have their maximal absorption in the 600 mμ region whereas the *R*-pigments do not show any absorption at

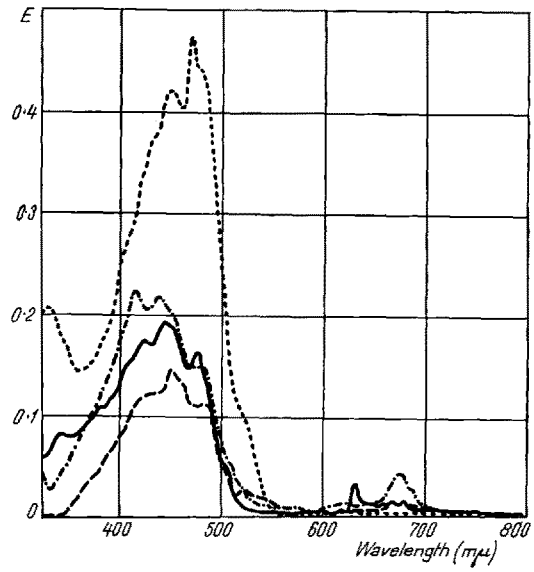

Fig. 4.–Yellow pigments.
Absorption spectra in acetone. ——— Y_1; —·— Y_2; ····· Y_3; —— Y_4.

all at this wavelength (Fig. 5). Therefore Phycobilins were excluded. Carotenoids may, however, also have red

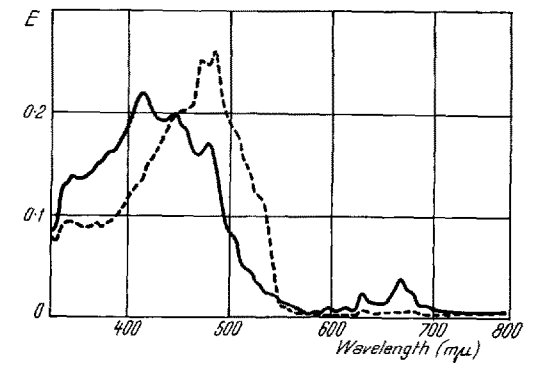

Fig. 5.–Red pigments.
Absorption spectra in acetone. —— R_1; ——— R_2.

colours, and the absorption curve of R_1 make it probable that the compound belongs to this group and also is

Absorption maxima of pigments in acetone

	Main peaks mμ	Accessory peaks mμ
Green pigments		
G_1	410, 672·5	540, 615
G_2	412·5, 675	540, 615
G_3	410, 670	540, 615
G_4	415, 680	375, 510, 545, 620
G_5	417·5, 677·5	505, 542·5, 620
Yellow pigments		
Y_1	450	430, 485
Y_2	417·5, 440	480, 675
Y_3	330, 450, 470	
Y_4	445	345, 425, 477·5
Red pigments		
R_1	410	345, 445, 477·5
R_2	470, 485	345

related to luteol of fucoxanthol. The nature of R_2 is uncertain; the major absorption of this pigment lies about 30 mμ further toward longer wavelengths with two maxima, 470 and 485 mμ.

A survey of main and accessory absorption maxima for the compounds described is given in the Table. The final identification of the different pigments found in the gyttja would necessitate the isolation of the compounds in a solid state which is only possible by large scale separation on absorption columns.

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Geological Survey of Denmark, Charlottenlund, and Institute of Plant Physiology, University of Copenhagen, June 25, 1955.

Zusammenfassung

Etwa 100000 Jahre alte, interglaziale Gyttja aus Rodebäk im westlichen Dänemark (Riss–Würm-Interglazialzeit) wurde mit Äther extrahiert und die Extrakte mittels Papierchromatographie getrennt und untersucht. Es wurden grüne, gelbe und rote Pigmente gefunden. Aus den Absorptionsspektren und der Fluoreszenz dieser Stoffe ergibt sich, dass die grünen Pigmente Chlorophyllderivate, die gelben (sowie wenigstens eines der roten) Karotinoide sind. Die Gyttja enthielt sehr wenige bestimmbare Pflanzenreste, hauptsächlich nur Pollen und Diatomeen. Die Farbstoffe müssen von lebenden Pflanzen im interglazialen See, in erster Linie Algen, herrühren, und haben infolge Lichtabschluss und niedrigem Redoxpotential sowie niedriger Temperatur ausserordentlich gute Aufbewahrungsbedingungen gefunden; normalerweise werden solche Stoffe schnell abgebaut.

