Separation within the Homologous Series of *n*-Alkyl Sulphates, *n*-Alkyl Pyridium and *n*-Alkyl Trimethyl Ammonium Halides by Paper Chromatography

Methods have been described for the identification of synthetic detergents in admixture with three or four dyestuffs utilizing an ascending chromatogram[1,2]; but these procedures do not allow the separation of mixtures of detergents, neither do they permit the separation of members of a homologous series.

Work carried out in these laboratories has clearly shown that it is possible to effect a complete separation of the normal saturated alkyl sulphates when the adjacent members differ in chain length by two carbon atoms. Mixtures of *n*-octyl, *n*-decyl and *n*-dodecyl sulphates have been separated by ascending paper chromatography using 15 per cent aqueous ethyl alcohol, while 40 per cent aqueous ethyl alcohol has been found effective in separating mixtures of *n*-tetradecyl, *n*-hexadecyl and *n*-octadecyl sulphates. The chromatograms were run at 30° C. using Whatman No. 1 paper.

The position of the spots of the anionic material can be shown by spraying with an aqueous solution of pinacryptol yellow after drying in a current of warm air. When the sprayed chromatogram is examined under ultra-violet light the spots of anionic material fluoresce a bright orange on a pale yellow background. The minimum amount of material capable of detection by this means is of the order of one microgram. Preliminary tests have indicated that this method of separation is applicable to other classes of sulphated and sulphonated detergents.

A parallel investigation of cationic materials has indicated that paper chromatography is capable of effecting the separation of the homologous series of *n*-alkyl pyridinium halides and also the *n*-alkyl trimethyl ammonium halides. The ascending chromatogram of these materials is carried out at 30° C. using 30 per cent aqueous ethyl alcohol containing 5 per cent of concentrated hydrochloric acid. The position of the spots of cationic material is shown by spraying the air-dried chromatogram with an aqueous solution of rhodamine *BS* containing a commercially available optical bleach. The sprayed chromatogram is then exposed to ammonia vapour to neutralize any mineral acid present and examined under the ultra-violet lamp. The spots of the cationic material are seen to fluoresce a bright vermilion on a white background. The method is sensitive to approximately one microgram of cationic material.

It will be appreciated that these methods allow the differentiation of other classes of organic compounds which by means of chemical reaction may be converted to surface-active compounds of the above-mentioned types.

Further investigations on the qualitative and quantitative aspects of the paper chromatography of other classes of anionic and cationic compounds continue, and the results will be published elsewhere at a later date.

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[1] Kopaczewski, W., *Chimie and Industrie*, **67**, 761 (1952).
[2] Blandin, J., and Desalme, R., *Bull. Mens. ITERG.*, **8**, 69 (1954).

Reproduction of Mumps Virus in the Chorio-allantoic Membrane

Little is known about the reproduction cycle of mumps virus. This seems strange, as some of the techniques available for influenza virus should be applicable to this virus, which is in many ways similar in its reactions. The method introduced by Hoyle[1,2] in his study on the multiplication of influenza virus in egg membranes, however, did not yield clear-cut results with mumps virus. The main difficulties were the slow adsorption of the virus to the reproductive cells, the relatively high proportion of free (vital) virus particles not taking part in the reproduction process during the experiments and the relatively low reproduction velocity. This last factor made it necessary to expand the experiments to more than thirty hours, and consequently the experiments had to be divided into many parts. The comparability of the infection-, hæmagglutination- and other titres is impeded by this division of the experiment.

In five series of experiments eggs were inoculated with 0·1 ml. of a dilution of freshly harvested allantoic fluid of the mumps strain 'Enders'. To measure the infection titre of the inoculum a series of tenfold dilutions was made and three eggs were injected with 0·1 ml. of each dilution. Every half-hour after the infection the allantoic fluids of four eggs were tested for hæmagglutination power, and the chorio-allantoic membranes were harvested. These membranes were ground with quartz sand and brought into suspension with saline (containing penicillin and streptomycin). A series of tenfold dilutions was made and with each dilution three eggs were inoculated. With the same material the amount of complement-fixing antigen was measured. As the whole series of experiments could not be carried out at the same time the series was cut into parts of four hours each. With each part a reference test was carried out half an hour after the inoculation of the eggs.

The following results were obtained (Fig. 1).

Complement-fixing antigen could not be detected before 18½ hr. from inoculation.

Hæmagglutinin did not appear before 19½ hr. from the beginning of the experiment.

Infective material remains detectable throughout the experiment. The infection titre decreases from 2½ hr. after inoculation until it reaches a minimum after 5 hr., to remain low until 14½ hr. after injection. From then onward infection titres increase very slowly. No secondary phase of decrease of infection titres can be detected within 30 hr. The absence of complement-fixing antigen between 21½ hr. and 28 hr. after the beginning of the experiment may be due to technical error. Hæmagglutinin titres show a steady increase not followed by a secondary decrease.

In consecutive experiments the above-mentioned results could not always be repeated. In the case when the initial infection dosage was low no hæmagglutinin or complement-fixing antigen could be detected. In the case when the initial infection dosage was high (10^5 or more), no decrease in infection titre was detectable and the gain in infectivity-rate was relatively low.

In order to get more clear-cut results, we used another experimental arrangement. Chorio-allantoic membranes of nine days old chick embryos were cut into a great number of pieces each of about 0·01 sq. in. After washing in saline, the pieces were put into a Petri dish containing a mumps virus suspension in saline (infection titre 10^{-3}–10^{-4}). After twenty