humidified air atmosphere continuously supplied with a flow of CO_2 sufficient to keep the *p*H at 7·4 in the culture medium.

After varying periods of cultivation, pieces were removed from the culture and they were examined histochemically, using the chromaffin reaction or formaldehyde vapour-induced fluorescence for the demonstration of catecholamines[5], or by electron microscopy after fixation in Palade's solution and embedding in 'Epon'. Pieces of fresh adrenal glands were similarly treated and served as controls.

The intensity of the chromaffin reaction weakened in the medullary cells during the first 1–3 days of culture. Thereafter the reaction intensity was again similar in the surviving cells and in fresh controls. After 2–3 weeks' culture, the chromaffin reaction of the medullary cells of new-born rats was sometimes even more intense than normal. Formaldehyde-induced fluorescence (Fig. 1) also indicated at least normal catecholamine content in pieces cultured for up to 60 days. Although many cells thus clearly retained their ability to store and possibly to synthetize catecholamines, other cells obviously died during cultivation, especially in cultures of pieces taken from adult animals; pieces from new-born rats survived better.

The ultrastructure of the cultured cells (Fig. 2) corresponded to that of the controls. Droplets containing catecholamine, and surrounded by a membrane, were of typical structure, as were other cytoplasmic organelles, such as mitochondria.

Cultures of this kind may facilitate the investigation of, for example, direct effects of drugs on adrenal medullary cells.

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HISTOCHEMISTRY

Staining of Bacteriophage Nucleic Acids with Acridine Orange

The use of the fluorescent stain acridine orange for the quick identification of viral nucleic acids has been described by Mayor and Hill[1]. In this valuable technique colour differences permit differentiation between double-stranded deoxyribonucleic acid (DNA), on one hand (fluoresces green), and ribonucleic acid (RNA) and single-stranded DNA on the other (fluoresces red). The latter nucleic acids can be separated by their respective sensitivities to RNase and DNase. While the procedure of Mayor and Hill is simple compared with the techniques of biochemical analysis, it involves numerous treatments and the use of fluorescence microscopy. Furthermore, acridine orange staining is particularly sensitive to *p*H levels, and, because of this, some difficulty may be experienced in obtaining the correct colours. In addition, the procedure does not differentiate between single-stranded DNA and RNA, using the stain alone. This communication describes some improvements and modifications with these points in mind. Direct viewing under ultra-violet light is substituted for the fluorescence microscope, the pre-staining treatment is simplified, and possible unreliability due to *p*H sensitivity is eliminated.

The method is as follows. The reagents required are Carnoy's fixative, 0·1 M citric acid, 0·15 M Na_2HPO_4, 1 per cent acridine orange in water and buffered saline (Na_2HPO_4, 1·27 g/l.; KH_2PO_4, 0·41 g/l.; NaCl, 7·36 g/l.; *p*H 7·2).

Droplets of a virus suspension (10^{10}–10^{12} particles/ml. in buffered saline) are dried down on to microscope slides. The slides are transferred to Carnoy's fixative for 5 min, then rinsed in absolute alcohol and dried in a stream of warm air. The material is stained with acridine orange for 5 min in the following modified McIlvaine's buffer: 6 ml. 0·1 M citric acid; 4 ml. 0·15 M Na_2HPO_4; 0·1 ml. 1 per cent acridine orange; *p*H 3·8. The slides are rinsed in 6 ml. 0·1 M citric acid + 4 ml. 0·15 M Na_2HPO_4, and transferred to 0·15 M Na_2HPO_4 for 15 min. Excess liquid is shaken off and the slides are examined under an ultra-violet lamp (wave-length 2570 Å), the colour of the smears being noted. If the smears are red, the slides are placed in 0·1 M citric acid and examined in ultra-violet light at intervals of 1, 2 and 3 min, the colour changes being noted.

The results obtained with various bacteriophages, etc., are summarized in Table 1.

Table 1

Specimen	Nucleic acid	Na_2HPO_4	Citric acid
Phage *T*4	2-stranded DNA	Yellow-green	—
Phage *Ēl*[2]	Unknown	Yellow-green	—
Mouse liver DNA	2-stranded DNA	Yellow-green	—
Mouse liver DNA (denatured)	1-stranded DNA	Flame-red	Stays red but fades noticeably
Phage φR^3	1-stranded DNA	Flame-red	Goes pale green then fades
Phage *ZJ*/2[4]	1-stranded DNA	Flame-red	Stays red but fades noticeably
Phage α12*	Unknown	Flame-red	Fades completely
Phage *ZIK*/1[4]	RNA	Flame-red	Stays red, does not fade
Phage *ZJ*/1[4]	RNA	Flame-red	Stays red, does not fade

*A filamentous form similar to *ZJ*/2.

It can be seen that viruses with double-stranded DNA are quickly distinguished from others by their colour after treatment with phosphate. The green is bright and quite unmistakable, though there is sometimes a trace of red in the centre of the smear if the phage concentration is high. If green is obtained there is obviously no need for treatment with citric acid. In fact, this causes the colour to change to red and fade. Both single-stranded DNA and RNA phages produce flame-red colour after phosphate treatment. The colour is almost crimson and there is no trace of green. The citric acid treatment helps to distinguish these two types of nucleic acid. The criterion is that RNA phages do not fade over the 3-min period, whereas the single-stranded DNA phages fade noticeably and may be accompanied by a change to green. While this test is to a certain extent subjective, it can be used to confirm other indications as to the type of nucleic acid. For example, a phage which is subject to plaque inhibition by RNase[4], the red fluorescence of which does not fade in citric acid, undoubtedly contains RNA. If additional confirmation is required, however, Carnoy-fixed slides may be treated with RNase or DNase[1]. Subsequent staining will produce no fluorescence with the appropriate nucleic acid.

The procedure must be strictly followed for satisfactory results and virus preparations should be free from contaminating nucleic acids.

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