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AUTOFLUORESCENCE OF FOSSIL POLLEN AND SPORES
WITH SPECIAL REFERENCE TO AGE DETERMINATION AND COALIFICATION

BY

PIETER VAN GIJZEL

PHOTOPLATE I

Fluorescence colours of fossil pollen grains, spores and Foraminifera (after van Gijzel, 1966, negatives by courtesy of Leitz, G.m.b.H., Wetzlar, Germany):

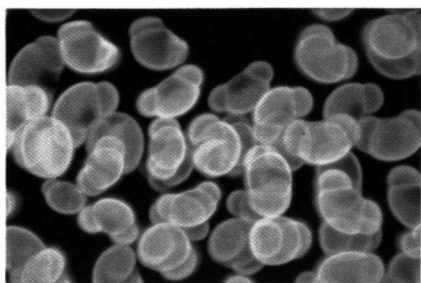


Fig. 1. Recent pollen grains of *Pinus sylvestris*; protoplasm absent from the blue grains and partly so from the white ones.

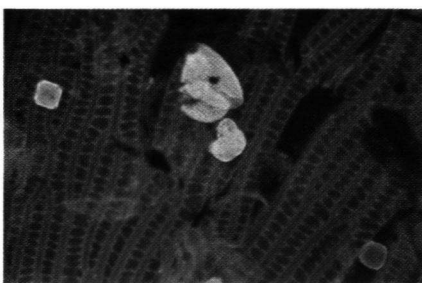


Fig. 2. Cells of a *Sphagnum* leaf and pollen grains of *Picea* (greenish-yellow), Ericaceae (yellow) and *Alnus* (light yellow and orange).

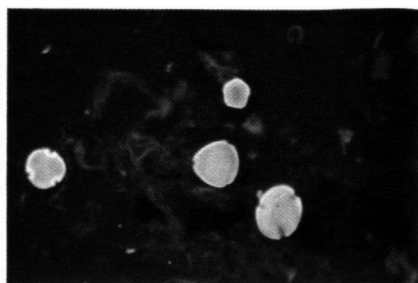


Fig. 3. Pollen grains of *Fagus* (orange- and greenish-yellow), *Alnus* (yellowish white) and *Tilia* (white).

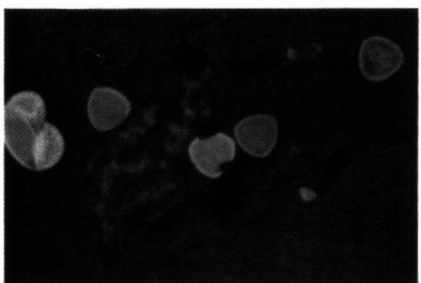


Fig. 4. Pollen grain of *Pinus sylvestris* (dark green) and *Sphagnum* spores (blue and dark orange). (fig. 2—4: preparation of young *Sphagnum* peat from Ekamp, N. Netherlands).

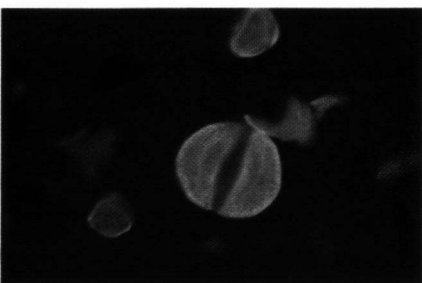


Fig. 5. Pollen of *Pinus haploxylon*-type (green) and Myricaceae (greenish-yellow) from humic clay of Puentes, Spain. Age: boundary Oligo-Miocene.



Fig. 6. Bisaccate pollen grain from Bathonian clay of Le West, France.

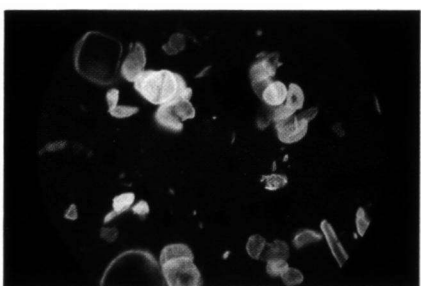


Fig. 7. Some autochthonous pollen grains of *Pinus sylvestris* (bright white) with rebedded Tertiary pollen of *Pinus sylvestris*, *Pinus haploxylon*-type and other forms (dark brownish yellow) from the Elsterian banded clay of Glindow near Berlin, Germany. Age: Pleistocene.

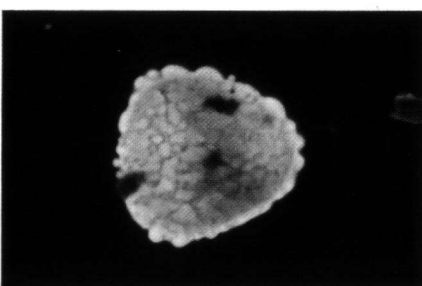


Fig. 8. *Cingulatisporites marxheimensis* (orange yellow) from Palaeocene clay of Elmpt, S. Netherlands, slightly coalified.



Fig. 9. *Pseudofusulina*, a large foraminifer from limestone of the Isle of Timor, Indonesia. Age: Permian.

Magnification: fig. 1—5, 7: objective 25x; figs. 6, 8: obj. 40x Apo; fig 9: Photar 63 mm. Darkfield condensor. Colourfilm: Kodak Ektachrome, H. S., Daylight-type. Leitz Ortholux fluorescence microscope with Orthomat microscope camera.

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ABSTRACT

In the present study the primary fluorescence phenomena of fossil pollen and spores are described. This new method in palynology is based on a large number of fluorescence microscopical observations and spectrophotometrical determinations of palynomorphs from deposits of various type and age. It resulted in three principles: the relationship between fluorescence colour to type or form of pollen and spores (Plate I and figs. 21—22), the change in their fluorescence colour from blue or green to red or brown with increasing geological age (Plate II, III and fig. 24) and a similar colour change with increasing rank of coal of the embedding deposits (fig. 33). These phenomena appear to be in accordance with other fossilization and coalification studies of fossil palynomorphs by various authors.

For the preparation of pollen samples and the microscopical determination of fluorescence colours some special techniques have been adapted or developed.

The discoveries of fluorescence palynology can be applied to various questions, as, for instance, the study of pollen morphology and corrosion susceptibility and the age determination of those deposits, for which conventional pollen analysis fails. Such datings of Cenozoic rocks can be carried out with an accuracy of more than 80 %. As an example a number of age determinations of contaminated sediments is given (Plate V). Besides, fluorescence palynology may be used to determine the rank of coal of palynomorphs in coalified rocks in that part of the coalification series, ranging up to a fixed carbon content of about 70 %.

The explanation of the fluorescence phenomena described, meets still great difficulties, due to the inadequate knowledge of the chemical nature of the walls of fossil pollen and spores. Once again it is proved by this study that fossil palynomorphs are less resistant to fossilization and coalification than has been previously assumed.

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I. INTRODUCTION

PURPOSE OF THE INVESTIGATION

The subject of this study concerns the phenomena of a little known property of fossil pollen and spores: viz. the fluorescence, which is closely related to the physical and chemical nature of their walls, and this can be applied to the study of some palynological problems. The themes developed in this paper are: what happened to these microfossils after their deposition, how does fluorescence change in geological time and how can these changes be applied in palynology?

After burial in the soil, exines and exospores were involved in the sequence of processes of humification, fossilization, biochemical and often of geochemical coalification. The great majority of remains of pollen and spores is very well preserved in deposits under anaerobic conditions and only those with a very weak exine will disappear.

Their resistance to these processes makes possible the investigation of vegetational history and stratigraphy. The pollen-containing deposits may be correlated with the stratigraphy of similar deposits elsewhere,

in combination with other geological investigations. In general it may be assumed that the vegetation of a sedimentation area is reflected in the pollen content of a deposit, if the circumstances were favourable for their fossilization. The vegetational changes will then be reflected in the changing pollen composition by which zones in a pollen diagram can be distinguished. Palynological age determinations and correlations are based mainly on these zonations and on the climatological and stratigraphical evidence of the microscopical plant remains.

Unfortunately, it is not always so easy practice as one might think. Every palynologist knows from his own experience that in the diagrams the zonation may be absent or poorly developed, due to various factors.

In the first place, contamination with allochthonous or older pollen grains may occur during sedimentation, which results in an incorrect age determination. The dominance of Tertiary pollen associations in Pleistocene clays in the N. Netherlands is an extreme example of this sort of contamination. The clays then seem to

be Tertiary in age owing to this outnumbering of the autochthonous components.

The facies of a sediment is an important factor. In the pollen rain the autochthonous pollen can predominate over that of the surrounding area. Changes in the latter will not be expressed then in the pollen content of the samples. This phenomenon appears repeatedly in lignites, in which the pollen from the dense marsh forests is predominant. Only in sediments deposited in open water, the pollen from vegetations occurring at higher altitudes is present in such quantities, that stratigraphical conclusions can be drawn.

Other factors to be taken in account are climatological differences. The vegetational changes during the Pleistocene of temperate areas, e.g. N.W. Europe, are typical and may be compared with other paleoclimatological data. The Pleistocene vegetational history of Spain (Menéndez Amor and Florschütz, 1962, 1964) for instance and that of Colombia (van der Hammen and Gonzalez, 1964) may also be compared with paleotemperature curves of sea water. Such a comparison still meets with difficulties (van Gijzel et al., 1967). C 14 datings can only be carried out on the uppermost part of the sequence of Pleistocene layers; consequently, a correlation with the Middle and Lower Pleistocene subdivisions of various regions is hardly possible (Chapter VI).

Moreover, pollen and spores may be selected by corrosion, as a result of the oxidation and biochemical activity in the soil during and after the sedimentation. This corrosion mainly occurs in coarse deposits such as sands and humic sandy soils under dry conditions. For this reason the older pollen zones may not be represented in the pollen diagrams of such deposits (Havinga, 1962; Chapter VIII).

Some other problems of age determination, in connection with pollen analyses, arose. For deposits which are younger than 50,000 years and without a distinct palynological character, radiocarbon datings can lead to correlation, but dating and correlation of older deposits still meet with great difficulties (Chapter V). In the present publication these questions are investigated from a new point of view, i.e. the phenomenon of fluorescence of pollen and spores and its changes during geological time.

PRINCIPLES OF FLUORESCENCE PALYNOLOGY

Some years ago, the present author tried to find a means to distinguish autochthonous and secondary pollen for dating contaminated sediments in the N. Netherlands. Fluorescence-microscopical techniques proved to be very useful in solving this problem. Fossil exines observed under UV-light were found to show more or less brilliant fluorescence colours.

In some preliminary notes the outlines of the phenomena were described and the application of fluorescence microscopy to palynology was summarized (van Gijzel, 1961, 1963, 1966). It was then stated that three principles are to be distinguished. (1) Fresh and subfossil pollen grains and spores show various

fluorescence colours, dependent on type or species (Chapter IV), (2) differences in colour appear in each pollen type at different ages (Chapter V) and (3) a relation exists between the fluorescence and the rank of coal in coalified rocks (Chapter VII).

All these phenomena are closely related to the chemical character of the pollen and spore walls, which is still poorly understood (Chapter VIII).

It soon became apparent to the author that this technique could be applied extensively in palynology, on the condition, however, that the observed colours are registered objectively by means of a method, which is verified with a standard in order to make analyses reproducible. A method was required therefore, which was sensitive and accurate enough to enable measurements of such small objects as fluorescing pollen grains (Chapter II).

Besides it appeared that the preparation techniques, used in the laboratory to separate the pollen and spores from other sedimentary constituents, are very important for fluorescence microscopy. The use of acids influences the fluorescence colours harmfully and is therefore to be avoided (Chapter III).

In this study, dealing with the most important aspects of fluorescence palynology, much attention is given to the description of the colours at various geological ages, from which the relations of fluorescence palynology are derived (Chapter IV and V). The applications of this phenomenon to the afore-mentioned palynological and chronostratigraphical problems are extensively investigated (Chapter V—VI), but the use of fluorescence in pollen morphological studies had to be left out of consideration. For a historical review of fluorescence palynology one should refer to van Gijzel (1967 a).

The localities of the rock samples, analysed for this study, are shown in the map of fig. 10. The material is partly registered in the collection of the State Museum for Geology and Mineralogy, Leiden (see Appendix).

In the course of the investigations it became clear that the causes of the observed phenomena could not be easily explained. The complex chemical nature of the sporopollenine, the main constituent of the fossil spore- and pollen-walls, is very difficult to examine and it was only possible to present some hypotheses as to the causes (Chapter VIII).

Nevertheless, it may be possible that this study will be a stimulus to the continued application of fluorescence techniques in palynology and other geological studies, for instance, to micropaleontology (van Gijzel, 1966). But most of all it may be a contribution to a better knowledge of the processes, determining the preservation of pollen and spores, in connection with palynological practice. Still, comparatively little is known about its theoretical basis. Although fluorescence is a fascinating subject, it is not only the more theoretical aspects but the practical applications that should be considered. In the first place fluorescence is geologically and palynologically important. In the present study, therefore, more emphasis is laid on the

application of these phenomena than on the theory behind it.

PREVIOUS INVESTIGATIONS

Till now the fluorescence of pollen and spores had seldom been studied, although many investigations on the fluorescence of other plant substances have been published.

The first, who observed fresh pollen under UV-light,

between fluorescence and the fossil pollen types, as investigated by the present author, is in clear accordance with a similar relationship among fresh pollen as found by Berger.

Some time later, Asbeck (1955) studied fresh pollen under UV-Light with other purposes in mind. It appeared to him that the exine screens the very sensible chromosomes in the pollen cell against the UV-light of the sun. He noticed that some transparent

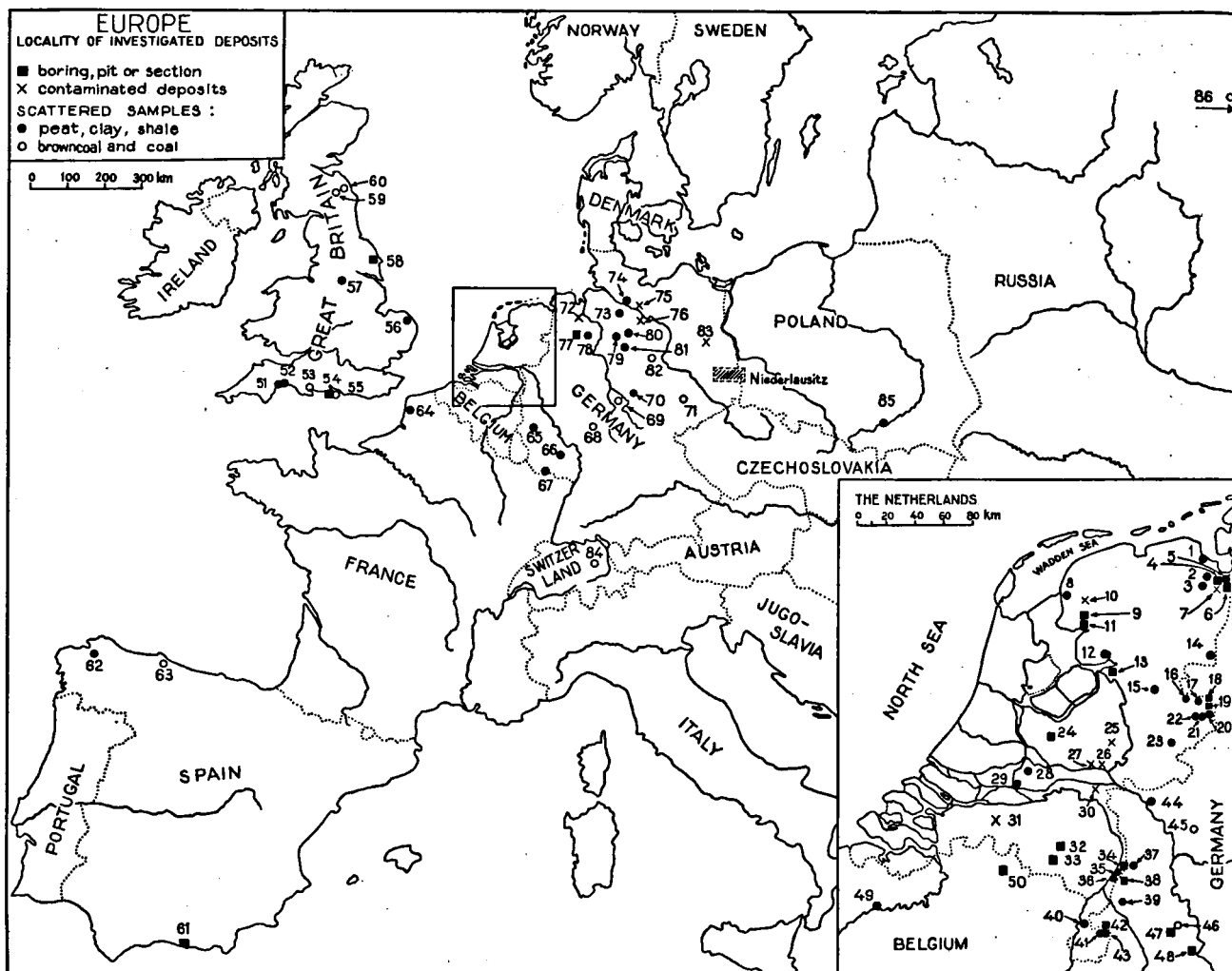


Fig. 10. The numbers correspond with the localities in the tables of samples (see Appendix).

was Berger (1934). He investigated various pollen grains in connection with hay-fever and stated that they show characteristic fluorescence colours, which are more or less constant and typical for every type. However, these analyses concerned living pollen only, which still contains protoplasm, the bright fluorescence of which is dominant over that of the exine. Consequently, the observed colours were of mixed origin and his descriptions cannot be applied to fossil material, as the intine disappears during fossilization and the exine only is fluorescent. The relationship

pollen species, showing a white colour in daylight, are fluorescent in ultra-violet radiation and that those of other colours often do not fluoresce. However, neither Berger, nor Asbeck observed the fluorescence of the pure exine.

Sitte (1960) noticed the differences in fluorescence of the fresh exines of some species and found that a bright blue colour of *Selaginella* spores is changed into yellow-brown by acetolysis. In accordance with this statement is the fact, that the use of acids in the preparation of pollen slides has a similar effect (Chapter III).

As the exine of fresh pollen is fluorescent, one would expect fossil pollen grains to possess the same property. Only a few investigators have observed this phenomenon in fossil remains.

The first study of fossil pollen and spores under the UV-microscope was made by Maier and Wetzel (1958). They discovered the luminescence of some microfossils such as *Hystriosphera* (acritarchs) and other organic remains, but found no indication of the fluorescence of pollen and spores. They later concluded that, in general, fossil animal remains show a clear luminescence, while plant substances only possess this property in the fresh state and lose their luminescence when they are fossilized (Wetzel, 1959; Maier, 1959).

As is apparent from the author's present and earlier investigations, there is no doubt that fossil pollen and spores are fluorescent in UV-light (van Gijzel, 1961, 1963), with the exception of those from highly coalified deposits. This phenomenon even occurs in similar deposits and of the same (Tertiary and Mesozoic) ages as those from which the samples of Wetzel and Maier originated. The absence of fluorescence in the objects, which they observed, was probably caused by the preparation of the samples in HF and by oxidation — these two being the greatest enemies of fluorescence — and partly by the use of an embedding medium, being self-fluorescent to such an extent that it prevented the observation of the weak fluorescence of the pollen. Furthermore, one cannot agree with the opinion of Wetzel and Maier that fossil plant and animal remains can be distinguished on the basis of luminescence. The present author has found that dinoflagellates, considered non-luminescent by Wetzel and Maier, show bright green or yellow fluorescence colours. Other fossil plant remains of various origins are fluorescent as well: for instance *Pediastrum*, Fungi, wood, the massulae of *Azolla* species, etc., and even those of pre-Quaternary age (van Gijzel, 1963). The luminescence of fossil Algae, moreover, has been found by Jacob (1961a, 1961b) in some clayey bituminous rocks and by Wolf (1966) in boghead coal. The fluorescence intensities of all these plant remains are in most cases no less than those of the bright green-coloured acritarchs, which should belong in the opinion of Wetzel and Maier to the animal kingdom (see Chapter V).

In the papers of some other investigators, the fluorescence of fossil pollen in lignite samples has been mentioned and illustrated, but without closer examination (see among others: Jacob, 1961a; Eder and Fritsche, 1963).

Shellhorn et al. (1964) were the first to use fluorochrome (acridine orange) in the search for fresh and fossil pollen in soils and deeper layers, but they restricted their paper mainly to a description of the methods used.

THE PRINCIPLE OF FLUORESCENCE

Fluorescence is the property of matter to emit light under the influence of an exciting light (visible or

U.V.). The emitted light is, in general, of longer wave-length than the exciting light. If the fluorescence glow persists for an appreciable time after the stimulating rays have been cut off, this afterglow is termed phosphorescence. Both phenomena are termed luminescence. The use of the latter term for the fluorescence phenomena by many authors is incorrect and gives rise to confusion.

The present study concerns fluorescence only, phosphorescence being left out of consideration as it has not been observed in fossil pollen and spores.

The fluorescence emission spectrum of a specimen is the sum of fluorescence spectra of atoms and molecules.

Two kinds of visible fluorescence are distinguishable: *autofluorescence* or *primary fluorescence*, which is a property of the substance itself and is not activated by staining, and *secondary fluorescence*, excited by microchemical reactions, caused by staining liquids (known as fluorochromes) such as eosin, fuchsin, fluorescein and many others, for which various elements, moreover, show a specific affinity.

Autofluorescence may show splendid colours and has appeared to be the most important for age determination. Investigations on the secondary fluorescence of fossil pollen and spores are in progress and will not be dealt with here.

The spectral distribution of fluorescent substances may range from ultra-violet into infra-red (see among others: Goodwin, 1953; Krieg, 1955). Of the absorbed UV-energy, most is dissipated as heat and a part is emitted in the visible region of the spectrum. The fluorescent light of fossil pollen and spores is also polychromatic. The absorbed UV-light is mainly derived from the Hg-band at 365.5 nm and, to a much lesser extent, from the faint bands at 313 and 334 nm (see Chapter II, fig. 13).

Fluorescence in UV-light has been applied on a large scale in chemistry and technical sciences for analysing certain minerals, organic and inorganic chemicals, metal-organic compounds, etc. Many substances emit a typical fluorescence, which provides an effective method for quantitative and qualitative analyses, even in extremely small concentrations.

Fluorescence is, moreover, an important property of substances in living organisms, such as in plants and the human body. The various colours of organs and tissues are usually produced by an assortment of compounds. Different cell structures in plants show characteristic fluorescence colours, depending on their chemical nature, e.g. cuticle whitish, epidermis blue, phloem bundles blue, xylem violet or blue-green, chloroplasts red, etc.

It is remarkable that fossil plant substances often show fluorescence. The majority of the tissues left in Young *Sphagnum* Peat are fluorescent, the cell-walls of *Sphagnum* leaves themselves showing faint blue or green colours (Photoplate I: fig. 2). In older deposits this fluorescence is hardly visible and only the palynomorphs and some other remains continue to fluoresce. Fluorescence is often the key to the isolation and

identification of many biochemical constituents by means of special techniques (Goodwin, 1953). The phenomenon of fluorescence has been applied successfully in physiological studies. For the application of the techniques and microscopy of fluorescence, reference should be made to the following studies, dealing with various aspects of the application to investigations of organic matter: Haitinger (1938), Bukatsch (1914), Bräutigam and Grabner (1949), Goodwin (1953), Gottschewski (1954), Clark (1961) and many others.

USE OF FLUORESCENCE MICROSCOPY IN GEOLOGY

In the past the use of fluorescence in earth sciences has been restricted to certain fields only. Prziham (1962) used fluorescence for the identification and the study of the distribution of organic remains in natural inorganic material. It has been applied for instance, in geochemistry, to the fluorescence analysis of hydrocarbon combinations in connection with the migration of petroleum and it has been used in the study of the origin of oil by means of the fluorescence

microscopy of bituminous rocks (Jacob, 1961b). Investigations of facies and diagenesis of organic deposits under the UV-microscope were made by Wetzel (1939, 1959, 1962), Maier (1959) and Haberlandt (1942). Overbeck (1964) investigated the fluorescence phenomena of plant fossils and molluscs, in particular those of skeletal substances which are found in animal fossils only.

Sacchi Viali (1962, 1964) investigated the fluorescence of vertebrate teeth and some other macrofossils and this opened up a new field in the study of palaeobiochemistry.

In mineralogy, luminescence has proved to be a useful property for the detection and identification of minerals and gemstones (see among others: Gleason, 1960). They often show brilliant fluorescence colours: adamite, green; calcite, red, white and yellow; scheelite, bluish white; wollastonite, orange; uranium minerals, green; fluorite, blue etc.

It is, therefore, not surprising that microfossils with tests, consisting of minerals, such as Foraminifera and Bryozoa, appear to be fluorescent as well. Their colours may be analysed by means of the fluorescence photometrical methods, described in the next chapter (van Gijzel, 1963, 1966).

II. MICROSCOPICAL EQUIPMENTS FOR FLUORESCENCE ANALYSIS

THE BEREK FLUORESCENCE PHOTOMETER

Principle and equipment

The fluorescence colours, described in this study have been determined mainly by means of the Leitz fluorescence microscope in combination with an adapted Berek photometer as applied by Ruch to investigate the fluorescence of cytochemical substances (Ruch and Bosshard, 1963). It appeared to be very useful as well for fluorescence measurements of microfossils (van Gijzel, 1966).

This method is based on visual observations, therefore it is suitable only for the visible part of the spectrum. The human eye is very sensitive for differences in light intensities and is even able to compare minute light sources. This property depends only on the sensitivity of the eye at different wave-lengths (reaching its maximum in the yellow-orange).

It allows to compare the faint light of an object to a standard source, both observed at the same colour by means of filters.

The Berek-photometrical equipment used for the fluorescence palynology is mainly identical to that described by Ruch and Bosshard (1963), but has slightly been modified for this purpose. The Berek photometer is placed on top of the phototube of a Leitz Ortholux fluorescence microscope (fig. 11).

The high-pressure mercury vapor lamp (Philips CS 150) is used as UV-light source. The "long-wave" UV-light is split up by a special mirror into an exciting

and a reference beam, the latter reaching only 20 % of the total radiation. The exciting beam passes through the base of the microscope and excites the fluorescence of the object, of which the emitted light is led into the photometer. The reference (secondary) beam radiates a thin plate of uranglass, which is placed in the narrow vertical tube, entering the photometer from behind. The fluorescence of this uranglass forms the standard. The optical paths are sketched in fig. 12.

The excitation and barrier filters used in the microscope, are very important for the exact observation and determination of fluorescence colours. Their characteristics are summarized schematically in fig. 13. Pollen and spores show more difference in fluorescence under UV-excitation (in german: "UV-Anregung") than under blue excitation ("Blau-Anregung"), which is confirmed by microspectrographical analyses. Therefore the combination of blue-excitation and barrier filters has not been used for the present study. The filters of the photometer (fig. 14) have another function: they are used for measuring the fluorescence intensities. Their bandwidth in wave-length ("half-value-width" HW) and transmission (τ) are shown in fig. 14, and table I. These values are taken into account at the calculations of the spectra.

UV-spectroscopy makes special demands with regard to the optical equipment of the microscope. Only objectives with a high numeric aperture, which are corrected for spherical and chromatical aberrations

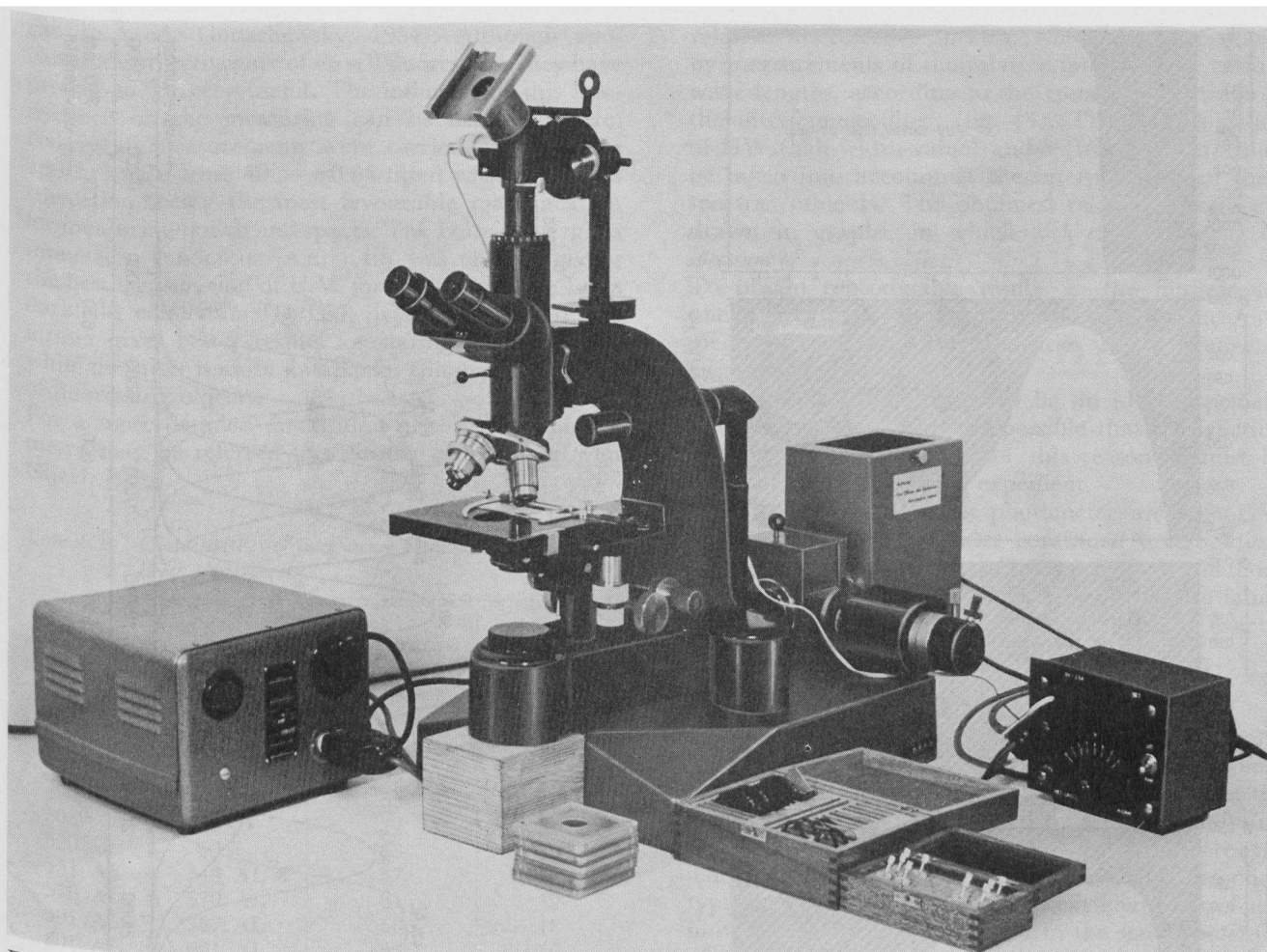


Fig. 11. Leitz Ortholux fluorescence microscope with the Berek photometer and a reference beam for fluorescence photometry.

