shorter wave-lengths, for example,  $0.42 \mu$ , a discriminable step could be observed before  $0.46 \,\mu$  was reached, although the observation was difficult owing to the low intensity.

The results given here represent experimental data which unequivocally confirm König's original statement that the central fovea is dichromatic and tritanopic. It is difficult to explain why his observations were received with such doubt for so many years, unless the reason lay in the difficulty of locating the small test field on the fovea and maintaining it in that position for more than a very short time. With the test fields used in the present experiments, any deviation from direct fixation immediately caused a breakdown in the matches obtained : whether this would have been true with still smaller fields it is impossible to say without further experiment. Prof. H. Hartridge's observations and those of one of us (E. N. W.) with painted test fields suggest that it may not be so. With regard to Prof. Hartridge's conclusions<sup>5</sup> about the dichromatism of retinal areas other than the central fovea, it is likely that colour matching experiments may confirm this provided the test field is of suitable size, yet we believe that the characteristics of the central fovea differ significantly from those of the retinal areas in its immediate Experiments are in progress to neighbourhood. investigate this question.

We wish to express our thanks for the continued support of the Medical Research Council.

<sup>1</sup> Willmer, E. N., Nature, **153**, 774 (1944). <sup>2</sup> Pirenne, M. H., Nature, **154**, 741 (1944); **155**, 177 (1945).

<sup>8</sup> Stiles, W. S., Nature, 155, 177 (1945).
<sup>4</sup> Thomson, L. C., Nature, 155, 177 (1945).

<sup>6</sup> Hartridge, H., Nature, 155, 391 and 657 (1945).
<sup>6</sup> König, A., "Gesammelte Abhandlungen Physiol. Optik.", 353 (1903).
<sup>7</sup> Parsons, J., "Introduction to the Study of Colour Vision", 84 (Cambridge, 1924).

<sup>8</sup> Wright, W. D., Trans. Opt. Soc., 29, 225 (1927-28); J. Sci. Inst., 16, 10 (1939).

<sup>9</sup> Wright, W. D., Med. Res. Counc. Spec. Rep. Ser., No. 139 (1929). <sup>10</sup> Pitt, F. H. G., Med. Res. Counc. Spec. Rep. Ser., No. 139 (1929).
 <sup>11</sup> Pitt, F. H. G., Med. Res. Counc. Spec. Rep. Ser., No. 200 (1935).
 <sup>12</sup> Pitt, F. H. G., Proc. Roy. Soc., B, 132, 101 (1944).
 <sup>12</sup> Walters, H. V., and Wright, W. D., Proc. Roy. Soc., B, 131 (1943).
 <sup>13</sup> Wright, W. D., Nature, 151, 726 (1943).
 <sup>14</sup> Weight, W. D., Proc. Roy. Soc., B, 132 (1943).

<sup>14</sup> Walters, H. V., Proc. Roy. Soc., B, 131, 27 (1942).
 <sup>14</sup> Wright, W. D., and Pitt, F. H. G., Proc. Phys. Soc., 46, 459 (1934).

## ESTER WAX: A NEW EMBEDDING MEDIUM

## By DR. H. F. STEEDMAN

## Department of Zoology, University of Glasgow

IN view of the many disadvantages of paraffin wax as an embedding medium, a search was made among natural and synthetic fatty acid esters for a more suitable material. No single substance was found which had all the necessary requirements, but a mixture was finally produced using diethylene glycol distearate as the main ingredient. A complete account of this work will be published elsewhere.

A formula suitable for most types of tissue is as follows:

Diethylene glycol distearate	* •	••		82 gm.
Ethyl cellulose, low viscosity	• •	••	••	4,,
Ricipoleic (octadecanediol) dia	retate	••	••	o "
Themerers (connection) and		••	••	0 11

The following are among the physical characters of this mixture:

Melting point
Section range
Ribbon range
Compression after
flattening

4-20  $\mu$  at a room temperature of 66° F. 4-15  $\mu$  at a room temperature of 66° F.

7.6 per cent at  $10 \mu$ .

48° C.

