PDD_{50} doses of interferon were added to the cultures which received a multiplicity of vaccinia virus of about 2 PFU/cell and the per cent inhibition of virus yield plotted, a parallel curve was obtained (Fig. 1). Thus, the dose-response curve relating virus yield also seemed to be sigmoidal. For each dose of interferon employed, the per cent inhibition of virus yield was less than the corresponding inhibition of plaque formation.

Discussion. Gifford, Toy, and Lindenmann (2) have suggested that the total plaque area is a measure of the total amount of virus and found a relationship between total plaque area and interferon concentration. The log total plaque area decreased linearly with increasing log interferon concentration. The inhibition of total plaque area was a more sensitive measure of interferon than the depression of plaque count. However, the present study indicates that virus yield is less sensitive than plaque numbers to inhibition by interferon. This might be related to the findings of Ho(3) that viral inhibition caused by interferon could be overcome to a certain extent by increasing multiplicities of infection. In the studies reported here, approximately 2 PFU/cell were employed. However, Lockart (personal communication) does not find a difference in viral yield when different multiplicities of Western equine encephalomyelitis (WEE) virus are employed. These differences may be due to differences inherent in the virus-host cell system. For example, interferon decreases virus yield and increases the latent period in vaccinia and poliovirus-infected chick cells(2,3). In WEE infection, only virus yield seems to be affected(8). Moreover, Lockart, Sreevalsan and Horne(7) found the effect of interferon on virus yield to be more sensitive than its effect on plaque inhibition. These differences serve to emphasize how units of interferon activity may vary according to the assay procedure.

A recognition of the sigmoidal nature of the dose-response curves is important because it indicates that complete inhibition of virus synthesis is difficult to achieve since 100% inhibition is approached asymptotically.

Summary. The effects of interferon on vaccinia virus yield and on plaque formation were compared in chick embryo tissue cultures and the latter procedure was found to be more sensitive. The upper portion of the dose-response curve relating inhibition of virus yield to interferon dose seemed to be sigmoidal.

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Immunoelectrophoretic Analysis of Serum Proteins in Agar and in Agarose. (30520)

ESTHER M. LEISE AND CARL G. EVANS (Introduced by J. L. Fahey)
Immunochemistry Section, Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md.

Brishammar(1) using agarose prepared by Hjerten(2) presented one comparison of the immunoelectrophoretic behavior of enolase in this medium and in agar. It was stated that the lines of precipitation in agarose were clearer, with no halo effects and no false lines of precipitation. Sullmann(3), on the other hand, prepared agarose by the method of Hjerten, and compared the reactions of undiluted and diluted human serum with rabbit antisera to β_{2M}-cryoglobulin and to gamma globulin. He stated that weak precipitin lines appeared much clearer in agarose and electroendosmosis was somewhat diminished but that lines obtained with normal serum and polyvalent anti-human sera were about the same on both supporting media.

These observations led us to study the immunoelectrophoretic behavior of serum protein antigens in agarose. We were particularly interested in the possible advantages of agarose because of difficulties encountered in the interpretation of immunoelectrophoretic analysis (I.E.A.) in agar of weakly precipitating antigen-antibody reactions. In addition to the advantages reported by these workers, we have found that in I.E.A. carried out in agarose, instead of agar, there is little or no electroendosmosis and more lines of precipitation are obtained with a given mixture of serum protein antigens. The separation of proteins is improved and the clarity of resolution of precipitin arcs, particularly in the alpha and beta globulin areas, is superior.

The experiments cited in this report demonstrate the superiority of agarose over agar for I.E.A.

Materials and methods. In all experiments the gels were dissolved in 0.05 M tris (tris(hydroxymethyl)aminomethane) buffer, pH 8.6, containing 0.1 g of merthiolate per liter of buffer. Agar (Special Nobel Agar, Difco) was used at a concentration of 1.5% (w/v). Agarose, a neutral, uncharged galactose polymer derived from agar, was obtained from 2 different sources, one (M) from Mann Research Laboratories* and the other (S) Seakem obtained from Bausch and Lomb.† The sulfate content of agarose (S) is less than 0.5%. Both preparations of agarose were used at a concentration of 0.75% (w/v). To dissolve agarose (M) it was necessary to heat the suspension for 10 minutes at about 120°C under a pressure of 15 lb per square inch. By contrast, agarose (S) was easily soluble at 100°C under atmospheric pressure. The I.E.A. patterns obtained with both agarose preparations, however, were very similar. At the concentrations used the agarose preparations were more transparent than agar. Agarose (S) was used for most of the work here. To obtain a gel firm enough to use on plates larger than microscope slides, it was necessary to increase the agarose concentration to 1%. The results obtained at this concentration appear to be comparable to those obtained at the lower concentration(4).

