pared from pigeon livers. The only noteworthy modification was that frozen liver pieces were pulverised before addition of cold acetone (JÄNNES[1]).

The "transacetylase enzyme" prepared by the author was able to acetylate at most 65% of sulfanilamide in the reaction mixture when boiling water of yeast or rat liver was used as standard.

The author had no possibility to operate with commercial coenzyme A standards. The following results were obtained in these assays.

Effect of vitamin B_{12} on the pantothenate synthesising ability of *E. coli* strain No. 1.

Vitamin B_{12} per ml of medium	Synthesised pantothenic acid per ml of medium
0·0 γ	0·09 γ
0·3 γ	0·04 γ

The effect of vitamin B_{12} on the pantothenate synthesising ability of this strain was very clear.

Assay of coenzyme A content of cells of this strain gave the following results:

Vitamin B_{12} per ml of medium	Sulfonamide acetylation readings with Beckmann Photometer Wavelength 545 mμ					Coenzyme A in Lipmann units per g of dry bacteria
	Blank test	Maximum acetylation	Readings with boiling water from the bacteria			unit
			ml			
			0·05	0·1	0·3	
0·0 γ	0·90	0·36	0·71	0·59	0·39	285
0·3 γ			0·71	0·60	0·42	300

In this experiment, the acetylation readings were almost the same and no notable difference in the amounts of coenzyme A in bacteria cultivated with and without vitamin B_{12} could be detected.

A larger addition of vitamin B_{12} had no effect in this respect:

Vitamin B_{12} per ml of medium	Coenzyme A in Lipmann units per g of dry bacteria
0·0 γ	290
1·0 γ	280

The author also isolated strain No. 2 of *E. coli*, which had the following properties:

Vitamin B_{12} per ml of medium	Synthesised pantothenic acid per ml of medium
0·0 γ	0·06 γ
0·3 γ	0·02 γ

[1] L. JÄNNES, Ann. Acad. Sci. Fenn. Suppl. *61*, 39 (1954); Exper. *10*, 31 (1954).

The corresponding values for coenzyme A were:

Vitamin B_{12} per ml of medium	Coenzyme A in Lipmann units per g of dry weight
0·0 γ	305
0·3 γ	310

According to these experiments, the effect of vitamin B_{12} concerns only the synthesis of the amount of pantothenic acid which is liberated by the cells to the medium. It appears probable that the bacterial cells satisfy their own need of pantothenic acid for the synthesis of coenzyme A. The cultivation time in my experiments, however, was short, only 24 h, and data concerning experiments with a longer cultivation time are not available. The growth of the cells was unaffected by the addition of vitamin B_{12}. The observation of MAAS[1] that there exists in *E. coli* an enzyme which is capable of synthesising pantothenic acid from β-alanine and pantoic acid and is not dependent on coenzyme A, is not in disagreement with my results.

SAXENA, GHOTEK, and AGARWALO[2] noted in 1954 that vitamin B_{12} causes a similar effect on the synthesis of thiamine in the metabolism of *E. coli*. These effects have as yet no explanation and therefore deserve further investigation.

J. JÄNNES

Department of Medical Chemistry, University of Helsinki, April 4, 1955.

Zusammenfassung

Auf vitaminfreien Nährböden wurden *Escherichia-Coli*-Stämme kultiviert, die bei Zusatz von Vitamin B_{12} einen deutlichen Rückgang der Abgabe von Pantothensäure in das Nährmedium zeigten.

Die Bestimmung des Coenzym-A-Gehaltes der Bakterien ist mittels der Methode von KAPLAN und LIPMANN durchgeführt worden. Es ergab sich, dass keine grösseren Schwankungen des Coenzym-A-Gehaltes der Bakterien vorkamen, obgleich der Gehalt an freier Pantothensäure in der Nährflüssigkeit deutlich abnahm.

[1] W. K. MAAS, J. Biol. Chem. *198*, 23 (1952).

[2] K. C. SAXENA, S. GHOTEK, and S. C. AGRAWALO, Exper. *10*, 488 (1954).