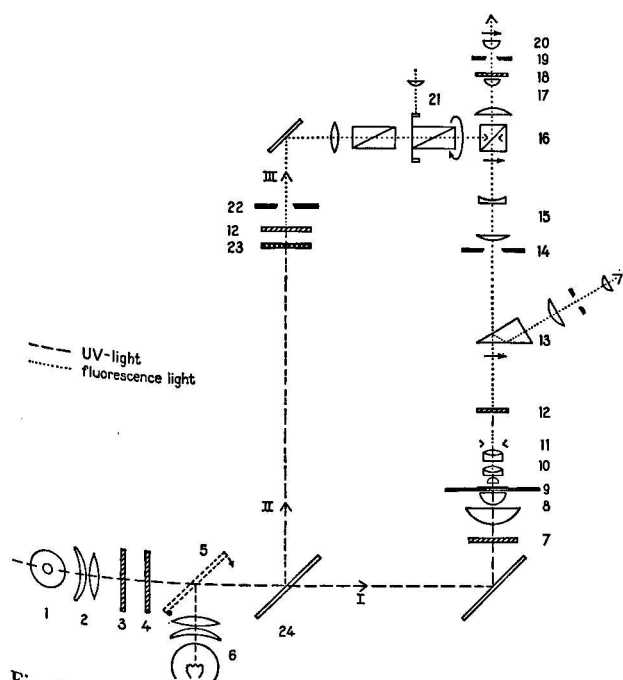


Fig. 12. Scheme of optical system of the Leitz Ortholux fluorescence microscope with Berek photometer.

1—13: Fluorescence microscope,
14—24: photometer with accessories.

1. high pressure mercury-vapor lamp (Philips CS 150, fig. 13). 2. collector. 3. heat barrier filter KG 2. 4. excitation filter UG 1, 4 mm. 5. removable mirror for illumination with (6) low voltage lamp. 7. red barrier filter BG 38 (fig. 13). 8. quartzglass condensor. 9. specimen. 10. objective with (11) upper focal plane. 12. UV-barrier filter UV-abs. 13. binocular observation with a removable prism. 14. fixed measuring diaphragm in the real image. 15. lense system to project the upper focal plane of the objective in the (16) cubic prism of the photometer entrance tube. 17. divided photometer ocular. 18. spectral filters (fig. 14). 19. image of eye-piece diaphragm (14) in the eye-point. 20. removable lense for adjustment of the object. 21. polarization prisms for measuring, one prism is rotatable and provided with a nonius division for reading. 22. iris diaphragm. 23. fluorescing uranglass (2 mm) producing the standard beam (III). 24. special mirror separating the reference UV-beam (II) from the exciting beam (I).

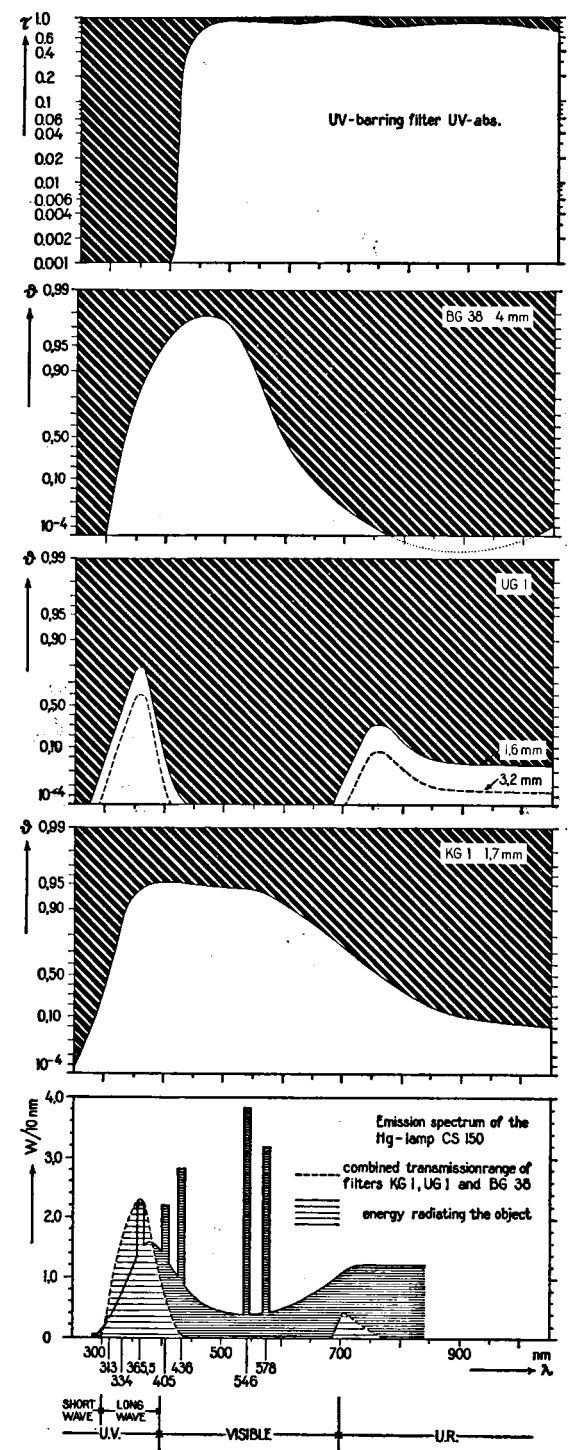


Fig. 13. Schematic picture of filters used for UV-light fluorescence microscopy.
 v = true transmission degree λ = wave-length in nm
 τ = transmittancy nm = nanometer (1 nm = 10^{-6} mm)
Curves of the filters according to Schotts' "Filterbuch".

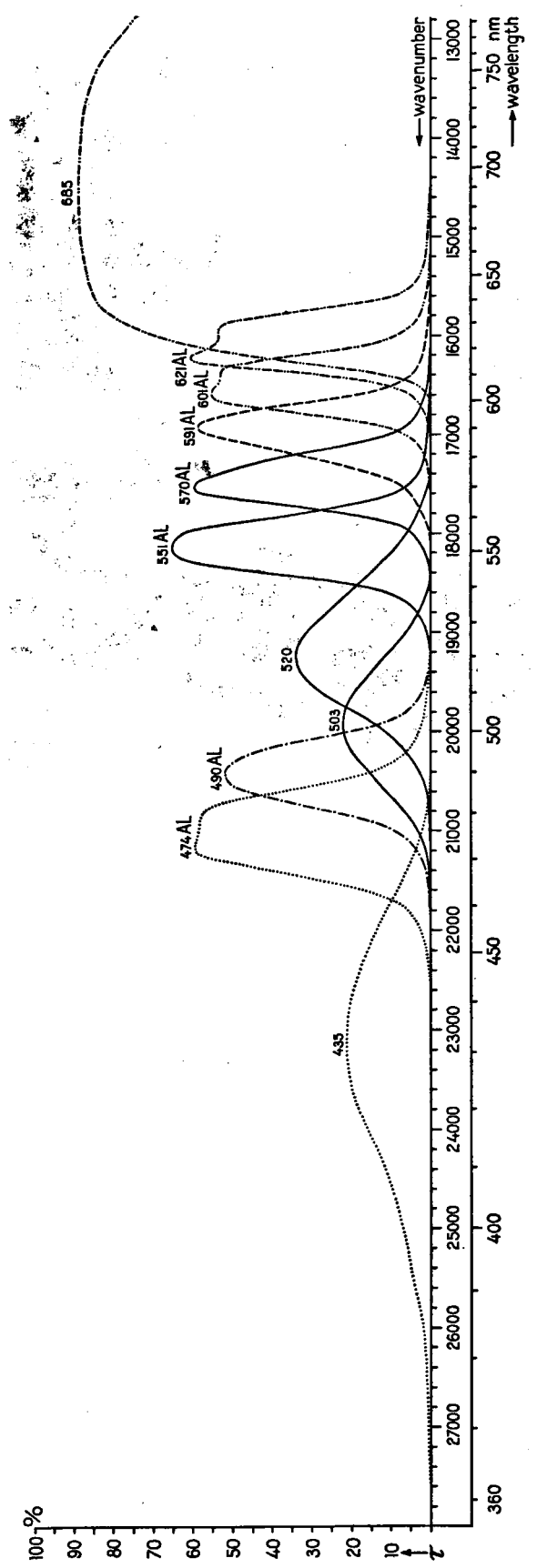


Fig. 14. Transmission of interference filters at various wavelengths, used for measurements by means of the Berek photometer.

can be used (Gottschewsky, 1954). Although apochromate objectives are often self fluorescent, they have proved to be very useful. The influence of this fluorescence on the measuring can be neglected here. Nearly all measurements were carried out with the apochromate lense $40\times / 0.95$ fitted with coverglass correction, being the most favourable magnification for measuring pollen and spores. The Leitz quartzglass immersion condensor (n.a. 1.40) was chosen, giving the best transmission of U.V. for the object. The Leitz darkfield condensor (D 0.80) is as useful, but the former gives better results for stating a colourscale, while the latter is more suitable for colourphotography of fluorescing objects.

For a more detailed description of the Berek photometry may be referred to a former publication (van Gijzel, 1966).

TABLE I. Characteristics of interference filters used in the Berek photometer

| λ in the graphs | fabr. nr. | max. trans- mission in % | Hw in nm |
|-------------------------|-----------|-----------------------------|----------|
| 435 | 45 | 21 | 50 |
| 474 AL | 474 AL | 58 | 21 |
| 490 AL | 488 AL | 53 | 18 |
| 503 | 50 | 22 | 35 |
| 520 | 53 | 33 | 38 |
| 551 AL | 549 AL | 67 | 20 |
| 570 AL | 570 AL | 61 | 19 |
| 591 AL | 587 AL | 58 | 18 |
| 601 AL | 601 AL | 53 | 19 |
| 621 AL | 621 AL | 60 | 25 |
| 685 | 64 | 89 | 150 |

All filters are made by Schott Jenaer Glaswerk, G.m.b.H., Mainz, W. Germany.

Procedure and reproducibility

The object light is projected in the ocular of the photometer, after passing through a fixed diaphragm of ca 20μ diameter, which screens off the faintly illuminated environs of the object. Through this ocular two halves with a different illumination are visible, of which the left shows the object light and the right represents the reference (standard) beam. By placing a narrow bandfilter ("interference filter") between the eye and the ocular, the two halves show an equal colour. The intensity of the standard beam can be changed by means of two polarization prisms and be brought into balance with the object light. This equality is achieved as soon as the entire circular picture is equal bright throughout. According to Ruch and Bosshard (1963) the relative fluorescence intensity F of the object can be calculated then from the rotation angle α of one polarization prism α according to the formula $F = \sin^2 \alpha$.

The fluorescence photometrical method delivers

relative fluorescence spectra, which are determined by measurements of the relative intensities at various wave-lengths, according to the transmission range of the interference filters (fig. 14). The various values of HW (half-width-value) and τ (transmission) must be taken into account at the interpretation of these spectra (table I). The obtained relative spectra are drawn in graphs, in which each curve represents the spectrum of a single object.

To obtain reproducible results in the fluorescence photometry it is necessary to calibrate the measurements by expressing the intensities with reference to a standard.

Uranglass did not appear to be an ideal expedient for this purpose, since it is possible that its structure may be inhomogeneous. For this reason it must be considered a provisional expedient.

Calibration of the Berek photometer and the UV-microspectrograph is under continued investigation. In principle, the spectra, obtained by both methods can be expressed in the spectrum of a wolfram lamp, of which the emission at all wave-lengths is exactly known.

Fluorescence colour and spectral ratio

The Berek fluorescence spectra of fossil pollen and spores nearly always show a curve with two or more peaks: one in the blue-green and one or more in the yellow-orange range, separated by a broad minimum between 500 and 570 nm, which is always present. These maxima are specific both for different pollen types and geological ages (see next chapters). Their mutual proportion is expressed in the *spectral ratio* Q , which is calculated by dividing the sum of relative intensities at 495 and 500 nm (green) by that one of 601, 621 and 685 nm (yellow-orange, see also Table II). Errors in the measurements are reduced by the use of this ratio.

Accuracy of the measurements

Ruch and Bosshard (1963) noticed that the accuracy of measuring with their fluorescence photometrical method may be enlarged by repeating each observation of the rotation angle and by calculating the average α . They found that, when the observations are repeated twice, the variation of the average α amounts ca 3 %. The accuracy of measurements, made with the modified version of this instrument, proved to be somewhat higher as a result of the use of filters with a smaller transmission, by which no difference in colour between object light and reference occurred (van Gijzel, 1966).

All determinations in the present paper have been repeated at least twice. Table II (see Appendix) shows the average deviation of α for one object, measured with various filters used.

In order to obtain comparable results the adjustment of the instrument has to remain unchanged, as soon as an optimal adjustment has been chosen.

It will be obvious that it is not allowed to change the adjustment of the instrument, as the most favourable

one is chosen. Accurate and reproducible results can be obtained only under standard conditions during the measuring. The accessories of the instrument must be properly centred and the maximal amount of UV-light must be concentrated on the object. Measurements at different magnifications may not be compared without more ado, due to chromatical differences of the lens systems. Invariable diaphragms are used in the base of the photometer and above the uranglass to measure always equally. All objects in a slide were chosen arbitrary and all preparations were analysed in a changing order.

As appears, however, from microspectrographical analyses of uranglass and fossil exines (see fig. 19), the maxima in the green and yellow parts of the

built very simple, it appeared to be as accurate as photo-electrical methods. It is a comparatively inexpensive method; moreover it can be used as well for reflection measurements in coal petrography and for a simple determination of absorption of tungsten light by pollen and spores. The pollen preparations need no quartz object and cover glasses or glycerine immersion lenses, as distinct from the UV-absorption measurements.

THE UV-MICROSPECTROGRAPH

Equipment and procedure of the measurements

To amplify the aforementioned shortcomings in range of the Berek-fluorescence-photometry and to test the

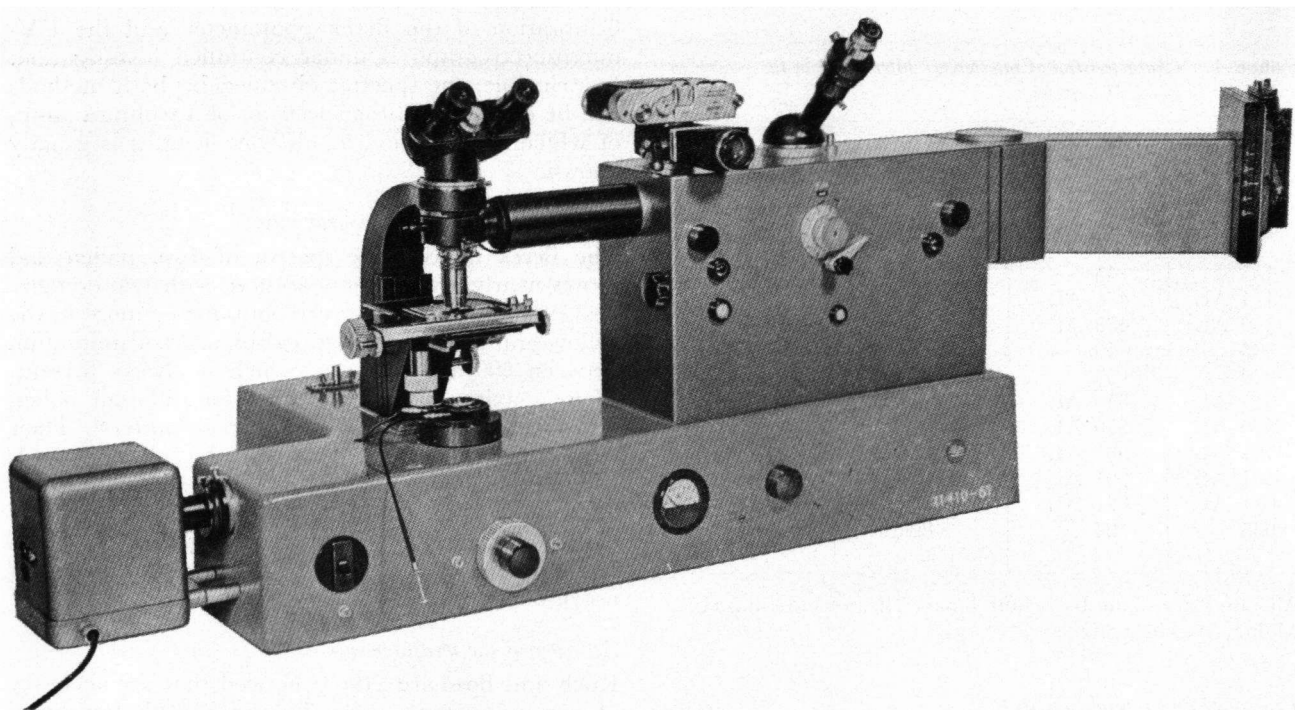


Fig. 15. The Leitz UV-microspectrograph.

complete exine spectra between 500 and 575 nm are not apparent in the Berek fluorescence spectra, due to the fact that the maximum of the uranglass emission is situated in that region.

In spite of this limitation this relative method proved to be very useful for a preliminary description of the fluorescence phenomena at fossil pollen and spores. Another disadvantage of the Berek-photometry is situated in its principle of visual observation, by which its measuring range is limited to that part of the spectrum, for which the human eye is sensitive.

Besides the work is very tiring, because it must be done in a dark room.

On the other hand this method possesses some great advantages. Although the Berek photometer has been

relative spectra obtained, some fluorescence spectra have been analysed by means of the Leitz UV-microspectrograph, designed by Ruch (1960, 1961). Besides, this instrument is used for a number of absorption measurements of the same objects. For these investigations only quartz object- and cover glasses have to be used. The results of these analyses are discussed in Chapter V and VIII.

This instrument (figs. 15 and 16) forms a combination of a reflecting microscope with a spectrograph, by which the spectrum for various wave-lengths is simultaneously projected on a photographic plate. The following light sources are used:

(1) A water-cooled hydrogen lamp (type WHS 200 by Kern & Sprenger, Göttingen). This UV-light source

has the advantage of a flat and continuous spectral intensity curve in the particularly important 220–350 m μ range. It has been stabilized with special care against voltage fluctuations. A precision-adjustable square stop in the beam path illuminates and masks the field of measurement produced by the spectrograph slit. Spectral filters for the UV- and the visible range may be introduced between the field stop and the illuminating prism.

chromatic picture of object and slit on the plate of the spectrograph. This slit picture serves for wave-length calibration and the location of the object strip transmitted by the slit.

The Laborlux microscope built into the microspectrograph, is fitted with a reflecting condensor and reflecting objective (optical paths: left in the scheme), which are achromatic, extending through the entire wave range from 220 to 700 nm. The reflecting objec-

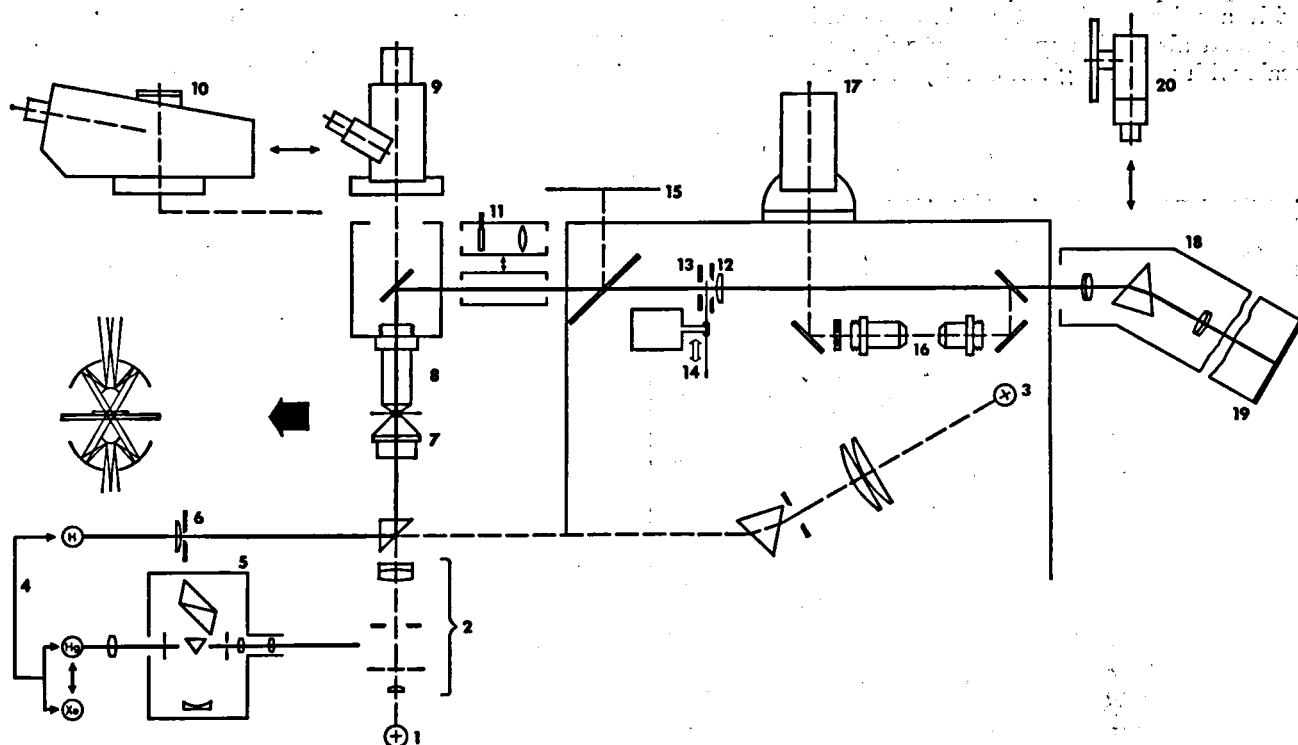


Fig. 16. Optical scheme of the Leitz Microspectrograph.

1. low-voltage filament lamp. 2. illuminating system for phase contrast. 3. mercury vapor lamp. 4. spectral lamps. 5. monochromator. 6. field stop. 7. reflecting condensor. 8. reflecting objective. 9. binocular photo tube. 10. UV-image converter. 11. eyepiece tube with suppression filter. 12. spectrograph slit. 13. iris diaphragm. 14. rotating sector stop. 15. film plane for photomicrography. 16. phase contrast system. 17. phase contrast focussing microscope. 18. spectrograph. 19. photographic plate (see fig. 17) or photomultiplier (20).

The hydrogen lamp can be replaced by other light sources such as a low-voltage lamp for absorption spectra in the visible range, and a xenon or mercury high-pressure lamp for fluorescence excitation. A linear mirror monochromator can be inserted possibly between these light sources and the microspectrograph, e.g. to study the fluorescence excitation at various wave-lengths.

(2) A 6 volt/25 amp. tungsten lamp for searching and focussing the object in the visible range. A stop with green filter can be inserted in its beam path for observation with the phase contrast system of the microspectrograph.

(3) A mercury vapour lamp, of which rays from the 253.7 nm Hg line are transmitted along the beam path as monochromatic UV light. It produces a mono-

chromatic Q. Glyc. 300/0.85 S has been used for the photographs of absorption spectra in the UV range.

After focussing the object in the binocular tube, the image formed by the objective is deflected into the slit plane of the spectrograph component by means of a quartz prism.

The specimen is embedded between a 0.5 mm quartz object slide and a 0.8 mm quartz cover glass with the use of glycerin immersion, possessing the same refractive index, for absorption measurements in the UV range. The spectrograph slit can be removed from the beam path; its width is adjustable. An image of the slit is formed on the spectrograph plate at unit magnification through a dispersing prism and one quartz-fluorspar achromat lense, both in front and behind it. For photoelectric measurements the photo-

graphic plate holder can be removed and exchanged by a photomultiplier holder. Furthermore, an iris diaphragm is placed immediately in front of the slit for circular measuring fields. Suppression filters can be introduced into the object beam path for determinations of fluorescence in the visible and near-UV-range.

Evaluation of the density spectra

The spectrophotograph (fig. 17) must be evaluated with a microdensitometer. This evaluation of the recorded absorption spectrum requires a calibration scale with which a given density of the plate in any

point of the spectrum can be expressed into the appropriate extinction value in the object plane. For this purpose a rotating sector stop with 20 extinction steps ($E = 0.1 - 2.0$) can be moved into the beam path directly in front of the slit of the microspectrograph. An example of densitometric evaluation of an UV absorption spectrum is shown in fig. 18A. The recording has been made parallel to the direction of spectral dispersion for the object point reproduced in the slit. By means of the reference spectra, the spectral extinction curve of the object can be plotted (fig. 18B). With the microspectrograph a fluorescence spectrum is obtained after changing the hydrogen lamp by a

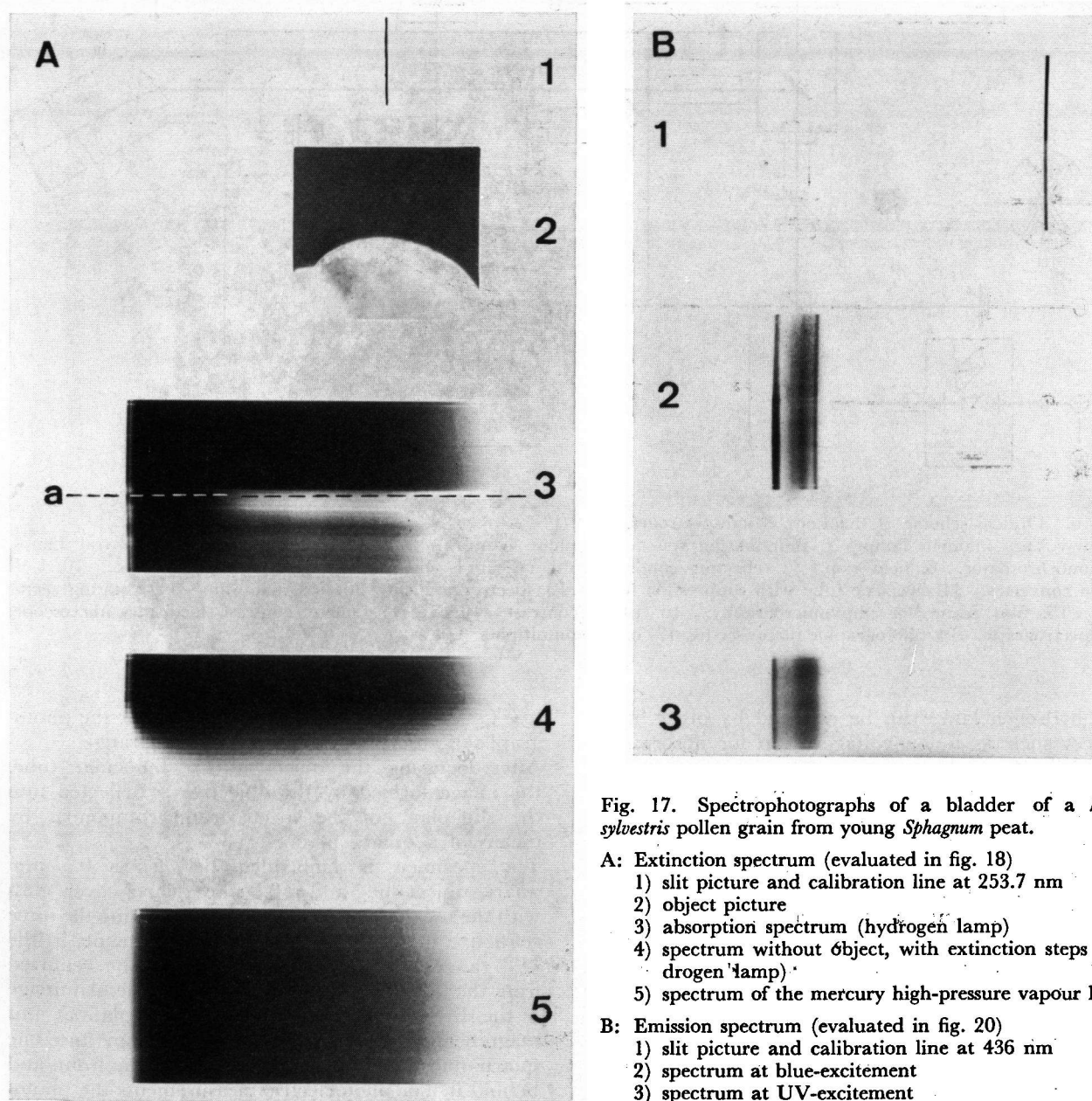


Fig. 17. Spectrophotographs of a bladder of a *Pinus sylvestris* pollen grain from young *Sphagnum* peat.

