glycosides, methylated polysaccharides and acetal derivatives of sugars are readily detected by spraying with a solution (5 per cent) of 1-naphthol in 10 *N* sulphuric acid.

The paper electrophoresis experiments were carried out in a 'sandwich' type of apparatus[2,3]. The glass paper (made with 3 A. fibre and no organic binder)[1], held between two thin insulating sheets of polythene, is placed on a sheet of foam rubber (0·5 in. thick) and clamped between two sheets of plate glass (0·25 in. thick) by means of two simple wooden clamps[2]. The foam rubber enables an even pressure to be applied to the paper[3]. Since evaporation is prevented, cooling need not be applied except when dealing with proteins, in which case the upper glass plate is replaced by one to which has been cemented a 'Pyrex' dish and through which cold water can be circulated[2].

For electrophoretic analysis of carbohydrates, 0·1 *M* sodium tetraborate was used as the buffer, a voltage of 600 was applied and separations were carried out for 0·5–3 hr., depending on the nature of the substances being examined. Ethylene glycol, D-glucose, tetra-O-methyl D-glucose or its methyl glycoside were used as reference compounds. After the separation, the paper, still containing borate buffer, was placed on a glass plate and dried in the oven at 100° and, while still hot, the reagent was applied as a spray in the usual way. With the 1-naphthol reagent mono-, oligosaccharides, methyl sugars, glycosides, sugar acetals, polysaccharides and methyl polysaccharides were located by the appearance of dark blue spots on a white background, while with alkaline permanganate all oxidizable compounds were detected by the appearance of well-defined white or pale yellowish brown spots on a pink or greenish-pink background. The same glass fibre paper chromatogram may be subjected to the alkaline permanganate treatment (this detects all the oxidizable components), and then with the acidified 1-naphthol reagent (this detects sugar derivatives that escape permanganate oxidation).

Many other substances, including the amino-acids, proteins, sterol glycosides, organic acids and phenols, can be separated in the same manner and readily identified by taking advantage of the fact that a wide variety of reagents and conditions may be used for locating the substances on the glass papers.

Using glass instead of cellulose paper, the possibility of combination between polyhydroxy compounds and the paper through a common borate ion is impossible, whereas this is not necessarily the case when cellulose paper is employed. Hence, structural deductions from borate complex formation, as revealed by electrophoresis, are likely to be more reliable when glass paper is used instead of cellulose paper. The method has proved useful in the separation and comparison of *cis* and *trans* C_5, C_6, and C_7 saturated cyclic diols and other cyclic and straight-chain polyols. The steric effect of neighbouring groups and of the nature and size of rings on vicinal hydroxyl groups has also been investigated.

It will be apparent that this method will also simplify certain quantitative determinations which, hitherto, have had to take into consideration material extracted from the cellulose filter papers, and it is also likely that electrophoresis on thick sheets of glass paper will prove to be useful for preparative work.

The glass filter paper can be re-used after a suitable washing treatment, and a further saving can be effected by using glass paper only for the central portion of the chromatogram, with overlapping extensions of cellulose filter paper to make contact with the buffer solution.

Further details of this work will appear elsewhere.

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[1] O'Leary, M. J., Hobbs, R. B., Missimer, J. K., and Erving, J. J., *Tappi*, **37**, 446 (1954).
[2] Briggs, D. R., Garner, E. F., Montgomery, R., and Smith, F., *Anal. Chem.* (in the press).
[3] Cf. Kunkel, H. G., "Methods of Biochemical Analysis", Ed. D. Glick, p. 141, Interscience, New York (1954); Lederer, M., "Introduction to Paper Electrophoresis and Related Methods", Elsevier Publishing Co., New York (1955).