Electrophoresis was performed at room temperature in an LKB apparatus(5), at a constant current of 4 ma per plate (6 slides) for 60 minutes at a starting voltage of 300. Tris buffer 0.05 M, pH 8.6, was used in electrode vessels and to wet the connecting wicks. Antisera were added to the troughs immediately after electrophoresis. The plates were kept in a humid chamber for 48 hours at room temperature to permit development of precipitin lines. The slides were then washed, dried and the fixed proteins were stained with naphthol blue black.

Antisera to human serum were prepared by injecting rabbits with increasing doses (0.1-1.0 ml) of alum precipitated serum(6). The rabbits were bled one week after the 5th injection (short course) and again one week after 10 more injections (long course). The rabbit antiserum to guinea pig serum was produced after 25 injections of alum precipitated serum and was obtained from Dr. Tibor Borsos.

Results and discussion. In Fig. 1 patterns in agarose are on the left and the identical reaction in agar is on the right. The advantages in the use of agarose over agar are clearly demonstrated in reactions with short course antisera (Fig. 1, Slides A). There are more lines on agarose which are better separated and of better definition. There is an increase in the mobility of an alpha globulin component toward the anode in the agarose medium leaving clearly visible precipitin arcs near the antigen well. These precipitin reactions near the antigen well are not observed in the reaction in agar. This difference in I.E.A. patterns in agar and agarose is typical of the difference in I.E.A. patterns observed

* Mann Research Laboratories, Inc., New York.

† Bausch and Lomb, Inc., Washington, D.C.

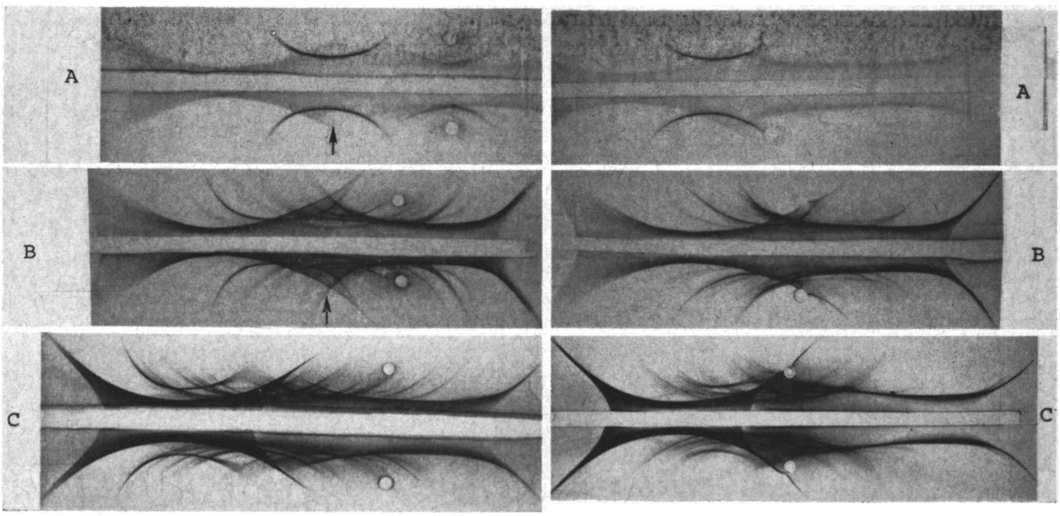

FIG. 1. Comparison of immunoelectrophoretic reactions in agar and in agarose. A. Reactions of 2 human sera with a rabbit antiserum to human serum produced after a short course of immunization (5 injections). Arrow indicates shift of arc in alpha globulin region in agarose. B. Reactions of 2 human sera with a rabbit antiserum to human serum produced after a longer course of immunization (15 injections). Arrow indicates greater clarity in beta globulin region in agarose. C. Reactions of 2 guinea pig sera with a rabbit antiserum to guinea pig serum produced after a prolonged course of immunization (25 injections).

with all human sera tested with many short course antisera.

With antisera produced after a longer course of immunization the advantages of agarose are also evident (Fig. 1, Slide B, left). There are more clearly distinguishable precipitin lines in agarose, especially in the alpha and beta globulin areas. Patterns are not only clearer because of improved electrophoretic separation of components in agarose but precipitin arcs in a given area are more clearly separated.