A: Extinction spectrum (evaluated in fig. 18)

- 1) slit picture and calibration line at 253.7 nm
- 2) object picture
- 3) absorption spectrum (hydrogen lamp)
- 4) spectrum without object, with extinction steps (hydrogen lamp)
- 5) spectrum of the mercury high-pressure vapour lamp

B: Emission spectrum (evaluated in fig. 20)

- 1) slit picture and calibration line at 436 nm
- 2) spectrum at blue-excitement
- 3) spectrum at UV-excitement

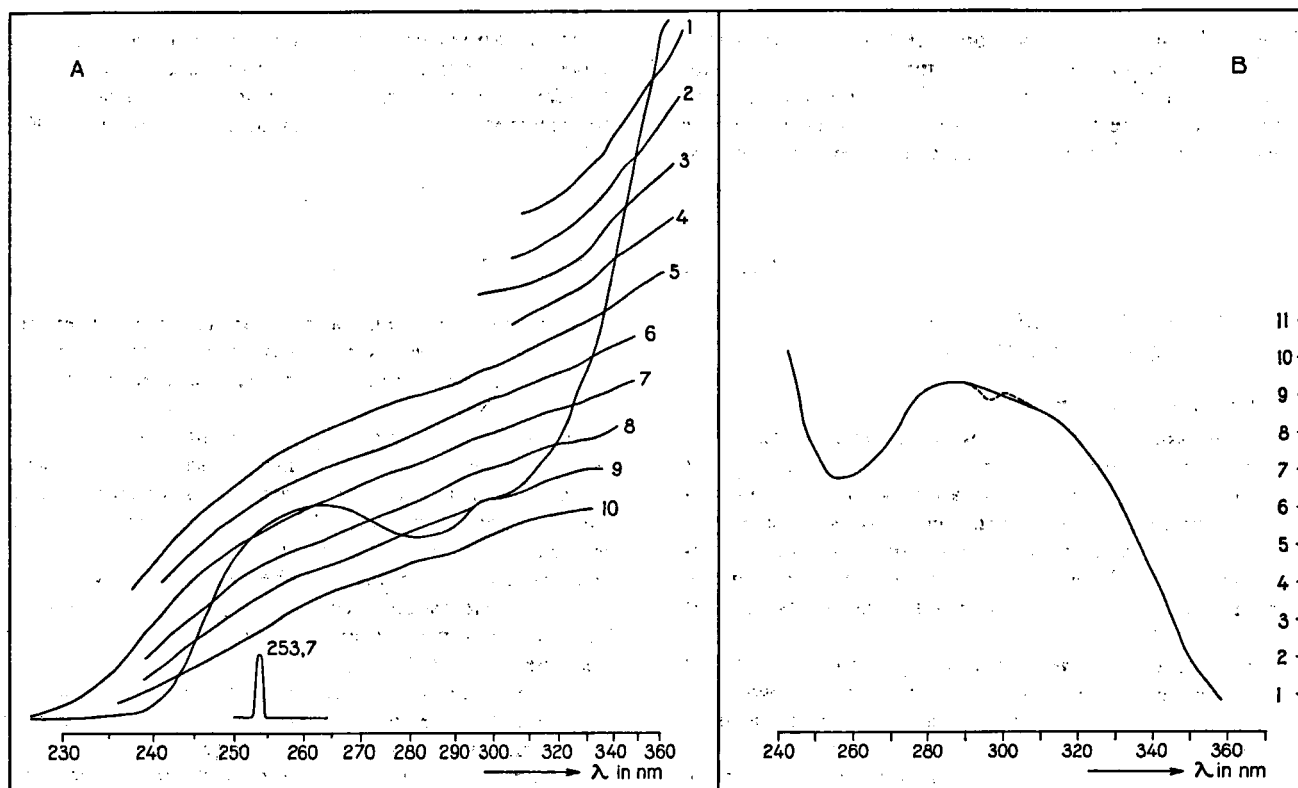


Fig. 18. Spectral absorption of the *Pinus sylvestris* pollen grain of fig. 17.

A) densitometer curves (1—10) of the spectra of an object point (see broken line a in fig. 17) and the sector steps with extinctions
B) spectral extinction curve of this object point, constructed from the densitometer curves

high pressure mercury vapor lamp (Philips CS 150 or Osram HBO 200). Densitometric recording of the photographic plate delivers uncorrected fluorescence spectra (fig. 19a), for which the wave-length scale is fixed by the line of 435,8 nm in the mercury vapor spectrum. These fluorescence spectra can be corrected by means of the spectral sensitivity curve of the photographic plate. Then the intensity of the emission at each wave-length is expressed in percentages of the film density (fig. 20). Comparison of these fluorescence spectra with those obtained by means of the Berek photometer shows clearly the limitations of the Berek photometry, as has been noticed above. The coincidence of the green and yellow minima in the relative spectra (and all other ones given in this study) with the maximal emission in the uranglass spectrum (fig. 19a, above) is responsible for the absence of both peaks in that region in the relative spectra, being distinct in microspectrographical analyses.

Comparison of all these spectra, measured on microscopical objects (fig. 19a) with those, obtained from fresh spores in suspension, suggests that the fluorescence spectra of palynomorphs show only one maximum instead of four. This subject is still under investigation now.

Accuracy of the measurements

The accuracy of the microspectrograph depends on a number of various factors such as the extinction range,

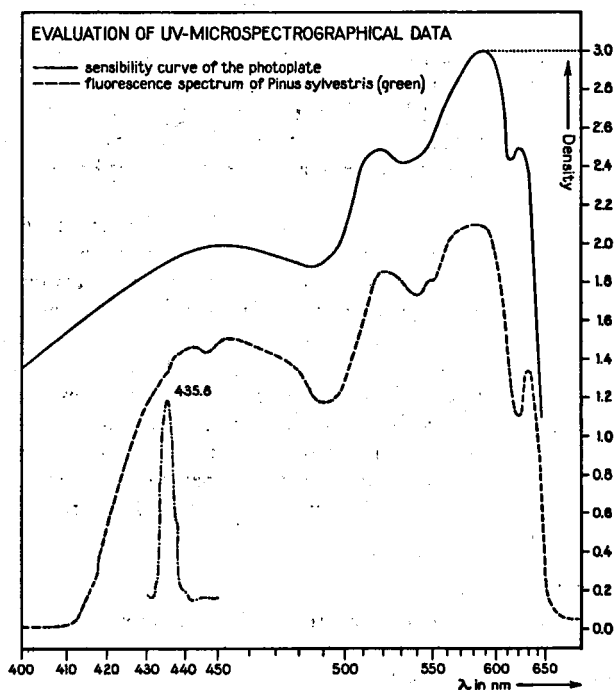


Fig. 20. Evaluation of an UV-microspectrographical fluorescence curve, based on the sensitivity curve of the photoplate used (Illford, FP 3). By expressing the fluorescence values at various wave-lengths in percents of the photoplate sensibility, the calibrated fluorescence spectrum is obtained. Photoplate curve by courtesy of Illford Comp.

the uniform slit illumination, the homogeneity, grain size and development of the emulsion of the photoplate, the accuracy of the densitometric evaluation and the object properties. Therefore it is impossible to give general data. However the measuring error with test

solutions appeared to be of the order of ca 2 % of the measured extinction value. For the accuracy and the detailed description of the microspectrograph may be referred to the publications by Ruch (1960, 1961).

III. PREPARATION METHODS

PREPARATION PROCEDURES FOR FLUORESCENCE PALYNOLOGY

Pulverization of the samples

It is necessary in many cases to pulverize slightly the sample in a mortar before further treatment. The pestle must be handled very carefully to avoid destruction of pollen. But for hardened rocks such as shale, browncoal, coal and limestone, this means of pulverization is insufficient. To disassociate the mineral grains from each other, various treatments can be applied:

The so-called *benzene method* delivers good results for fluorescence studies. Its procedure is as follows:

- (1) The sample is placed in a drying apparatus to remove the water from the pores in the rock pieces,
- (2) After cooling the sample, a sufficient quantity of liquid is introduced, which possesses a surface tension lower than that of water, as, for instance, xylene or benzene. The tumbler is shaken now and then until the light liquid has filled all pores of the rock.
- (3) After the particles have settled down, the liquid is poured off and hot water is added to the sample instantly. Then the water molecules try to replace those of the light liquid from the rock pores, resulting in a strong pressure, which will break up the rock fragments into separate grains (see also Staplin et al., 1960).
- (4) The sample is filtrated and dried. This treatment can be repeated until the sample is pulverized completely. The sample is ready now for further treatment. Another method is *ultrasonic disintegration*. It is used on a large scale for our purpose, for which various equipments exist (Stevens et al., 1960; Dumait, 1962a, 1962b; Gibson, 1963; Streel, 1964). Exines and exosporia can resist without serious damage, ultrasonic vibrations with a frequency up to 20,000 cycles/sec for 10 min. Repeated ultrasonic treatment, however, must be carried out very carefully to avoid destruction of pollen.

Further treatment

We will pass now to a description of preparation of various deposits, such as coal, browncoal, hardrock, peat, clay and sand.

Browncoal and *coal* are treated previously by means of oxidation with 10 % warm H_2O_2 for 10 min. **Do not boil!** Coalified rocks must always be oxidized to break the pollen away from the rock particles. This treatment must be done carefully, in order to prevent oxidation of the pollen grains as much as possible. Therefore,

during the treatment a regular control under the microscope is necessary. Oxidation with hydrogen peroxide is preferred rather than a treatment with acids as HNO_3 .

After cooling of the sample the peroxide must be removed. Centrifuge and wash the sample twice with aqua destillata. Further treatment is similar to that used for peat and clay, and is used only in case sand or clay are present in the browncoal or coal.

Peat samples are treated in the following way:

- (1) Boil the sample for 10 min with 10 % NaOH. Meanwhile some water is added to prevent higher alkaline concentrations.
- (2) Sieve the sample through a coarse and a fine sieve with meshes of min. 200 μ . Transfer the filtrate simultaneously to a glass centrifuge tube.
- (3) Centrifuge and wash twice with aqua destillata. For peat and browncoal without inorganic material this procedure will suffice.

Clay, sand, diatomite and other sediments with inorganic matter are initially treated with the first three steps of the peat treatment. The removal of minerals must be carried out as follows:

- (1) The sample is washed with 96 % alcohol to remove the water. Centrifuge and repeat this washing. If some water is left in the sample when it is brought into a bromoform alcohol solution, the latter becomes an emulsion and will prevent the mixing of the bromoform with the sample.
- (2) Add some bromoform, diluted in alcohol, with a s.g. of 2.0 until the tube is filled half. Stir the mass until no clods are left. This takes a long time and can be accelerated by means of ultrasonic vibration of the mixture or by means of a special glass tube shaker, by which the lumps will disintegrate.
- (3) Centrifuge rather short (3 min) at low speed (1500 r/min). The minerals will settle down on the bottom of the tube, while the pollen is floating.
- (4) Pour off the liquid with the pollen into another centrifuge tube and fill it with alcohol (96 %). Stir the liquid. The specific gravity of the mixture will be reduced so much that the botanical substances settle down on the bottom. Centrifuge at high speed (5000 r/min) and long (10 min). If necessary this separation may be repeated once or more.
- (5) Wash several times with alcohol (96 %) and centrifuge to remove the bromoform. Water cannot be added instantly, because emulsion can be formed.
- (6) Wash several times with distilled water and centrifuge to remove the alcohol.
- (7) Turn the tube upside down to allow the water

to leak out from the sample. Water would cause a drying up of the permanent pollen slides afterwards. Add some drops of pure glycerine (water-free) and stir the mixture.

For the preparation of *hardrocks* after the pulverization of the sample the same procedure is followed.

Preparation of the slides

The slides must be prepared in the usual way with the utmost care. The object-glass and cover-slip are cleaned very carefully, at which no dust may left on the glass (dust is self-fluorescent!). The use of fluorescent media for conservation, as glycerine gelatine, silicone oil, canadabalsem or plastics causes an undesirable illumination of the picture of the UV-microscope. But this picture has to be as dark as possible for the observation and measurement of the fluorescence. Water-free, chemically pure glycerine, being non-fluorescent, has proved to be most suitable. **Do not stain the residue** with saffranine or other liquids, which may cause secondary fluorescence (Hatinger, 1938; Krieg, 1955).

The greatest care and accuracy is required in collecting, preparing and studying of the samples. The cleanliness in a palynological laboratory must be equal to that in a bacteriological one. This maxim, given by Faegri and Iversen (1950) holds the more for the study of fluorescence palynology.

INFLUENCE OF THE TREATMENT ON FLUORESCENCE

For the separation of pollen grains, spores and other plant remains from minerals and humic substances (humic acids) in sediments, numerous methods are used (see Faegri and Iversen, 1950; Brown, 1960; Staplin et al., 1960; and other papers). However, nearly all of them concern treatments with acids or strong oxidizing agents. Although their action on the exines and exosporia, have generally been considered as neglectable, it appeared to the present author that these procedures may have an important influence on the chemical properties of sporopollenine. Mechanical techniques are preferable for this separation. This is in accordance with the experience of Felix (1963), who stated that mechanical disintegration of shales without the use of acids contributes to an improved recovery of pollen, spores and other microfossils.

The use of the well-known acetolysis method of Erdtman (1954, 1960) and similar contact of pollen with acids as HF, HCl, H₂SO₄ and HNO₃ causes a change in fluorescence colour towards the red part of the spectrum. By such treatments the colour of, for instance, *Pinus sylvestris* grains, changes from green to yellow, orange or brown and the total intensity of the fluorescence decreases. Sitte (1960) already noticed that the use of acetolysis at the preparation of fresh *Selaginella* spores changes the fluorescence from blue to brown. As appears to the present author, similar changes occur at many other types of palynomorphs in fresh condition. Besides, it appeared that the

activity of acids and other strong reagents works more or less selective; certain types are corroded more than other ones and a large variation in fluorescence occurs for grains of the same type.

Extensive experiments have been made on samples of Holocene clay and peat, which confirm this statement. In comparison with that part of the samples, treated with NaOH only, the boiling with HF for longer than three minutes resulted in a remarkable different pollen composition. Mainly *Pinus sylvestris*, *Tilia* and *Alnus*, being the most resistant, increased in percents. Therefore the author cannot agree with the opinion of Faegri and Iversen (1950) and other investigators, who supposed that organic remains as pollen are not, or only to a small degree, damaged by HF.

Oxidation of material is used at the maceration of browncoal and coal for the preparation of pollen slides (Potonié, 1931; and other papers). It causes a similar change in fluorescence colour, but to a lesser extent if the treatment has been stopped at the right moment, i.e. as the oxygen can attack the grains too strongly. This is in accordance with the oxidation experiments on palynomorphs from browncoal, carried out by Kirchheimer (1933a, 1933b, 1934).

The preparation of browncoal and coal with strong cleansing reagents as NaClO, transforms the fluorescence in an opposite direction; for instance fresh grains of *Alnus*, normally yellow or white fluorescent, are changed hereby towards green or blue. Other types show the same phenomenon. Although this reagent is very useful for the preparation of such rocks, it is unsuitable for fluorescence studies.

Washing the samples insufficiently with water after the oxidation procedure and heating afterwards with NaOH may result in similar blue colours. It is not clear what happens at the violent reaction, occurring when the sample, centrifuged only and still containing much hydrogen peroxide, is brought into NaOH. Possibly a natrifaction of the sporopollenine takes place.

Although the treatment with acids can be replaced completely by other methods, the oxidation method cannot be avoided in all cases in order to pulverize strongly indurated rocks for the fluorescence study. The influence of alkaline solvents like NaOH, KOH, bromoform and alcohol on the fluorescence of fossil palynomorphs can be neglected. To diminish or eliminate the possible undesirable influence of the solvents used, all treatments have been carried out as much as possible under standardized conditions: equal duration of preparation, concentration of reagents and sequence of treatments.

Very clean pollen slides are obtained by the use of bromoform instead of HF and HCl for the separation of the pollen and other plant remains from the mineral particles, according to the procedure, described by Urban (1961), Felix (1963) and other authors. Then heating with HCl can be avoided, because the lime is removed as well. This method is similar to that used

in sediment-petrology before long and is based in principle on the differences in specific gravity between minerals and plant material. In a solution of bromoform in alcohol with a specific gravity of 2.0 or somewhat lower (min. 1.75), the plant substances remain floating. Nearly all the minerals will settle down at the bottom of the centrifuge tube. This treatment is very suitable for fluorescence studies and has been used, if necessary, for all samples.

The mentioned preparation methods have been previously tested by comparative fluorescence analyses of samples of various sediment types and ages. This test was made of the following samples: a young *Sphagnum* peat, a humic Holocene clay, a strongly humified Pleistocene peat, an Eocene and a Paleocene browncoal and a black humic Cretaceous clay. Each

sample was split up into equal parts, which were prepared in different ways. They were treated respectively either only with distilled water, or NaOH, or HF and NaOH, or HCl, HF and NaOH, or NaOH, alcohol and bromoform, or H_2O_2 and NaOH, or NaClO and NaOH. It appeared that distilled water, alkaline solvents, alcohol and bromoform may only be used without drawbacks. Chemical treatment must be limited as much as possible. Oxidation, if inevitable, must be carried out very carefully and held under control by testing a drop of the sample from time to time under the microscope. After each treatment the sample must be washed repeatedly with water to remove the suspended and solved compounds, which may cause an adverse effect on the microscopical observations.

IV. FLUORESCENCE OF FRESH AND SUBFOSSIL PALYNOMORPHS

PREVIOUS STUDIES

Berger (1934), Asbeck (1955) and some other allergologists established that various types of fresh pollen grains and spores show different fluorescence colours, dependent on type or form. They are caused by the combined fluorescence of the various substances of the living pollen cell, in which that of the protoplasm is dominant. This is shown clearly, for instance, by fresh exines of *Pinus sylvestris* (Photoplate: fig. 1). The protoplasm, showing a white fluorescence colour, can be more or less removed by treatment of the grains with KOH. The fluorescence colour of the exine, light blue, then becomes visible. In view of the fact that during fossilization the exine appears to be resistant to destruction, it is to be expected that the pollen walls will remain fluorescent.

Observations on the fluorescence of subrecent palynomorphs have been made from thin *Sphagnum* layers, situated some centimeters below the surface of a living peat bog and other peat formations or from young, slightly humified, *Sphagnum* peats. As the protoplasm has disappeared, their fluorescence colours are considerably different from the fresh pollen grains described by Berger and Asbeck. *Pinus sylvestris* pollen, for instance, shows more bluish colours in fresh condition, as against the bluish green or dark green fluorescence of subrecent grains (see Pl. III: uppermost samples and fig. 1 and 4).

It appeared that subrecent palynomorphs show more or less bright fluorescence colours, which are different in the various forms. From these phenomena the first principle of fluorescence palynology has been previously derived (van Gijzel, 1961, 1963). It can be defined more exactly now as follows:

Similar to the differences in fluorescence between fresh pollen and spores the subfossil and fossil palynomorphs show a more or less typical fluorescence colour.

These phenomena will be described below by means of extensive analyses of the various forms.

The terms fossil and subfossil have been used in this study with regard to the state of preservation, being a concept, different from that generally used by geologists. Pollen grains are considered as subfossil or fossil when the protoplasm and other living substances have disappeared; when the pollen walls have been humified the pollen is considered as fossil. Recent and subrecent are terms, which concern geological time of less than 10,000 years.

It appeared furthermore that other plant remains e.g. leaf fragments and cuticles, resin, wood and cork tissues which are preserved and fossilized in the soil, frequently remain fluorescent, even when these are from older Pleistocene peat and clay or Tertiary browncoal.

FLUORESCENCE COLOUR DETERMINATIONS

The fluorescence picture of palynomorphs from the so-called young *Sphagnum* peats is clearly illustrated by the figs. 2—4 of the Photoplate. As examples of such deposits have been chosen the peats occurring at Ekamp¹) near Winschoten, N. Netherlands (prep. nr. EK 65/L) and at Kloosterhaar in the E. Netherlands (prep. nr. KH/JV/2). The figures show that spores possess blue fluorescence colours, whereas the pollen grains are green, white, yellow, orange or pink. In certain cases they show a rather large variation in fluorescence colour, due to differences in their state of preservation. This variation may even have been caused by autoxidation before burial in the soil (see Chapter VIII). The fluorescence of these objects is

¹) For palynological data see van Gijzel (1967c).

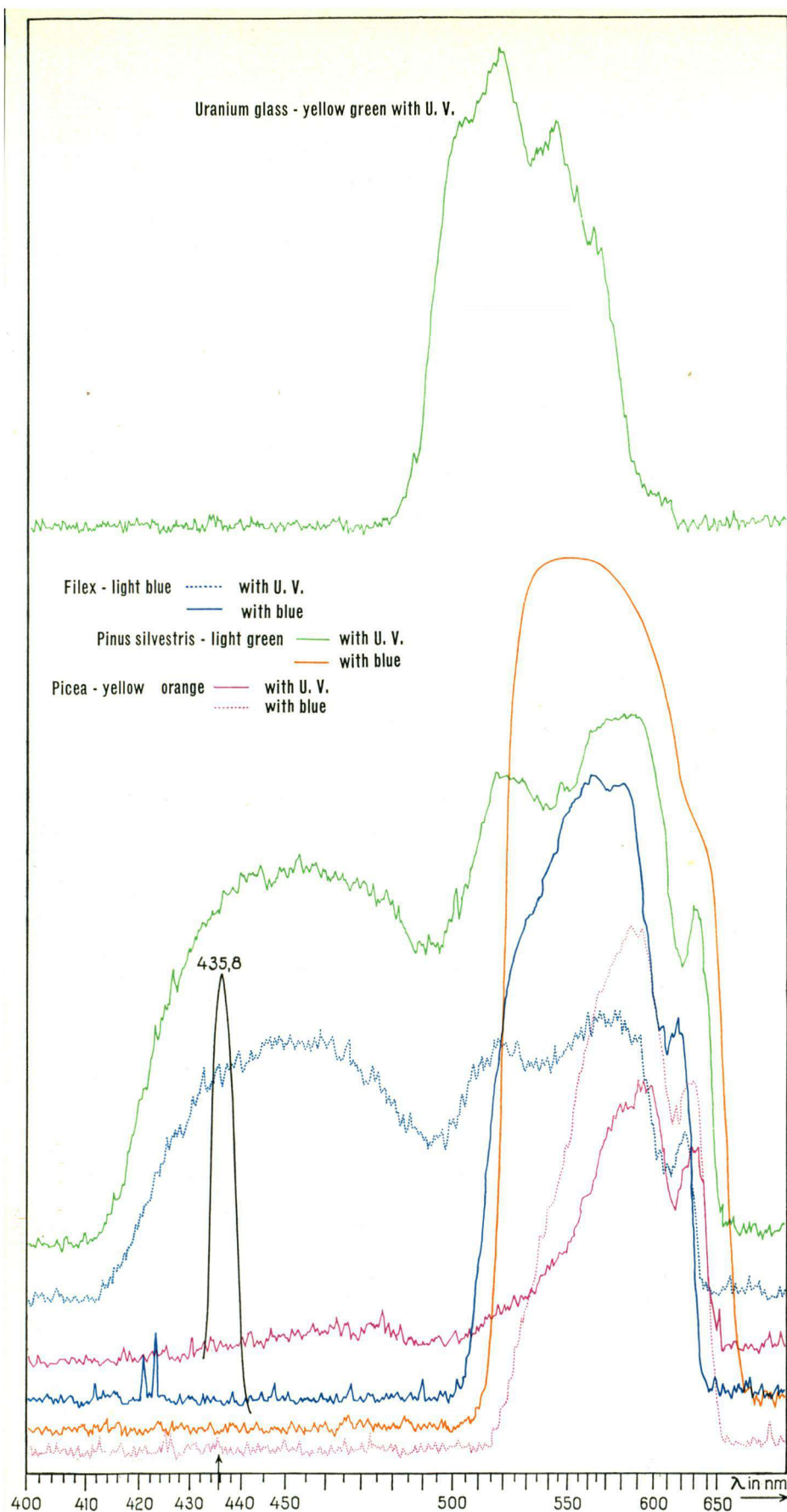


Fig. 19. Uncorrected fluorescence spectra at UV- and blue-excitation of uran glass (top) and some fossil pollen grains and a fern, determined by means of the Leitz UV-microspectrograph (after van Gijzel, 1966).

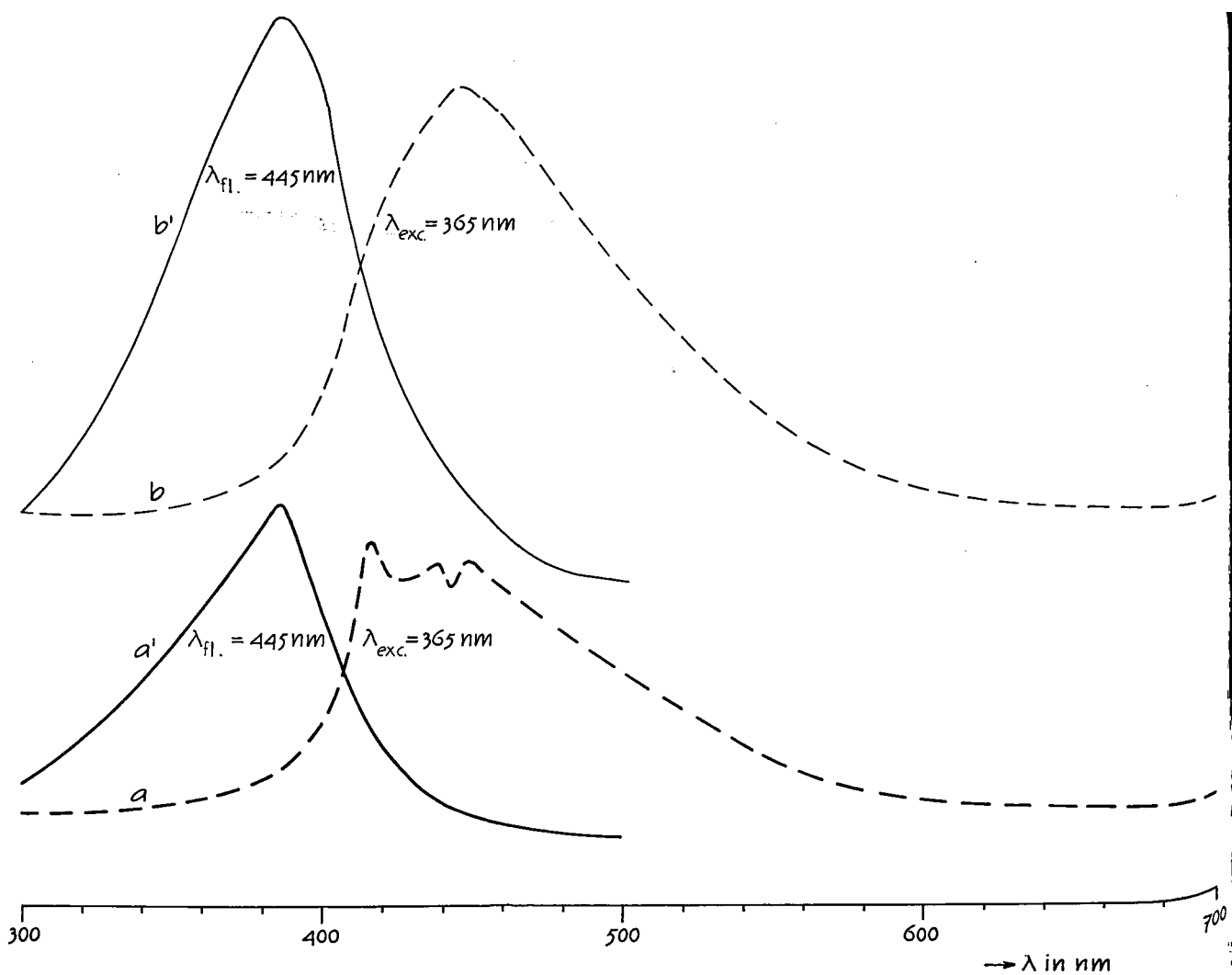


Fig. 19b. Fluorescence and excitation spectra of fresh spores of *Lycopodium*, measured in suspension in aqua bidistillata, by means of a Fluorispec fluorescence spectrophotometer (made by Baird — Atomic Inc., Cambridge, Mass., U.S.A.).

- a) fluorescence spectrum, excited at 365 nm; spores treated previously with acetolysis and KOH,
 - a') excitation spectrum belonging to the fluorescence at 445 nm; spores treated similar to a,
 - b) fluorescence spectrum, excited at 365 nm, spores treated with KOH only,
 - b') excitation spectrum, belonging to the fluorescence at 445 nm; spores treated similar to b.
- The fluorescence colour of these spores is similar to the blue colour of *Filix* spores (fig. 19a).

similar to, or but slightly different from that of subfossil grains from living peat bogs.

In order to describe the fluorescence colours more objectively than is possible by means of the human eye, a number of relative Berek fluorescence spectra have been determined, as summarized in Plate I and some other figures. It appears that spores of Filices and Sphagna are blue in ultra-violet light, while pollen grains of *Pinus sylvestris*, *Abies*, *Carpinus*, Cyperaceae, Chenopodiaceae and Ericaceae are greenish white, or yellowish green in fluorescence; *Alnus* and *Fagus* yellowish white; *Picea*, *Quercus*, *Tilia*, *Ulmus*, *Betula*, *Myrica* and *Corylus* yellow or orange and the Poaceae (Gramineae) orange or pink.

The difference in spectral composition between the blue, green and yellow coloured objects, is formed by a gradual shifting of the main intensity from blue to red, which is expressed by the gradual decrease of the maxima at 474 and 488 nm in favour of those at 601 and 621 nm. The striking difference between the spectra in blue spores and the pollen of the orange or pink Poaceae (Gramineae) gives a clear impression of the range in fluorescence colour to be encountered.

In plate I the spectra have been arranged in a fluorescence series from blue to red. A similar gradual change also occurs in each pollen type with increasing geological age (see next chapter and Plate II). The similarity of the spectra is remarkable, for these always show two distinct maxima. These analyses, carried out by means of the Berek photometer are in accordance with the UV-microspectrographic determinations of some palynomorphs from the Ekamp peat (fig. 19 in Chapter II). These spectra show a more complete picture of the fluorescence emission with four instead of two maxima. When one compares the spectra of a blue fern, a green *Pinus* and an orange *Picea*, respectively, it is clearly observable that the blue and green maxima diminish gradually in favour of the two maxima in the yellow, orange and red part of the spectrum. Both types of spectra have been measured and calculated according to the procedures, described above, in which each line represents the fluorescence spectrum of one specimen.