The Action of Hydrogen Peroxide on Amino Acids in Presence of Iron Salts and its Bearing on Photolysis of Amino Acids

Earlier studies[1] on the action of FENTON's reagent on amino acids showed that they are deaminated and converted to aldehydes and corresponding carboxylic acids. Recently JOHNSON *et al.*[2] have shown that α-keto acids are formed by the action of FENTON's reagent on α-amino acids. They have further pointed out that certain enzymatic processes can be simulated by reactions involving free radicals *in vitro*. Therefore the importance of the study of the action of FENTON's reagent on the amino acids is obvious.

We have observed during our experiments that the amino acids undergo a series of complicated changes by the action of hydrogen peroxide in presence of iron salts. A typical experiment carried out to study the action of FENTON's reagent was as follows.

To 0·2 cm³ of 0·1 *M* solution of amino acid was added dropwise 0·2 cm³ of 0·1 *M* ferrous sulphate solution and the volume was made up to 1·8 cm³ with distilled water. To this 0·2 cm³ of H_2O_2 (0·1 *M*) was added and the tube shaken well for about 3 min. Controls were also kept with H_2O_2 alone and also with ferrous sulphate in absence of hydrogen peroxide. After vigorous shaking, the tubes

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[2] G. R. A. JOHNSON, G. SCHOLES, and J. WEISS, Science *114*, 412 (1951).

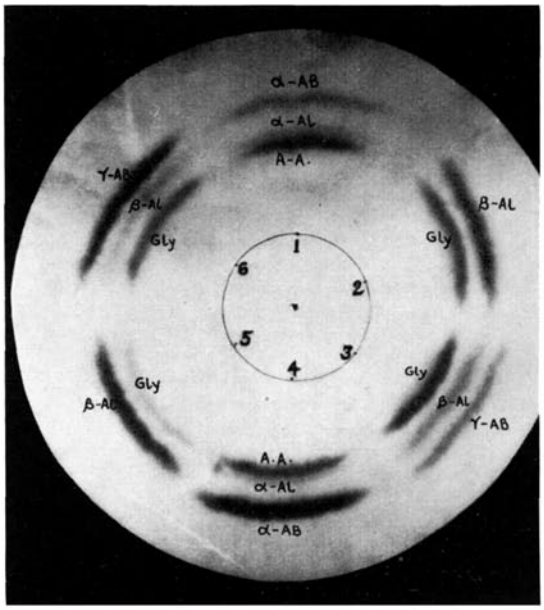

Fig. 1.–Chromatogram showing the action of hydrogen peroxide on amino acids in presence of ferrous and ferric salts (Temperature 24–25°C).
(1) α-aminobutyric acid + H_2O_2 + $FeSO_4$
(2) β-alanine + H_2O_2 + $FeSO_4$
(3) γ-aminobutyric acid + H_2O_2 + $FeSO_4$
(4) α-aminobutyric acid + H_2O_2 + $FeCl_3$
(5) β-alanine + H_2O_2 + $FeCl_3$
(6) γ-aminobutyric acid + H_2O_2 + $FeCl_3$
(α-AB = α-aminobutyric acid, β-Al = β-Alanine, α-Al = α-Alanine, γ-AB = γ-aminobutyric acid, A.A = Aspartic acid, Gly = glycine)

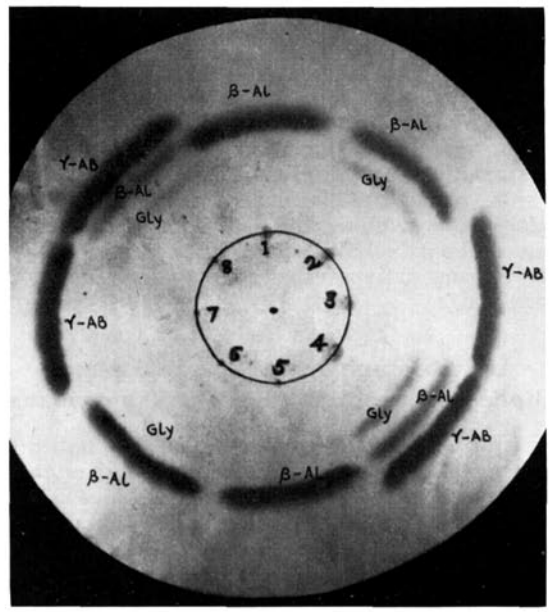

Fig. 2.–Chromatogram showing the photodegradation of amino acids.
(1) β-alanine + $FeCl_3$ in dark
(2) β-alanine + $FeCl_3$ in sunlight
(3) γ-aminobutyric acid + $FeCl_3$ in dark
(4) γ-aminobutyric acid + $FeCl_3$ in sunlight
(5) β-alanine + $FeSO_4$ in dark
(6) β-alanine + $FeSO_4$ in sunlight
(7) γ-aminobutyric acid + $FeSO_4$ in dark
(8) γ-aminobutyric acid + $FeSO_4$ in sunlight
(β-Al = β-alanine, γ-AB = γ-aminobutyric acid, Gly = glycine).

