then stored in opaque bottles at room temperature over calcium chloride. In use the papers are wetted with distilled or tap water and pressed against the suspected seminal stain. A positive reaction is shown by an intense purplish colour.

Discoloration on storage proceeds only slowly and we have found the papers to be usable up to a year from manufacture, with a little activity remaining up to two years (storage temperature $15^{\circ}\text{C} \pm 5^{\circ}\text{C}$). Storage at 55°C . for some weeks is possible, showing that temperature will not be a limiting factor for use in tropical countries.

A modification of the test is to interpose wet filter paper between the stain and the test-paper instead of placing them in direct contact. This possesses the advantage of reducing contamination of the seminal stain by the reagent (this is especially important where serological tests are afterwards to be applied): it permits also the use of test papers which, although discoloured by long storage, are still usable. This is because extracts from the stain and test paper pass into the clean filter paper and react there. The insoluble coloured decomposition products remain on the test paper.

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Detection of Hæmoglobin, Hæmoglobin-Haptoglobin Complexes and other Substances with Peroxidase Activity after Zone Electrophoresis

HÆMOGLOBIN and complexes of serum haptoglobins with hæmoglobin, have peroxidase-like activity which permits their detection in small amounts. For this purpose benzidine^{1,2}, *o*-tolidine², guaiacol³ and leucomalachite green⁴ have been employed. However, none of these substances has proved entirely satisfactory. The colours given by benzidine and tolidine fade within minutes⁵, especially when applied to starch-gel strips, and both reagents give appreciable background staining on paper. Instability of benzidine and guaiacol colours used to detect horse-radish peroxidase after paper electrophoresis has been noted also by Jermyn and Thomas⁶, who reported that *o*-anisidine, *p*-aminodimethylaniline and hydroquinone were even less satisfactory as reagents for the detection of peroxidases. Malachite green, formed from leucomalachite green, is more stable; but we have found the reagent much less sensitive than either benzidine or tolidine.

In studying the haptoglobins of human serum we have sought a more satisfactory peroxidase colour reaction and have compared a number of likely substances. The reagents in each case were prepared by dissolving 100 mgm. of the test substance in 70 ml. of ethanol and adding 10 ml. of 1.5 *M* acetate buffer (pH 4.7) and 18 ml. of water. Immediately before use 2 ml. of hydrogen peroxide (100 vol.) was

Fig. 1. Starch-gel electrophoretic strips (borate buffer, pH 8.6; ref. 7) of human serum containing added hæmoglobin. *a*, stained with naphthalene black to show proteins; *b*, stained with dianisidine reagent to show hæmoglobin-haptoglobin complexes and photographed after 2 weeks. *Hb*, hæmoglobin; *Hb-Hp*, hæmoglobin-haptoglobin complex. Arrow indicates point of insertion

added. The composition of the reagents was thus similar to the benzidine reagent used by Moretti *et al.*¹. After electrophoresis, paper or starch-gel strips were placed in the reagent for 15 min. and were then washed in three changes of water.

Results are shown in Table 1 and indicate that *o*-dianisidine was the most satisfactory reagent. It gives an intense brown colour in zones having peroxidase activity. After washing and drying, the reactive areas on paper become brown-pink while the paper shows a very pale pink background. In starch-gel strips the coloured areas remain unaltered for at least a month while the background remains perfectly white. Fig. 1 illustrates a starch-gel electrophoresis strip of human serum containing added hæmoglobin.

Table 1. COMPARISON OF PEROXIDASE REAGENTS

Substance*	Sensitivity† (10^{-2} $\mu\text{gm./cm.}^2$)	Comment
<i>o</i> -Dianisidine	5	Colour stable
<i>o</i> -Tolidine	10	Marked background colour fades in hours
Benzidine	20	Colour fades in minutes
<i>p</i> -Anisidine	40	Colour fades in minutes
Amidopyrine	>80	—
Leucomalachite green	>80	—
Guaiacol	>80	—

* All materials were obtained from British Drug Houses, Ltd.

† Minimal concentration of hæmoglobin detectable on paper.

While most of our experiments were carried out with hæmoglobin or with hæmoglobin-haptoglobin complexes, we have also found the dianisidine reagent satisfactory in the detection of peroxidase activity in extracts of horse-radish (*Cochlearia armoracia* L.) and other plants.

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