These phenomena are very important in relation to the problems of the chemical nature of sporopollenine (see Chapter VIII). The walls of fresh and fossil palynomorphs do not appear to be fundamentally different in chemical composition, but only in the proportions of certain omnipresent compounds.

The spectra of Plate I show just like the colour pictures of fig. 2—4, that a certain variation in fluorescence colour exists in the forms studied. The specimens measured were chosen in such a way that the largest variation was included. For technical reasons only part of the spectra could be reproduced in the plate and figures; those which coincided are represented by one curve only. As a result the spectra of *Myrica* (nr. 50), *Betula* (nr. 65), *Corylus* (nr. 41) and *Alnus* (nr. 53) are illustrated in Plate I (left below), while the Cyperaceae (fig. 21) are represented by another curve (nr. 60). The spectrum for the Cheno-

podaceae (nr. 55) is an average, derived from three spectra, which could not be separately represented.

The Berek photometrical determinations of a number of grains of the Myricaceae and Betulaceae have been summarized in fig. 22. It appears from these spectra and numerous visual observations that the following differences in fluorescence colour can be distinguished: *Myrica* pollen grains show a smaller variation in colour

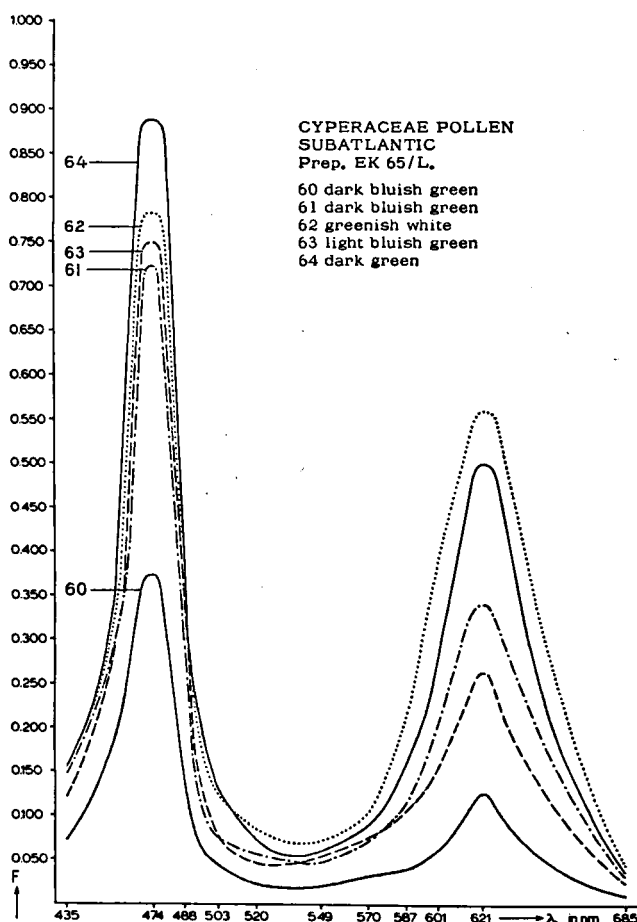


Fig. 21.

than those of *Betula*. On the other hand they differ only slightly from those of *Corylus*, which possess an equal colour intensity as *Myrica* grains. The total fluorescence intensities of *Myrica* and *Corylus* are larger than those of *Betula*, which shows more orange colours, in contrast to the white and yellow of *Myrica* and *Corylus*. *Alnus* pollen shows more white and green colours in comparison with the yellowish shades of *Myrica* and *Corylus*.

These differences become the more distinct by calculations of the spectral ratio according to the method described above. Together with the Q values of other forms measured, they can be arranged in a fluorescence series or colour scale (Table III), which may

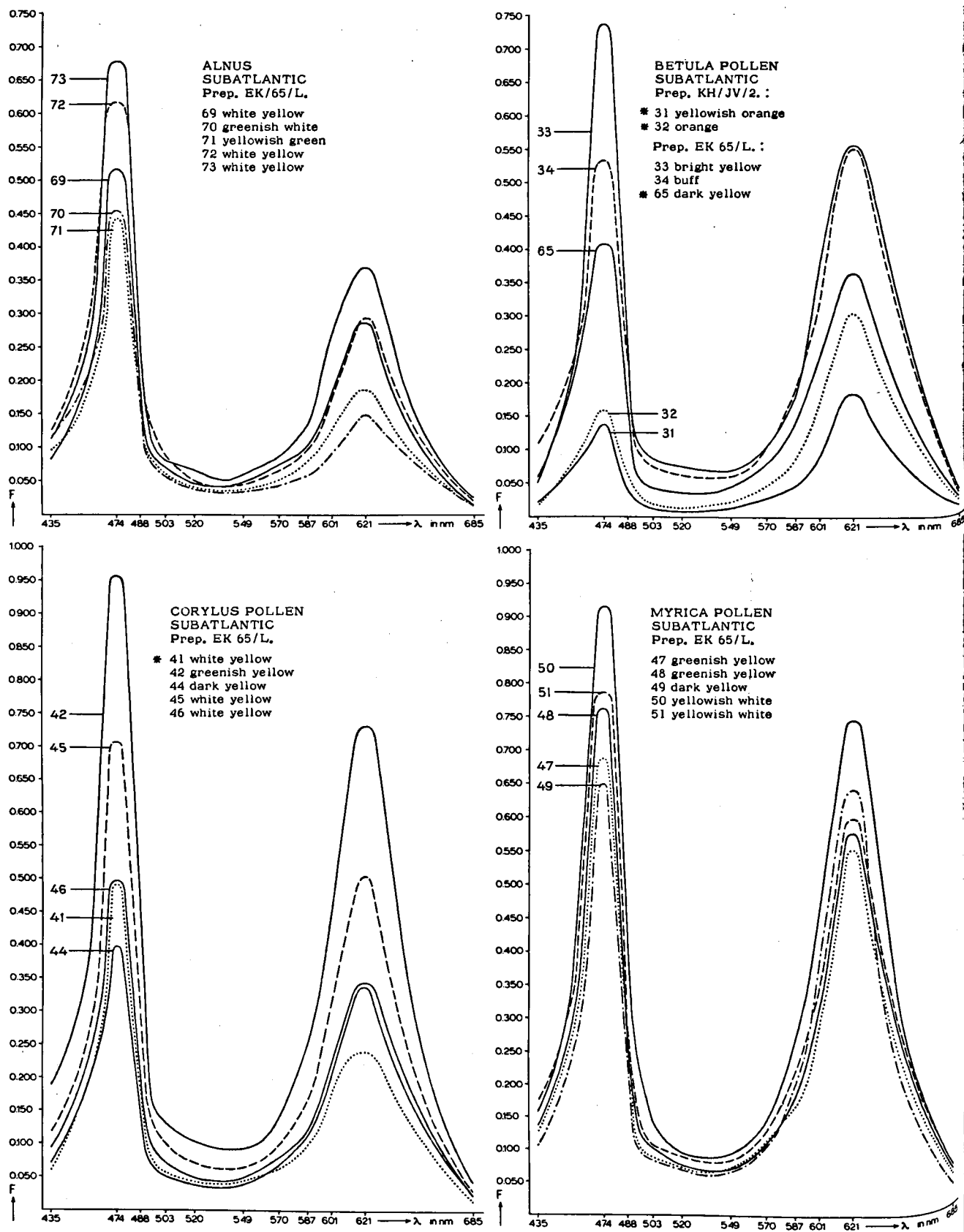


Fig. 22.

be compared with the corrosion susceptibility series of fresh pollen and spores, published by Havinga (1964). It appears that a general agreement exists between both series. Blue and green fluorescing palynomorphs are more resistant to oxidation than the yellow and orange coloured ones, whereas those which are white occupy an intermediate position. Although some differences in the arrangement of both series occur, the general trend appears to be similar. The question arises whether fresh pollen and spores, which have been oxidized by treatments in the laboratory, may be directly compared with those, obtained from young *Sphagnum* peats, which have been fossilized and changed chemically in another way. More extensive investigations on fresh and fossil material are necessary in order to obtain more data to amplify the fluorescence series. It may be important to make measurements on oxidized pollen material, which has also been treated in other ways.

TABLE III. Corrosion susceptibility and spectral ratio of some fresh and fossil palynomorphs

| Corrosion susceptibility of fresh pollen and spores, increasing downwards (after Havinga, 1964): | Average Q values of the fluorescence of some palynomorphs from young <i>Sphagnum</i> peats: | |
|--|---|-----------|
| | <i>Sphagna</i> | 4.38 (8) |
| <i>Lycopodium</i> | Filices | 4.25 (8) |
| | Cyperaceae | 2.28 (7) |
| <i>Pinus sylvestris</i> | <i>Pinus sylvestris</i> | 2.21 (11) |
| <i>Tilia</i> | <i>Abies</i> | 1.84 (6) |
| <i>Alnus</i> | <i>Alnus</i> | 1.69 (10) |
| <i>Corylus</i> | <i>Tilia</i> | 1.67 (5) |
| <i>Myrica</i> | <i>Carpinus</i> | 1.65 (5) |
| <i>Betula</i> | Chenopodiaceae | 1.46 (3) |
| <i>Carpinus</i> | Ericaceae | 1.45 (8) |
| <i>Populus</i> | <i>Picea</i> | 1.44 (4) |
| <i>Quercus</i> | <i>Corylus</i> | 1.34 (5) |
| <i>Ulmus</i> | <i>Myrica</i> | 1.24 (8) |
| <i>Fagus</i> | <i>Fagus</i> | 1.13 (4) |
| <i>Fraxinus</i> | Gramineae | 1.08 (7) |
| <i>Acer</i> | <i>Betula</i> | 0.86 (5) |
| <i>Salix</i> | | |

(8) = number of measurements on each form

In the table below those forms of which less than three determinations are available have been left out of consideration, since these types show a rather large variation in fluorescence colour. Moreover, more measurements of the types mentioned are required before a more accurate arrangement of this fluorescence series can be presented.

APPLICATIONS

It is important to point out the practical use of fluorescence microscopy to the analysis of pollen preparations, when these are very poor in pollen. These can be more easily studied under ultraviolet light, in particular when a darkfield microscope condensor is used. The light and coloured grains can be found rapidly in the dark image projected by the microscope, even when the preparations are from older deposits, unless the material from these has been coalified so strongly that the fluorescence is weak or absent. After the detection of an object, the illumination can be easily switched from ultra-violet to normal for identification. Not only small concentrations of pollen grains, but those of other plant remains, e.g. *Pediastrum*, sporangia of *Azolla*, spores of Fungi, specimens of acritarchs (Hystriochosphaeridae) and dinoflagellates can be easily detected and counted in this way.

The importance of the first principle of fluorescence palynology is restricted to pollen morphological studies in which determination of the spectral ratio can be used for distinguishing pollen species. A number of grains of related species have then to be measured in order to be able to give an accurate and significant average Q value for each of them. Care must be taken, however, that no acetolysis or any treatment with acids is used in the preparation of the slides in the laboratory. The number of measurements of the spectral ratio will be somewhat smaller than with fossil material, due to the smaller variation in fluorescence colour of fresh exines.

In conclusion it ought to be pointed out that by means of the ultra-violet microscope the recognition of the various layers composing the exine may be facilitated and yield results important for pollen morphology. The applications mentioned are under further investigation.

V. FLUORESCENCE AND GEOLOGICAL AGE

INTRODUCTION

From the study of contaminated sediments by means of fluorescence microscopy it appeared that a certain relationship exists between the fluorescence colour and geological age of fossil pollen and spores. This is considered the second principle of fluorescence palynology (van Gijzel, 1961, 1963). In the first stage of the present investigations some experiments were carried out to test the utility of fluorescence microscopy with

regard to palynological purposes. The differences in fluorescence colour between Holocene palynomorphs occurring in a young *Sphagnum* peat and those in a Tertiary clay, the residues of which were mixed with each other, gave rise to the assumption that such a relationship exists.

Continued studies of more than one hundred deposits of different ages and of various origins now furnish proof of this supposition and result in many new data.

The results are summarized and discussed in this chapter which deals with the description of the phenomena of fluorescence colour change.

PRESENTATION AND EVALUATION OF DATA

With regard to the interpretation of the figures and diagrams reproduced below, a short explanation is given.

The procedure for measuring and calculating is described in Chapter II. At least eight pollen grains in each slide have been tested. The number depends on the variation of fluorescence colour in each sample. More or less weathered deposits, in which corroded pollen occurs, have been left out of consideration with regard to the calculations of the average fluorescence values per sample. These samples have been indicated in the diagrams with an asterisk.

In the figures and diagrams each line represents the fluorescence spectrum and each dot the spectral ratio of the pollen grain measured. All objects were chosen arbitrarily, unless the preparations contained but few pollen grains.

The colour of the curves and dots, in Plate II and III, resembles the fluorescence colour observed in each pollen grain. The boundaries between the colour groups merge into each other, due to the fact that visual observations of fluorescence colours under UV-microscope are always a question of personal judgement. It appeared to be very difficult to establish visually whether a pollen grain is, for instance, either greenish yellow or yellowish green or white.

After continued investigations it appeared that it is sufficient to determine the spectral ratio Q — a new term which is introduced and defined in Chapter II — instead of the complete fluorescence spectra. This proportional number is represented as the horizontal scale in the standard diagrams. It expresses the proportion of the blue-green and orange-red part of the spectrum of each fluorescent pollen grain measured. The number of the sample (in code) refers to the deposit studied. The Q values of each sample are plotted on a thin horizontal line behind it. The average spectral ratio-values of all samples of a certain geological age are presented in a separate column. For geological and other data of the material, reference should be made to Table VII (see Appendix). The localities of the deposits studied are indicated in fig. 10 (Chapter I).

Geochemical coalification has an important influence on the fluorescence colour of fossil palynomorphs (see Chapter VII). One therefore meets with great difficulties in finding for the standard diagram a sufficient number of non-coalified deposits from Mesozoic and Palaeozoic eras. They may often have been originally buried more or less deep in the subsoil, even when they are found at the present erosion surface. The average spectral ratio-values for ages older than the Cenozoic have therefore to be regarded as preliminary. Finally, for the statistical evaluation of fluorescence data, an attempt has been made to find a statistical

model, that should show how much Q determinations are needed per sample. This appeared to be at least 6 to 8 per sample, depending on the variation in fluorescence colour.

GEOLOGICAL TIME-SCALE

The time-scale used in Plate III, demands some explanation. For practical reasons the absolute time boundaries are plotted on a logarithmic scale. The geological ages have been derived from various papers and data.

The absolute pre-Quaternary dates are those according to Holmes (1959). Some years ago, new data became available in the so-called "Geological Society Phanerozoic time-scale 1964", i.e. a list abstracted from various chronostratigraphical papers.¹ They differ not much from those mentioned by Holmes.

The ages for the Upper Pleistocene and Holocene time units are based on generally accepted C 14 dates. The problem of the actual limits of the Lower and Middle Pleistocene glacials and interglacials still constitutes an important problem, to which more attention will be paid below. In the diagram of Plate III those absolute time lines have been chosen, which are in accordance with the highest known values, viz. those given by Eberl (1928). The Pleistocene sequence of glacials and interglacials, introduced by Zagwijn (1957) and other investigators, is used here as no generally accepted nomenclature exists for this epoch. Some informal terms have been used for the subdivision of various Tertiary epochs. These have been printed in italics in Plate III. These are used since for continental deposits (the great majority of the samples studied here), another stratigraphical nomenclature is necessary than that for marine sediments.

DESCRIPTION OF FLUORESCENCE COLOURS AT VARIOUS AGES

The second principle of fluorescence palynology

This can be defined as follows:

With increasing geological age all fossil palynomorphs show a gradual change in fluorescence colour from blue, green or white towards orange, red or brown colours, followed by extinction of the fluorescence.

The gradual change is effected by the decrease of the blue-green maxima of the fluorescence spectra in favour of those in the yellow, orange and red part of the spectrum. The relative spectra, obtained by means of the Berek photometer appeared to be in full accordance with the UV-microspectrographical observations.

Change in fluorescence of vesiculate forms

Vesiculate pollen grains belong to a group of palynomorphs, showing very clearly the change of fluorescence colour with increasing geological age. Recent

¹ Published in: Quart. J. géol. Soc. London, 120, s, p. 260—262.

grains show a blue or green fluorescence. Consequently, with increasing age nearly the whole visible spectrum is available for the colour change, contrary to other recent palynomorphs, which initially show yellow or orange fluorescence colours. Besides, many vesiculate grains are generally present in humic deposits and these forms furthermore show a very long stratigraphical range.

The relation of fluorescence colour and geological age is closely tied up with the following assumptions: The fresh exines of Tertiary and older *Pinus* species probably showed originally a fluorescence colour similar to that of recent *Pinus sylvestris*. Should this be the case, then *Pinus sylvestris*-like types from those ages may be compared directly with younger *Pinus sylvestris* pollen. Furthermore *Abies* and *Pinus haploxylon*-types from the Cenozoic are similar in fluorescence to *Pinus sylvestris*. Their change of colour during the Mesozoic may consequently be considered a direct continuation of the colour change of *Pinus sylvestris* forms.

The fluorescence of all these forms changes as follows in time. The Holocene is characterized by a predominance of green and white colours, the Pleistocene by white and yellow, the Tertiary by bright yellow and the first distinct appearance of orange, the Mesozoic and Palaeozoic by dark yellow and orange with the continuous appearance of brown, announcing the final extinction of fluorescence. With increasing age the total intensities gradually decrease, but far less regularly than the change in spectral composition. The decrease of intensity is therefore unsuitable for use in palynology.

The colour change phenomenon is clearly illustrated in Plate II by a number of Berek photometrical spectra. The same gradual change appears from the complete UV-microspectrographical spectra in fig. 23. All determinations of the spectral ratio are summarized in the standard diagrams. Plate III gives a general idea and fig. 24 the complete picture of the Pleistocene. The second principle of fluorescence palynology is expressed by two curves: the average Q connection line (striped) and the general tendency line (full). Both curves have been calculated in the Cenozoic for *Pinus sylvestris* and *haploxylon* types only, in older eras for all vesiculate forms measured. Only the Quaternary *Picea* grains differ much in fluorescence colour from the other vesiculate grains. The general tendency is a parabolic regression curve, calculated by means of the so-called arithmetic method of the smallest averages. Its curved course may be evidence as to the chemical nature of the fluorescence phenomena (see Chapter VIII). The area of the range of fluorescence colour groups shows the different variation in spectral ratio for the various deposits studied.

Palynologists, engaged in palaeoclimatological studies, must notice, that the oscillating line, connecting the average Q values, cannot be interpreted like climatic or normal pollen curves. A sudden rise or fall in the connecting line has nothing to do with climatic changes. Similarly the regressive line reflecting the general tendency in fluorescence cannot be interpreted in detail.

Both curves prove the direct relationship between the fluorescence colour and the geological age of the vesiculate forms. At least thirteen fluorescence colour groups can be distinguished in the standard diagrams, viz. recent, subrecent, Holocene, Upper-, Middle- and Lower Pleistocene, Pliocene, Miocene, Oligocene, Eocene with Palaeocene, Upper Mesozoic, Middle with Lower Mesozoic and finally the Palaeozoic. Some of the Holocene, Pleistocene and Upper Tertiary groups may be subdivided as demonstrated for the Pleistocene in fig. 24.

The point in time of complete extinction of fluorescence which is brought about by the geological age only, is still unknown due to the lack of suitable material. It must be situated somewhere in pre-Carboniferous time, because in a number of Carboniferous rocks fluorescent palynomorphs have been found, and in some older deposits fluorescence appeared to be absent.

Only part of the material, collected for this study, has been measured. Selection of samples for fluorescence analysis has been made for practical reasons such as the presence of suitable pollen forms and the degree of coalification.

Geochemical coalification may have strongly influenced the fluorescence of palynomorphs. This fact must be taken into account when one considers Tertiary and pre-Cenozoic strata, composed of either lignite or coal. Mesozoic and Palaeozoic deposits, studied here, are in all probability not coalified to such an extent that the real Q values for these eras must be considered to amount much more. With regard to the Permian and Carboniferous it may be assumed that the Q values are at most 0.10 on the small side, but this is not important for the general trend. More data must become available, however, before it will be possible to establish the lowermost course of the general tendency line exactly.

The results of these investigations may, consequently, be applied to age determinations of deposits in which conventional pollen analysis fails to be reliable (see below). Eighty five deposits have been measured in order to compile a standard diagram for vesiculate forms. Fourteen of these deposits show divergent average Q values, being either too high or too low compared with other samples of the same age. This means that the accuracy of this method amounts to at least 83 %.

A number of sedimentary properties may be responsible for these differences. Values which are too low may be caused by weathering of a sediment during or after its deposition. This can be confirmed by the rather bad state of preservation of the pollen grains and spores. Numerous corroded pollen grains occur, for instance, in the Lateglacial peat of Lutterzand in the E. Netherlands (prep. nr. LTZ/A), which outcrops in the bank of the river Dinkel, some metres above the normal water level in summer. Another example is the Waalian clay at a depth of 97 m in the boring Eindhoven (prep. nr. EH 97), at which depth an old erosion surface has been considered to be present (personal communication by Dr. W. H. Zagwijn).

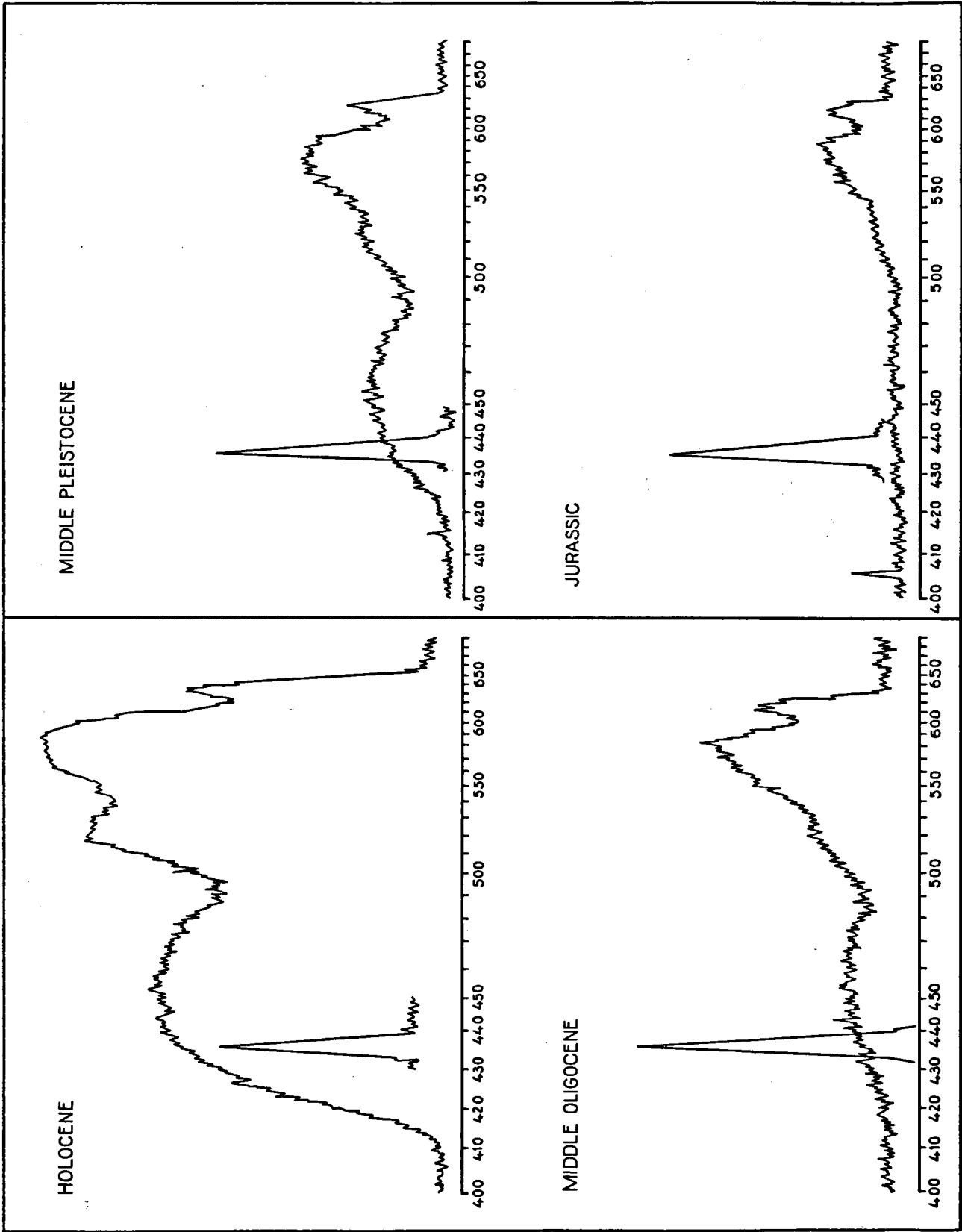
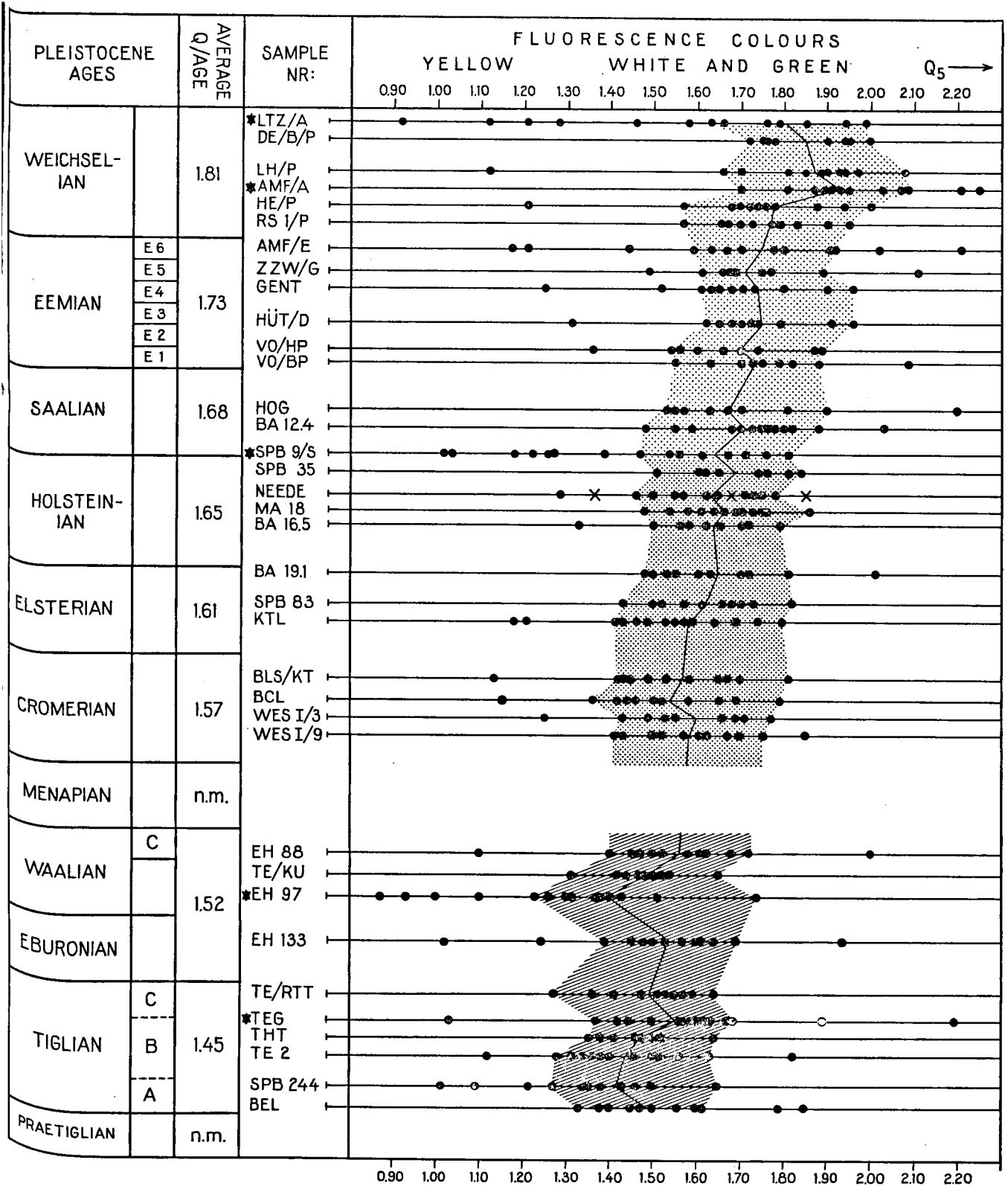


Fig. 23. Fluorescence spectra of some vesiculate pollen grains of various ages, obtained by means of an UV-micro-spectrograph.



SPECTRAL RATIO OF PINUS SYLVESTRIS FROM THE PLEISTOCENE

Fig. 24.

Corrosion of pollen and spores occurs repeatedly in older deposits as well, for instance in coals (Wilson, 1961, 1964). The chance of meeting with corroded pollen is greater in coarse sediments such as sand and loam than in fine deposits like clay and peat, in particular when they have been situated above the groundwater level for some time. A more intensive air circulation then results in damaged exines.

On the other hand, no differences between the fluorescence colours of pollen exines preserved in either clay or peat or diatomite were observed. It is remarkable, however, that in many cases small differences in fluorescence seem to exist between pollen grains from clay and from browncoal in the same Tertiary areas. More research is necessary in order to establish whether the action of geochemical coalification on clay and browncoal results in different fluorescence of the pollen grains.

