

cells and may also be extra-cellular. They stain well with Giemsa and may also be detected when numerous with Leishman's stain. Other workers who have kindly examined infected blood smears concur with our opinion that the organisms are *Eperythrozoa*. (We wish to acknowledge the assistance of Dr. June Thurston, Molteno Institute, University of Cambridge, in this respect.) While we are as yet uncertain of the species concerned, it seems likely that the parasite is *Eperythrozoon parvum*.

The organisms have been found in seven splenectomized pigs less than three months old. They have been seen in the blood within nine days of splenectomy and have remained evident for at least seven days. Initially few in number, they increase until a peak infection is reached, then gradually regress. Recrudescences occur, and in one case this was associated with a mild anaemia (haemoglobin-level reduced by 3 gm./100 ml. and red blood cell counts reduced by 2 million per c.mm.) and a mild fever. A number of attempts at culture on bacteriological media and in developing chick embryos, together with transmission to small laboratory animals, have been unsuccessful. Three pig-to-pig transmissions have been accomplished. A detailed account of these experiments will be published elsewhere.

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<sup>1</sup> Splitter, E. J., *Science*, **111**, 513 (1950).

### Unexpected Anomalies in the Behaviour of Neutral Red and Related Dyes

THE colour base of neutral red, 2-amino-8-N,N-dimethylamino-3-methylphenazine, together with 2-amino-8-N,N-dimethylaminophenazine (the colour base of 'neutral violet' in German usage), the amphoteric 8-amino-2-phenazino, and 2-amino-7-N,N-dimethylaminophenazine have been made for the first time by unambiguous means: bomb-tube reactions of aqueous ammonia with the corresponding chloro- or bromo-N,N-dimethylaminophenazines, or with the halogenated 2-phenazino<sup>1</sup>. The phenazines were made by ring closure through the nitro group of the corresponding 2-nitrodiphenylamines, according to the method of Waterman and Vivian<sup>2</sup>.

None of the four compounds stained two species of *Amoeba* (*A. proteus* and *A. dubia*), or cells of sarcoma 37 ascites tumour when they were dissolved in dilute hydrochloric acid and the solutions brought to approximate neutrality. The staining power, on the other hand, was retained by the purified colour base of commercial neutral red (obtained by passing the crude dye twice through basic alumina), when this purified sample was likewise dissolved in dilute hydrochloric acid and the solution neutralized.

Analytical figures for carbon and hydrogen of this latter sample corresponded closely to the theoretical for aminodimethylaminomethylphenazine; the infra-red spectrum of the purified commercial base was ambiguous with regard to that of the synthetic 2-amino-8-N,N-dimethylamino-3-methylphenazine. This line of investigation is being continued. The melting points were different, however, for the base from the commercial dye melted with decomposition

at 241–245°, while the synthetic base melted without decomposition at 166–167°. Also, the latter gave a brown colour with concentrated sulphuric acid, while the base from the commercial dye gave a green. In addition, the yellow solution of the synthetic base in ether showed a marked green fluorescence on exposure to ultra-violet light which was absent from the ether solution of the less-soluble purified commercial base.

Significantly, it was found possible to obtain forms of all three of the synthetic 2,8-substituted colour bases which gave vital stains when dissolved in dilute hydrochloric acid. These forms were made by dissolving the colour bases in concentrated sulphuric acid, diluting, and precipitating with ammonium hydroxide. The finely divided products were isolated by centrifuging. It is significant that the 2-amino-7-N,N-dimethylaminophenazine did not give a stain when subjected to this process.

The colour bases so obtained contained varying amounts of water, depending on the humidity of the atmosphere in which they were spontaneously dried; but in the instance of the colour base of neutral red, and presumably for the others, this water was not essential to the staining properties, which were retained undiminished when the compound was dried at 110°.

There apparently exists a third form, also, of the colour base of neutral red at least; for dissolving the synthetic compound in concentrated sulphuric acid and neutralizing directly by concentrated ammonium hydroxide, without prior dilution, produces a non-staining form which cannot be converted to the stain by subsequent treatment with sulphuric acid, dilution, and neutralization by ammonium hydroxide.

Both of the first two forms have been shown to be monomers in methanol solution by the Signer method<sup>3</sup>.

Further investigation is being undertaken to determine the nature of the structural differences between the three forms.

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<sup>1</sup> Vivian, *J. Org. Chem.*, **21**, 565 (1956).

<sup>2</sup> Waterman and Vivian, *J. Org. Chem.*, **14**, 239 (1949).

<sup>3</sup> Clark, *Indust. Eng. Chem., Anal. Ed.*, **13**, 820 (1941).

### Separation of Carbohydrates by Electrophoresis on Glass Filter Paper

IN recent studies on the paper electrophoresis of carbohydrate compounds, we have found that replacing the cellulose filter paper by paper made from glass fibres<sup>1</sup> greatly facilitates the identification of sugars and their derivatives, especially those which are difficult or impossible to detect on cellulose paper.

Sugars, methylated sugars, sugar alcohols, lactones, sugar phosphates, neutral and acidic polysaccharides may be detected by spraying with a solution (0.5 per cent) of potassium permanganate in *N* sodium hydroxide. The more stable methylated methyl