Ether Soluble Pigments in Interglacial Gyttja

It has been demonstrated that certain circumstances may favour the preservation of plant pigments. Thus TREIBS[1] identified a series of chlorophyll- and haemin-derivates in mesozoic oil-slate, coals etc.; FOX *et al.*[2] showed that carotenoids were present in marine sediments 8000 years old. Recently VALLENTYNE[3] described three chlorophyll degradation products from freshwater sediments aged up to 11,000 years from Canadian lakes.

[1] A. TREIBS, Liebigs Ann. *509*, 103 (1934); *510*, 42 (1934); *517*, 172 (1935); *520*, 144 (1935).

[2] D. L. FOX, D. M. UPDEGRAFF, and D. G. NOVELLI, Arch. Biochem. *5*, 1 (1944).

[3] J. R. VALLENTYNE, Canad. J. Bot. *33* (1955) (in the press).

In the course of recent borings in interglacial freshwater deposits carried out by the Geological Survey of Denmark a gyttja deposit of a remarkably green colour was encountered near Rodebaek in westernmost Jutland. As a preliminary examination revealed that chlorophyll might be responsible for the colour a special investigation of this problem was made.

Origin and Age of the Gyttja. The interglacial deposit Rodebaek I was originally investigated by JESSEN and MILTHERS[1]; the following is a brief summary of their description. The lake deposits were formed in a kettlehole in boulder clay belonging to the penultimate glaciation (Saale-Riss) approximately 125 m across and up to 12 m deep. The general sequence in the deepest part of the basin is,

0 – 0·7 m	Postglacial peat
0·7– 4·7 m	Deposits belonging to the last glacial period (under reinvestigation)
4·7– 5·2 m	Interglacial *Sphagnum* peat
5·2– 6·5 m	Interglacial forest peat
6·5– 7·1 m	Interglacial coarse detritus mud
7·1–10·5 m	Interglacial greenish mud (the stratum under consideration here)
10·5–	Boulder clay.

The deposit was not reached by the last glaciation and is up to the present day essentially undisturbed.

Pollen analysis of the samples for the present investigation show that they belong to the lower part of pollen zone *f* by JESSEN and MILTHERS, which represents the climatic optium of the interglacial period. They consist of a very fine-grained, slightly sandy detritus gyttja. Chemical analysis gave 44·6% loss on ignition, 40·9% Fe_2O_3, 5·0% Al_2O_3, 0·2% Mn, 8·4% silicates, and traces of CaO. The iron has been present to a large degree as ferrous carbonate[2]. Dry matter content was 60·4%, and pH 6·3–6·8. Macroscopical plant remains are very scarce; aquatics identified from this stratum by JESSEN and MILTHERS are: *Batrachium aquatile* L., *Ceratophyllum demersum* L. var. *apiculatum* Cham., *C. submersum* L., *Najas marina* L., *Nuphar luteum* L., *Nymphaea alba* L., and *Potamogeton natans* L. Microscopical examination of unprepared material revealed that the gyttja consists of a structureless organic mass in which only a few pollen grains and diatom shells can be distinguished. In a sample treated with H_2O_2 the following diatoms were the most common: *Cymbella lanceolata* (Ehr.) Van Heurck, *Epithemia turgida* (Ehr.) Kütz., *Gomphonema acuminatum* Ehr., *G. constrictum* Ehr., *Gyrosigma attenuatum* (Kütz.) Rabh., *Navicula placentula* (Ehr.) Grun., *Pinnularia gibba* Ehr., *Synedra ulna* (Nitzsch.) Ehr. together with a number of resting spores of Chrysomonadales and sponge spicules. It is evident that the bulk of the organic matter was formed by a planctonic algal community, the remains of which was not preserved during the fossilization process.

The age in years of the last interglacial period has been estimated by various methods. ZEUNER[3] gives the figures 180,000–120,000 years based on MILANKOVIC's solar radiation curve. Its applicability has been questioned *i.a.* by WOLDSTEDT[1]. FLINT[2] gives a tentative estimation of 210,000–100,000 years, but in view of recent C^{14} dates these figures are certainly too large. ARRHENIUS[3], finally, gives the figures 160,000–70,000 years but there is at the present little agreement as to how the material should be interpreted, cp. WISEMAN[4]. On the whole, the subject is not quite clear, but an estimated age of about 100,000 years of the Rodebaek gyttja samples cannot be entirely wrong.