Another example of the difference in clarity of lines and dispersion of components on the two media, even with a hyperimmune polyvalent antiserum, is represented in Fig. 1, Slides C. This is in contrast to the findings of Sullmann who did not observe any differences with polyvalent antisera. However, he did state that his agarose preparation "probably contained some acid polysaccharide." This may have been sufficient to alter its value as a superior supporting medium.

Comparisons were made of the I.E.A. reactions in agar and agarose of plasma with antisera to specific plasma components. Some antisera were obtained from a commercial source‡ and some antisera to the immunoglobulins IgG, IgA and IgM were obtained from Dr. William Terry.

With prealbumin antiserum, where the concentration of this component was low, the precipitin arc in agar was hardly visible, whereas in each case these weak reactions were clearly visible in agarose. Wider arcs of increased density and clarity were always obtained in agarose with reactions between plasma and antisera to fibrinogen.

With antisera to the immunoglobulins IgG and IgM the reactions in agarose had greater clarity. Although reactions in both media appeared similar, these components did not move as far toward the cathode in agarose as they did in agar. With 2 antisera to IgA, this immunoglobulin appears as a double arc in agarose extending from the point of origin and directed toward the anode. In agar this reaction appears as a single heavy line extending from the antigen well toward the cathode. With one serum from a patient with IgA myeloma protein the precipitin arcs in both media appear to be directed toward the

‡ Lloyd Brothers, Inc., Cincinnati, Ohio.

anode, but in agarose this component moved a much greater distance toward the anode in the same period of time.

Summary. Several antigen-antibody precipitin systems were compared by immunoelectrophoretic analysis using agar and agarose as the supporting medium. Electroendosmosis was reduced in agarose, mobility of components was more rapid, lateral separation of alpha and beta globulin components was increased and better definition of individual precipitin arcs in this medium was observed. Some precipitin reactions which failed to develop in agar gave clear reactions in agarose. The reverse was never observed. There were some individual antigen-antibody reactions in which there appeared to be little change in either the shape or position of the precipitin arcs on either medium, but increased clarity was always observed on the agarose medium. The advantages of agarose for I.E.A. when increased sensitivity is required, as in quantitating antigens or in testing for homogeneity of purified proteins, should be considered.

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Influence of Some Nonionic Surfactants on Pancreatic Lipase Activity. (30521)

E. P. JOROLAN AND B. W. JANICKI
Basic Microbiology Research Laboratory, Veterans Administration Hospital, Washington, D.C.

Earlier investigations have demonstrated that Triton WR-1339, a nonionic surfactant, induced hyperlipemia when injected into laboratory animals(1-5). The elevation of blood lipids is believed to result from inhibition of the host's lipid catabolic processes(2-3). It has been reported that plasma lipoproteins of Triton-treated rabbits(6) and guinea pigs (7) are resistant to the action of lipoprotein lipase. Furthermore, normal guinea pig plasma lipoproteins which are incubated with Triton are also resistant to the enzyme(7). Similar treatment of a coconut oil emulsion with Triton shows that the clearing action of pancreatic lipase is impaired(8). These results suggest that the detergent might alter the original substrate molecule so that an entirely different substrate is formed that would be resistant to the action of a lipolytic enzyme. The present study was conducted to evaluate this possibility and to gain similar information concerning two other chemically related nonionic surfactants, Macrocyclon and HOC-60.

Materials and methods. A 50% coconut oil emulsion, (Ediol, Schenlabs Pharmaceuticals, Inc., New York), was used as the substrate in all experiments; the stock solution was diluted to 1% with 0.15 M saline-phosphate buffer (pH 7.4) to prepare daily working solutions. Commercial pancreatic lipase (General Biochemicals, Chagrin Falls, Ohio), prepared as a 20 mg/ml solution in saline-phosphate buffer, was used as the enzyme. Three nonionic surfactants (polyoxyethylene ethers of octyl phenol) were used. Triton WR-1339 was obtained from Winthrop Laboratories, Rensselaer, N. Y.; Macrocyclon and HOC-60 were obtained from Dr. R. J. W. Rees (National Institute for Medical Research, Mill Hill, London). In the report describing the preparation of the latter two surfactants(9), Macrocyclon was listed as HOC-20 (GN/57) and HOC-60 was coded as GN/102. Work-