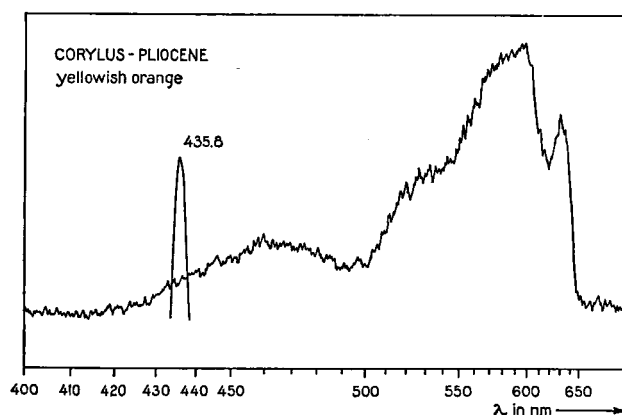


Fig. 25. Fluorescence spectrum of a *Corylus* pollen grain from the Pliocene, obtained by means of an UV-micro-spectrograph.

The fluorescence values of certain samples which were found to be too high for their geological age, are not so easy to explain. The processes of chemical transformation of the sporopollenine under the influence of geological time apparently have been delayed in certain deposits for unknown reasons.

Fluorescence of other forms

With increasing geological age, other forms of fossil palynomorphs show changes in fluorescence, similar to the colour series of Plates II and III for vesiculate pollen. A number of relative fluorescence spectra of other types from certain geological periods has been published previously (van Gijzel, 1963). UV-micro-spectrographical analyses demonstrate this phenomenon in more detail. Fig. 25 shows the fluorescence spectrum of a Pliocene *Corylus* pollen grain, which shows much resemblance with that of an orange-yellow *Picea* from the Holocene (fig. 19, orange curve). The spectrum of a Holocene *Corylus* pollen grain is similar to other yellowish white spectra, as, for instance, that of a Middle Pleistocene *Pinus sylvestris*

(fig. 23). It appears from a comparison of such spectra, that those pollen grains from young deposits which show yellow or orange fluorescence colours, e.g. *Corylus*, can shift in colour with increasing age, but to a lesser extent than in forms showing green or white colours, e.g. *Pinus* and *Abies*. This is especially true for spores which are dark blue in recent and subrecent material.

Recent and subrecent palynomorphs often show large differences in fluorescence colour, which have been described above (Chapter IV). With increasing geological age these differences diminish gradually and finally disappear almost entirely. In slides from the oldest rocks investigated here, viz. those from the Carboniferous, various forms of palynomorphs still show differences in fluorescence colour.

Wilson (1961, 1964) described the selective susceptibility of fossil spores from coal to corrosion and increase of temperature. With increasing rank of coal, some forms become opaque earlier than others. Where old erosion surfaces are present in coal, the spores appeared to be corroded selectively. The fluorescence phenomena appear to be in accordance with this selective susceptibility.

Photoplate I illustrates the colour change of other forms of palynomorphs, while fig. 5 shows a picture of a Tertiary pollen slide containing grains of *Pinus haploxylon* type and some Myricaceae. They all show fluorescence colours which are less different than those of grains of *Pinus sylvestris* and *Myrica* in the Young Holocene. Tertiary Myricaceae are more yellow and less white than younger ones. In fig. 8 a Palaeocene spore is illustrated, showing a yellow fluorescence colour. It may be assumed that this grain originally possessed a blue fluorescence.

Fluorescence of acritarchs and dinoflagellates

The fluorescence of fossil acritarchs and dinoflagellates was compared to that of palynomorphs. A number of spectra were determined by means of the Berek fluorescence photometry, the results of which are shown in Plate IV. The ages of the deposits studied, range from Middle Pleistocene to the Blue Lias (Jurassic). Some of the slides were also used for measurements of vesiculate pollen. It appears from these spectra that no relation exists between fluorescence colour and geological age of these microfossils. This is in accordance with many other visual observations. The orange, yellow and green colours occur together at nearly every geological age. A relationship between the fluorescence colour and type or species has not been established up to now, but the possibility cannot be excluded. This could explain the rather large variation in colour even in those specimens of old age, compared with fossil palynomorphs.

The influence of geological time, which is different from that on fossil palynomorphs, may be due to the fact that sporopollenine differs fundamentally in chemical nature from the tests of acritarchs and dinoflagellates, which consist of a chitinous substance. It

appears to be much more resistant to the action of coalification and geological time than sporopollenine. In certain cases the fluorescence colour is determined by the state of preservation. Brown and orange coloured microfossils occur simultaneously with more or less corroded palynomorphs as in contaminated deposits. Damage during the transport of the rebedded material may be the cause of a change of fluorescence colour as well.

APPLICATIONS TO AGE DETERMINATIONS

Fluorescence analyses for long-distance correlation

As stated in Chapter I, palynological age determination and correlation sometimes meet with great difficulties. This is the result of various factors, e.g. contamination with rebedded material, the facies of the sediment, climatological differences and corrosion. Fluorescence palynology opens up the possibility of age determinations and correlations, in particular for Cenozoic beds older than 50,000 years. In that era the differences in fluorescence values are larger than those of older eras. It may be successfully applied, under the condition, however, that the pollen grains are well-preserved and are not too strongly coalified. Besides, a large number of measurements on *Pinus sylvestris* grains, or pollen which is comparable in fluorescence colour and stratigraphical range, must be made. The variation in fluorescence colour and the average Q values can be compared with those of standard diagrams such as Plate III and fig. 24.

How fluorescence palynology may be applied to the study of these problems, will now be shown more extensively by some examples. The first is the long-distance correlation of Pleistocene sequences. Those from Padul (Spain), previously investigated by Menéndez Amor and Florschütz (1964), and from the Sabana de Bogota (Colombia, S. America), studied by van der Hammen and Gonzalez (1964), are compared with the Pleistocene pollen stratigraphy of the Netherlands, which has been established by Zagwijn (1961, 1963). The uppermost parts of the climatic curves are in accordance with each other and these correlations are supported by a number of 14 C dates. Difficulties arise, however, in the correlation of the older parts of these diagrams, for which the application of fluorescence palynology appeared to be useful (van Gijzel et al., 1967). For more details on palynological data, interpretations of the diagrams and climatic curves, reference should be made to that and the other papers mentioned.

Long-distance correlations can be effected by comparing fluorescence measurements according to the procedure mentioned above. A large number of spectral ratio determinations of these sequences are compared with those of the standard diagrams, based in many cases on deposits previously studied by Zagwijn. The results are summarized in fig. 26 and Table IV.

In the Colombian sequence, grains of the vesiculate *Podocarpus* were measured, the fluorescence values of

which are similar to those of *Pinus sylvestris*. In this table the arithmetic averages of the fluorescence analyses of groups of samples, belonging to certain supposed ages, were calculated. These averages for Q were placed in the table in the same position as those of the standard values. Not only in the standard diagram, but also in the two sequences, the change in fluorescence with increasing geological age is obvious. The divergent averages for some samples and ages may be caused by the action of corrosion, for instance, in the Eemian lake-chalk of Padul, the figures of which were left out of consideration here.

On the basis of fluorescence analyses the following correlation appears to be possible. The similarity between the Weichselian and Saalian glacials of Padul and N.W. Europe is evident. The data from the deeper layers give support to the opinion that a pre-Cromerian interglacial may be present in the Padul section at a depth of about 60–70 m. The correlation with the diagram of the Sabana de Bogota appears to be more complicated. The values of the lowermost part of this sequence are in accordance with the standard values of the Tiglian in Europe. This supports the idea that the Tiglian interglacial was represented here. In the interval between 95 and 125 m, the fluorescence figures suggest the presence of the Cromerian interglacial.

Other applications

Another example of the successful application of fluorescence palynology with reference to age determination is found in the study of contaminated Pleistocene deposits, the results of which are to be discussed in the next Chapter. Furthermore it may be applied, for instance, to the dating of scattered samples from borings or deposits, dredged out of the subsoil and in general to those beds, in which typical pollen associations or zonations in the pollen content are absent. Examples are pollen spectra from clay and peat, occurring in fossil skulls and bones of mammals, which have been dredged out of the river Meuse (the Netherlands) or from other localities where Pleistocene sands are exploited. Such spectra are often contaminated with rebedded material, which even may be of Mesozoic age. Numerous other examples of deposits, of which only a few samples could be obtained, can be easily found.

As stated above, fluorescence palynology can only be applied when the pollen grains are not corroded or coalified too strongly. In such cases the accuracy of fluorescence-palynological dating can amount to more than 80 %.

In addition, some other aspects of this method may be quoted here. As appears from the standard diagram of fig. 24, at least three groups of fluorescence colours can be distinguished in the Pleistocene: a Lower, a Middle and an Upper Pleistocene group. This number may even be extended to six groups: Weichselian, Eemian, Saalian with Holsteinian, Elsterian with Cromerian, Menapian with Waalian and Eburonian and a group of Tiglian with Pretiglian. This method

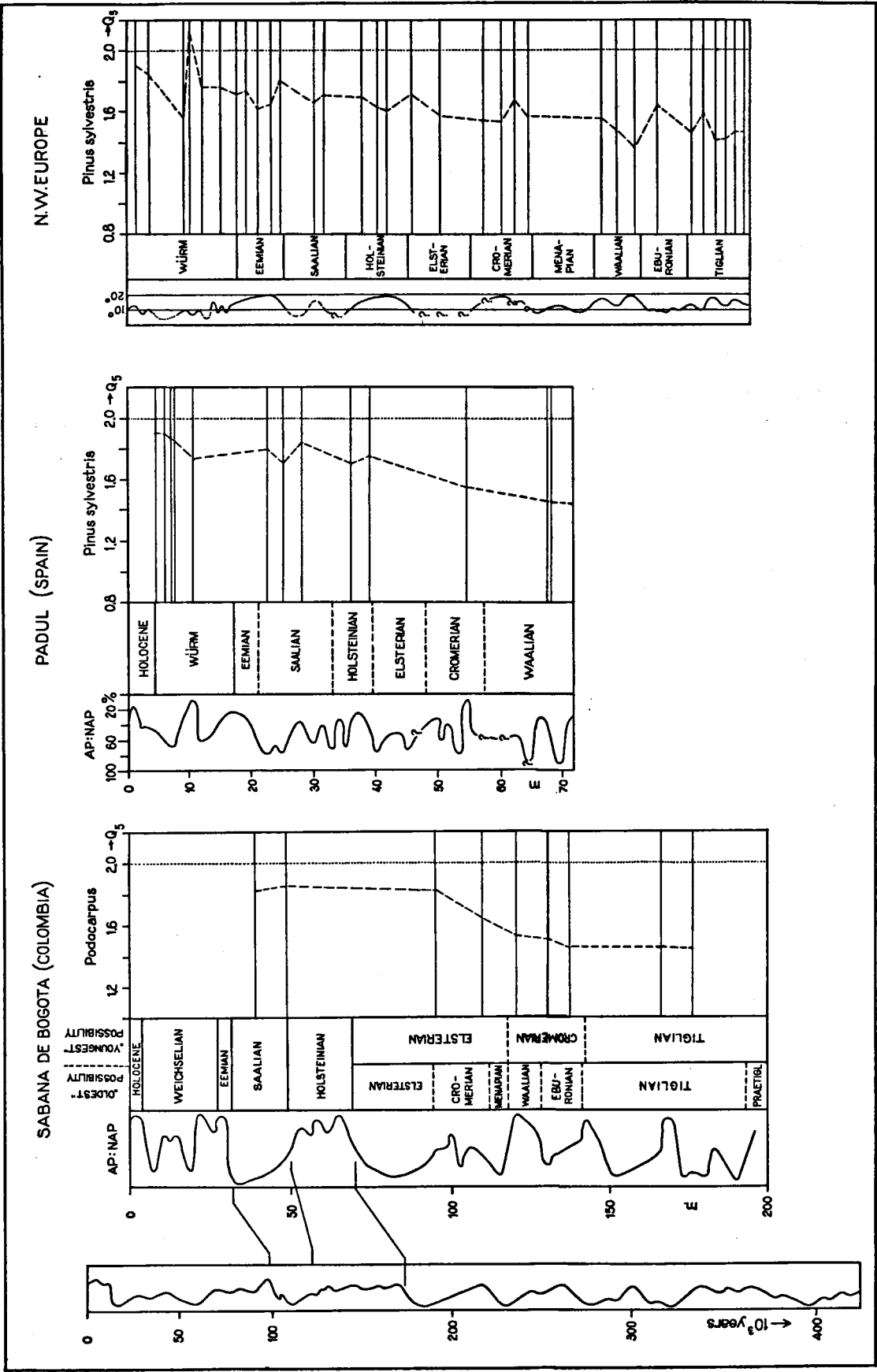


Fig. 26. Average values of the spectral ratio of fluorescent pollen grains used for long-distance correlation of climatic curves (after van Gijzel et al., 1967). The sea water temperature curve at the left is after Emiliani (1966); the stratigraphic subdivisions in the diagrams are after various authors (see text).

thus covers a range in age, between the lower limit of the 14 C method and the Pliocene, for which other absolute age determination methods exist. Some other new geochronological methods fill this gap, but they are still inadequate for determining the age of continental deposits. After some improvements to the procedure, fluorescence palynology may possibly furnish the solution to this problem.

Geochronology of the Pleistocene

It is possible that this method can contribute to the solution of another important problem, viz. the duration of the Pleistocene epoch. With regard to this a great controversy still exists.

Fluorescence data may contribute to the solution of this question. The samples from the Lower Pleistocene have been plotted in the absolute time scale of Plate III, according to the ages given by Eberl. With regard to the general tendency line, the Q averages for the Tiglian are situated somewhat too low. This may indicate that Eberl's figures may be somewhat too low as well. This could mean that the Plio-Pleistocene boundary is to be drawn at one million years or more ago. If so, then some of the oldest glacials and interglacials must have had a somewhat longer duration than assumed by Eberl. It appeared later on, however, that Eberl's opinion about the duration of the Weichselian glacial was too premature, as has been proved by

TABLE IV. Average values of the spectral ration of fluorescent pleistocene pollen grains¹

| Age | Northwestern Europe (<i>Pinus sylvestris</i>) average of Q | Padul (Spain) (<i>Pinus sylvestris</i>) depth in m | Sabana de Bogota | |
|-------------|--|--|------------------------------------|--------------|
| | | | (<i>Podocarpus</i>) prep. no. | average of Q |
| Weichselian | 1.81 (37) | 4.5—10.5 | | n.m. |
| Eemian | 1.73 (32) | 16.5—18.0 | | n.m. |
| Saalian | 1.68 (16) | 22.4—28.0 | 220/236 | 1.75 (12) |
| Holsteinian | 1.65 (26) | 35.7—39.2 | | n.m. |
| Elsterian | 1.63 (20) | | | n.m. |
| Cromerian | 1.57 (26) | 54.5 | 339/367 | 1.79 (12) |
| | | 67.5—71.2 | | |
| Menapian | n.m. | | | n.m. |
| Waalian | | | 389 | 1.47 (8) |
| Eburonian | 1.52 (42) | | 409/420 | 1.42 (14) |
| Tiglian | 1.45 (46) | | 483/492 | 1.43 (14) |
| Practiglian | n.m. | | | n.m. |

1. Numbers in brackets indicate the number of objects measured; n.m. = no measurements; the preparation no. of the Sabana de Bogota are from Van der Hammen and Gonzalez (1964), (after Van Gijzel, et al., 1967).

The geochronological data are mainly based on the curve of changes in the radiation of the sun during the last one million years, calculated by Milankovitch and others. Among the numerous interpretations of this curve, Eberl (1928) mentioned the highest value for the lower boundary of the first glacial, situated at 761,000 years. In Plate III this figure has been drawn as the Plio-Pleistocene boundary.

On the other hand, it is remarkable that for this boundary the radio-active age determinations delivered figures of at least one million years (Holmes, 1959) or much more (1.5 m.y. according to the Geological Society Phanerozoic time-scale 1964). Furthermore, during the last few decades palynologists have succeeded in establishing a complete picture of the vegetational history of the Pleistocene. In the author's opinion the possibility that one or more glacials and interglacials should have been overlooked is highly unlikely.

14 C datings. On the other hand, no indication exists that the Lower Pleistocene stages were of longer duration than those in the Upper Pleistocene.

The situation of the Plio-Pleistocene boundary is obviously a very complex problem. Many more fluorescence palynological data are needed, however, to find a synthesis, which is in accordance with both absolute dating figures and palaeoclimatological studies.

DISCUSSION OF RESULTS

In reviewing the fluorescence data with increasing geological age, some critical remarks have to be made.

1. A number of samples studied, show average Q values, diverging too much from the general tendency and the fluorescence figures of the time unit to which they belong. In order to increase the accuracy of this method, it is necessary to carry out a larger number

of determinations on comparable deposits. A study of the causes of the fluorescence phenomena described is needed therefore. This refers in particular to the large variation in fluorescence colour of fossil palynomorphs in certain sediments.

2. As stated above, the average *Q* values for the Mesozoic and Palaeozoic must be established more exactly by using samples of selected material, which are not or only slightly coalified. Such deposits are not easy to find.

3. The disadvantages of the Berek photometer must be taken into account in the interpretation of the fluorescence data. More accurate figures of the spectral ratio may be obtained when intensities in the green and yellow range of the spectrum can be measured.

4. In spite of these restrictions, fluorescence palynological dating is delivering reliable results. It needs, however, further investigation. Much care must be taken in sampling, preparing and measuring of the material.

VI. FLUORESCENCE AND DATING OF CONTAMINATED SEDIMENTS

THE CONTAMINATION PROBLEM

In the Introduction it was stated that the problem of pollen contamination was the starting point of the present fluorescence investigations. Contaminated deposits represent an old and complex question. It is useful to quote the essential part of earlier attempts at reaching a solution.

Contamination takes place when *autochthonous* pollen grains and spores produced by the vegetation in and around the area of sedimentation are mixed during their deposition with *secondary* or *redeposited* specimens. This reworked material is found in different quantities, varying from the one case with a small amount consisting of a few species to the case in which the allochthonous components may entirely predominate over the autochthonous components.

Palynological dating and correlation are based mainly on the presence of typical pollen assemblages and zonations in pollen diagrams, by which the vegetational and climatological history is reflected. These palynological characteristics may become unclear or even disappear by contamination with either older and reworked pollen and spores or with contemporaneous material from vegetations situated at a great distance from the site of sedimentation. Even a small amount of such material may prevent the establishment of zonations.

The quintessence of the question is how to distinguish the non-contemporaneous secondary pollen and spores from the autochthonous grains in case that both belong to the same form or species. For instance, in a mixture of Miocene and Pleistocene elements, the Tertiary representatives are for the greater part recognizable and can be left out of consideration. But how does one ascertain which number of such forms as e.g. *Pinus sylvestris*, *Picea*, *Abies*, *Alnus*, *Betula*, *Carpinus*, *Myrica*, belongs to the autochthonous and which to the reworked part of a pollen spectrum?

Thomson (1935) described a contamination from older interglacial layers in Late Glacial deposits.

Iversen (1936) tried to find a solution in his paper "Sekundäres Pollen als Fehlerquelle". He analysed the boulder clay of Egebjerg, Fyn, Denmark, which contains numerous pollen grains of thermophilous trees. A large number of them also occurs as con-

tamination in the overlying Late Glacial deposits. The amount of redeposition could be determined by subtracting the quantitative pollen composition in the boulder clay from that in the younger layers. The remaining pollen spectra corresponded to the autochthonous vegetation and resulted in an uncontaminated diagram.

Iversen's method is, however, very limited in its possibilities. In general it can only be used if the origin of the contamination is known and is situated nearby as was shown above, or if part of a layer has remained uncontaminated and the vegetation has not changed during sedimentation.¹ In that case only is it permitted to assume a constant average pollen content, which may be subtracted from the reworked spectra.

No difficulties arise when the contaminations are so different in age, that the autochthonous components have nothing in common with the secondary species. Such old reworked pollen sometimes occurs in the Pleistocene deposits of the river Meuse in the S. Netherlands, in Middle Tertiary marine clays and glauconitic sands in the Netherlands and N.W. Germany and in deep-sea sediments, which are only contaminated with Mesozoic or older material.

In most cases, however, the origin, age and amount of contamination are difficult to estimate with any degree of accuracy. The source may have been removed by erosion and then calculations according to the Iversen method become impossible (see Davis, 1961, and others). Besides, the source deposit itself possesses in general a certain variation in its pollen content and an average pollen content will not be easy to find. The homogeneous dispersion of pollen grains in boulder clay, caused by the kneading and mixing activity of the inland ice, forms a favourable exception.

Furthermore, selection of secondary pollen during erosion and transportation over long distances in river or sea water may occur and the chance of more sources of contamination increases in such a case, delivering

¹ For example in the Upper Saalian or Lower Eemian clay of Amsterdam—Slotermeer in the Netherlands, analysed by Zagwijn (personal communication).

reworked pollen in variable amounts. It will therefore be obvious that by means of conventional methods qualitative comparison at best is permitted and that successful quantitative analyses are favourable exceptions.

Thomson (1952) remarked that rebedded pollen can be expected mainly in all fine-grained clayey sediments, where they sustain destructive alkaline reactions and oxidation. He mentioned the clay of Satzvey in Germany as an example, the lower part of which is without doubt of a Middle Tertiary age. But the clay in the overlying browncoal conglomerate contains numerous Lower Tertiary species, resulting in pollen spectra which are comparable to the Lower Eocene of Helmstedt. Thomson presumed that the well-known "helle Schichten" (light bands), often occurring in lignites and fimminites (lake deposits, consisting mainly of pollen exines) might be the source of reworked material, rebedded in the clay and resulting in a domination of the secondary pollen over the autochthonous pollen content.

Contamination can be caused, however, by long distance airborne transport of fresh pollen as well. Since Erdtman made his trip across the Atlantic (1937), it has been generally accepted that airborne pollen grains can be transported over long distances. Such pollen, especially bisaccate grains as in the conifers, may have their origin far away. Aario (1940), for instance, found in surface samples from the tundra vegetation of Lapland tree pollen, mainly of conifers, but of *Tilia* as well, which must have been transported by the wind over hundreds of kilometers and was finally deposited in the tundra of Northern Europe. Such contamination is not recognizable if it consists of the same pollen species as occurring in the autochthonous vegetation far from the area of sedimentation. In general its influence will be rather small. This aspect of the contamination demonstrates however that it is not easy to define this phenomenon exactly. When does one consider airborne pollen secondary and where does one draw the limits of the area of origin of the autochthonous group? On the other hand, what is the minimum difference in age, necessary to establish whether a pollen diagram shows contamination or not? The question naturally arises whether the occurrence of *Picea* and *Abies* pollen in the Upper Holocene pollen diagrams of the Netherlands has to be considered a contamination either by air or by water transport (see below).

If considerable differences in age may be assumed, the contamination problem can be investigated successfully by the application of fluorescence palynology, on the condition, however, that for each important pollen form various groups of fluorescence colour can be distinguished. Only then it is certain if a grain belongs to the autochthonous part or not. Fluorescence palynology produces, consequently, a direct method of testing autochthony contrary to the indirect method of Iversen. Its procedure and use will be described below and illustrated by some examples of fluorescence analyses of contaminated Pleistocene deposits.

EXAMPLES OF CONTAMINATED SEDIMENTS IN LITERATURE

The increasing importance of the study of contamination phenomena is apparent from the steady increase in the number of publications on this subject. It therefore appears useful to review some of the most important studies, of which those by Iversen (1936) and Thomson (1935, 1952) were already mentioned above.

Pflug (1963) attempted to solve the problem of rebedded pollen at his review of the pollen analysis of salt deposits on the Gulf Coast (U.S.A.), which were carried out earlier by Jux. Pflug examined the original preparations, which show a considerable contamination, among others by means of fluorescence techniques but he kept silence upon the method used. Examples of long distance pollen transport are to be found in some Holocene clays from the Netherlands and N.W. Germany. With the lithological change from peat into brackish clay the values of *Pinus sylvestris* increase rapidly, whilst in many cases *Picea* and *Abies* appear simultaneously in small numbers, though they must have been absent in the Atlantic and Subboreal vegetation of these areas (Overbeck and Schmitz, 1931; Polak, 1936). A similar occurrence has been found by Florschütz and Jonker (1939) in a clay and peat section at Wijk bij Duurstede, The Netherlands. Particles of these clays, deposited by the river Rhine, settled down simultaneously with conifer pollen derived from the forests of the upper Rhine region, where *Picea* and *Abies* composed the most important elements of the vegetation. In all these cases, however, it is uncertain whether the sum total of this pollen had been transported by sea or river water only, because it occurs repeatedly in small percentages in the peat as well.

Much more attention has been paid in the literature to contamination with older reworked pollen. An extreme example is the dominance of Tertiary pollen associations in Pleistocene clays in the N. Netherlands to such an extent (60 % or more), that these clays often seem to be of Tertiary age, as for instance the so-called "potklei" (pottery-clay). The contamination prevented the finding of a palynological correlation of the stratigraphical sequence in this area with that of the Lower and Middle Pleistocene in the S. Netherlands (van Gijzel, 1961, 1963).

In the central part of the Netherlands contamination has been reported for the Middle Pleistocene at Loenermark (Polak et al., 1962), at Zweiersdal and Duno (Teunissen and Florschütz, 1957; Teunissen, 1961) and for the Late Glacial sand and lake-marl of the "Bleeke Meer" (Polak, 1963).

Marine and brackish sediments often appear to be contaminated, as, for instance, the Plio-Pleistocene clays in the harbour pit of Antwerp (Hacquaert, 1960) and the marine Miocene glauconitic sands and the Oligocene "Septarienton" (septaria clay) in the subsoil of N.W. Germany and the Netherlands.

Some examples in other areas may be quoted. Davis (1961) studied the problem of rebedded pollen in Late

Glacial sediments of Taunton, Mass. (U.S.A.) by means of Iversen's method and concluded that the mixed autochthonous and secondary pollen content could not be interpreted successfully. Cushing (1964) found pre-Quaternary microfossils in Late-Wisconsin silty sediments of East-Central Minnesota (U.S.A.). Moreover they contain corroded pollen, derived from inwash of soil from the surrounding slopes. Subtraction appeared here again inadequate to distinguish both groups.

Rebedded remains of lignites and other deposits from the Upper Pliocene were found at Fürstenhagen near Hessisch-Lichtenau in W. Germany by Brosius (1958). They were redeposited in the Lower Pleistocene under periglacial circumstances. It appeared difficult, however, to establish the degree of contamination due to the presence of only a few typical Tertiary species.

Recently a large number of contaminated sediments have been reported from the U.S.S.R. Chiguryayeva and Voronina (1960) described rebedded microspores of various ages (Palaeozoic to Cenozoic) in Upper Pleistocene deposits in the North Caspian area. They tried to distinguish the secondary pollen by staining and other treatments, but the corrections of the spectra obtained, appeared to be unsatisfactory. Ralska-Jasiewiczowa (1960) noticed that Pleistocene layers at Zablocie on the river Bug were partly contaminated with numerous grains from the underlying Tertiary. Arkhipov and Matveyeva (1960) reported the occurrence of much redeposited pollen from the Jurassic and Cretaceous (max. 45 %) and numerous individual grains of Tertiary origin in the Pleistocene pre-Samarova deposits in the Yenisei region of the West Siberian Lowland.

It is remarkable that contamination occurs so often in continental Pleistocene deposits. Various factors may be responsible for this. In the first place the chemical weathering and other destructive processes by which the pollen grains can be damaged, were not as intensive in a cold or temperate climate as in a (sub-)tropical one. Consequently the reworked pollen may have more easily sustained the destruction by erosion and transport during the Pleistocene glaciations, than in warmer epochs. Secondly, the thick and extensive Tertiary browncoal layers contain an abundance of pollen and spores. Thus a great opportunity existed during the Pleistocene — an epoch with strong glacial and river erosion in many regions — for these deposits to become a prey of river or inland-ice erosion and for this reason to become the source of contamination in the succeeding glacials. Many Tertiary browncoals in N.W. Germany appear to have been partly or completely eroded during the Pleistocene and supplied their pollen to the rivers (see below).

The mechanical destruction during river transport influenced the state of preservation of the rebedded pollen to a lesser extent than did the chemical weathering. An analogy is found in the conservation of other rebedded microfossils as for instance Bryozoa in boulder-clay, which due to their small size, better

resisted the strong kneading by the inland-ice than the weathering of this clay after its deposition (Veenstra, 1963). Fossil pollen and spores in boulder-clay show a similar resistance with respect to mechanical destruction. They resist the less destructive transport by river water rather easily.

Recently the study of contamination has been enlarged in scope by the development of marine palynology. During the last few years deep-sea sediments have become a new field for palynological investigation, by which pollen diagrams of Holocene and even Pleistocene sediment sequences may be obtained. Pollen and spores have been deposited more or less continuously on the ocean floor, contrary to continental sedimentation where erosion and sea-level changes gave rise to incomplete lithological successions. It might therefore be expected that pollen analysis of deep-sea sediments, where the influence of erosion is to be neglected, might be a means of establishing complete climatic curves for the Quaternary.

In many cases, however, it appeared that these deposits contain a mixture of reworked pollen grains and spores, ranging from the Palaeozoic to the Quaternary (Groot and Groot, 1966; Stanley, 1965, 1966a). This contamination hampers the investigation of climatic curves considerably. Even sediments from the sea floor at small depths contain a large amount of rebedded pollen, as has been found by Zagwijn and Veenstra (1966) in the North Sea, where Mesozoic and Paleozoic miospores often reach more than 25 %. As pointed out by Groot (1964, 1966) it is very important to find a solution to this contamination problem, in order to obtain reliable pollen diagrams and climatic curves. It is also important for the future use and development of marine palynology in connection with other investigations of these sediments, for instance the study of Foraminifera and sea-water temperature determinations, that this problem cleared up.

Stanley (1965, 1966a, 1966b) tried to distinguish the secondary material from the non-reworked grains by means of staining with safranin "O" liquid. He found four groups of contamination, based on the decreasing susceptibility for staining with increasing geological age: Palaeozoic-Lower Mesozoic, Mesozoic, Upper Mesozoic and Lower Tertiary and the group of Quaternary and recent. This statement is in accordance with the results of the first experiments on the contamination problem, made by the present author, from which appeared, however, that the staining method is in fact inadequate, as it does not give any information on contamination within the Cenozoic era. Besides this the limits of Stanley's groups are very vague. Nevertheless it means an important advance in the search for a solution to the contamination problem, which is one of the most difficult questions in palynology.