Methods. In the field the samples were immediately transferred into a thermos-flask. Parts were also stored in dark glass jars. Later comparison of gyttja stored in this way did not differ in pigment composition from gyttja stored at original temperature. The colour of freshly collected gyttja was dark moss-green. On storage the gyttja became brownish on parts exposed to air.

5 g of non-oxidized sample was shaken in a flask containing glass beads with 5 ml of ethyl ether and filtered through Jena glass-filter 3G3. A clear yellowish extract was obtained which was concentrated *in vacuo* to approximately half volume. Chromatograms were developed on Whatman paper no. 1. The method employed is described by SIRONVAL[5] and is a modification of BAUER's[6] method for chromatography of chlorophylls. The developer consists of benzene : petrolether : acetone 100 : 25 : 20. Chromatograms were run descending in dark 70 cm high glass chambers in an atmosphere of petrolether at a constant temperature of 15°C. After satisfactory separation of the pigments the paper strips were examined partly in daylight, partly under an ultra-violet lamp for determination of fluorescence. Fluorescence spectra were not determined. Visible and fluorescent spots on the chromatograms were separated and transferred to test tubes and a few ml of acetone added. The test tubes were kept in the refrigerator overnight before absorption measurements. These were made in a Beckman quartz spectrophotometer model DU using 10 mm glass cuvettes. Readings were made with 5 mμ intervals between 325 and 800 mμ.

Results. Three types of pigments were found on chromatograms prepared from ether extracts from different levels of the gyttja layer. These are divided according to their colour into green, yellow and red pigments. Figure 1 shows characteristic results of the chromatography. There are five green spots (G_{1-5}), four yellow (Y_{1-4}), and two red (R_{1-2}). In some samples different other ether-soluble pigments were present, but usually these were barely recognizable. The material presented in this report only comprises pigments that have been found repeatedly and being so strong that subsequent reextraction from the chromatograms for measurements of absorption spectra was successful. Minute quantities of non detectable pigments very probably act as contaminants in the detectable pigments; this should be kept in mind in the comparative study of absorption curves. Connecting the starting point and G_3 (see Fig. 1) a faint green longitudinal band with red fluorescence was always seen, but the spots G_1 and G_2 were distinct spots on this background. G_3 was, however, only

[1] K. JESSEN and V. MILTHERS, Danmarks Geologiske Undersögelse, II raekke, *48*, p. 1–379 (1928).

[2] Analysis by K. SKOUSBÖLL-HANSEN, *Geological Survey of Denmark*.

[3] F. ZEUNER, *Dating the Past* (Methuen & Co. Ltd., London, 1946).

[1] P. WOLDSTEDT, *Das Eiszeitalter I*, 2. Aufl. (Ferdinand Enke Verlag, Stuttgart, 1954).

[2] R. F. FLINT, *Glacial Geology and the Pleistocene Epoch* (New York: John Wiley & Sons Inc., London: Chapman & Hall, Ltd. 1947).

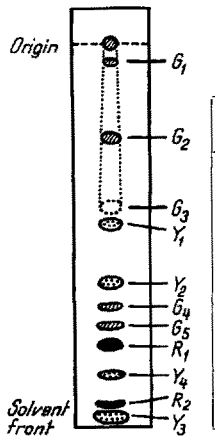
[3] G. ARRHENIUS, Rep. Swedish Deep-Sea Exped. 1947–48 *5*, fasc. 1, 187 (1952).

[4] J. D. H. WISEMAN, Proc. roy. Soc. [A] *222*, 296 (1954).

[5] C. SIRONVAL, Bull. Soc. roy. Bot. Belg. *85*, 285 (1953).

[6] L. BAUER, Naturwissenschaften *39*, 88 (1952).

recognized as a separate spot on its strong fluorescence. All other spots by the chromatograms were well separated and were recognized by colour and fluorescence (R_1 showed no fluorescence). R_f values and relative strength of the pigments in visible and ultra-violet radiation will be found in Figure 1.



