

The following experiment was carried out in an attempt to differentiate between five different strains of fowl-plague virus using one of the sulphonphthalein dyes, namely, phenol red. The virus strains used were:

Fowl-plague, original strain	$PFC_2P_{11}C_{88}$
Fowl-plague, attenuated strain,	$PFC_2P_{11}C_{88}P_{88}C_1$
Fowl-plague, reactivated strain,	$PFC_2P_{11}C_{88}P_{88}C_{15}$
Fowl-plague, mice strain,	$PFC_2P_{11}C_{88}M_{88}C_1$
Fowl-plague, tyroid strain,	$T_{111}$

The experiment was carried out in the following steps: (1) A 1:1,000 solution of the dye in 0.01 N sodium hydroxide was prepared. (2) 3 ml. of 10<sup>-2</sup> solution of the virus in phenol red was inoculated into the allantoic cavity of three eleven-day old eggs; three chick embryos inoculated with phenol red alone served as controls. (3) The inoculated embryos were incubated at 37° C. for 48 hr. and the allantoic fluid of each egg was aspirated separately and tested for freedom from bacterial contamination. (4) The colour intensity of the sterile chorio-allantoic fluid was determined, arbitrary units being given to designate this intensity; ++++ indicated the original deep red colour of phenol red, while 0 represented complete discharge of the red colour. All degrees of intergradation which existed between these two extremes were given respective arbitrary units between these two.

The results obtained from the above experiment are presented in Table 1.

Table 1. EFFECT OF FIVE DIFFERENT STRAINS OF FOWL-PLAGUE VIRUS ON PHENOL RED IN CHICK EMBRYOS

Virus strain	Colour intensity of chorio-allantoic fluid (arbitrary units)
Phenol red (control)	++++
Fowl-plague, original, $PFC_2P_{11}C_{88}$	++++
Fowl-plague, attenuated, $PFC_2P_{11}C_{88}P_{88}C_1$	+++
Fowl-plague, reactivated, $PFC_2P_{11}C_{88}P_{88}C_{15}$	++
Fowl-plague, mice, $PFC_2P_{11}C_{88}M_{88}C_1$	++±
Fowl-plague, tyroid, $T_{111}$	±

The results in Table 1 show that the five strains of fowl-plague virus used in this experiment possess varied powers of discharging the colour of phenol red in allantoic fluids inoculated with each of them separately. Thus the reactivated strain induced a notable decrease in the colour intensity of the allantoic fluid as compared to the controls. The change in intensity was much less when the dye was inoculated together with the attenuated strain, whereas the original strain of fowl-plague virus failed to induce any change in the colour intensity. The change in colour by the mice strain was of intermediate nature between that induced by the attenuated and reactivated strains, while the tyroid strain induced almost complete discharge of the colour of the infected allantoic fluid.

Determination of the pH-value of the infected chorio-allantoic fluids showed that the decrease in the colour intensity was accompanied by a similar decrease in the pH value. Thus, the chorio-allantoic fluid inoculated with the original strain which showed a deep red coloration very similar to that of the control gave a pH-value of 8.0, while that infected with the tyroid strain had a pH value of 6.3, which would explain the complete discharge of colour of phenol red by this virus. This result is, however, contrary to the findings of Kunst (*loc. cit.*), who claimed that there was no change in the pH of the allantoic fluid during the development of swine influenza and human influenza A viruses, though they induced different changes in the colour intensities of the allantoic fluid inoculated with a mixture of each of them and a series of sulphonphthalein dyes.

It is suggested that this method, if worked out thoroughly, might serve as an easy means of separating closely related virus strains.

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<sup>1</sup> Kunst, H., *Nature*, **167**, 368 (1951).

### A Selective Method for the Staining of Negri Bodies in Histological Brain Sections

SEVERAL techniques have been devised for the demonstration of Negri bodies in histological preparations; but many of them, in my experience, proved to be sometimes capricious and not always reliable. Hitherto, in this laboratory routine examinations for rabies are carried out by staining sections of the hippocampus with a slightly modified Heidenhain's iron hæmatoxylin. This technique was found to be extremely reliable, and Negri bodies appear as blue-black well-defined structures, often containing black granules and vacuoles. However, as the whole background is greyish-black and red blood corpuscles, glial cells and leucocytes stain black also, some confusion may occasionally be encountered while differentiating Negri bodies. In view of this drawback, another method has been evolved, which gives very selective, durable and constant staining of Negri bodies.

The method described below is based upon picro-Mallory modifications of Lendrum and McFarlane<sup>1</sup> and McFarlane<sup>2</sup>. The picro-Mallory techniques were originally devised for the staining of connective tissue; however, advantage has been taken of the principles of the method in order to demonstrate Negri bodies.

The method is as follows. Stain paraffin sections in Ehrlich's hæmatoxylin (5 min.). Blue in tap water for 2 min. Orange G  $\frac{1}{2}$  per cent in saturated aqueous solution of picric acid (1 min.). Wash in tap water until only erythrocytes remain stained yellow. Rinse in distilled water and stain for 10 min. in the following solution: acid fuchsin (0.5 gm.), phosphotungstic acid (0.5 gm.), 1 per cent acetic acid (100 ml.). Rinse in distilled water and differentiate for 5 min. in the following solution: phosphotungstic acid (2 gm.), phosphomolybdic acid (2 gm.), saturated aqueous picric acid (70 ml.), absolute alcohol (30 ml.). Rinse in distilled water and then in 1 per cent acetic acid. Stain for 15 min. in 1 per cent anilin blue in 2 per cent aqueous acetic acid. Rinse in 1 per cent acetic acid, dehydrate, clear and mount.

Negri bodies are stained purplish-red with blue granulations, cytoplasm of neurones bluish, nucleoli dark purple and erythrocytes yellow.

I wish to thank the Director of Veterinary Services, Nyasaland, for permission to publish this communication.

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July 30.

<sup>1</sup> Lendrum, A. C., and McFarlane, D., *J. Path. Bact.*, **50**, 381 (1940).

<sup>2</sup> McFarlane, D., *Stain Tech.*, **19**, 29 (1944).