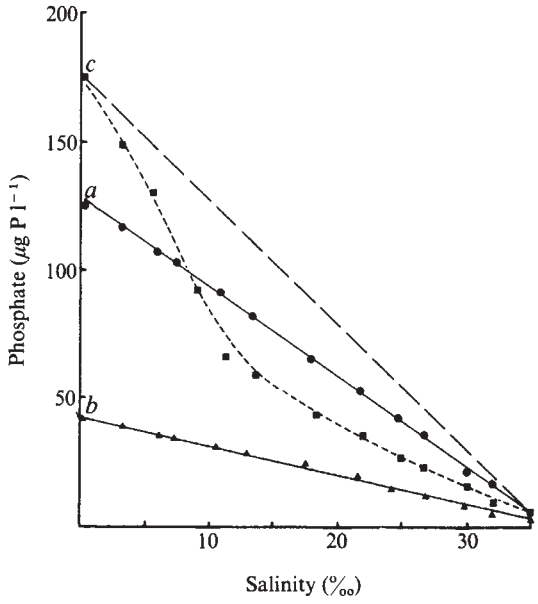

Fig. 3 Concentrations of filterable phosphate observed during addition of seawater to different synthetic solutions in a laboratory model system. Initial solutions were prepared as follows: *a*, phosphate added after iron; *b*, phosphate added before iron; *c*, phosphate added after humic acid and iron. The initial solutions were stirred overnight then used unfiltered.

weakly bound iron seems able to interact with dissolved phosphate. The presence of cations of Na, K, Ca and Mg, which are able to complex phosphate, is expected to increase the stability of dissolved phosphate in real river waters compared with the synthetic starting solutions[21].

A consequence of the observed behaviour of phosphate is that the proportion of phosphate removed from solution in river water, before it enters the sea, will be largely controlled by the sequence and concentrations of the additions of iron, humic acid and phosphate to the river. In rivers exceptionally high in iron and humic acid, up to about half of the dissolved phosphate may be removed by inorganic processes in the estuary. For rivers near the world average concentration of iron[12], dissolved phosphate is likely to show conservative chemical behaviour in the estuary, or removal to only a small degree.

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1. Jitts, H. R. *Aust. J. mar. Freshwat. Res.* **10,** 7 (1959).
2. Sholkovitz, E. R. *Geochim. cosmochim. Acta* **40,** 831 (1976).
3. Boyle, E. A., Edmond, J. M. & Sholkovitz, E. R. *Geochim. cosmochim. Acta* **41,** 1313 (1977).
4. Mackay, D. W. & Leatherland, T. M. in *Estuarine Chemistry* (eds Burton, J. D. & Liss, P. J.) 185–218 (Academic, London, 1976).
5. Van Bennekom, A. J., Berger, G. W., Helder, W. & De Vries, R. T. P. *Neth. J. Sea Res.* **12,** 296 (1978).
6. Stefansson, U. & Richards, F. A. *Limnol. Oceanogr.* **8,** 394 (1963).
7. Butler, E. I. & Tibbitts, S. *J. mar. Biol. Ass. U.K.* 52, 681 (1972).
8. Pomeroy, L. R., Smith, E. E. & Grant, C. M. *Limnol. Oceanogr.* **10,** 167 (1965).
9. Stirling, H. P. & Wormald, A. P. *Est. Coast. Mar. Sci.* **5,** 631 (1977).
10. Wormald, A. P. & Stirling, H. P. *Est. Coast. Mar. Sci.* **8,** 441 (1979).
11. Lu, J. C. S. & Chen, K. Y. in *Chemistry of Marine Sediments* (ed. Yen, T. F.) 181–222 (Ann Arbor, Ann Arbor, 1977).
12. Figures, G., Martin, J. M. & Meybeck, M. *Neth. J. Sea Res.* **12,** 329 (1978).
13. Smith, J. D. & Longmore, A. R. *Aust. J. mar. Freshwat. Res.* **31,** 119 (1980).
14. Blutstein, H. & Smith, J. D. *Wat. Res.* **12,** 119 (1978).
15. Murphy, J. & Riley, J. P. *Analyt. chim. Acta* **27,** 31 (1962).
16. Stookey, L. L. *Analyt. Chem.* **42,** 779 (1970).
17. Coonley, L. S., Baker, E. B. & Holland, H. O. *Chem Geol.* **7,** 51 (1971).
18. Murray, J. W. & Gill, G. *Geochim. cosmochim. Acta* **42,** 9 (1978).
19. Pickard, G. L. & Felbeck, G. T. *Geochim. cosmochim. Acta* **40,** 1347 (1976).
20. Sinesi, N., Griffith, S. M., Schnitzer, M. & Townsend, M. G. *Geochim. cosmochim. Acta* **41,** 969 (1977).
21. Stumm, W. & Morgan, J. J. *Aquatic Chemistry* 518 (Wiley-Interscience, New York, 1970).