	R_f	Visual light		Ultra-violet radiation	
		Colour	Strength	Fluorescence	Strength
G_1	0·06	green	+++	grey-red	++
G_2	0·26	green	++ -	red	++
G_3	0·44	—	—	red	+++
Y_1	0·49	yellow	+ +	grey-red	+
Y_2	0·64	yellow	++	grey-red	+
G_4	0·70	green	+	red	+++
G_5	0·75	green	+	red	+++
R_1	0·81	pink	++	—	—
Y_4	0·88	yellow	+	grey-red	++
R_2	0·96	pink	++	grey-red	+
Y_3	0·99	yellow-orange	++++	grey-red	+++

Fig. 1.–Chromatogram of ether-soluble pigments from gyttja.

Green pigments. The five green pigments isolated seem to be closely related. They all have their blue maxima in the 410–420 mμ region and their red maxima between 670 and 680 mμ (Fig. 2 and 3). However, the ratio max. blue/max. red differs considerably from compound to compound: $G_1 = 4{\cdot}3$, $G_2 = 3{\cdot}7$, $G_3 = 4{\cdot}0$, $G_4 = 2{\cdot}7$ and $G_5 = 2{\cdot}3$. Although it seems difficult to identify the pigments in comparing data from known pigments isolated from different sources, cp. RABINOWITCH[1], it could be stated that the five green compounds are derivates or degradation products of chlorophylls originating mainly from phytoplancton which populated the interglacial Rodebaek lake about a hundred thousand years ago. It is astonishing that the pigments have been so well preserved as the samples examined under the microscope were almost completely without cell structure. But as the cells from the photosynthetic layer of the water settle to form the bottom sediment both the amount of light and oxygen decrease considerably, and also the temperature will be low and constant in the bottom sediment. Conditions for autocatabolism have thus been unfavourable, and degradation of the pigments by anaerobic microorganisms apparently does not take place.

The green pigments, except G_1, all showed a bright red fluorescence of high intensity when the chromatograms were examined in ultra-violet radiation. G_1 showed a type of fluorescence which is also characteristic for chlorophyll *b* when examined on paper after chromatographic separation. Only the periphery of the spot appears bright red, whereas the centre could be described as being red with a heavy grey shade covering the colour.

Yellow pigments. The absorption curves of the yellow pigments Y_1, Y_2 and Y_4 indicate that fucoxanthol and luteol or related compounds are present (Fig. 4). The bulk absorption region of these compounds is between 400 and 500 mμ with three characteristic peaks, the position of which depends on the solvent used, cp. KARRER and JUCKER[2]. The fourth yellow pigment Y_3, on the chromatogram situated near the solvent front, seems to be a mixture of compounds mainly consisting of

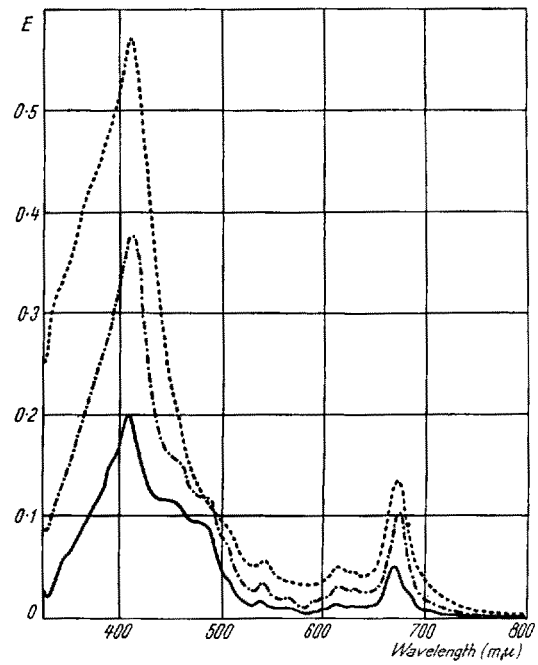


Fig. 2.–Green pigments (1).
Absorption spectra in acetone. ····· G_1; —·—·— G_2; ——— G_3.

β-carotene. The further separation of these pigments by means of paper chromatography was not successful.