Spectrum of the Cyclopentadienyl Radical

The great stability of benzene has aroused much interest in the properties of other planar symmetrical cyclic species of formula $(CH)_n$. For even values of n, only cyclo-octatetraene is stable, and is of limited interest owing to puckering of the ring[1]. The odd series can exist only as radicals or ions and, while several calculations have been made of their resonance energies[2–4], only $C_7H_7^+$ has been observed spectroscopically[5], although the acidity of cyclopentadiene[6] provides evidence for the stability of the ion $C_5H_5^-$. Like benzene, both these species have six π-electrons and are expected to have higher resonance energies than the 38 k.cal. predicted for the cyclopentadienyl radical (C_5H_5)[3,4].

Experiments have been carried out on the flash photolysis of both cyclopentadiene and 'ferrocene' (biscyclopentadienyl iron) in the gas phase using the apparatus previously described[7]. Pressures of 1–10 mm. of cyclopentadiene or 1 mm. of ferrocene diluted with 250–750 mm. of inert gas (nitrogen or carbon dioxide) to limit the rise in temperature were used. In all these mixtures, the spectrum shown in Fig. 1 was observed during the photolysis but disappeared rapidly, its half-life being less than 10^{-4} sec. This spectrum consists of two heads at 29,581 and 29,911 cm.$^{-1}$, which are relatively sharp, having widths of about 10 cm.$^{-1}$; this spectrum must presumably be due to the C_5H_5 radical, since it is difficult to visualize any other transient species except atoms which would be formed directly in the photolysis of both these compounds. Furthermore, recent calculations by McEwen and Longuet-Higgins[8] using a semi-empirical molecular orbital treatment predict an E_1'' ground-state for this radical, with an A_2'' first excited state to which a transition would be allowed (transition moment symmetry—E_1'). Neglecting second-order interactions, they find an excitation energy of 4·02 eV. for this state, which is in strikingly good agreement with the observed values of 3·67 and 3·70 eV. Also, they predict that there will be no other states lying below 6 eV., which is in accordance with the absence of further absorption bands above the limit of observation at 2400 A.

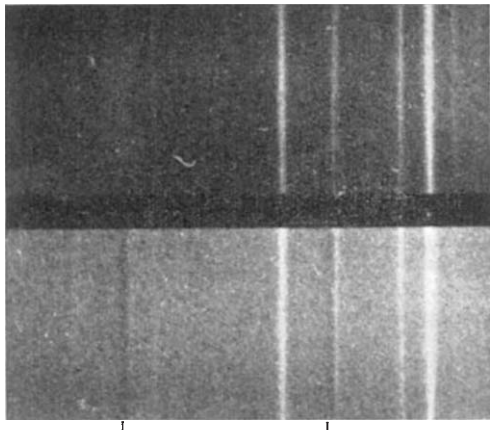

Fig. 1. Absorption spectrum attributed to the cyclopentadienyl radical

The decay of the C_5H_5 radical is accompanied by the appearance of featureless continuous absorption below 3500 A., due to a stable product which has not yet been investigated. In the absence of an inert gas, the rise in temperature due to the light energy absorbed by either cyclopentadiene or ferrocene is insufficient to produce appreciable thermal decomposition; under these conditions, the continuous absorption by the products appears without the intensity of the C_5H_5 spectrum reaching the limit of detection. This increase in the rate of the reactions removing the radical indicates that they have an appreciable activation energy, which is not surprising in view of the high resonance energy expected for C_5H_5. During this photolysis in the absence of inert gas, ferrocene shows strong absorption from iron atoms in the sub-levels of the (x^5D) and (a^5F) states, the latter being intense enough to suggest that the iron atoms might be liberated in this state.

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[1] Karle, I. L., *J. Chem. Phys.*, **20**, 65 (1952).
[2] Hückel, E., *Z. Physik*, **70**, 204 (1931).
[3] Roberts, J. S., and Skinner, H. A., *Trans. Farad. Soc.*, **45**, 339 (1949).
[4] Franklin, J. L., and Field, F. H., *J. Amer. Chem. Soc.*, **75**, 2819 (1953).
[5] Doering, W. von E., and Knox, L. H., *J. Amer. Chem. Soc.*, **76**, 3203 (1954).
[6] Thiele, J., *Ber.*, **33**, 666 (1900).
[7] Norrish, R. G. W., Porter, G., and Thrush, B. A., *Proc. Roy. Soc.*, A, **216**, 165 (1953).
[8] McEwen, K. L., and Longuet-Higgins, H. C. (to be published).