The diethylene glycol distearate should be heated and filtered before the mixture is made. It should be kept in a molten condition in an oven for two days to allow any free diethylene glycol which it may contain to settle down. The ester may then be decanted.

Ricinoleic diacetate is unobtainable in Great Britain and has to be made from ricinoleic alcohol (octadecanediol) and acetic anhydride. Should these materials be difficult to obtain, castor oil may be used instead.

To make up the mixture put the diacetate into a porcelain pot and add about 15 gm. of the distearate. Heat until the distearate is melted and then add the cellulose. Heat until this is dissolved and then add the rest of the distearate and the stearin. The cellulose dissolves only at a high temperature (approximately 100° C.) and solution should take place with the minimum possible of the two other solids present.

Ester wax is soluble in most alcohols, ethers, esters, ketones, hydrocarbons, chlorinated hydrocarbons and natural oils. The following have been found suitable as clearing agents as well as solvents :

> Dioxan. Ethylene glycol mono ethyl ether ('Cellosolve'). Ethylene glycol mono butyl ether. Diethylene glycol mono butyl ether. Cedarwood oil.

As with paraffin wax, a pre-embedding bath of solvent and ester wax is recommended before placing the specimen in ester wax. The time which each specimen requires is the same as when using paraffin wax.

L-pieces are recommended for block-making, and as the block is cooling with the specimen in it the hollow which is produced in the centre due to shrinkage should be filled in with drops of molten ester wax. Rapid cooling of the block by placing it in, but not submerged in, cold water, produces a slightly better block than one cooled at room temperature.

Ester wax is harder than paraffin wax, and in trimming, thinner cuts should be made to prevent chipping. A one-sided razor blade is most suitable.

Sections should be cut at a slower speed than for paraffin wax sections. If cut too slowly poor ribboning results; if cut too fast the sections will crinkle considerably. The crinkles will go when the sections are flattened, but even then the fewer the better. Experience soon indicates the correct speed.

The most important property of ester wax is that of 'ribbon staining'. Instead of flattening on water, as with paraffin wax, ester wax sections may be flattened on stain solutions which easily penetrate the wax and stain the sections. The stain is then drained off, the slide irrigated with water to remove the excess stain, and the sections may then be dried in an oven. To make this clearer the steps are as follows:

1. Flood an albumened slide with methylene blue solution; Loeffler's formula full strength or diluted up to 1 in 10,000. A weak solution is preferable because of the later removal of excess stain which dries on the slide.

2. Lay the ribbon on the stain solution and flatten in by placing the slide on a warm plate or on the surface of warm water at about  $4\bar{0}^{\circ}$  C. until the wrinkles in the wax disappear.

3. Drain away the excess staining solution. Lower the slide below the surface of distilled water in a petri dish. Gently agitate the slide until it is quite free from methylene blue, and then raise it out of the petri dish with the sections on it. Drain away most but not all of the water. Place the slide in an oven at about 40° C. until dry. It is important to dry at this temperature or slightly higher or the sections will be wrinkled. After one hour or even less, the sections will be dry enough for dissolving the wax, differentiating, and counter-staining.

The sections or ribbons may be flattened on water if preferred and treated as paraffin ribbons.

Removal of wax may be done in xylol, which will dissolve the wax without dissolving the methylene blue. It does not dissolve the ethyl cellulose very quickly, however, and a mixed solvent of xylol, ethylene glycol mono ethyl ether ('Cellosolve'), and ethyl acetate is recommended. Should the sections have been stained progressively to a point at which no further extraction of methylene blue is necessary the following mixture is suitable:

'Cellosolve'		••	••	10 per cent
Ethyl acetate	••	••	••	45 ,, ,,
Xylol	••	• •	• •	45 ,, ,,

The wax will be removed in about five minutes and the sections may then be transferred to pure xylol, and mounted with balsam in the usual way.

Generally sections are overstained and differentiated in a mixture similar to the above but with a greater proportion of the stain solvent—'Cellosolve'. In some cases pure 'Cellosolve' alone may be needed to remove the methylene blue from the tissues. Extraction is stopped by placing the slide in the 10 per cent 'Cellosolve' mixture.