Changes in spruce composition following burial in lake sediments for 10,000 yr

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Organic matter in lake sediments is a mixture of biological materials from a number of modern sources plus variable amounts of recycled detrital material. Hence, it is usually difficult to distinguish input variations over time from the diagenesis of organic matter. Comparison of old material of known biological origin with younger material helps resolve this problem. We have compared the compositions of wood and needles from a modern white spruce (*Picea glauca*) with those from a white spruce buried for 10,000 yr in a lake bottom. Although the tissues are structurally well preserved, some chemical changes are evident in the old samples. Total fatty acid concentrations decrease by over 90%. Sterol and hydrocarbon concentrations are similar in the modern and 10,000-yr-old wood, but the concentration of sterols is lower in the old needles. Cellulose components in the wood have decreased relative to lignin components, although both types of materials remain in high concentration in comparison to other organic components.

The types of chemical alterations which biological materials experience during early diagenesis in geological settings are important to biogeochemistry. A particularly interesting approach which has been used to describe some of these changes has involved chemical analysis of fossil materials, including bones[1], hair[2], corals[3], and marine plankton[4,5]. In addition, fossilized fruit[6], leaves[7] and wood[8,9] have been studied.

We collected samples of wood and needles from a living white spruce (*P. glauca*) at The University of Michigan Matthaei Botanical Gardens near Ann Arbor. Similar samples were obtained from a tree which had been drowned and buried still erect in a lake bottom 10,000 yr ago. This lake was formed by glacial damming near present-day Marquette, Michigan, and was then quickly filled with sediment[10]. Lipid components of wood and needle samples were extracted in a Soxhlet apparatus with benzene–methanol for 48 h and by alkaline hydrolysis of the extracted samples in methanolic 0.5M KOH in benzene. The two extracts are analogous to the unbound and bound lipids released from sediments by a similar treatment[11]. Each extract was fractionated by thin-layer and column chromatography into fatty acid, sterol, and aliphatic hydrocarbon fractions which were analysed by gas chromatography. Cellulose and lignin components of the modern and ancient wood samples were isolated by hydrolysis with 5M NaOH at 170 °C for 12 h followed by extraction of the degradation products with ethyl acetate. These were analysed as their trimethylsilyl derivatives by GC and GC-MS.

As shown in Table 1, the concentrations of all of the needle components and some of the wood components are lower in the 10,000-yr-old samples than in the modern ones. If the biochemical processes of the white spruce have not changed over the past 10,000 yr, then these differences are due to alterations which have occurred in the older samples during burial in the water-saturated sediment.

Fatty acid concentrations show the greatest differences, and these reflect degradation of acids originally present in the wood

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and needles. The unbound fractions have greater decreases in concentration than do the bound fractions. Because the unbound acids are extracted readily with solvent, it is probable they exist in forms that are vulnerable to both solution and microbial attack in an aqueous environment such as a lake sediment. There are some differences between the distributions in the wood and needle samples. For example, *n*-tetradecanoic acid is abundant in both the modern and old needles, but not in either wood sample. Also, *n*-docosanoic and *n*-tetracosanoic acids are important components of the unbound acids in the needles, but not in the wood. Another major difference is that the bound acid fraction is an order of magnitude more abundant in the needle samples.

Some of the fatty acid changes are shown in Fig. 1. These compositions are of wood samples; comparable changes were observed in the needles. Relative proportions of unsaturated acids are less in the older wood, whereas the contributions of *anteiso* methyl-branched acids indicative of microbial processes increases. Longer-chain *n*-alkanoic acids seem to be degraded less in the bound acids. Similar differences have been found in fatty acids extracted from modern and 12,000-yr-old Lake Huron sediments[12] and evidently are due to diagenesis rather than changes in input.

Unbound sterol concentrations of the needles indicate a time-related loss of the same magnitude as found in the fatty acids. However, the bound sterol concentration increases from essentially zero to 55 $\mu g\, g^{-1}$, suggesting conversion of some unbound sterols to bound forms. A similar conversion has been reported in young sediments[13,14] and also seems to occur in the wood samples. In the wood, this is accompanied by a small increase in the concentration of unbound sterols. Because diagenetic formation of sterols from other wood components is unlikely, the apparent increase in concentration suggests preferential losses of proteinaceous and saccharide materials, with the result that sterols comprise a larger portion of the old wood. Sterols are evidently only mildly reactive in wood. This also appears to be true in sediments[13–15].