Fluorescence palynology appears to be a useful expedient for contamination investigations. This will be shown here by fluorescence analyses of a number of strongly contaminated Pleistocene sediments from

the N. Netherlands, as for example the above-mentioned "potklei" and older deposits, of which the source of the rebedded material could be established. Some examples of contamination to a minor degree are given in addition.

EXAMPLES OF FLUORESCENCE PALYNOLOGICAL DATING

Pleistocene clays in the N. Netherlands and N.W. Germany a) *Previous studies*

A vast area, extending from the Waddenzee and the former Zuiderzee in the N. Netherlands up to the

and muscovite and often showing structures resembling varves (fig. 27). Where they are situated just below the boulder clay, they have been pushed by the Saalian glaciers, which gave rise to the very complicated structures in the sand lenses within the clay. Such features are exposed, for instance, in the clay pit of the "Lauenburger Ton" of the Bookholzberg (fig. 28). Similar structures are found in the "potklei" as well (van Gijzel, 1967b).

Various opinions exist about the age of this complex, which has been supposed to be Late Elsterian, Holsteinian or Early Saalian. In the subsoil of the Hamburg area these beds are known to be separated from the Saalian boulder clay by layers of marine



Fig. 27. Bottom of the clay pit Basedow near Lauenburg upon Elbe, N.W. Germany, showing the Lauenburger Ton complex, consisting of an alternation of fine sands and thin clay layers with fine folds (left and right) and small faults (centre) as ice-pushed structures.

town of Lauenburg, E. of Hamburg in N.W. Germany, contains in the subsoil under the Saalian boulder clay a complex of fine sand and clay deposits, which is known as "potklei" (pottery clay) and in Germany as "Lauenburger Ton" (clay of Lauenburg). By German geologists it has been considered as a guide-horizon for the Middle Pleistocene in this area (for a summary see Woldstedt, 1955). These sediments reach a thickness of tens of metres or more; they are black, brown or dark grey, very humic and rich in pollen. Sometimes they are finely layered or banded by an alternation of clay and fine sands, being rich in biotite

Holsteinian, from numerous borings to a depth of 20 to 50 m below surface (Schucht, 1908; Gripp, 1933). It is assumed that the clay might have been deposited in a glacial basin during the Late Elsterian, possibly in melt water, that was brackish due to the upward movement of salt domes under the pressure of the inland-ice cover (Gripp, 1952).

The equivalent of this complex in the N. Netherlands locally reaches a thickness of 100 m or more, for instance near Winschoten, Assen and Sneek, where it is situated between coarser Middle Pleistocene sands and gravels. Sediment-petrological studies of the beds

underneath the Saalian boulder clay in this area pointed out that the "potklei" complex belongs to the Middle Pleistocene, although the monotonous picture of its heavy-mineral diagrams cannot be compared with the mineral associations of the surrounding formations (Edelman, 1933; Zonneveld, 1958; and other papers). Besides it contains an abundance of Tertiary pollen, which suggests a Pliocene or Miocene age.

The first palynological investigation of the "potklei" complex was carried out by Brouwer (1948), who considered that so far as its uppermost part is concerned

clay, in which numerous Reuverian representatives appeared to occur. Nevertheless, no doubt existed about the Middle Pleistocene age of this clay. Geological investigations by ter Wee (1962) proved this again and advanced new arguments for an Elsterian age of its upper part.

It appeared to the present author that the amount of contamination must be estimated to be 60 % or more (van Gijzel, 1963). The method of Iversen is not to be used here, as pointed out by Brouwer (1948), because the sources of contamination are not exactly known. Hydrogeological study, made by Gischler



Fig. 28. Clay pit Kamern in the Bookholzberg near Oldenburg, N.W. Germany. Strongly ice-pushed Lauenburger Ton with curved sand lenses. Remains of Saalian boulder clay just below surface on top of the weathered uppermost part of the clay complex (horizontal lines represent excavation surfaces).

it should have been deposited during the interstadial of the Saalian glacial. Sedimentation took place in erosion gullies, that were formed in the preceding cold stadial of this glaciation. The pollen diagrams are rather monotonous with a considerable amount of contamination from Tertiary pollen and hystrichosphaerids. A correlation of these diagrams with the Holsteinian interglacial vegetational succession as found by Brouwer (1948) in the borings of Bantega, Bergumerheide and Spannenburg, appeared to be impossible.

Some time later Florschütz (in van Heuveln, 1959) paid special attention to the secondary pollen in this

(1967), proved that when the "potklei" attains great thickness it belongs to at least two gully systems, considerably different in age.

Only a short review of this problem is given here. For more information reference should be made to the papers mentioned and to a special study by the present author (van Gijzel, 1967b). Some attention must be paid here, however, to the origin of these fine sediments and their secondary pollen.

b) Sources of contamination

Pollen grains, spores, acritarchs, some indeterminable remains of diatoms and very rarely reworked tests of

Balanus have been preserved in this clay; no other fossils (except wood fragments) are to be found. This points to the fact that their origin must be found in continental deposits, being very rich in pollen and mainly fine-grained. In the author's opinion this problem can be solved as follows:

In the Lausitz region and adjacent areas, situated S.E. of Berlin in E. Germany, various Upper Tertiary clays, fine sands and lignite seams are present in the subsoil, and extend over a vast area (fig. 29). In many places they fell a prey to erosion, as appears from the map of fig. 30 (after Cepek, 1960 and

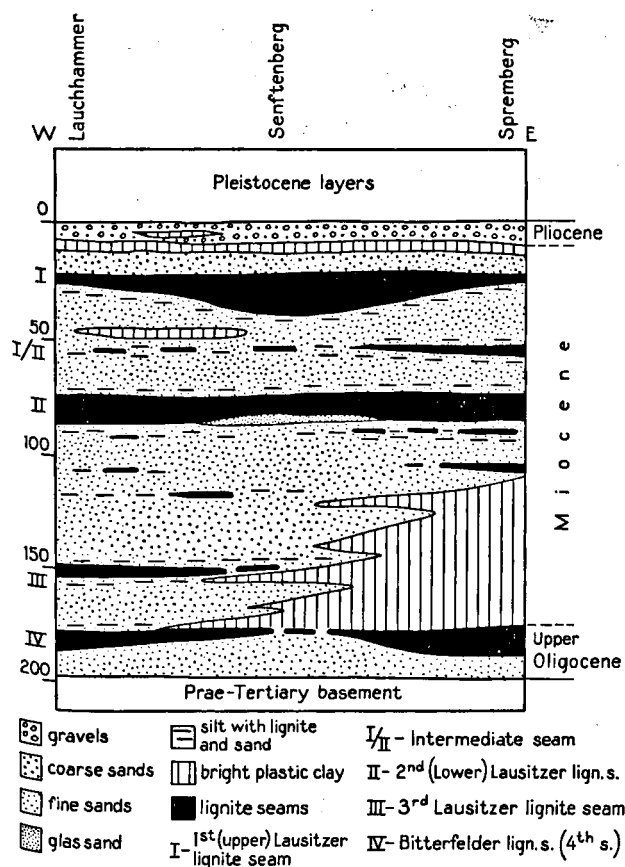


Fig. 29. Stratigraphical sequence of the Tertiary in the Lausitz browncoal region, E. Germany (after Mehner, 1960).

Mehner, 1960). The uppermost lignite seam (first Lausitz seam) and attending sediments are often lacking in places where ancient Pleistocene river valleys are situated, while their extension is larger in the non-eroded plains between them. The geological map of N.W. Germany shows an extensive pattern of these ancient river valleys (called in German: "Urstromtäler"), possessing an E—W direction from the river Vistula in the East to the Netherlands in the West and connecting the great Polish and N. German rivers Vistula, Oder, Elbe and Weser with the glacial valleys in the N. Netherlands. After their formation during Pleistocene glacials these valleys were filled up, mainly with coarse sand and gravel. Their origin is to be explained as follows:

When in a glacial, for instance in the Elsterian, the inland ice moved from Scandinavia southwards, these rivers were dammed up and forced to divert their lower course through new valleys leading in the direction of the North Sea. These valleys were cut in Pleistocene and Tertiary substrata. Increased erosion resulted from the simultaneous lowering of both the sea level and the erosion base of these rivers. These rivers carried away a large quantity of clay, sand and lignite, mainly of Miocene and, if present, of Pliocene age. In numerous open-cast mines in the lignites and in borings in this area these Pleistocene erosion gullies (fig. 31) are found (Wagenbreth, 1960; Hultzs, 1960; Viète, 1960), and in some cases the second Lausitz lignite seam lying at greater depth has been partly removed by erosion (fig. 32).

When the Scandinavian glaciers advanced further to the west and the south, the German rivers had to pass by the N. Netherlands, where they continued their gully formation. It may be assumed that during the next phase of glacier advance the North Sea basin was dammed up by the ice barrier and became a great meltwater lake, in which the fine material from E. Germany was deposited. This process was followed by the filling up of the "potklei" and Lauenburg clay gully system. After the retreat of the glaciers the rivers resumed their original course.

In principle these events may have taken place in each glacial stage, on the condition, however, that the glaciers extended far enough to the south in order to bend the river courses westwards (which did not occur in the last glacial, the Weichselian) and far enough to the west to dam up the North Sea. A natural barrier in the Dover Strait was present to block its southern exit. This was apparently not the case during the Saalian glacial. We know fine-grained basin deposits, though on a limited scale and very locally, indicating that no blockage occurred.

In the Elsterian and the older Pleistocene glacials the circumstances for the sedimentation of such contaminated deposits on a large scale were more favourable, as appears from the numerous occurrences of secondary pollen in the deeper borings in the N. Netherlands. The more or less strong contamination of these clays and sands prevented the establishment of a correlation with the Middle and Lower Pleistocene pollenstratigraphy in the S. Netherlands, in which Zagwijn (1957, 1960) described the wellknown sequence of glacials and interglacials.

Procedure for measuring and evaluation of data

The measurements and calculations of the spectral ratio Q were carried out according to the procedure described in Chapter II. In order to distinguish various groups of contamination, a number of pollen grains had to be measured, viz. of each sample at least 20 grains of *Pinus sylvestris*. Although the pollen grains appeared to be generally wellpreserved, the slides sometimes contain a certain amount of corroded pollen, which has been left out of the calculations of the average Q values per group. Only those grains of

ANCIENT RIVER VALLEYS IN THE NIEDERLAUSITZ BROWNCOAL AREA

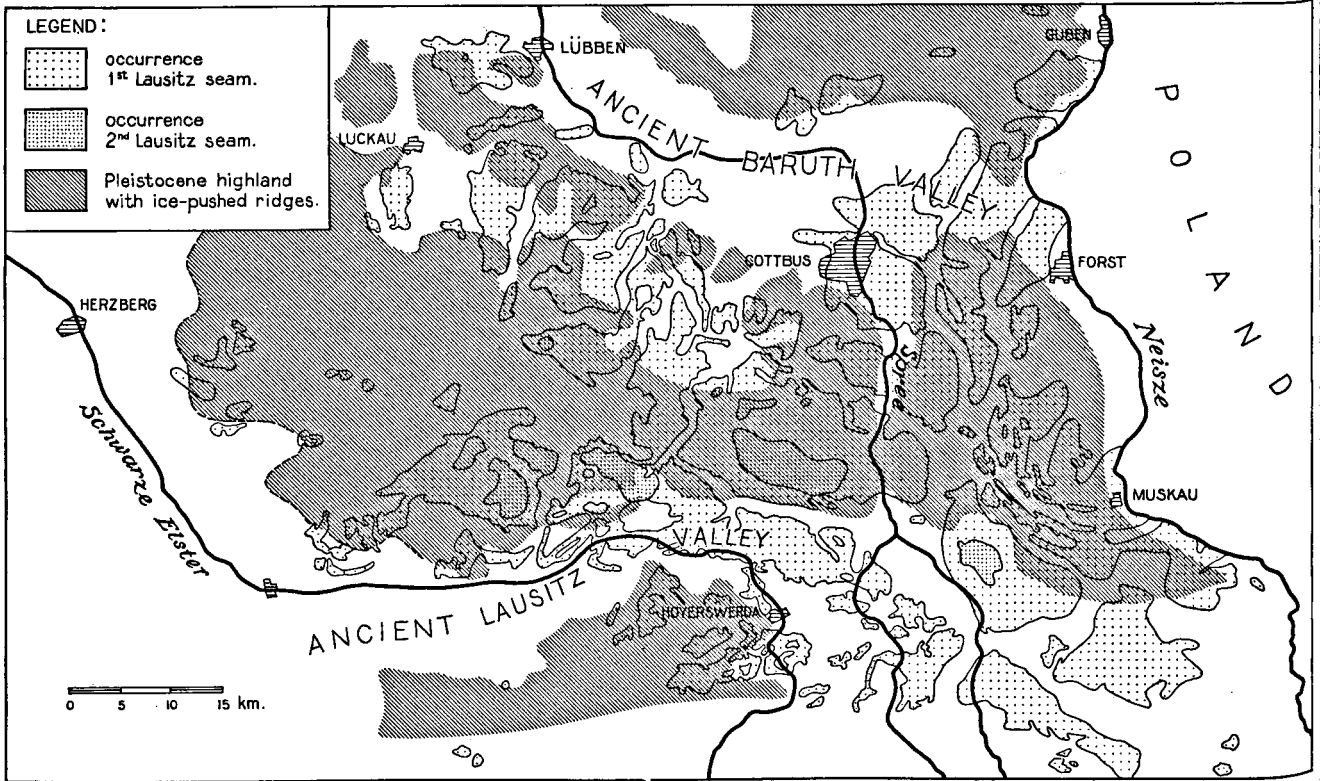


Fig. 30.

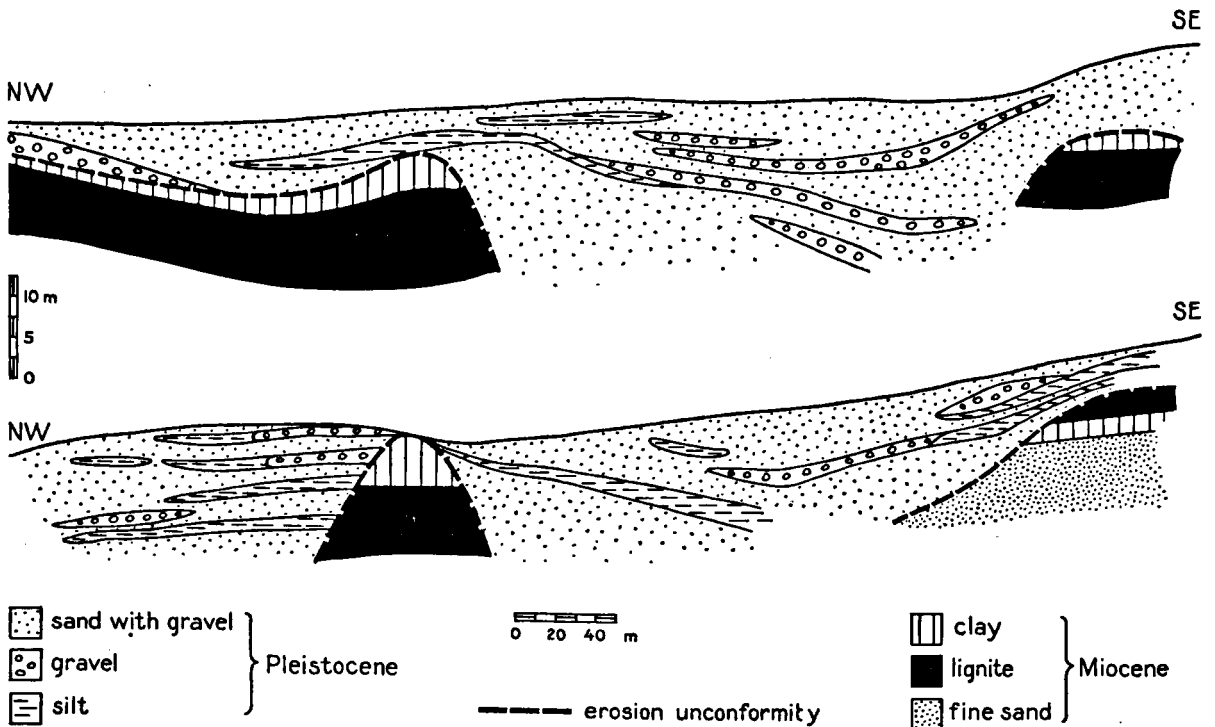


Fig. 31. Section through the "Senftenberg ancient Elbe valley" near Hoyerswerda-Bernsdorf with gravel and sands, covering ice-pushed and eroded Miocene beds (after Hultsch, 1960).

which the sculpture showed no considerable corrosion have been used.

All analyses have been summarized in plate V, in which each dot represents the spectral ratio of one object. The results of the measurements of a sample are recorded on a vertical line. In contaminated deposits the pollen show a greater variation in fluorescence colour and spectral ratio, compared to those of the standard diagram (plate III and fig. 24). This variation reached down to the lowermost part of the colour scale. One or more groups of Q values will occur, corresponding to the visually observable fluorescence-colour groups of each pollen form.

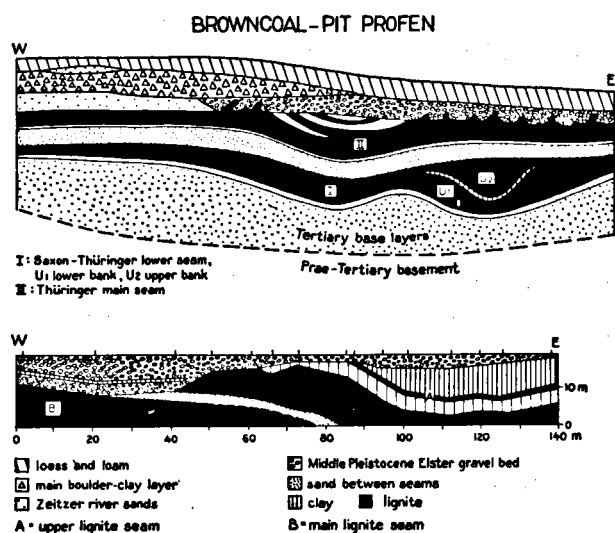


Fig. 32: Top: general review of browncoal exposure of Profen with browncoal seams, being partly ice-pushed and eroded in the upper part. Below: detail section of exposure nr. 4 in this pit (after Wagenbreth, 1960).

In each case the uppermost group reflects the real age of the samples investigated; the groups of lower Q values represent rebedded pollen. The geological age of each group is determined by the average Q value per group. These averages of Q values are connected by a line.

The following points must be taken into account in the interpretation of the figures. The number of measurements in each group does not indicate the percentages of each contamination group in the pollen content of a sample. These percentages have to be established by means of extensive countings under UV-illumination. The boundaries of the groups have been drawn in general in such a way that a group includes about 80 % of the Q values in a certain range. In a separate column on the left, the standard average Q values for the various Pleistocene and Tertiary ages are given. They have been derived from the diagrams of spectral ratio and geological age. The large group of coalified and corroded pollen does not correspond with the ages in the stratigraphical column, due to the decrease of Q as a result of coalification and corrosion. They are united in one group with the

lowest values, because in this region it not always appears possible to distinguish whether the colour of a grain is determined by coalification or by corrosion. Pleistocene deposits have only been chosen as examples, because the difference in fluorescence between the various ages is greater in Pleistocene than in older deposits.

The diagram largely speaks for itself although it seems worthwhile pointing out some of the conclusions that may be drawn. For geological and other data regarding the samples reference should be made to Table VII (Appendix).

Fluorescence analyses

a) The Lauenburger Ton in N.W. Germany

The following samples of this clay have been studied: Lauenburg (nr. 12) from the type-locality and Bookholzberg (nr. 18) near Oldenburg, W. of Bremen. The presence of Q values, corresponding to the Elsterian-Cromerian group, confirms the opinion that the clay complex is of an Elsterian age. The contamination was formed by an important group of Lower Pliocene to Upper Miocene pollen and a considerable number of coalified ones. These results are in accordance with the assumed origin of these fine deposits in the Lausitz area.

Besides, the large amount of rebedded pollen in the Lauenburger Ton and the related Dutch "potklei" is admissible by the supposition that they have been deposited during a glacial. The arctic vegetation produced only a small number of pollen grains compared to the abundance of Tertiary specimens supplied by the eroding German rivers. The autochthonous representatives thus became outnumbered by a mass of rebedded grains during their sedimentation in the glacial gullies. The Q values of the rebedded coalified pollen of *Pinus sylvestris* are in accordance with those of the lignites from the Rhine area in W. Germany (Q is less than ca 1.10). The lignites from both areas are of the same age and rank of coal. Therefore it may be assumed that the spectral ratio of the pollen of the Lausitz area will be similar to that of the Rhine area. The occurrence of Tertiary forms of pollen and spores and those types found in Tertiary as well as recent sediments in the samples measured have been summarized in Table V, in which a separate column shows the form groups, found in the uppermost lignite seams from the Spreetal pit in the Lausitz area (Schneider, 1965). Much pollen of conifers is present there together with alnoid, ulmoid and tricolporate groups and numerous *Filix*-like spores. Besides these, many grains of the Taxodiaceae, Cupressaceae and nysoid pollen occur. These forms are found in the Lauenburger Ton and "potklei" as well.

b) Banded clay of Gindow

Although the banded clay (in German "Bänderton") of Gindow (near Berlin) does not belong to the Lauenburger Ton complex, occurring more to the W. only, this clay is also strongly contaminated in a

similar way. A photograph of this kind of contamination is given in the Photoplate (fig. 4), showing the great differences in fluorescence colour between the white autochthonous and dark yellow or brownish secondary *Pinus* grains.

It has been supposed that this clay was deposited in an ice-carved basin after the retreat of the Saalian inland ice (Diener, 1960), but due to the lack of certain data this dating has to be considered provisional. The underlying sands contain contaminated pollen spectra and the entire complex is situated on a boulder clay basement. Fluorescence measurements give the impression that an Elsterian age of the banded clay is more admissible. The contamination appears to be somewhat younger than in the Lauenburger Ton; but here again much coalified pollen occurs, according to the observations of Diener.

c) *Peat and clay of Adendorf*

Near Adendorf, S. of Lauenburg but on the other bank of the river Elbe, a clay layer of some metres thickness is found overlying a thin peat deposit (30 cm). Although the clay is similar in lithology to the Lauenburger Ton, it might be attributed to the Holsteinian interglacial fresh water beds, which are present in many places in this part of N.W. Germany (see Gripp et al., 1941).

Fluorescence analyses indicate that this clay must be younger than the Lauenburger Ton, although it contains a slight contamination of coalified *Pinus sylvestris* pollen in contrast with the underlying peat, in which no distinct groups of rebedded material can be distinguished. It is possible, however, that the grains of *Pterocarya*, *Tsuga*, *Pinus haploxylon*-type and a few other remains which may be of Tertiary age, have been derived from eroded young Tertiary deposits. They are, however, only found in small percentages.

d) *The "potklei" of the N. Netherlands*

Two samples of the thick "potklei" complex near Winschoten have been studied: one from a depth of 3.9 m and one from 99 m. The upper clay contains an Elsterian-Cromerian group, the deeper one shows somewhat lower values for the autochthonous pollen group, comparable with a Menapian to Eburonian age and corresponding in fluorescence to the "potklei" from the boring Sneek at a depth of 125 m. All these clays are contaminated with Mio-Pliocene- and coalified pollen, similar to the Lauenburger Ton. The upper part of the "potklei" of Winschoten may be correlated with the Late Elsterian of Lauenburg, which supports the original assumption of the German investigators and the opinion of ter Wee (1962).

The upper sample from the boring Sneek (31 m) shows higher Q values for the autochthonous pollen than the "potklei", and resembles those of the Holsteinian. This result is in agreement with the pollen diagram of this boring by Brouwer (1948), who found above this depth a number of pollen spectra belonging to the Holsteinian and consequently different from the underlying "potklei" spectra. This sample, moreover,

does not consist of the typical black humic "potklei", but of a grey sandy loam. It seems to be slightly contaminated and corroded pollen is apparently present.

The results of the fluorescence determinations confirm the above-mentioned suggestions, that the deposition of "potklei" has not been limited to a short time-interval of the Elsterian glacial, but may have occurred in at least one older glacial as well. The name "potklei" has therefore to be considered a facies name, which at the same time includes the relationship between its deposition in ancient glacial river valleys and its infilling with rebedded Tertiary sediments.

e) *Other Pleistocene clays in the N. Netherlands*

The boring of Spannenburg represents a number of clay layers alternating with fine and coarse sands. Most spectra contain rebedded pollen grains with the exception of those above the level of ca 60 m. The uppermost part of the Spannenburg diagram, published by Brouwer (1948), is in accordance with the Holsteinian pollen zones. Below 60 m however, the pollenstratigraphical succession as known from the S. Netherlands, by the studies of Zagwijn (1957, 1960), may (and then only partly) or may not be recognizable. Fluorescence determinations of some strongly contaminated samples from this boring have been carried out in order to get an impression of the age of the deeper clay layers.

With increasing depth four fluorescence colour groups in the autochthonous pollen can be distinguished: viz. at 61 m a Holsteinian group, at 150 m an Elsterian-Cromerian group, at 187.5 m the group corresponding with the upper part of the Lower Pleistocene and at 229 m a Tiglian-Praetiglian group. At a depth of 200 m and more the marine equivalent of the Tiglian beds, the so-called Icenian deposits have been found by Brouwer (1948). This is supported by the results of the fluorescence measurements. Besides this, in the fine sandy clay at 219 m, the present author found a number of fossil diatoms, mainly belonging to brackish water and tidal flat species, together with some species known from the Tertiary only. This points to the supposition that during the Tiglian the coast of the Icenian sea must have been situated near Spannenburg. At the mentioned depths the contamination of the samples appears to be of different ages. Above the Tiglian sediments coalified pollen occurs. The differences in the other groups of rebedded Tertiary material cannot be explained, due to our lack of knowledge with regard to the presence and distribution of ancient river valleys in the Lower Pleistocene in N.W. Germany.

f) *Clays in the central Netherlands*

As appears from the fluorescence determinations, the clay at Nijmegen (34.5 m) must be regarded as having been deposited during the Holsteinian interglacial. It contains a considerable contamination with Pliocene and Miocene pollen, which might have been derived from Tertiary clays and browncoals, present in a vast

area between the river Meuse and the Rhine district. The same clays and browncoals probably also occur at a small depth below this clay.

The banded clay at Loenermark, deformed by ice movement, is reported to have been deposited in a warm-temperate climate during the Cromerian or Waalian interglacial (Polak et al., 1962). The uppermost group of Q values are fully in accordance with this supposition. The clay is contaminated with Pliocene, Miocene and coalified pollen. For this the same source must be responsible as for the Nijmegen clay, as the river Rhine mainly deposited the Pleistocene sediments in this area. This may be also the case in the following examples.

The deposits of Zweiersdal and Duno are situated on the S. border of the Veluwe area, which consists of Middle Pleistocene ridges formed as a result of ice movement. Both have been pollenanalytically investigated (Teunissen and Florschütz, 1957; Teunissen, 1961). The clay at Zweiersdal was initially considered to belong to the Holsteinian or possibly the Tiglian. The latter possibility later became untenable (Teunissen, personal communication). Fluorescence analysis supports the opinion that its age is Holsteinian. The Lower Pleistocene elements among its pollen content must be considered a contamination. These might eventually be derived from the Upper Pliocene. The Duno clay belongs to the Cromerian, as appears from the Q determinations which is in accordance with the opinion of Teunissen and Florschütz. It shows a contamination with a distinct Lower Pleistocene fraction.

g) The clay of Terhaagen (N. Belgium)

This example of contaminated deposits forms the most simple one of all the samples studied, as the sources of redeposition are known and situated nearby. In a clay pit near Terhaagen the base of the exposed layers is formed by the Middle Oligocene Rupelian clay, covered by Middle Miocene glauconitic sands containing a number of typical species of small Foraminifera (de Meuter, 1965). On top of these sediments a layer of variegated sand occurs which is in its turn covered with another sand layer showing krypturbatic structures. This latter must belong to the Pleistocene. It was uncertain, however, whether the second sand had to be considered Lower Pleistocene as well. Fluorescence analyses of pollen from the clay balls from the contact with the glauconitic sands, show that the spectral ratio values are too high for a Pliocene age. They point in the direction of a Lower Pleistocene age with contaminating pollen derived from the underlying Miocene sands and Oligocene clay. The Q values for both secondary groups are in accordance with those of the glauconitic sands and the Rupelian clay, which have been measured separately (see Plate III). These Tertiary deposits may have been eroded at a site close to this clay pit. In this case only small quantities of rebedded clay and sandy material occur.

APPLICATIONS TO OTHER CONTAMINATION STUDIES

After the description of these Pleistocene examples it is obvious that fluorescence palynology may be successfully applied to the age determination of contaminated deposits of younger and older beds, under the condition, however, that the autochthonous and secondary pollen differ so much in age that they can be distinguished under UV-illumination and that both groups are not too strongly coalified. Countings under normal and UV-illumination remain essential if one is to compare the results of the measurements with other data. For the examples analysed here only conventional qualitative analyses are given.

The accuracy of the age determinations, based on a comparison with the standard values of the spectral ratio of Plate III and fig. 24, amounts to at least 83 %. In the contaminated examples measured here, this degree of accuracy even appeared to be slightly higher. Only a few samples show values in the secondary groups which are slightly too low, as for instance the Upper Pliocene group of Zweiersdal and the M. Miocene group of Terhaagen. Nevertheless, this method makes it possible to date deposits, of which the age could not be determined easily with certainty. In addition some other possibilities of contamination dating may be quoted.