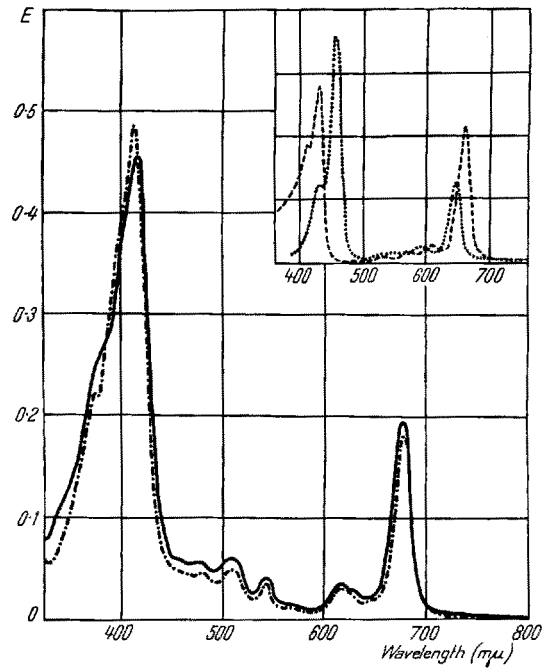


Fig. 3.–Green pigments (2).
Absorption spectra in acetone. —·—·— G_4; ——— G_5. Inserted absorption spectra in acetone of chlorophyll *a* (— — —) and chlorophyll *b* (· · · · ·).

Red pigments. The spots R_1 and R_2 in normal light appear reddish or pink, and the presence of phycobilins was therefore suspected. Phycobilin pigments, however,

[1] E. RABINOWITCH, *Photosynthesis*, Vol. II/1 (Interscience, New York, 1951).

[2] P. KARRER and E. JUCKER, *Carotinoide* (Birkhäuser Verlag Basel, 1948).

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

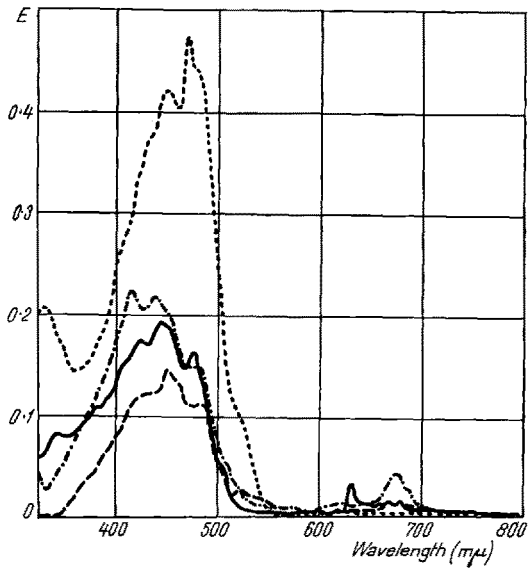


Fig. 4.–Yellow pigments.
Absorption spectra in acetone. ——— Y_1; —·— Y_2; ····· Y_3; —— Y_4.

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

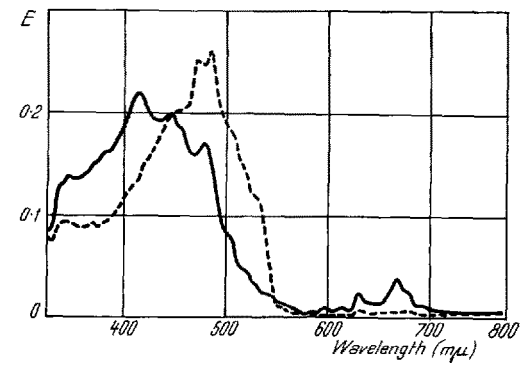


Fig. 5.–Red pigments.
Absorption spectra in acetone. —— R_1; ——— R_2.

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.

S. TH. ANDERSEN and K. GUNDERSEN

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

[1] H. D. DAKIN, J. Biol. Chem. *1*, 171 (1905). – C. NEUBERG, Biochem. Z. *20*, 531 (1909). – H. WIELAND and W. FRANKE, Ann. Chem. *457*, 1 (1927).

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