Although at lower concentrations than the sterols, aliphatic hydrocarbons behave similarly. The concentration of unbound hydrocarbons is lower in the old needles than in the new, but higher in the old wood than in the modern sample. Unlike the sterols, no conversion of hydrocarbons from the unbound form to the bound form is evident. In view of the lack of reactive functional groups in saturated hydrocarbons, this is not surprising. Normal alkanes from C_{16} to C_{37} are present in these samples, and no major compositional changes are found between the 10,000-yr-old and modern samples.

The changes in the lipid components of the needles suggest that the unbound lipids are easily removed or degraded over geologically short periods of time in sediments. Much of this material may exist as a waxy coating on the needles which is exposed to microbial degradation, chemical weathering and dissolution. Unbound lipids in wood are internal structural components and therefore not as available to similar attack as are the needle-coating lipids. Even so, wood fatty acids are destroyed or converted to non-acid forms relatively quickly. Evidently, preservation of fatty acids in sediments requires special circumstances, such as anoxic conditions and rapid burial.

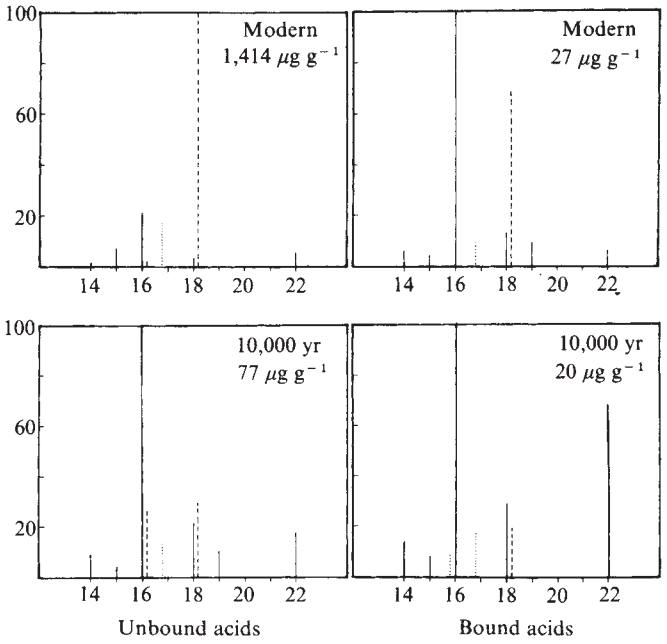

Fig. 1 Comparison of fatty acids extracted from modern and 10,000-yr-old spruce wood. Unbound acids released by Soxhlet extraction; bound acids by alkaline hydrolysis of extracted wood. Compositions are relative to major acid component (100%). Acids are displayed by number of carbon atoms. Solid lines represent *n*-alkanoic acids; dashed lines *n*-alkenoic acids; dotted lines *anteiso* acids.

Table 1 Extractable components of modern and 10,000-year-old wood and needles from the white spruce *Picea glauca*

	Wood			Needles		
Component	Modern	Old	Δ	Modern	Old	Δ
Fatty acids						
unbound	1,414	77	−1,337	2,785	114	−2,671
bound	27	20	−7	362	237	−125
total	1,441	97	−1,344	3,147	351	−2,796
Sterols						
unbound	311	339	+28	2,159	107	−2,052
bound	2	11	+9	0	55	+55
total	313	350	+37	2,159	162	−1,997
Hydrocarbons						
unbound	27	36	+9	146	54	−92
bound	1	1	0	1	1	0
total	28	37	+9	147	55	−92
Cellulose plus lignin	157	192	+35			

Concentrations of fatty acids, sterols and hydrocarbons are given in μg per g dry wood; cellulose plus lignin components are in mg per g. Δ, Difference between modern and old concentrations.

The cellulose plus lignin component of the old wood is at a higher concentration than in the modern wood. As suggested in the case of wood sterols and hydrocarbons, this indicates less alteration of these materials relative to other, more labile components. Lactic acid is the most abundant compound found in these samples. Oxalic and succinic acids are present as hydrolysis products of cellulose[16]. Their proportions relative to each other are the same in both samples. Vanillic acid and ferulic acid are also found and are derived from lignin[16,17]. The contribution of cellulose components, represented by oxalic and succinic acids, decreases relative to that of lignin components, represented by vanillic and ferulic acids, in the old wood. The ratio of these components changes from 2.2 in the modern wood to 1.2 in the old, indicating a selective loss of cellulose compared to lignin over this 10,000-yr period of burial.