In rocks older than the Quaternary the source of contamination will be less easily found in most cases. A direct method, viz. the fluorescence analysis is therefore badly needed. It is important to be able to distinguish reworked material from the autochthonous elements, in order to avoid an incorrect statement of the stratigraphical range and the palaeogeographical distribution of the palynomorphs involved. Since pollen grains of *Classopollis* have been found in Pleistocene clays, deposited by the river Meuse in Limburg (S. Netherlands), it will be obvious that these and other Mesozoic forms may be expected in younger rocks.

Even when contamination is not expected it may be detected by means of this method. The presence of reworked material may be expected in general when different fluorescence colour groups for each pollen form are found. The group showing the highest Q value represents the autochthonous vegetation.

The growing importance of marine palynology has been mentioned above. Deep-sea sediments often show contaminations with secondary pollen of different age ranging from Palaeozoic to subrecent. There are so many sources of contamination here that only our direct method is appropriate in establishing whether each pollen grain or spore belonged to an autochthonous vegetation or to the secondary material. Thus the pollen diagrams of deep-sea cores have to be corrected and for a solution of these problems fluorescence palynology may be very useful. These and other applications to contamination studies are under investigation by the present author.

VII. FLUORESCENCE AND GEOCHEMICAL COALIFICATION

INTRODUCTION

When a peat layer is progressively buried under sedimentary accumulations, it passes into lignite, which, on its turn, can be transformed into bituminous coal and further into anthracite. During this transformation the chemical composition of the plant substances is fundamentally altered. The carbon content of the remaining plant material relatively increases and other chemical compounds are progressively decomposed. These chemical changes are called coalification. The coalification series is characterized by the sequence peat — lignite — subbituminous coal — bituminous coal — anthracite, in which a general relationship between the successive stages exists. With regard to this series two fundamentally different mechanisms are distinguishable: (1) transformation by biochemical processes which is called *biochemical coalification* and (2) *geochemical coalification* (considered by the International Committee for Coal Petrography as metamorphism, see below).

Biochemical coalification takes place at or nearby the surface in the soil under atmospheric pressure and temperature and ends probably at the soft lignitic stage. It is caused by microorganisms occurring in peat and other humic deposits by which the less resistant plant substances are decomposed. It may have had an important influence on the chemical change of fossil palynomorphs during geological time, which resulted in a change in fluorescence colour of exines and exosporia (see next Chapter).

After a peat is lignified it may become involved in geochemical coalification. The fundamental chemical changes, occurring in the deposit, are the disappearance of nitrogen, a diminution of the oxygen and hydrogen content. The volatile matter yield (% V.M.¹) and the "fixed carbon" (% F.C.¹) relatively decrease and increase respectively. The fixed carbon content is used by the present author to express the rank of coal, except for soft browncoals. Rank is the stage reached by plant material after coalification; it represents the degree of coalification.

In fact the F.C. content and other chemical characteristics of coals mentioned do not reflect exclusively the degree of coalification, but also vary somewhat with their original petrographic and organic composition. In order to exclude the influence of variable petrographic compositions, the properties of the maceral vitrinite — in particular its optical reflectivity (e.g. Kötter, 1960; International Committee for Coal Petrography, 1963) — are used in some recent studies on coalification as a dependable parameter of rank (e.g. Patteisky and Teichmüller, 1960).

The degree of coalification has been long known to increase in vertical sections in sedimentary basins ("Hilt's rule"). Investigations of the regional increase

in coalification have shown that the rank of coal is controlled mainly by the factors of temperature (i.e. maximum depth of burial and geothermal gradient), duration of exposure to temperature increase (M. and R. Teichmüller, 1954; Karweil, 1956; Patteisky and Teichmüller, 1960), and to a lesser extent by loading pressure, structural pressure and the presence of radioactive material in the buried rocks. For more details regarding the coalification problem and the factors governing its processes, reference should be made to the recent review papers of M. and R. Teichmüller (1954, 1958, 1965, 1966), M. Teichmüller and Thomson (1958), Karweil (1956), van Krevelen (1961), Kröger (1966), Hedemann and Teichmüller (1966) and many others.

Some remarks have to be made on the terminology used in coalification studies. In the literature one may find the term carbonization incorrectly used instead of coalification. Carbonization, however, is the result of rapid heating of coal in the laboratory or industry or by igneous intrusions into coal seams. The term coalification is used here according to the terminology accepted by the International Committee for Coal Petrography (1963).

This Committee and many authors consider coalification as a process of metamorphism. The physical conditions of geochemical coalification are largely similar to those of late diagenesis (epidiagenesis) or "burial metamorphism". A distinct boundary between diagenesis and metamorphism cannot be drawn, but browncoals and low-rank coals may be considered to be diagenetic. The relation between rank of coal and late diagenetic or burial metamorphic alteration in associated sedimentary rocks has been reviewed by M. and R. Teichmüller (1966) and Kisch (1966a, 1966b).

INFLUENCE OF GEOLOGICAL TIME AND GEOCHEMICAL COALIFICATION ON THE FLUORESCENCE OF PALYNOMORPHS

In the preceding chapters of the present study much attention has been paid to the important influence of geological time on the chemical nature of fossil palynomorphs, as appears from the change in their fluorescence colour. It has become known from the work of various authors that the chemical character of exines and exosporia has been influenced also by geochemical coalification (Wilson, 1961, 1964; Gutjahr, 1966). Therefore we will pass now to a discussion of the importance of both time and rank of coal. After a short review of the influence of geological time, the change in fluorescence as a result of geochemical coalification will be discussed.

Influence of geological time

It appeared that the fluorescence of fossil palynomorphs changes gradually with increasing age from

¹) All V.M. and F.C. percentages throughout the text are given on a dry, ashfree basis (d.a.f.).

colours at lower wave-lengths into colours at higher wave-lengths. In Chapter V this phenomenon has been described by means of a large number of fluorescence measurements of vesiculate pollen grains from various ages, ranging from the Paleozoic up to the Recent (Plate III). Fresh pollen grains of *Pinus sylvestris* show light blue or bluish white fluorescence colours. Those from the Holocene are dark or light green or bright white in ultra-violet light. The Pleistocene grains of this type show more light green and yellowish green or greenish yellow colours. In the Tertiary the *Pinus sylvestris*-type is mainly white and yellow in its fluorescence, while orange colours appear regularly. In the Mesozoic and Paleozoic the vesiculate grains show mainly orange and brown colours.

This great change includes almost the entire visible spectrum, except the violet and dark blue region. As the fluorescence is closely related to the chemical character of palynomorphs, it may be assumed that their chemical nature has been considerably changed by geological time. As will be discussed in the next Chapter, this change may be a result of biochemical coalification. From this large scale of fluorescence colour change with increasing age, it may be concluded that time forms the most important factor in the process of chemical alteration of palynomorphs. All samples from the Miocene and younger, mentioned in Plate III and fig. 24, belong to deposits, which have been only slightly buried in the subsoil. Their rank has never reached the lignitic stage. Therefore the influence of temperature on this process may be neglected.

Influence of geochemical coalification

It has become well known that with increasing rank of coal, fossil pollen grains and spores from coal beds or deep sections change in colour in normal light from yellow to light brown and then to dark brown. Their light absorption increases gradually (Gutjahr, 1966). They finally become black as soon as the exines and the exosporia have been totally transformed into coalified particles. In coals with an F.C. content of approx. 75 or 80 % or more, they have become indeterminate.

Some experiments have been carried out in the laboratory in order to reproduce the coalification phenomena of fossil palynomorphs. Kirchheimer (1933a, 1933b, 1935) tested fresh *Lycopodium* spores under high temperature and pressure. Similar experiments on spores from Eocene browncoal resulted in a chemical decomposition of the exosporia. Kirchheimer suggested that the damage which he observed on spores from Eocene browncoals, has been caused in a similar way by coalification. Gutjahr (1966) subjected fresh pollen grains of *Quercus robur* to a temperature test. Heating at various temperatures above 100° C at various exposure times showed that the colour of these pollen grains changes with higher temperatures. In the present author's opinion, however, such experiments have a limited value, as exposure time and

natural conditions in the lithosphere can hardly be reproduced in the laboratory.

In order to determine the influence of geochemical coalification, a number of fluorescence determinations of palynomorphs from browncoals and coals of various age have been made. The rank of coal of the samples has been obtained by means of measurements of the optical reflectivity of vitrinite macerals in the same samples. The determination of the reflectivity has been carried out, according to the directions of the International Committee for Coal Petrography, by means of the photo-electrical method, and using the curves, published by Kötter (1960).

Fig. 33 shows a number of fluorescence measurements on palynomorphs of various age and rank ¹). The fluorescence values from Upper Tertiary browncoals have been separately plotted on a somewhat stretched horizontal scale in the left part of the figure, because the Miocene and Eocene browncoals coincide in rank. The reflectivity values of the samples are average values, calculated from at least 25 measurements on vitrinite or collinite. For localities and other data of the samples, reference should be made to Table VIII (Appendix).

The results of these comparative measurements must be interpreted very carefully, due to the presence of scattered samples and the small number of fluorescence measurements and samples. It appears, however, from the general decrease in spectral ratio with increasing reflectivity, that a correlation between fluorescence and reflectivity may be assumed.

It appears that the change in fluorescence with increasing rank runs parallel to the change with increasing age (Plate III). An increase in rank, however, resulted in a much smaller change of fluorescence of palynomorphs in low-rank deposits (from yellow to orange or brown) than that caused by time (from blue or white to yellow). As will be discussed below, time may be a more important factor than rank in the process of chemical alteration of palynomorphs from low-rank deposits.

Influence of corrosion

Another factor that may play a role at the change in fluorescence of palynomorphs must be mentioned, viz. the corrosion. Kirchheimer (1935) and Wilson (1964) already called attention to the importance of corrosion to the study of coalified palynomorphs. It also causes a change in the fluorescence properties — even in material that has not been coalified (see next Chapter) — probably as a result of erosion of the browncoal or coal on ancient erosional surfaces, which were covered afterwards by younger sediments. A considerable number of corroded palynomorphs is present in two samples mentioned in fig. 33. The Eocene browncoal from Hampstead, Great Britain (sample VI/B), shows some corrosion, observable

¹) Reflectivity measurements kindly communicated by Shell Exploration and Production Laboratory, Rijswijk (Z.-H.), The Netherlands.

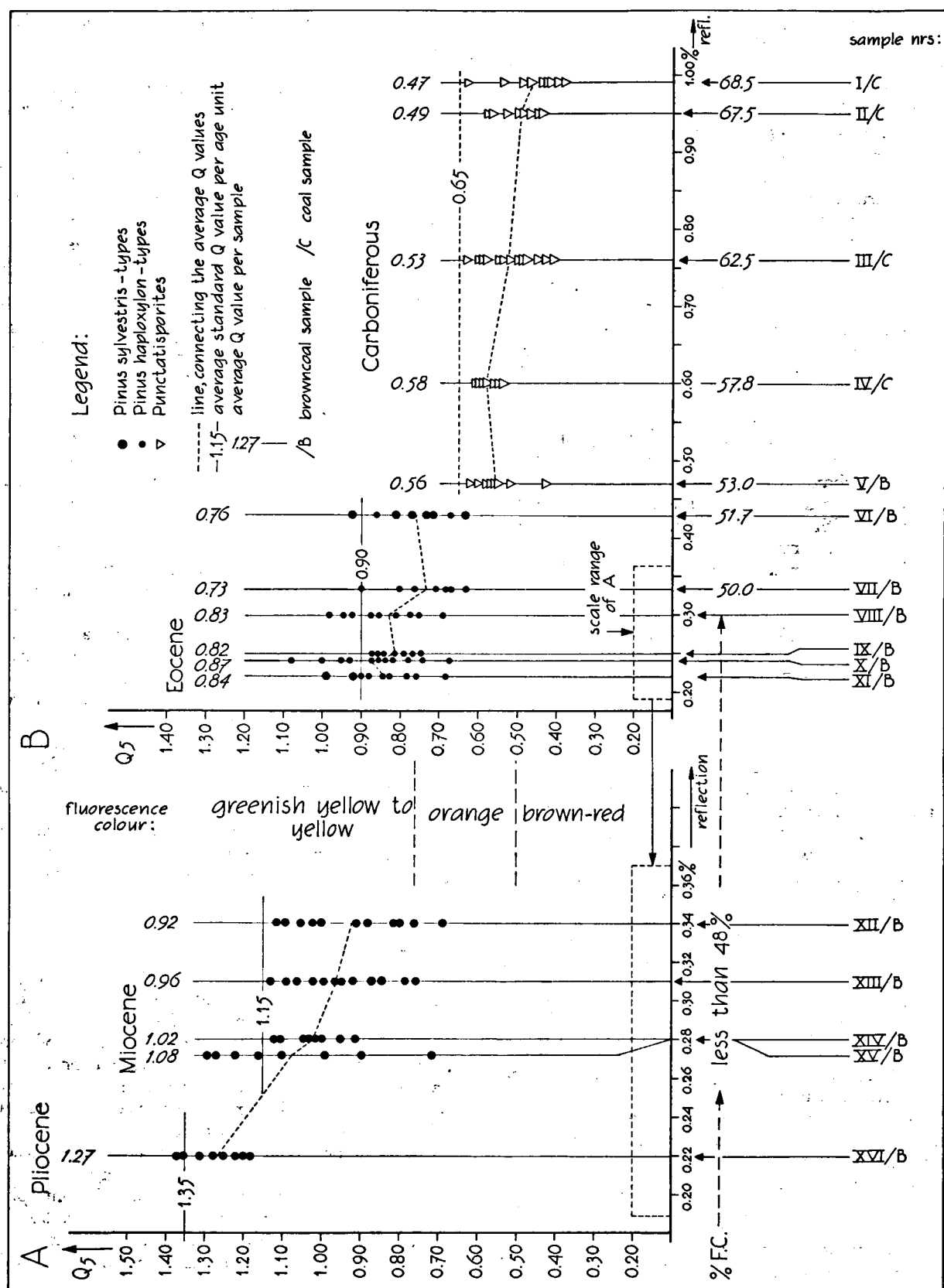


Fig. 33. Relationship between fluorescence of coalified pollen and spores to the reflectivity of vitrinite from brown coal and coal of various geological ages. A: Upper Tertiary brown coal B: Lower Tertiary and Carboniferous brown coal and coal.

from the brown colours of the pollen grains both in normal and ultra-violet light, and macroscopically visible by the brown coal as well. Of this and another sample, viz. the coal from Lippe, W. Germany (sample II/C), only the highest Q values have been drawn in fig. 33, and have been taken into account at the calculation of the spectral ratio averages.

RELATIONSHIP BETWEEN FLUORESCENCE AND RANK OF COAL

After the search for a correlation between fluorescence and optical reflectivity that has been mentioned above, it should be useful to investigate the relation between fluorescence and light absorption, in order

palynomorphs. The light absorption values have therefore been considered to be a measure of the rank of coal. The light absorption of palynomorphs appeared to increase with increasing depth of burial of the sediments, probably as a result of temperature increase.

Judging from the results of a small number of comparative measurements of fluorescence and light absorption (fig. 34), it seems that fluorescence and light absorption of palynomorphs may be correlated, whereby the fluorescence shifts towards the red part of the spectrum with increasing light absorption. The fluorescence measurements are plotted in fig. 34 on the vertical scale; the absorption values¹⁾ are given horizontally. A broken line connects the determinations

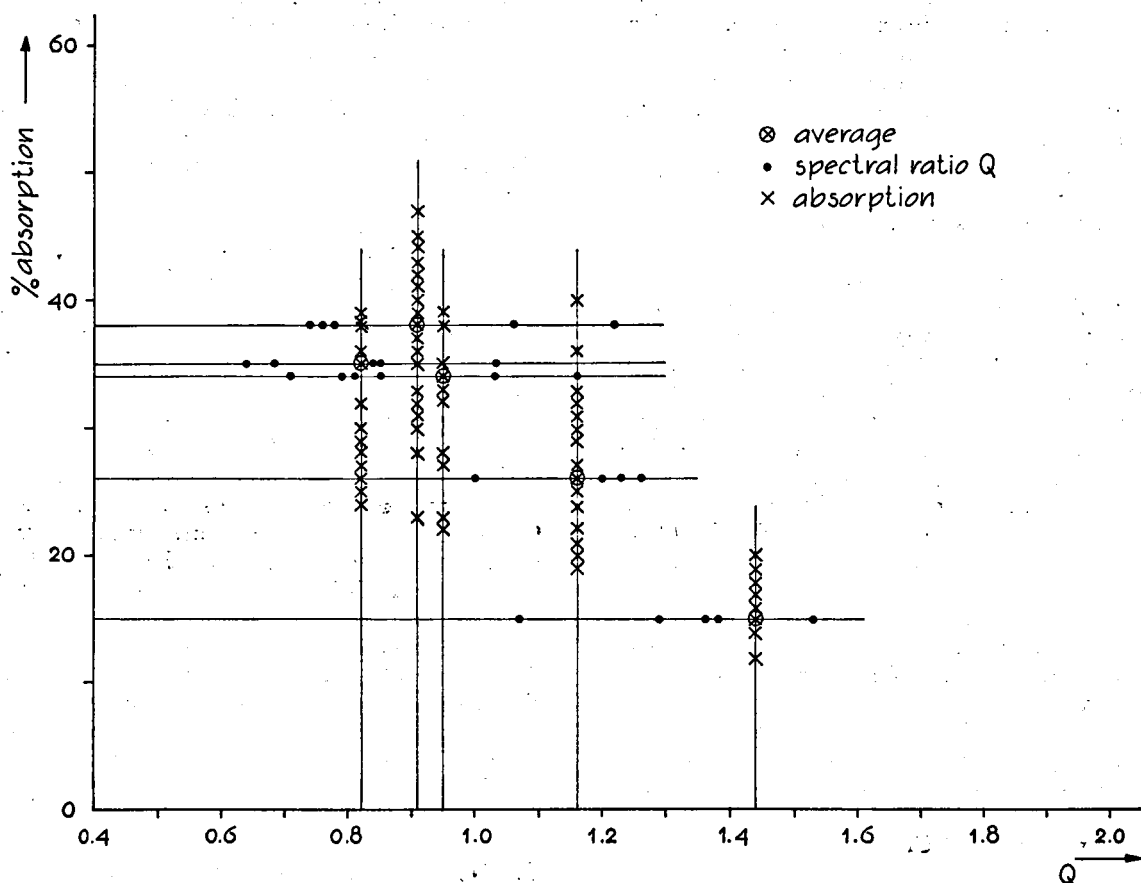


Fig. 34. Comparison of fluorescence and translucency of coalified pollen grains.

to obtain a complete picture of the correlation between fluorescence and rank of coal.

Relationship between fluorescence and light absorption

The increase in absorption of light by fossil palynomorphs with increasing rank has been investigated by Gutjahr (1966). He used equipment consisting of a photocell, fixed on the microscope body and connected with a separate four-stage amplifier. Gutjahr suggested that a correlation exists between the degree of coalification and the light absorption of

of palynomorphs of the same slide. The actual values of the spectral ratio Q are represented by dots; the absorption values are indicated with small crosses in order to show the spread which is found with either method. Each value represents the fluorescence or absorption value of one pollen grain or spore measured.

¹⁾ Light absorption measurements are kindly communicated by Shell Exploration and Production Laboratory, Rijswijk (Z.-H.), The Netherlands, determined according to the method by Gutjahr (1966).

The interpretation of fig. 34 meets difficulties due to the small number of fluorescence measurements and samples. More extensive investigations are needed to establish exactly the relationship between fluorescence and light absorption. Nevertheless, the figure suggests that light absorption and fluorescence may be correlated.

Relationship between fluorescence and rank of coal

The correlation between fluorescence and reflectivity, and between fluorescence and light absorption, can be summarized in a general correlation between fluorescence and rank of coal of fossil palynomorphs. In comparison with the large number of reflectivity data, which have been published by various authors, much more determinations of the fluorescence are needed for the continued investigation of this subject. Besides, geochemical coalification forms a complex problem, of which various aspects may be important for the study of the chemical character and the fluorescence of palynomorphs.

Some remarks have to be made with regard to figs. 33 and 34.

The comparative measurements of both figures suggest that the fluorescence determinations show only significant differences in the average Q of various samples in a range of the spectral ratio of approx. 0.90 or more. From the large spread in those samples with an average Q value of less than 0.90, the decrease in spectral ratio seems to be slight. On the other hand, the spread appears to be different in various samples. When fluorescence analyses will be used as a standard to the rank of coal of palynomorphs, it must be known whether these differences are significant or not. Besides, the question must be studied, why the spread is different for various rock samples of similar age and rank.

Fossil palynomorphs from coalified deposits seem to be "older" in fluorescence (fig. 33) than those from non-coalified rocks of the same age (Plate III). A slight increase in rank of younger (Tertiary) deposits may cause a greater change in fluorescence than that caused by a considerable increase in rank of older coalified rocks. This phenomenon may be related with the hypothesis of the chemical composition of sporopollenine (see next Chapter).

CONCLUSIONS

Some preliminary conclusions may be drawn from the fluorescence analysis.

In each group of a certain age in fig. 33 the fluorescence changes into orange or brown colours with increasing reflectivity (i.e. rank). The influence of geochemical coalification may be expressed in this figure by the broken line, which connects the average Q values. The factor time is represented for the ages mentioned by the standard average Q values, derived from Plate III.

The importance of time and rank for the fluorescence phenomena may be illustrated as follows:

(1) The largest shifting in fluorescence colour occurs in the low-rank part of the coalification series (peat and lignite), where geological time plays the prominent role at the chemical alteration of palynomorphs. A much smaller change in fluorescence took place at higher ranks between 48 and 75 % F.C.

(2) The Miocene lignites, mentioned in fig. 33, are nearly equal in age, but they differ in rank and fluorescence. The samples nrs. XII/B-XIV/B are taken from the upper part of the main browncoal seam ("Hauptflöz") in the Lower Rhine area in W. Germany, which belongs to the Lower Helvetian or Upper Burdigalian (von der Brelie, 1967). The lignite of Meisner (sample nr. XV/B) is somewhat older in age and can be correlated with the lower part of the "Hauptflöz", which has been deposited earlier in the Burdigalian (von der Brelie, 1967). Hence it appears that the decrease in spectral ratio of *Pinus sylvestris* pollen grains with increasing reflectivity of the vitrinite of these browncoals must have been caused by temperature rather than by difference in age.

(3) The fluorescence of exosporia of *Punctatisporites* from the Lower Carboniferous browncoal of the Moscow Basin (sample nr. V/B) appears to be only slightly different from that of similar spores from the more strongly coalified Carboniferous coals (samples nrs. I/C-IV/C). Apparently the fluorescence has been shifted here so much, that even a large increase in rank resulted only in a small decrease of the average Q .

(4) A comparison of the Moscow material with Miocene lignites from the Rhine area (samples nrs. XII/B-XV/B), on the other hand, shows a considerable difference in fluorescence of the palynomorphs. As in Carboniferous coals the fluorescence of vesiculate forms appears to be equal to that of *Punctatisporites* exosporia, the difference between Carboniferous and Miocene fluorescence cannot be explained by the fact that various forms from both ages were measured. The rank of coal differs not much in both cases, as they show only a small difference in reflectivity, but the difference in age of these browncoals amounts to approx. 300 million years. Therefore it may be assumed that the difference in fluorescence between the Miocene and Carboniferous palynomorphs is determined by time rather than by rank.

(5) No important difference exists between the fluorescence colour of vesiculate types and microspores of *Punctatisporites* from the same Carboniferous coals. Likewise the fluorescence colour of *Pinus sylvestris* pollen grains appears to be similar to that of *Pinus haploxylon*-types from Eocene and other Tertiary deposits. This supports the above-mentioned opinion (Chapter V) that the differences in fluorescence colour between various forms become gradually smaller with increasing age and ultimately disappear. Increase of rank has a similar result.

Resuming it may be established that the influence of time alone during biochemical coalification and of time and temperature during geochemical coalification result in a similar change in fluorescence colour

of palynomorphs towards the red part of the spectrum. It may be assumed that time forms the most important factor at the colour change in low-rank deposits and that both time and temperature are determining the fluorescence colour of palynomorphs in stronger coalified rocks, whereas time may have had a greater influence on the chemical nature of exines and exosporia in higher rank browncoals and coals than has been expected. It must be noticed, however, that a short exposure time with higher temperature may result in a more rapid disappearance of translucency and fluorescence of palynomorphs than a long exposure time at low temperature. Much more comparative investigations like those described in this Chapter, are needed to get a clear insight into this subject.

Regarding the application of fluorescence palynology

to coalification studies, the limitations of this method must be taken into account. Like the light absorption method of Gutjahr, fluorescence photometry covers a range of the coalification series, corresponding to an F.C. content of less than approx. 75 %, contrary to the reflectivity method, that can be used for the range between approx. 48 and 85 %. The disappearance of translucency and fluorescence coincides with the point at which the palynomorphs become unrecognizable.

Nevertheless, fluorescence palynology can be used in the study of low-rank coalified deposits. Besides, it may be useful to investigate the fluorescence of various types of coalified palynomorphs in relation to the question of the chemical and physical properties of sporopollenine (see next Chapter).

VIII. FLUORESCENCE AND CHEMICAL CHARACTER OF SPOROPOLLENINE

INTRODUCTION

The afore-described phenomena of fluorescence can be explained and discussed only when attention is paid to the chemical properties of the sporopollenine. In living plants many biochemical compounds and tissues show characteristic fluorescence colours, depending on their chemical nature and on the conditions during the observation (Goodwin, 1953 and others). The fluorescence of pollen and spore walls is also related to the chemical composition of exine and exosporium respectively.

This composition is difficult to study for palynomorphs, because they are very resistant and difficult to examine chemically. The reason why this fluorescence shows differences during geological history, must be due to changes in their chemical nature, caused by processes in the earth's crust. Such processes are very complicated and still poorly understood, though this subject was studied extensively by many investigators. In order to explain the processes and factors, by which the fluorescence phenomena are governed, the chemical nature of fresh and fossil sporopollenine must be known exactly. It must be established whether one or more fluorescent components in the exine and exosporium are present, how these are composed and how they change or disappear. It appears, however, from the data in literature that the exact chemical nature of fresh and fossil palynomorphs is in fact unknown. The fresh ones, showing bright fluorescence, have been investigated by numerous workers. Though many properties of these objects have become known and some investigators even gave brutto formulae for their composition, these studies deliver not any information on the origin of fluorescence. On the other hand more extensive analyses were made of fossil megaspores from the Carboniferous in which strongly fluorescent substances were found, like resin, paraffine and mainly naphthene,

but these spores are not fluorescent at all! Unfortunately data of exines from intermediate ages are not available. Nevertheless it is useful to pay some attention here to the facts and suppositions of the chemical data, the fossilization processes and their connection with the fluorescence phenomena. So much has been published, however, about coalification, fossilization and chemical analysis that the present knowledge of these subjects must be discussed here in a short review, in which only the most important studies are mentioned. For a complete information, reference should be made to a special paper (van Gijzel, 1968), dealing also with various aspects of the fluorescence phenomena.

PREVIOUS CHEMICAL ANALYSES

Fresh pollen and spores

Erdtman (1954) remarked in his "Introduction to Pollenanalysis" that fresh pollen and spores possess a high resistance to most chemical reactions, except to oxidation and strong heating, and are insoluble in nearly all solvents. Thanks to these properties fossil pollen and spores may be well preserved in deposits under anaerobic conditions, but on the other hand their resistance makes the study of the chemical composition far from easy.

Zetzsche and his co-operators succeeded in isolating an insoluble substance of fresh pollen and spores from the easily soluble constituents as fat, proteins, acidic and colouring matter. It was termed sporonine or sporopollenine and should be characteristic for exosporia and exines, respectively (Zetzsche and Huggler, 1928; Zetzsche and Vicari, 1931; Zetzsche and Kälin, 1931). Its presence has been established in fossil megaspores from the kaustobiolithes of Moscow and Tasmania (Zetzsche, Vicari and Schärer 1931). Zetzsche and Vicari even gave brutto formulae

for this substance, which is different in composition for various species of pollen and spores. After Zetzsche it is highly unsaturated and reacts easily with halogens like bromine. The difference in chemical nature between the fossil megaspores and fresh *Lycopodium* spores should be caused by microbiological action. Recently, more data have become available by various studies. Rowley (1962) noticed that the resistance to acetolysis¹⁾ is regarded at present as a positive test for sporopollenine. Shaw and Yeadon (1966) found that membranes of *Lycopodium clavatum* spores and *Pinus sylvestris* pollen are similar. They consist approximately of cellulose (10–15 %), an ill-defined "xylan" fraction (10 %), a lipid fraction (55–65 %) and a lignin-like fraction (10–15 %). Heinen (1960, 1963) and Linskens (1962) supposed that sporopollenine should be related with cutine, which is the main constituent of cuticles.

Fossil megaspores

Furthermore fossil megaspores only have been subject of chemical study. The fact that certain coal-layers often show an abundance of fossil spores and possess a divergent content of volatile matter drew the attention of coal chemists. However, literature on coal chemistry contains only a few data on the composition of these megaspores.

Zetzsche was the first one who succeeded in co-operation with Vicari and Schärer (1931) in separating and analysing fossil megaspores, occurring in an oil-shale of Tasmania and a browncoal of the Basin of Moscow. He pointed out that biochemical reactions during the coalification left the (undetermined) oxygen of the sporopollenine undisturbed, but changed on the other hand the amount of hydrogen and hydroxyl groups. The fossil exosporia from both deposits should show a decrease in these components. Later on, these suppositions were confirmed by the experiments of Zetzsche and Liechti (1937), at which fresh exines of *Lycopodium* and *Corylus* could be changed in a similar way by chemical processes.

The analyses of Macrae and Sprunk and co-workers are far more extensive.

Sprunk, Selvig and Ode (1938) investigated megaspores from a Michigan spore coal in the U.S.A. They noticed that former workers have separated spores from coal by chemical treatments, but the results of analyses of such spores are subject to criticism, because the spores are affected by the chemicals used. Therefore they cleaned the spores mechanically. The gas, distilled from megaspores, contained a high content of hydrogen, methane and ethane. The amount of carbonized residue proved to be low.