were kept for about 10–15 min at room temperature (24–25°C) and the reaction mixtures were examined for the presence of amino acids by two-dimensional paper chromatography (solvents: butanol-acetic acid-water, 40:10:50 followed by either water-saturated phenol or pyridine-water, 80:20) and also by the circular paper chromatographic technique. The interference by iron salts was overcome by exposing the spot to liquor-ammonia when the iron complex was rendered more mobile and moved with the solvent front (solvent: butanol-acetic acid-water). The chromatogram showing the degradation of amino acids by H_2O_2 in presence of ferrous and ferric salts is given in Figure 1. The amino acids studied were α- and β-alanine. α-amino butyric acid, γ-amino butyric acid, aspartic and glutamic acids and serine. The products formed were the same irrespective of whether ferrous or ferric salts were used. β-Alanine and serine gave glycine; γ-amino butyric acid in turn gave β-alanine and glycine, Glutamic acid gave rise to only aspartic acid, whereas in the case of α-alanine and aspartic acid, ninhydrin-positive products could not be detected. From α-amino butyric acid, the formation of aspartic acid and α-alanine was observed. The amino acids were stable in presence of H_2O_2 alone at room temperature.

It is well-known that when ferrous and ferric salts are added to H_2O_2, it goes through a series of reactions giving rise to free radicals[1]. We have found that the amino acids are degraded by FENTON's reagent only when the molar concentration of ferrous salts is equal to or less than that of H_2O_2. The absence of reaction when the molar concentration of ferrous ions was greater than that of H_2O_2 may be explained by the fact that in this case the regeneration of free radicals by chain mechanism is restricted[1].

The reactions reported here have a striking resemblance to the photolysis of amino acids in presence of TiO_2[2] and to the action of H_2O_2 at 100°C[3]. Considering the close similarity between the action of FENTON's reagent and the photosensitized action of TiO_2 on amino acids, it can be suggested that during the photolysis of amino acids by sunlight in presence of TiO_2, H atoms and OH radicals are generated by dissociation of the water molecule and that the OH radicals are responsible for the oxidative reactions reported earlier[2].

While studying the photolysis of amino acids, it was observed by us that in addition to metal oxides like TiO_2 and ZnO, ferrous sulphate and ferric chloride also acted as efficient photosensitizers and gave the same type of degradative reactions with some of the amino acids, namely, γ-amino butyric acid and β-alanine (Figure 2).

Further studies on the role of these photosensitizers in the oxidative degradation of amino acids are in progress.

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Department of Biochemistry, Indian Institute of Science, Bangalore 3, India, January 17, 1955.

[1] F. HABER and J. WEISS, Proc. Roy. Soc. [A] *147*, 332 (1934). – W. G. BARB, J. H. BAXENDALE, P. GEORGE, and K. R. HARGRAVE, Trans. Faraday Soc. *47*, 462, 591 (1951).

[1] W. G. BARB, J. H. BAXENDALE, P. GEORGE, and K. R. HARGRAVE, Trans. Faraday Soc. *47*, 462, 591 (1951).

[2] K. V. GIRI, G. D. KALYANKAR, and C. S. VAIDYANATHAN, Naturwissenschaften *40*, 440 (1953); *41*, 88 (1954).

[3] Y. MATSUO, Nature *171*, 1021 (1953).

Zusammenfassung

Es wurde der Abbau von Aminosäuren durch Wasserstoffsuperoxyd in Gegenwart von Eisensalzen untersucht. Papierchromatographische Analyse zeigte, dass dabei aus γ-Aminobuttersäure β-Alanin und Glycin, aus β-Alanin und Serin Glycin entsteht. Die Bildung von α-Alanin und Asparaginsäure aus α-Aminobuttersäure und von Asparaginsäure aus Glutaminsäure konnte ebenfalls festgestellt werden. Diese Resultate werden in Zusammenhang gebracht mit dem Mechanismus der Photolyse von Aminosäuren in Gegenwart von TiO_2.

Activité staphylostatique de quelques diphénols, naphtols et chalcones nouveaux

Au cours de nos recherches sur les mécanismes de l'action antibactérienne des substances organiques en relation avec leur structure moléculaire[1], nous avons été amenés à examiner l'activité staphylostatique de nombreux composés nouveaux appartenant à trois familles chimiques: diphénols, naphtols et chalcones. En effet, dans ces trois groupes, on avait déjà trouvé des dérivés fortement bactériostatiques[2].

