzerland). – This work was financed by a grant (No. F. 8-5/63 G) from the University Grants Commission.

Biochemical Genetical Studies on Host-Parasite Relationship[1]. Pathogenicity of Two Mutants of *Fusarium vasinfectum* Atk. on Cotton Plants

During our studies on the pathogenicity of *Fusarium vasinfectum* on the cotton plant *Gossypium arborium*, we have isolated a pair of nutritionally deficient mutants by nitrous acid treatment of the conidia. The pathogenicity of these strains when compared with the normal type has yielded interesting results.

The nitrous acid treatment was carried out as follows: A suspension containing $5 \cdot 10^8$ conidia/ml was obtained in 0.1 *M* acetate buffer of pH 4.4, from a ten-day-old slant culture of *Fusarium vasinfectum* in Czapeck's agar medium. 1 ml of 0.5 *M* sodium nitrite was added to 9 ml of the spore suspension, and at the end of 12 min it was plated out in agar medium. The colonies that came up after 48 h of incubation were tested for their nutritional requirements by the auxanographic method, keeping Czapek's medium as control. Two mutants were isolated, one a partial requirer of nicotinic acid and the other requiring para amino benzoic acid (PABA) for growth.

The pathogenicity of the mutants was tested by different methods. They were grown in Richard's medium with appropriate concentrations of the nutrients needed. The parent strain was also grown in Richard's medium, alone and in the presence of the vitamins to serve as controls. At the end of 28 days, the cultures were filtered and the pH of the filtrate was adjusted to the alkaline side. It was then extracted with petroleum ether repeatedly and the ether extracts were concentrated. Cut shoots of wild susceptible variety of cotton (K_6, Cotton Karunganni, *Gossypium arboreum*) were incubated for 8 h in test tubes containing 5 ml portions of the concentrated extracts diluted to 10 ml with water. Incubation of cut shoots in 10 ml water served as controls. It was observed that in the case of the nicotinic acid deficient strain, culture fluids produced very early wilting as compared to the normal, and that the PABA requirer could produce no wilting. Further, the presence of nicotinic acid or *p*-amino benzoic acid in the growth medium of the parent strain revealed no change in its action on normal wilt production. Also, the partial requirer of the nicotinic acid strain produced a blue pigment when grown in agar medium.

Tests on the pathogenicity of the mutant strains to the cotton plants were carried out in vivo also. The mutant strains and the parent wild type were cultured on separate sand-oat medium with appropriate levels of the respective vitamins. On germination of K_6 seeds in soil (sterilized at 22 lb/in^2 pressure for 2 h) mixed with 5% of 21-day-old sand-oat cultures has shown severe wilting in plants that received the nicotinic acid requirer. Normal parent strain caused wilting in seven days irrespective of the presence or absence of the vitamins. No wilting occurred in plants treated with PABA requiring mutant strain, inoculum.

[1] No. III. Scheme sponsored and financed by the United States Department of Agriculture under USPL 480 funds.

[2] G. F. PEGG, Ann. Bot. *26*, 219 (1962).