The most extensive analyses were made by Macrae (1943), who investigated spore exines from a bituminous coal from the Upper Beeston seam in West Yorkshire, Gr. Britain, by means of thermal de-

composition experiments. First the spores were extracted with hot acetone in an atmosphere of nitrogen. The extract, containing resins, appeared to be strongly fluorescent. The thermal decomposition of the exines yielded a high content of heavy oils, consisting of carboxylic acids, phenols, bases, and neutral oils. The latter contained many components as unsaturated hydrocarbons, paraffins and naphthenes.

Van Krevelen and Schuyer brought these analyses in connection with other investigations. In their handbooks of coal chemistry (van Krevelen and Schuyer, 1957; van Krevelen, 1961) they noticed that the reaction mechanism of the coalification is extremely complicated from a chemical point of view. Its final product, coal, possesses a complex, chemically heterogeneous structure, which often cannot be determined exactly. The chemical composition of cuticles, spore membranes and corky tissue, which are closely associated with each other, show an intermediate position between those of plant waxes and of lignin. Van Krevelen and Schuyer used the method of statistical constitution analysis by which the average structure of a substance can be determined. They supposed that the spore membranes, etc. are combinations of lignin units with polymerization products of waxy alcohols and waxy acids, built up according to a pattern, similar to wood, which consists of complexes of cellulose with lignin as binding agent. The cellulose should have been replaced in the spores by the strongly water-repellent waxy alcohols.

Conclusions.

Some critical remarks must be added to the cited analyses. Although the work of Zetzsche and his co-workers commands much respect, their conclusions must be considered as rather insufficiently founded. The insoluble residue of fossil spores begins to decompose at 200° C or slightly lower, as appears from the work of Sprunk and others. But Zetzsche analysed the samples at a temperature of 300° C, while the analyses of others were made at much higher temperatures (up to 500° C).

Zetzsche treated the pollen and spores with phosphoric acid to extract the cellulose and with hydrofluoric acid to remove the sand particles, while other investigators avoided these treatments. The question arises if these methods did not influence the results of Zetzsche's analyses. It seems not improbable that sporopollenine consists of various compounds, which are not mentioned by Zetzsche. His purpose of analysis, moreover, was quite different from that of Sprunk and Macrae. Hence it appears that the conclusions of Zetzsche are not simply comparable with those of the other authors and that the various analyses cannot be reconciled with each other.

Furthermore the question arises, how much the results of the analyses are influenced by the fact that the distillation was carried out at rather high temperatures, by which it becomes uncertain if all the components found were really present in the examined exines.

¹⁾ The term acetolysis is not used here in the chemical sense, but means a pollen preparation method after Erdtman (1954, 1960).

Hence, it appears that fossil exines possess a very complex character. Probably the fresh ones are more complicated in nature. Recapitulating, we may say that the composition of exines is still unknown and therefore it is impossible to define Zetzsche's term sporopollenine exactly. The present author uses this term in the sense that it includes all chemical components of palynomorphs, which are different from other plant constituents such as cutine, cellulose, etc. Taking the fluorescence phenomena into account, it cannot be proved that the fluorescent resins, extracted from the Carboniferous megaspore walls can be responsible for the fluorescence of the whole exines, because as far as known megaspores are not fluorescent at all. This also holds for other substances of the exosporium, found by Sprunk and Macrae, some of which are strongly fluorescent. On the other hand it may be possible that similar compounds, being absent here but present in younger exines, bring about the fluorescence. Another cause may be formed by the presence of lignin in the exines, except in the most strongly coalified ones (van Krevelen and Schuyer, 1957; van Krevelen, 1961, 1963). It is well known that this substance is fluorescent (see Brauns, 1952). Fluorescence of fossil algae in boghead coal was found by Wolf (1966). She supposed that the fluorescence should be related to the situation of the C-bonds of the organic compounds.

Other chemical data

The photochemical process of autoxidation, by which fresh pollenine takes up oxygen and fades if it is subjected to prolonged action of air, was discovered by Zetzsche and Kálin (1931). In the author's opinion it plays only a minor role at the fossilization of pollen, but may be responsible for the large variations in fluorescence colour of subfossil pollen.

Recently, interesting details of the properties of sporopollenine have become known by electron-microscopical studies. Afzelius (1956) sectioned fresh pollen for this purpose and observed that the exine can be affected by oxidation with benzoylperoxide at the preparation of the slides. Erdtman (1952) described a similar affection of spores by chlorination. In this reaction the sporopollenine should be transformed to chlorosporopollenine, changing its fine structure.

Chromic acid rapidly demolishes sporopollenine. The lamination and the amorphous granulation are destroyed and an almost hyaline appearance of the exine occurs (Afzelius, 1956). Williams, Backus and Russel (1951) supposed that a single macromolecule of sporopollenine should exist of approximately fifty units of sporonine, as proposed by Zetzsche. Its molecular weight amounts 60,000 to 100,000.

Larson et al. (1962) published an excellent review of the results of electron microscopy of fresh exines. It is certain now that in exines at least two layers can be distinguished, which are different in chemical nature and react differently on acetolysis treatment. This is in accordance with many fluorescence observations.

The ectexine and endexine show in many cases various fluorescence colours.

Other important properties of the exine have become known by the work of Havinga (1962, 1964) and Sangster and Dale (1961, 1964) on corrosion phenomena. Havinga stated a corrosion susceptibility series, based on results of experiments in order to explain the corrosion phenomena of fossil pollen and spores in the soil. Oxidation treatment resulted in a very marked corrosion of the grains in an acidic environment, but proved to be very slight in an alkaline solution. This series shows much resemblance with the fluorescence colour series of subfossil pollen and spores (see Chapter IV). This selective corrosion is also in accordance with the results of palynological comparison of sands and peats.

RELATIONSHIP OF FLUORESCENCE TO CHEMICAL CHARACTER

As was noticed above, both properties are closely related. Too little is known, however, about the chemical nature of palynomorphs to establish this relation exactly. Nevertheless, some suppositions about their chemical composition may be derived from the fluorescence phenomena.

The first hypothesis concerns a division of sporopollenine into two or more components, that may be derived from the fluorescence spectra, obtained by means of the Berek photometer and the UV-microspectrograph.

The fluorescence colour of a spore or pollen grain may be considered to be a mixture of two or more different fluorescence spectra: a blue to green spectrum and an orange to red one, which represent two or more different substances in the sporopollenine. The mutual proportion of these components should determine the fluorescence colour observed. The fluorescence of a pollen type at a certain geological age is determined then by the ratio of these substances, and may vary from type to type with differences, which should depend on the chemical composition and on the resistance against decomposition by geological processes. At increasing age this should result in a relative decrease of the bluish green substances in favour of the orangish red fluorescent compounds. This hypothesis is not supported, however, by the fluorescence spectra of fresh pollen and spores, measured in suspension (Chapter II, fig. 19b). Such spectra show one distinct maximum only in their curves, that may be situated at various wave-lengths. It is unknown whether these compounds are various modifications of one substance, sporopollenine, or not. It may be possible that they represent lignin and other compounds, mentioned by van Krevelen and Schuyer (1957), which should be present in the maceral exinite in coal. On the other hand, very small concentrations of contaminating elements in the sporopollenine may be responsible for the fluorescence phenomena. This important subject is still under investigation now.

Till more chemical data have become available, the term sporopollenine can be used for all palynomorphs. In the present study no distinction has been made into pollenine and sporonine, as done by Zetzsche. Probably the exines and exosporia are not fundamentally different in chemical character.

A second assumption is the constancy of the original fluorescence colour of all fresh spore and pollen walls during geological history, according to the geological principle of actualism. For instance Miocene pollen grains of *Pinus sylvestris*, when they have belonged to the same recent species, must have been bluish white in fluorescence before they became fossilized in the Miocene, just like at present. This supposition is in accordance with the conclusion of White (1933), who found no evidence that the principal peat forming plant compounds in the Paleozoic differed, even in any detail of their chemical composition, from those of the coal-forming vegetation from similar climates in later times.

The third hypothesis about the nature of fossil sporopollenine is the assumption that the change in chemical composition during geological time, resulting in a change in fluorescence colour, should be continuous, not discontinuous, as appears from the standard diagrams of Plate III and fig. 24 (see Chapter V).

Experiments to test preparation techniques for fluorescence palynology (Chapter III) deliver some information on these assumptions. It appeared that the use of acids and oxidation treatment causes a remarkable change in fluorescence colour towards the red part of the spectrum. Continued action of such reagents results finally in complete annihilation of palynomorphs. Oxidation experiments, made by Havinga and others, pointed out that sporopollenine is destroyed by strong oxidation or at least corroded. In UV-light such corroded grains show a divergent colour, similar to those from coarse sands. Similar changes occur in test preparations of the oxidation treatment (Chapter III).

An exact description of the term sporopollenine cannot be given now, because it contains a rather large number of organic compounds. Therefore the relation between chemical character and fluorescence still cannot be explained. Various causes of the fluorescence have to be taken into account.

RELATIONSHIP OF FLUORESCENCE TO FOSSILIZATION

Corrosion

After the sedimentation the exines can be affected by corrosion, being a factor which can hamper fossilization of pollen and spores. This mainly occurs in coarse deposits and takes place rapidly above the groundwater level and very slowly or not underneath this level.

Havinga (1962) and others paid special attention to this subject. Owing to oxidation and biological activity in the soil, a selective disappearance takes

place. Some pollen types are exceptional susceptible to corrosion. Pollen of *Quercus* and other types (Table III) is largely destroyed in sands under dry conditions, but is well preserved in a wet sandy soil. In pollen diagrams of sands the older pollen zonings often are not represented because their pollen has been destroyed entirely. Havinga (1962) stated that only a small number of the local tree pollen is preserved when a relative dry soil had a cover of oak forest during the Holocene.

Humification

When after sedimentation the conditions in a deposit were more favourable, the pollen and spores were more or less subjected to the sequence of the processes of humification, coalification and diagenesis.

In general the plant fragments firstly underwent humification or were transformed into peat. The genesis of most coal deposits starts in a living peat bog. The dead plant material settles down on the swamp bottom and is transformed into peat by microbiological activity. During the peat formation, decomposition products are formed, as for instance methane, the humates and humic acids, an important group of degradation products, the structure of which has not yet been clarified. An example of humification is the conversion of a *Sphagnum* bog into the slightly humified Young *Sphagnum* Peat.

When the humification begins, some instable and easily soluble constituents of pollen and spores as intine, fats, proteins, acidic and colouring substances begin to disappear. Under unfavourable conditions pollen grains with a rather weak exine are even completely destroyed.

The pollen surviving humification, is involved furthermore in the coalification process. In fact humification forms the first step in the coalification series.

Biochemical coalification

This study of fluorescence phenomena of fossil palynomorphs bears mainly on the coalification processes, in which they were involved. A clear distinction must be made between the biochemical and geochemical coalification, which differs fundamentally; the result of both is similar and their boundaries cannot always be drawn sharply.

Various authors stated that microorganisms are present in nearly every rock type, even under unfavourable conditions. These organisms cause the processes of biochemical coalification, during which the remaining weaker plant tissues and substances are further decomposed in so far as they survived peat formation and humification. This occurs for instance when a well-structured peat is broken down into a structureless mass with remains of very resistant fragments (wood, seeds, resin) only.

Many microorganisms are adapted to live under anaerobic circumstances as in peat and gyttja, and break down the plant fragments during the complex system of organic reactions, resulting in the formation of humates, humic acids and other compounds. At

increasing age this coalification results in a relative decrease of the H and O content of the deposit in favour of the number of C atoms, while the structure of the peat often disappears and volatile matter evades. The action of microorganisms causes decomposition of the carbon combinations as well, and may even bring about affection of the minerals in a deposit.

White (1933) established the gradual decomposition of plant substances at various water levels during the peat formation and summarized it in a coalification diagram (fig. 35), which shows various stages of biochemical decomposition. Plant material shows a divergent resistance to microbiological attack. At a

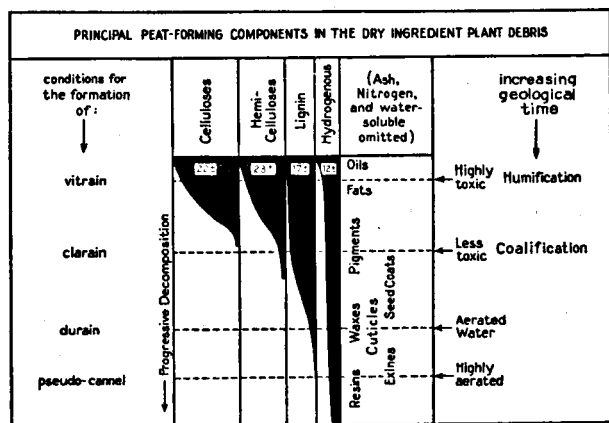


Fig. 35. Relative resistance to microbic decomposition and order of disappearance of principal peat-forming plant substances (modified after White, 1933).

decrease in acidity and toxicity of the environment during peat deposition an increase in the activity of microorganisms takes place, during which the material will be progressively decomposed. Successively the following substances will disappear: (1) oils, fats, celluloses, and part of the hemicelluloses, (2) the remaining hemicelluloses, pigments, part of the cuticles and seed coats, (3) the lignin, waxes and all seed coats and (4) part of the resins and exines and remaining cuticles. The most resistant fragments, as the resins and the exines, are partly preserved morphologically.

Although White's diagram concerns the relative resistance of principal plant components to subaqueous decay, determining in coals the type of coal macerals as vitrain, clarain, etc., it gives also an impression of the relative resistance of various substances to geochemical decomposition. It appears from this diagram that exines belong to the most resistant plant remains, during both biochemical and geochemical coalification.

The important role of biochemical coalification has become known by the geomicrobiological investigations of many authors. For a summary, reference should be made to the papers by Schwarz and Müller (1958), Abelson (1963) and Colombo and Hobson (1964) and

to various handbooks of organic geochemistry (Kuznetsov et al., 1962; Breger, 1963). It appeared that microorganisms are present not only in humic deposits, but in other sediments as well. They show an important activity in the processes of oil formation, coalification, oxidation and desulfurization in the lithosphere.

Van Krevelen and Schuyer (1957) and other investigators, however, believe that microbiological decomposition can only continue as long as Fungi and bacteria are capable of participating in the attack on the material. As Fungi do not occur below a depth of about 40 cm, browncoal formation cannot have been influenced by these organisms. Besides, in their opinion, the effect of bacterial action also rapidly decreases with increasing depth. At greater depths bacterial conversion should be completely impossible. Van Krevelen and Schuyer supposed that, after the humification stage and certainly after the browncoal formation, only metamorphosis can have played a role. This view, however, is not shared by all investigators. The processes of geochemical coalification are fundamentally different. The result of their action has been described above (Chapter VII).

CONCLUSIONS

Summarizing, it may be stated that, although a close relationship between fluorescence and chemical character of fossil palynomorphs must exist, it cannot be exactly established, due to our lack in knowledge of this character. Chemical analysis of fossil megaspores only delivers some information on the occurrence of fluorescent substances in exosporia. The fluorescence of pollen and spores may be caused by various organic compounds, in which possibly the situation of their C-bonds may play a role.

It appears from the interpretation of the fluorescence spectra that, in case more compounds are responsible for the fluorescence, they may possess various fluorescence colours. Their mutual proportion may determine the colour of pollen and spores in UV-light.

The question arises if and how the fluorescence phenomena of fossil palynomorphs are related to the aforementioned fossilization processes.

The standard diagrams of fluorescence and geological age show that the change in fluorescence colour of all types of pollen and spores possesses a gradual trend for those deposits, which are not coalified geochemically (Chapter V). Hence, it appears that geological time must be considered as the most important factor at the process of colour change in these deposits.

Time only, however, does not explain these phenomena. The chemical transformation of fossil exines and exosporia during geological time, which appears from the fluorescence spectra, forms a very complex question. Not only their complicated chemical character, but also the lack in knowledge about the role of microorganisms in their fossilization and transformation, makes it difficult to explain exactly what happened.

Biochemical coalification (including humification) may be responsible for the fluorescence phenomena at

increasing geological age for the following reasons: (1) geological time forms for both one of the most important factors, (2) both concern decomposition of plant substances, in which microorganisms may have participated, as Zetzsche presumed already for the alteration of sporopollenine in browncoal, (3) biochemical coalification and fluorescence colour change are wide-spread processes, occurring simultaneously in the same deposits.

On the other hand it may not always be expected that the biochemical activity has been constant during geological history. It may vary in different deposits and depends on temperature, pressure and ecological factors. This is not in full accordance with the gradual

change in fluorescence colour. But accessory chemical reactions, which came into action by microorganisms may form the real cause as well.

The small ups and downs in the average Q connection line in the standard diagrams may be caused by other factors as well, for instance by corrosion or other sediment properties.

The explanation of the fluorescence phenomena still presents a problem which calls for further investigation in order to obtain essential information about chemical nature and microbiological susceptibility of fossil sporopollenine. Therefore new data must become available.

IX. GENERAL CONCLUSIONS

Review of the results

It appears from this study that fluorescence microscopy, being a well-known technique, applied on a large scale in other sciences for quite a long time, now can be applied successfully to palynology as well, on the condition however, that equipments for microscopical fluorescence colour observation and determination are used, being sufficient accurate and sensitive. For the investigations, dealt with in this paper, an adapted Berek photometer and an UV-microspectrograph have been used, the results of which can be summarized in the following principles, which determine the primary fluorescence phenomena of fossil pollen grains and spores:

I. Each type shows at a certain geological age a specific fluorescence colour. The differences in colour are clearly observable mainly in material from Tertiary and younger deposits, as for instance is demonstrated by a number of fluorescence spectra of Young Holocene pollen grains and spores (Plate I). Differences in chemical nature between various forms must be responsible for it. The more or less variation in colour may be connected with the state of preservation of the pollen grains. At increasing geological age these variations decrease gradually.

II. Each type shows fluorescence colour changes with increasing geological age from the bluish green to the orange-red part of the spectrum. This relationship has been obtained from spectral fluorescence determinations of vesiculate pollen types, which were studied in about one hundred samples of sediments of various facies, ranging in age from subrecent to Upper Paleozoic. An explanation of this phenomenon may be found in the processes of humification and biochemical coalification. Complex chemical reactions and biochemical action may have changed the chemical character of the exines from the rocks involved in these processes, the final results of which appear from the fluorescence colours (Plate II and III). A relatively small number (ca 17 %) of the deposits measured show a fluorescence, being slightly

too high or too low in comparison with the general tendency. Here again the preservation of the exines may be important.

III. The third principle of fluorescence palynology has been derived from a number of fluorescence determinations of vesiculate pollen grains from lignites and coals of various stages in the coalification series. It concerns the change in fluorescence to red and brown colours with increasing rank of coal. The final stage at increasing age and coalification is the complete extinction of fluorescence, as the translucency of the exines has disappeared. This is in accordance with other coalification studies of fossil pollen grains and spores under normal illumination, carried out by Wilson (1961, 1964) and by Gutjahr (1966). The relationships between fluorescence colour and translucency of exines to reflectivity of coal have been investigated. Here again the relation between fluorescence colour and chemical nature of the pollen and spore walls is obvious.

IV. Furthermore it appeared that the preparation techniques of pollen slides for fluorescence microscopy may cause difficulties when strong oxidation or acids, as for instance fluoric acid, Schulze's treatment and acetolysis are used. Then a colour change appears to red or brown colours, similar to that with increasing age. A competitive examination of samples of various sediment types revealed some interesting facts about the following question.

V. The chemical character of fresh and fossil pollen and spore walls appears to be a very complicated problem. The fluorescence spectra suggest that sporopollenine may exist of at least two compounds, being not equal in resistance. Those, being orange or red in fluorescence colour, should be more resistant against oxidation, biochemical and geochemical coalification than the blue and green components. The chemical composition forms still an important question, however, as well as the changes in their nature by the above-mentioned geological processes.

Applications

In reference to the relations of fluorescence palynology the most important applications of these principles are as follows:

1. The difference in fluorescence colour of various forms of pollen grains and spores can be used in pollen morphological studies of fresh and fossil material. It may be valuable to observe the various layers of the exine of different types under the fluorescence microscope.

Continued study of fluorescence spectra of fresh and subrecent material may provide a useful tool in pollen morphology.

2. Age determination of deposits of unknown age, viz. from the Cenozoic, when conventional pollen analysis fails due to the absence of significant pollen associations or specific zonings in the pollen diagram. By comparison of fluorescence measurements with a standard diagram of fluorescence and geological age (Plate III) it has become possible to estimate the age of such deposits with an accuracy of more than 83 %. Fluorescence palynological dating can be applied in particular in the range between 70,000 years B.P. and the Middle Tertiary.

3. Deposits, contaminated with rebedded pollen, can be dated in a similar way. The greater the difference in age of autochthonous and reworked pollen, the more easily both groups can be distinguished (Photoplate, fig. 7). Besides, more groups of secondary material can be recognized, which is very important in case the sources of contamination are unknown. It has become an important question, occurring at any age. A number of examples of contaminated Pleistocene sediments could be dated successfully, as for instance the problems of the "potklei" and "Lauenburger Ton" in the N. Netherlands and N.W. Germany (Plate V), of which the age of the contamination sources could also be stated.

4. Similar fluorescence determinations can be used to make long-distance correlations, as has been attempted for Pleistocene sections from various continents (van Gijzel et al., 1967). It appeared that fluorescence changes gradually with increasing depth corresponding with increasing geological age. When comparing the spectral ratio values of vesiculate grains from various stratigraphical stages with the average fluorescence values of the standard diagram, dating and correlation are possible, on the condition, however, that a large number of measurements are made.

5. Determination of the rank of coal of coalified pollen bearing deposits may be carried out, unless they are so strongly coalified that exines can no longer be distinguished, (at an F. C. content of more than 75 %), because they have become black and indeterminable. This application may form — together with absorption measurements of exines according to the method of Gutjahr (1966), colour determinations by Wilson (1964) and reflection determinations of other coal substances — an expedient in coalification studies.

In general, the state of pollen preservation causes

great difficulties, as Wilson (1961) and Gutjahr (1966) pointed out, when pollen grains are coalified so intensely that they have become opaque and have lost any structure. Exines which are only less coalified, can be expected in deposits with a rank of coal in the range from high-volatile A bituminous coal or at a lower rank. Therefore, all these methods, including fluorescence palynology, are restricted in use up to this stage of the coalification series.

6. Fluorescence investigations of other palynological objects are possible, as, for instance, of acritarchs, hystrichosphaerids and dinoflagellates. The first mentioned are even more resistant than exines and show, due to their different chemical nature, no colour change with increasing geological age. They react differently upon the fossilization and coalification processes in comparison with fossil exines.

The main applications mentioned are now under continued investigation by the present author.

Discussion of the results

The question may arise, if all phenomena of fluorescence colour change with increasing geological age have been caused by biochemical or by geochemical coalification, in other terms, whether temperature and pressure have played an important role. There is no doubt, however, that their influence must be neglected there, for which the following arguments can be advanced:

Firstly, not any indication exists that factors as tectonical pressure or temperature have had an influence on the fluorescence colour change of the pollen grains from Upper Tertiary and Quaternary deposits which have been chosen for the standard diagrams. They are provided by sediments, being situated just below the surface, and, if they are obtained from borings, their depth amounts to less than 300 m. Above this level the influence of the temperature gradient in this part of the North Sea Basin (3° C/100 m) may be neglected. It may be excluded that a thick mass of overburdening rocks has covered these beds. In the collection areas magma intrusions or volcanic activity have been absent during the Cenozoic, and no strong tectonical movements occurred. Hence, it appears that regional metamorphism can be left out of consideration. Therefore geological time only must be considered to be the main factor in these fluorescence colour change phenomena.

Secondly, not any relation has been found between the fluorescence colour or intensity and the temperature of the atmosphere, which has fluctuated considerably and many times during the Cenozoic in N.W. Europe.

The results of this study accent the distinction between the biochemical and geochemical coalification. Although both have taken part in the formation of lignite and coal, they are very different in origin, nature and action. At the transformation of dead plant fragments into peat the first one only played a role. In biochemical coalification geological time forms a very important factor. In the geochemical coalification,

time, temperature and/or pressure are the main factors. The boundaries between both processes cannot be drawn sharply, because they pass often into each other. A browncoal or coal forms the final result of their combined action; but the part of each of the factors time, temperature and pressure cannot always be stated exactly.

Experiments, carried out in the laboratory to establish the separate influence of these factors will always be insufficient, because geological time can never be imitated. Nevertheless, such attempts may deliver

new informations about coalification problems. In the authors' opinion, much more may be expected from extensive and comparable studies of the natural conditions, under which peat, lignite and coal have been formed. By preference those deposits must be chosen, of which may be supposed that they have been altered by one of the mentioned factors only. One of the most important conclusions of the present paper is, that fluorescence palynology forms a useful expedient for these studies.

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RÉSUMÉ

L'utilisation en palynologie des techniques de fluorescence microscopique et de microspectrophotométrie, réunies sous le nom de fluorescence palynologique, fournit d'importantes contributions à l'étude des procès de fossilisation des palynomorphes fossiles. Les mensurations de la fluorescence, nécessaires au présent travail, ont été faites au moyen du microscope-photomètre de Berek et d'un microspectrographe à rayons u.v. Trois phénomènes de fluorescence primaire de pollen et spores fossiles ont été décrits: 1) la relation entre la couleur de la fluorescence et le type ou la forme des palynomorphes (Pl. I et fig. 21-22), 2) Le changement graduel de la couleur fluorescente, passant du bleu au rouge à mesure de l'âge géologique croissant (Pl. II, III et fig. 24), et 3) un changement semblable au précédent, résultant de l'augmentation du degré de carbonisation, par suite de la coalification géochimique des sédiments (fig. 33). Ceci est en rapport avec les résultats d'autres travaux portant sur la coalification et la fossilisation de pollen et spores fossiles.

L'application des principes de fluorescence palynologique a été illustrée par l'analyse des sédiments contaminés, au moyen de la fluorescence (Pl. V), afin d'en déterminer l'âge géologique, en cas que la méthode conventionnelle d'analyse du pollen ne saurait pas suffire. Cette détermination d'âge est possible avec une précision de plus que 80 %. Plusieurs autres possibilités d'application ont été traitées, comme par exemple la recherche sur la sensibilité à la corrosion des palynomorphes fossiles, la détermination d'âge de pollen différent à sédiments cénozoïques et l'étude sur la coalification de dépôts dans ce trajet des séries de coalification avec un contenu de carbone fixé moins de 75 %. Ces phénomènes ne peuvent pas s'expliquer, faute d'une connaissance suffisante du caractère chimique des exines du pollen et des spores fossiles. Il est apparu que celles-ci sont moins résistantes qu'on ne le supposait d'abord. Enfin, les résultats de cette nouvelle méthode palynologique sont passés la revue et discutés.

ZUSAMMENFASSUNG

Die neue Methode der Fluoreszenz-Palynologie gründet sich auf der Erscheinung der Primärfluoreszenz fossiler Pollenkörner und Sporen. Aus zahlreichen Bestimmungen mit Hilfe des Berek-Mikroskopphotometers und eines UV-Mikrospektrographen ergeben sich drei Prinzipien: (1) ein Zusammenhang zwischen Fluoreszenzfärbung und Art der Pollenkörner und Sporen (Pl. I und Fig. 21-22), (2) eine Verschiebung der Fluoreszenzfärbung fossiler Blütenstaubkörner von Blau oder Grün ins Rot oder Braun bei zunehmendem geologischem Alter (Pl. II, III und Fig. 24) und (3) eine ähnliche Fluoreszenzänderung bei zunehmendem Inkohlungsgrad der pollenführenden Gesteine (Fig. 33). Diese Ergebnisse stimmen mit bisherigen Veröffentlichungen über Fossilisation und Inkohlung fossiler Palynomorphen überein. Die Fluoreszenz-Analyse erfordert spezielle Methoden für die Aufbereitung von Pollenpräparaten und die mikroskopische Fluoreszenzphotometrie. Zu diesem Zweck wurden Verfahren entwickelt oder verbessert. Die Entdeckungen der Fluoreszenz-Palynologie können zur Klärung verschiedener Probleme angewandt

werden, z.B. auf die Pollenmorphologie, die Untersuchung der Korrosionserscheinungen von fossilen Palynomorphen, und zur Altersbestimmung von Ablagerungen wofür die konventionelle Pollenanalyse nicht ausreicht. Solche Datierungen, z.B. von känozoischen Gesteinsproben, können mit einer Präzision von mehr als 80 % durchgeführt werden. Zum Beispiel wurden verschiedene Altersbestimmungen von sekundäres Pollen enthaltenden Sedimenten gegeben (Pl. V). Die Fluoreszenz-Palynologie kann eventuell zur Bestimmung des Inkohlungsgrades fossiler Pollenkörner aus Gesteinen der Inkohlungsreihe mit einem fixierten Karbongehalt bis 75 % angewandt werden. Eine Erklärung der Fluoreszenzerscheinungen wird nicht gegeben weil die chemische Zusammensetzung fossiler Exinen noch fast unbekannt ist. Aus diesen Untersuchungen ergibt sich deutlich dass während der Fossilisation und Inkohlung fossile Palynomorphen eine geringere Resistenz zeigen als bisher allgemein angenommen wurde. Schliesslich werden die Ergebnisse und Möglichkeiten dieser neuen Methode diskutiert und zusammengefasst.

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ERRATA

Fig. 19 should be Fig 19a

To the subscription of fig. 19b should be added:

The spectra are uncorrected for the sensitivity of the photomultiplier (I P28) and the monochromator transmission. These spectra suggest that the real fluorescence spectra have a broad emission band between 400 to beyond 600 nm with a maximum between 400 and 460 nm.

The subscription of fig. 20 should be changed as follows:

The sensitivity curve of the photoplate used (Ilford, FP 3) and the uncorrected fluorescence spectra of a *Pinus sylvestris* pollen grain (also given in fig. 19a) are roughly proportional to each other, which suggests the absence of pronounced maxima in the region 430-570 nm.