Sections can be counter-stained in erythrosin or eosin dissolved to saturation in the following :

'Cellosolve	·	••	••		20 c.c.
Ethyl ace	tate	••		••	40 ,,
Xylol	••	••	••	••	40 ,,

This process takes place simultaneously with differentiation of methylene blue or other stain and with the removal of wax. Sections may first of all have part of the wax removed in the 10 per cent 'Cellosolve' mixture, or in pure 'Cellosolve' if heavily overstained with methylene blue, and then may be transferred to the counter-stain. In this the tissue will continue to lose a trace of methylene blue and any remaining wax will be dissolved. When staining is satisfactory the slide is placed in a lower 'Cellosolve' mixture (10 per cent or less). This will prevent any further extraction of methylene blue and will also slightly intensify the erythrosin staining. Finally the slide is transferred to pure xylol, and mounted in balsam.

Clean differentiation of both stains and perfect control throughout all operations are the principal advantages of the method. Other stains may be employed, but methylene blue and erythrosin have, up to the present, been the most satisfactory.

To prevent the methylene blue fading the sections should be mounted in 'Sira' (Stafford Allen & Co., London) and not baked. Such preparations will last for years.

The general outline of the method has been given above. In practice the following solutions are found to cover any combination of wax removal, differentiation of the first stain and application of the counter-stain which may be required :

July	28,	1945	Vol.	156
------	-----	------	------	-----

		1	2	3	4	5	6	7	8
'Cellosolve'	••	-	-	5	10	20	40	80	100
E. acetate	••	-	-	47	45	40	30	10	-
Xylol	••	100	100	48	45	40	30	10	-
No 5 sh	hlm	howa	onwthroain	+0	an transition				

No. 5 should have erythrosin to saturation.

The following reagents are not usually supplied by the general chemical supply houses, but may be obtained from the sources given : diethylene glycol distearate (Messrs. A. Boake Roberts, "Ellerslie", Buckhurst Hill, Essex); ricinoleic alcohol (octadecanediol) (Imperial Chemical Industries, Stocktonon-Tees); ethyl cellulose, low viscosity (Messrs. J. M. Steel, Kern House, Kingsway, London).

## THE BRITISH COUNCIL

THE last annual report of the British Council, which covered the year ending March 31, 1944 (see Nature, 155, 58; 1945), well indicated the importance of the work of the Council, not only in the war effort but also for the establishment of cultural relations in times of peace. The Council's work in making British contributions to science better known abroad and promoting contacts between British men of science and those of other countries, particularly since the establishment of its Science Department four years ago, has become so important that the tenth anniversary of the inauguration of the British Council in July 1935 should not be passed unmarked by scientific workers.

The present moment is therefore appropriate to recognize the work of its Science Committee and its Pure Science Panel, of both of which Sir Henry Dale is chairman, as well as its Panels for Medicine, Engineering and Agriculture. One of the earliest activities of the department was the publication of a four-page illustrated newsletter, Monthly Science News, in which accounts of research are presented in a form intelligible to non-scientific readers. Translated into French. Spanish, Portuguese and Arabic and reprinted in seven different countries, this has now a monthly circulation of 65,000. Compiled in collaboration with learned societies, professional bodies and the scientific and technical Press, Science Comment, a monthly compilation of abstracts and reviews started in 1943, is circulated to universities, libraries, etc., and scientific workers overseas to keep them informed of important publications in the scientific and technical field. A section has recently been added dealing with scientific The publication is used by booksellers as a films. valuable indication of British scientific publications likely to be in demand and in introducing technical and scientific periodicals to a wider overseas public.

Much has been done by the Council to enable British men of science to keep in touch with fellowworkers in other countries in the same field; the exchange of papers and specimens has been maintained and an ever-increasing number of requests for scientific information reaches the Council. Much material for Nature has been transmitted by the British Council. To facilitate the work in France, a scientific adviser has been appointed to the recently opened office in Paris. Scholarships are awarded by the Council to enable promising students to visit the United Kingdom and learn of British methods; since 1939 more than six hundred students have been brought from the Dominions, Colonies and many other countries.

During the War, some three hundred short-leave courses have been arranged at universities, technical