

Androstene 3 β -17 α -diol. The animals which received 1 mgm. of androstene 3 β -17 α -diol once daily for one week showed vital staining appearances similar to those of the controls. In those which received 1 mgm. daily for three weeks there was marked reduction in the number of vitally stained macrophages and also in the intensity of the vital staining in the spleen, liver and lymph nodes.

The above results indicate that in the male guinea pig testosterone propionate depresses the phagocytic activity of the reticulo-endothelial system during the first two weeks of treatment; but if the injections are continued the system gradually recovers during the third and fourth weeks. The effect of testosterone is, therefore, similar to that of cortisone. Androstene 3 β -17 α -diol is also shown to have a depressant action on the activity of the reticulo-endothelial system.

For this research we gratefully acknowledge the gift of androstene 3 β -17 α -diol from Dr. Tindall, of Organon Laboratories, Ltd.

R. S. SNELL
T. NICOL

Department of Anatomy,
King's College,
London, W.C.2.
Oct. 4.

¹ Nicol, T., Helmy, I. D., and Abou-Zikry, A., *Brit. J. Surg.*, **40**, 166 (1952).

² Nicol, T., and Snell, R. S., *Nature*, **174**, 554 (1954); **177**, 430 (1956).

³ Nicol, T., and Snell, R. S., *Nature*, **175**, 995 (1955).

Agar Clarification

THE clarification of agar has always presented technical difficulties. The problem has become even more acute with the application of agar gels as a supporting medium for the interaction of antigen-antibody systems. A clear, transparent gel is essential for reading and photographing the lines, often fine and faint, formed by the antigen-antibody precipitates. That the classical microbiological methods of clarification, for example, filtration through sand and paper pulp, egg albumin coagulation, are insufficient is obvious from the elaborate, laborious methods devised in attempts to produce clear agar^{1,2}.

A very simple procedure for satisfactory agar clarification is here described. It is an adaptation of a step used by Viswanatha and Liener in the purification of a proteinase³.

1 per cent w/v New Zealand agar is suspended in distilled water and dissolved by heating in a boiling water-bath. The solution is twice filtered rapidly through well-rinsed glass wool in a Buchner funnel to remove grossly insoluble particles. At this stage in our laboratory we add 1 per cent w/v sodium azide as a preservative, but this step is optional.

To the glass wool-filtered agar is added 1 or 2 per cent v/v of a mixture of equal parts of powdered bentonite and 'Hyflo Super-Cel' (Johns-Manville Co., Ltd.) and the whole shaken vigorously by hand to disperse the clarifying agents. The suspension is stored at 56° C. for several days, the clarifying agents being resuspended daily by gentle inversion of the bottles. When the cloudy flocculum is completely carried down from the supernatant by the bentonite-'Super-Cel' mixture the clarified agar is carefully decanted. Clarification is more quickly accomplished

with 2 per cent of the mixture, but the recovery of agar is smaller.

The clarified agar is filtered through a fluted Whatman No. 5 paper in a heated funnel into a bottle standing in hot water. The first 25 ml. or so are returned to the funnel for re-filtering. The filtered agar in the molten state has the colourless clarity of water, and only a faint, clean, unclouded opalescence when set in plates.

JOSEPH G. FEINBERG

C. L. Bencard Allergy Research Unit,
Beecham Research Laboratories, Ltd.,
Betchworth, Surrey.
Sept. 28.

¹ Ouchterlony, Ö., *Ark. Kemi, Min., Geol.*, **26**, B, 1 (1949).

² Bussard, A., and Perrin, D., *J. Lab. Clin. Med.*, **46**, 689 (1955).

³ Viswanatha, T., and Liener, E. E., *Arch. Biochem. and Biophys.*, **61**, 410 (1956).

Validity of Miley's Electrometric Method for the Qualitative Analysis of Oxidation Layers formed on Copper

IN a preliminary paper, published in 1951, L. de Brouckère, F. Bouillon and Y. Bouillon-Nyssen¹ showed that films formed on copper by anodic oxidation in molar solutions of sodium hydroxide contained, among other things, cupric hydroxide. This compound could be reduced electrochemically in 0.2 M solutions of ammonium chloride. The reduction potential was different from those of cuprous and cupric oxides, so that Miley's electrochemical method could, in principle, be applied to the qualitative analysis of mixed films. The various end-points were quite easy to localize.

The quantitative character of the electrochemical reduction of copper compounds in ammonium chloride solutions has been questioned by Campbell and Thomas², who suggested replacing it by potassium chloride, in which the copper oxides are completely insoluble. However, in this medium the potential-time curves are somewhat smoothed out, so that it is rather difficult to separate the various reactions occurring at the electrodes.

Ammonium chloride thus appeared to be the best electrolyte to use for qualitative analysis, potassium chloride being better suited for quantitative work.

Unfortunately, it has now been found that cupric hydroxide cannot be reduced in potassium chloride solutions, hydrogen being evolved from the very beginning of the electrolysis. A systematic study of these phenomena, which will be published shortly in the *Bulletin des Sociétés chimiques de Belgique*, has shown that copper hydroxide can only be reduced in such media as are able to dissolve it. Quantitative results can obviously only be obtained if the speed of reduction of the dissolved ions is greater than, or eventually equal to, the speed of dissolution.

We have been particularly interested in the reduction of copper compounds in solutions of sodium hydroxide as these have recently been used by several authors for analysing the films formed on copper by anodic oxidation³. Potential-time curves published in the literature generally have more than one horizontal part, and this has been interpreted as proving the complex nature of the films, which are supposed to consist of a mixture of cuprous and cupric oxides with some cupric hydroxide. We believe that this interpretation is not completely justified, especially