Full details of this work will be published elsewhere.

F. C. PEERS
Research Association of British Flour Millers,
Cereals Research Station, Old London Road,
St. Albans, Herts. Aug. 8.

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A New Spraying Agent for Paper Chromatography of Carbohydrates and Polyols

WHILE investigating the chromatographic separation of sugars upon filter paper impregnated with sodium borate buffer (0·05 *N* borax, *p*H 9·18) it was found that the carbohydrates showed up prominently on spraying with 80 per cent aqueous ethanol containing either 1 mgm./ml. of phenolphthalein or phenol red just made alkaline with sodium hydroxide. Phenolphthalein gave rise to white spots on a purple background and was rather more effective than phenol red, which gave yellow spots on a purple background.

It was considered that the change in *p*H consequent upon the formation of carbohydrate–borate complexes might be used directly. Preliminary studies led to the development of a spraying reagent consisting of 1 part 0·05 *N* sodium borate (*p*H 9·18), 2 parts phenol red (2 mgm./ml. in ethanol made just alkaline with sodium hydroxide) and 7 parts methanol. The reagent could also be made up in ethanol. This, however, caused some precipitation of borate, which might lead to variability of performance, although the ethanolic reagent has given uniformly good results in our hands. Phenolphthalein was an inferior substitute for phenol red, as the reagent had to be adjusted to such a *p*H that it just coloured the paper. This proved awkward and, since the indicator did not always spread evenly, owing apparently to adsorption on the paper, phenolphthalein is not recommended.

Although we have experienced no trouble from this cause, it should be noted that acidic or basic substances able to move in the solvents used might interfere. These could be removed by ion exchange resins. The method has, however, proved more satisfactory than the use of ammoniacal silver nitrate. Methanolic phenol red–borate gave good definition with 25–50 μgm. dihydroxyacetone, arabinose, xylose, fructose, glucose, lactose, mannose, sucrose, maltose, cellobiose, raffinose and a number of polyols.

Ascending chromatography on Whatman No. 4 paper was carried out for 15 hr. using developing solvents similar to those described by Jeanes, Wise and Dimler[1]. The distances moved by glucose were about 9·5 cm. in solvent *B* and 4·5 cm. in solvent *F*. Typical *Rg* values are quoted in the accompanying table for some polyols.

The method may also be of value when it is necessary to determine the position of sugars by a technique that leaves them unchanged, so as to make possible leaching out the spots for micro-determinations.

$$Rg = \frac{\text{distance moved by substance}}{\text{distance moved by glucose}}$$

Solvent	*B* *n*-Butanol : pyridine : water :: 3 : 2 : 1·5	*F* *n*-Butanol : pyridine : water :: 4 : 1 : 1
Dihydroxyacetone	1·84	3·38
Glycerol	1·61	2·58
Erythritol	1·35	1·85
Mannitol	0·91	0·92
Sorbitol	0·92	0·87
i-Inositol	0·39	0·19

Reference has been made by Bradfield and Flood[2] in a communication entitled "Soluble Carbohydrates of Fruit Plants" to the use of borate buffer containing bromcresol purple for similar purposes to the above.

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D. J. D. HOCKENHULL
Glaxo Laboratories, Ltd.,
Sefton Park, Stoke Poges, Bucks.

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Serological Activity of Globulin Fractions of Anti-*D* Sera separated by Paper Electrophoresis

IT seems that the existence of blocking, incomplete (non-blocking) and complete (saline) antibodies is generally accepted; but up to now little work has been done to widen our knowledge about serological activity of different fractions of anti-*Rh* sera containing those kinds of antibodies.

After preliminary examination of many immune sera, three anti-*D* sera were chosen. All these sera are very close to each other from the point of quantitative serological reaction, but at the same time they show marked differences in respect of the qualitative behaviour of anti-*D* antibody in carrying out various serological tests. The sera used are: No. 1286, containing blocking antibody (blocking titre 1/16, trypsin titre 1/32, albumin titre 1/16, saline titre negative, indirect *AHG* titre 1/128); No. 23530 with incomplete (non-blocking) antibody (blocking titre negative, trypsin titre 1/64, albumin titre 1/16, saline titre negative, indirect *AHG* titre 1/256); and No. 11871 with saline antibody (blocking titre negative, trypsin titre 1/64, albumin titre 1/64, saline titre 1/16, indirect *AHG* titre 1/128).

Electrophoresis on filter-paper strips (Whatman No. 2, 37 cm. × 5 cm.) was carried out to isolate α_1-, α_2-, β- and γ-globulin. The apparatus described by Flynn and de Mayo[1] was used with barbitone buffer *p*H 8·6; potential 350 V., for 3 hr., current 0·4 m.amp./cm. width.

In all experiments seven strips were used for each serum to be investigated; 0·03–0·04 ml. of serum was placed on each strip in a straight line 4 cm. long by means of a capillary glass pencil. After each run the strips, still wet, were cut into two parts and one of 37 cm. × 1·5 cm. was dried and stained with a solution of 1 per cent bromphenol blue in 95 per cent ethyl alcohol saturated with mercuric chloride. The strips were then washed in running tap water. The coloured strips were used for detecting the exact position of the different globulin fractions on the uncoloured parts of the strips. Each unstained strip was cut into fractions and the different globulins immersed in 1 ml. of saline (for each fraction) and eluted for 16 hr. at + 4° C. The filter paper was removed from the eluate by centrifugation and the eluates were examined, using blocking, trypsin, papain, albumin,