These data give relative rates of degradation of some types of biological compounds buried in lake sediments for short periods of geological time. In woody tissue, the order of alteration is fatty acids ≫ sterols > aliphatic hydrocarbons > cellulose > lignin. In spruce needles, the order is fatty acids = sterols ≫ aliphatic hydrocarbons. These relative rates can be useful for understanding the cycling of organic matter in young subaqueous sediments. Furthermore, this study describes some woody tissue changes which occur during the early stages of coalification.

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1. Dungworth, G., Schwartz, A. W. & van de Leemput, L. *Comp. Biochem. Physiol.* **53B**, 473–480 (1976).
2. Gillispie, J. M. *Science* **170**, 1100–1102 (1970).
3. Wehmiller, J. F., Hare, P. E. & Kujula, G. A. *Geochim. cosmochim. Acta* **40**, 763–776 (1976).
4. King, K. Jr & Hare, P. E. *Science* **175**, 1461–1463 (1972).
5. King, K. Jr *Nature* **252**, 690–692 (1974).
6. Hohn, M. E. & Meinschein, W. G. *Geochim. cosmochim. Acta* **41**, 189–193 (1977).
7. Dilcher, D. L., Pavlick, R. J. & Mitchell, J. *Science* **168**, 1447–1449 (1970).
8. Giannasi, D. E. & Niklas, K. J. *Science* **197**, 765–767 (1977).
9. Sigleo, A. C. *Science* **200**, 1054–1056 (1978).
10. Traver, R. *Am. Forests* **86**, 32–35, 51–54 (1980).
11. Farrington, J. W., Henrichs, S. M. & Anderson, R. *Geochim. cosmochim. Acta* **41**, 289–296 (1977).
12. Meyers, P. A., Bourbonniere, R. A. & Takeuchi, N. *Geochim. cosmochim. Acta* **44**, 1215–1221 (1980).
13. Lee, C., Gagosian, R. B. & Farrington, J. W. *Geochim. cosmochim. Acta* **41**, 985–992 (1977).
14. Nishimura, M. & Koyama, T. *Geochim. cosmochim. Acta* **41**, 379–385 (1977).
15. Nishimura, M. *Nature* **270**, 711–712 (1977).
16. Bourbonniere, R. A. thesis, Univ. Michigan (1979).
17. Hedges, J. I & Mann, D. C. *Geochim. cosmochim. Acta* **43**, 1803–1807 (1979).

Evidence for diploidy and mating in trypanosomes

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The question of whether any sexual process takes place at some stage of the life cycle of the trypanosome has often been raised[1,2] and crosses between different drug-resistant strains[3,4] have provided no convincing answer. Multinucleate forms of trypanosomes have been observed by electron microscopy[5,6] but their significance and origin remain obscure. As part of a study aimed at examining the speciation and genetics of the *Trypanosoma brucei* complex of trypanosomes, a series of isolates from a population of *T.b. brucei* have been screened for electrophoretic variation in 19 enzymes. The results of this survey, reported here, provide strong evidence that trypanosomes are diploid and undergo random mating and recombination.

The population used consisted of 17 stocks of *T.b. brucei*, collected in 1969–70 from Lugala, Uganda, by members of the East African Trypanosomiasis Research Organisation (EATRO). Fifteen of the stocks were obtained by injection of infected tsetse salivary glands into mice and subsequent storage of infected blood in liquid nitrogen. The remaining two stocks were obtained by injection of infected blood from wild game into mice, followed by storage in liquid nitrogen. Samples of these stocks were transferred to the Centre for Tropical Veterinary Medicine in Edinburgh. This material was made available by Dr A. Gray and preparations of trypanosomes were obtained by infecting laboratory mice with these stocks, followed by DEAE-cellulose chromatography[7] of infected blood. Extracts from such purified preparations were then subjected to starch gel electrophoresis and the gels specifically stained for the 19 enzymes listed in Table 1.

Electrophoretic variation was observed in eight of the enzymes, which can be subdivided on the basis of the type of variant banding pattern obtained on gels (Table 1). In type I, with, for example, isocitrate dehydrogenase, some stocks produce a single band of enzyme activity at one position on the gel (1CD-1), others produce a single band at a second position (1CD-2), and a third group of stocks produce three bands, two of which correspond to 1CD-1 and 1CD-2, respectively, the third (of double intensity) lying midway between 1CD-1 and 1CD-2 (Fig. 1*a*). In type II, with, for example, alkaline phosphatase, individual stocks may produce single bands at one of two alternative positions (AP-1 or AP-2), whereas other stocks produce two bands at positions corresponding to AP1-1 and AP-2 (Fig. 1*a*). Of the eight polymorphic enzymes found, three are of type I and five of type II (Table 1). Furthermore, four of the type II enzymes show more than two possible positions at which the bands may appear. The variants observed for the eight polymorphic enzymes are shown in Fig. 1*a* and *b*, together with the nomenclature used for their designation. With all these enzymes multiple-banded types were observed, triple banded in the case of type I and double banded in the case of type II.