A Test of the 'Redox' Hypothesis of Active Ion Transport

The concept that the electron transfer in the oxidation–reduction reactions of respiration constitutes the driving force for active ion transport has provided the basis for several hypotheses of ion transport[1–7]. All these have in common the quantitative limitation that no more than four univalent ions can be actively transported per molecule of oxygen consumed, because oxygen can accept just four electrons per molecule in its complete reduction.

To test this 'redox' hypothesis of ion transport, simultaneous measurements were made of active ion transport and oxygen consumption by isolated frog skin. Ussing and Zerahn[8] have shown that, with the same solution bathing both sides of an isolated frog skin, the short-circuit current measured, when a counter potential is applied across the skin so as to reduce the spontaneous membrane potential to zero, is equal to the net sodium transport through the skin. Hence the short-circuit current was taken as the measure of active sodium transport. Oxygen consumption was measured polarographically with a vibrating platinum micro-electrode[9].

A total of eighty-seven individual, simultaneous measurements of oxygen consumption and active ion transport was made on the ventral skins of nineteen frogs (*Rana temporaria*). The ratio of ions transported to molecules of oxygen consumed was found to range from 2 to 13 with a mean of 6·93 and standard error of the mean of $\pm$ 0·21. In all but five periods this ratio was above 4·0. The experimental error propagated into the determination of a single value of this ratio is estimated to be $\pm$ 9 per cent.

These results are incompatible with the 'redox' hypothesis of ion transport in which molecular oxygen is the electron acceptor. Similar results in this same tissue have recently been obtained by Zerahn[10].

Active sodium transport through the frog skin is augmented by neurohypophyseal preparations of mammalian origin[8]. We find in all but one of sixteen experiments that this effect is associated with a simultaneous increase of oxygen consumption by the stimulated membrane. Thus the mean rates of sodium transport for the sixteen periods just before and the sixteen periods just after addition of neurohypophyseal preparations were 1·69 and 2·76 μequiv./cm.^{2}/hr. respectively, the mean difference was 1·07 and its *S.E.* $\pm$ 0·089 and $P < 0{\cdot}001$. The mean figures for oxygen consumption for the same periods were 0·278 and 0·341 μmole/cm.^{2}/hr., the mean difference was 0·063 and its *S.E.* $\pm$ 0·0129 and $P < 0{\cdot}001$. If the one experiment in which no stimulation of oxygen consumption was observed is excluded, the mean ratio of increment of sodium transport to increase in oxygen consumption following stimulation of the skin with neurohypophyseal preparations was 21·1 $\pm$ 3·0 sodium ions transported per molecule of the increment of oxygen consumed. Though such a calculation is open to some criticism[11], this mean figure of the ratio of increase in sodium transport to increase in oxygen consumption represents the best estimate possible at present of the actual stoichiometric relationship of ion transport to oxygen consumption by the fraction of cells in this tissue engaged in the ion transport process.

The minimum energy required for the active ion transport process constitutes 8 and 10 per cent of the total energy available to the tissue from aerobic metabolism before and after stimulation with neurohypophyseal preparations, respectively, and 22 per cent for the increment of ion transport and oxygen consumption following hormonal stimulation. Thus no inconsistency is encountered when the energetics of the observed rates of ion transport are considered.

Previous attempts to evaluate the relationship of ion transport to oxygen consumption in this tissue have been made. Stapp and Lund[12] obtained values close to 4 but realized that, as they were not using a totally short-circuited skin preparation, their results gave only a minimal value. Linderholm[13] has estimated this relationship and his results give a value of 3·28. However, his oxygen and ion transport measure-