leucine (melting point 212–14° C. (dec.). Calc. for $C_{12}H_{20}O_3N_4$, N, 20·88 per cent; found N, 20·54 per cent).

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[1] Holley, R. W., and Sondheimer, E., *J. Amer. Chem. Soc.*, **76**, 1326 (1954). Fischer, R. F., and Whetstone, R. R., *ibid.*, **76**, 5076 (1954).
[2] Sheehan, J. C., and Hess, G. P., *J. Amer. Chem. Soc.*, **77**, 1067 (1955).

Electrophoretic Behaviour of *i*-Urobilin and Stercobilin

As the urobilinoid pigments *i*-urobilin and stercobilin are known to contain both basic and acidic groups, we have investigated their electrophoretic behaviour—yet unstudied—at various *p*H values. Paper electrophoresis was performed at 120 V., in acetate buffer (a mixture of *M*/5 acetic acid–sodium acetate buffer and 0·9 per cent sodium chloride solution) in the *p*H range less than 6·0, and barbital buffer (Michaelis sodium barbital–sodium acetate–hydrochloric acid buffer) at *p*H greater than 6·0; the positions of the coloured spots were observed directly during the procedure, but even minute quantities of urobilinoid pigments could be seen at the end of the run after spraying the papers with Schlesinger reagent (saturated zinc acetate solution in ethanol), and observing the green fluorescence in ultra-violet light. With the exception of the sharply defined *p*H value of 4·41 at which both urobilinoid pigments became immobile in the electric field, they moved towards the cathode at *p*H less than 4·41 and toward the anode at *p*H greater than this value, the direction of movement, which was the same for both pigments, being dependent only on the *p*H. However, the rate of movement of these materials differed at various *p*H levels: thus in buffers around *p*H 5·0 and at *p*H less than 4·41 stercobilin migrated faster toward the anode or cathode, respectively, whereas at higher *p*H values (8·6) urobilin was found to move faster than stercobilin in the anode direction.

As these substances are known to be ionized even at their isoelectric point[1], the basic and acid groups were found to be in equilibrium in the electric field around *p*H 4·41, but below this isoelectric point the direction of migration is determined by the ionized basic groups, and above this *p*H value by the ionized acid groups.

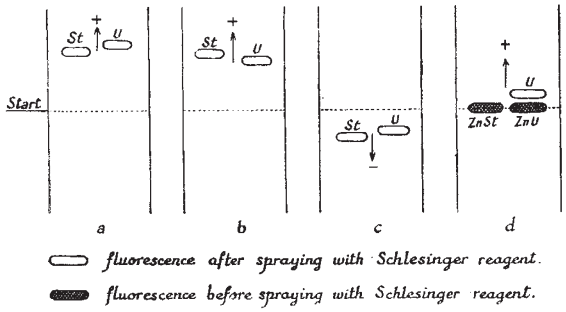

Fig. 1. Diagrams of electrophoresis paper strips. *St*, stercobilin; *U*, *i*-urobilin; arrow indicates migration in anodic or cathodic direction. Environment: (*a*) barbital buffer solution, *p*H 8·6; 7-hr. run; (*b*) acetate buffer, *p*H 5·5; 8-hr. run; (*c*) acetate buffer, *p*H 3·8; 4-hr. run; (*d*) electrophoretic behaviour of zinc complexes of urobilinoid pigments in barbital buffer, *p*H 8·6; 1-hr. run. Part of urobilin left the starting line

As the *pK* of stercobilin is known from spectrophotometric determinations[2] to be 8·0, at *p*H 8·0 only half the molecules of this substance are dissociated, whereas at the same *p*H *i*-urobilin characterized by *pK* 7·0 is far more completely dissociated, hence the greater mobility of the latter at *p*H 8·0, above its *pK*. This more acid character of *i*-urobilin and more basic property of stercobilin may be explained on the basis of the lactam structure of urobilinoid pigments proposed by Birch[3] and verified by Gray and Nicholson[4]. The NH-groups of the end rings attached to a conjugated system of double bonds in urobilin impart a greater acidity than the NH-groups in stercobilin behaving as ordinary amides. The nitrogen atom of an ordinary amide may more readily be protonated than the nitrogen atom of the end rings of urobilin; hence the faster migration of urobilin at high *p*H and of stercobilin at low *p*H.

However, the electrophoretic separation of stercobilin and *i*-urobilin is not yet sufficient for the practical isolation of either material from the other, but they may be isolated, with the aid of their electrophoretic behaviour, from mixtures containing the bile pigments.

The zinc complexes of the urobilinoid pigments were immobile during the electrophoretic procedure and could be detected at the 'start' position without spraying. However, their stability was limited, part of the pigments successively leaving the starting point and behaving as urobilinoids containing no zinc. The stability of zinc stercobilin proved more resistant to the action of the electric field than zinc *i*-urobilin.

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[1] Lemberg and Legge, "Hematin Compounds and Bile Pigments", 138 (Interscience Pub., New York, 1949).
[2] Gray, "The Bile Pigments", 36, 118 (Methuen and Co., Ltd., London, 1953).
[3] Birch, *Chem. and Indust.*, 652 (1955).
[4] Gray and Nicholson, *Nature*, **179**, 264 (1957).

A Possible Basis for the Anti-inflammatory Activity of Salicylates and other Non-Hormonal Anti-Rheumatic Drugs

RECENT studies have shown that sodium salicylate and acetyl-salicylic acid increase oxygen consumption in rats, rabbits and man[1–3]. Sproull has shown that both sodium salicylate and 2 : 4-dinitrophenol stimulate metabolism in mouse-liver slices[4], but has recently found a difference between the slopes of the concentration–response curves for the two compounds[5]. Reid has also directed attention to these similarities, and has shown that both compounds inhibit the growth of wheat coleoptiles[6]. Cochran[3] suggested that the stimulation of respiration by salicylate may be of fundamental importance in the therapeutic action of the drug.

2 : 4-Dinitrophenol is known to uncouple oxidative phosphorylation in mitochondrial preparations[7], and Brody[8] and Penniall *et al.*[9] demonstrated that sodium salicylate and acetylsalicylic acid also uncouple in very low concentrations. Smith and Jeffrey[10] pointed out that most of the previously observed effects of

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