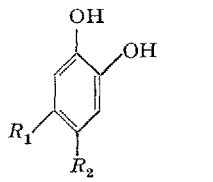
La méthode expérimentale utilisée dans les présentes recherches consiste à ensemencer des tubes de bouillon nutritif contenant la substance à examiner, avec une goutte d'une culture de 18 h de *Micrococcus pyogenes* var. *aureus* diluée au $1/10^6$. La pousse microbienne est suivie visuellement tous les jours pendant une durée de 3 ou 4 jours. Dans ces conditions, une étude effectuée sur 138 composés appartenant aux trois groupes chimiques sus-indiqués[3] a permis de déceler chez quelques-uns d'entre eux une activité staphylostatique importante (c'est-à-dire à des concentrations comprises entre 10^{-5} et 10^{-6}).

[1] Voir N. P. BUU-HOÏ et M. WELSCH, Exper. *10*, 248 (1954). – A. LACASSAGNE, N. P. BUU-HOÏ, F. ZAJDELA et N. D. XUONG, C. r. Acad. Sci. *231*, 89 (1950).

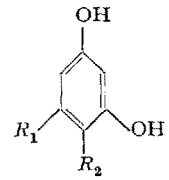
[2] Voir W. A. SEXTON, *Chemical Constitution and Biological Activity* (E. and F. N. Spon Ltd., London 1949), p. 249; pour les chalcones, voir E. SCHRAUFSTÄTTER et S. DEUTSCH, Z. Naturforsch. *36*, 430 (1948).

[3] La plupart des composés étudiés ont déjà été décrits chimiquement dans les publications suivantes: N. P. BUU-HOÏ, H. LE BIHAN, F. BINON et P. RAYET, J. Org. Chem. *15*, 1060 (1950). – N. P. BUU-HOÏ, H. LE BIHAN et F. BINON, J. Org. Chem. *16*, 185 (1951). – N. P. BUU-HOÏ, H. LE BIHAN et N. D. XUONG, J. Org. Chem. *16*, 987 (1951). – N. P. BUU-HOÏ, H. LE BIHAN, F. BINON et P. MALEYRAN, J. Org. Chem. *18*, 4 (1953).

Voici quelques-uns des résultats les plus significatifs ainsi obtenus (voir tableau I).

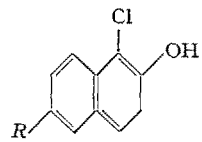


I; $R_1 = H$, $R_2 = -C(CH_3)_3$
II; $R_1 = Cl$, $R_2 = -C(CH_3)_3$
III; $R_1 = H$, $R_2 =$ 
IV; $R_1 = H$, $R_2 =$ 



V; $R_1 = H$, $R_2 = tert.\text{-}C_5H_{11}$
VI; $R_1 = H$, $R_2 =$ 
VII; $R_1 = Cl$, $R_2 =$ 

(Voir tableau II).



VIII; $R = -C(CH_3)_3$
IX; $R = tert.\text{-}C_5H_{11}$
X; $R =$ 

(Voir tableau III).

En résumé, il est montré que certains dérivés substitués du résorcinol et du β-naphtol, ainsi qu'une chalcone dérivée du thiophène, la *p*-chlorobenzal(5-nitro-2-acétothiénone), possèdent une activité staphylostatique élevée. Cette activité dépend, dans une grande mesure, de la structure moléculaire. Signalons par ailleurs qu'aucune des substances étudiées n'a montré d'activité bactériostatique contre *Escherichia coli*, en bouillon nutritif, et à la concentration de 10 γ/ml; et dans quelques cas, nous avons vérifié qu'il en est de même pour la concentration de 100 γ/ml.

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Tableau I. Groupe des diphénols

Substance	Formule	Concentration staphylostatique minimale en γ/ml
4-tertiobutylpyrocatéchine	I	retard de croissance à 10
5-chloro-4-tertiobutylpyrocatéchine	II	retard de croissance à 10
4-cyclohexylpyrocatéchine	III	retard de croissance à 10
4-(1-méthyl-1-cyclohexyl)-pyrocatéchine	IV	retard de croissance à 10
4-cyclohexylgaïacole		retard de croissance à 10
4-(1-méthyl-1-cyclohexyl)-gaïacole		retard de croissance à 10
chloro-4-tertiobutylgaïacole		5
bromo-4-tertiobutylgaïacole		retard à 10
4-tertioamylrésorcinol	V	5
4-(1-méthyl-1-cyclohexyl)-résorcinol	VI	1,25
5-chloro-4-(1-méthyl-1-cyclohexyl)-résorcinol	VII	1,25
2,7-dicyclohexylfluorescéine		retard à 10
2,7-dicyclohexyléosine		retard à 10