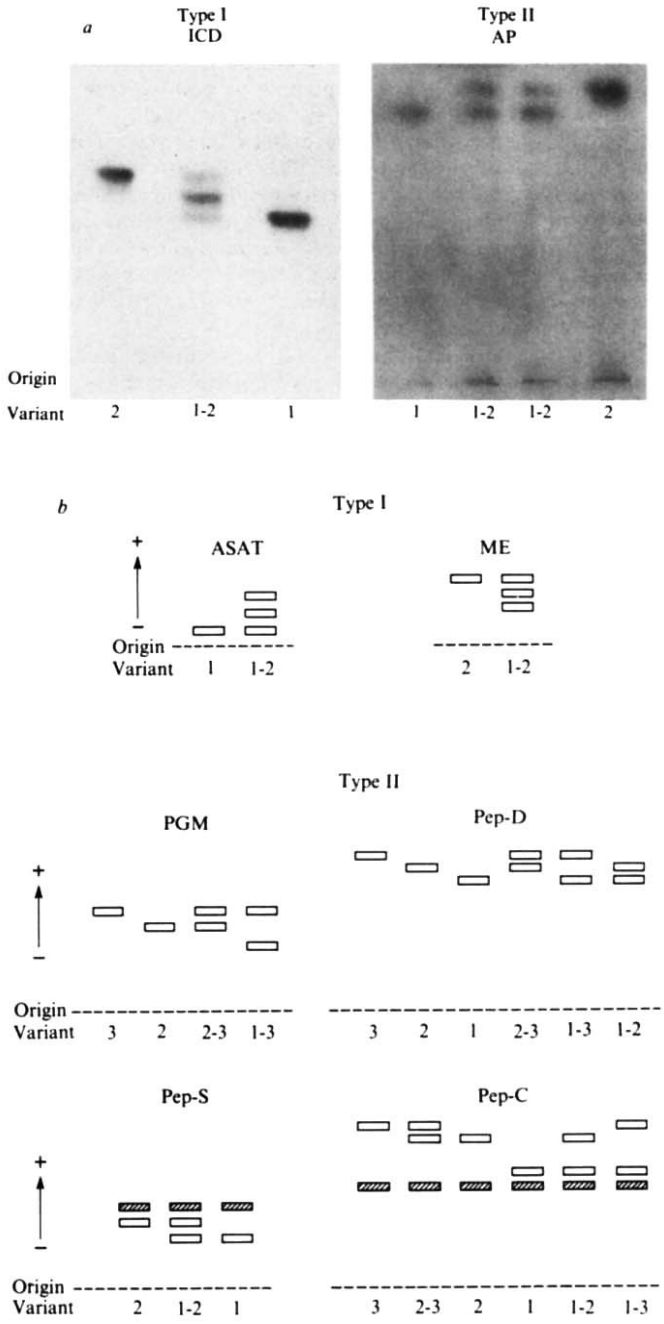

Fig. 1 Electrophoretic enzyme variation in isolates of *T.b. brucei*. *a*, Photographs of starch gel electrophoresis slabs stained for isocitrate dehydrogenase (ICD) and alkaline phosphatase (AP). Samples were run in 9% hydrolysed starch gels in 0.026 M Tris, 0.0033 M citric acid, *p*H 8.6, at 270 V for 3 h. The electrode buffer was 0.66 M Tris, 0.083 M citric acid, *p*H 8.6, and the enzyme activities were stained using published methods[16]. *b*, Diagrams of starch gel electrophoresis slabs stained for aspartate amino transferase (ASAT), malic enzyme (ME), phosphoglucomutase (PGM), peptidase-S (Pep-S), peptidase-C (Pep-C) and peptidase-D (Pep-D). The peptidases are designated on the basis of their di- and tripeptide specificities using the nomenclature described by Harris and Hopkinson[16]; the cross-hatched bands shown on Pep-S and Pep-C gels represent the two invariant peptidases (Pep-A and Pep-B, see Table 1) which overlap in their substrate specificities. Details of the electrophoretic conditions and activity stains will be published elsewhere. The variant bands observed are designated by numbers in order of increasing mobility.

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