Gelatin for Immunodiffusion of Basic Protein.* (32555)

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Gelatin was one of the first media used for immunodiffusion(1), but today it is very seldom employed. Such of its advantages as minimizing denaturation of labile antigens and providing water-clear gels at refrigerator temperatures usually are outweighed by its disadvantages of gel softness and the inconvenience of staining precipitin bands which appear within it. Hence, agar and its derivative, agarose, are much the more common gelling agents. But sometimes neither of these can be used; then gelatin should be remembered for its potential utility. Here we report an example of this which seems especially important because it involves as antigen a basic protein, a class of antigens of which there are many, but of which almost none have been analyzed by immunodiffusion; and because it shows how use of agar alone can lead to misinformation.

Materials and methods. Our antigen, methylated human serum albumin (MeHSA), was prepared from crystallized Pentex human serum albumin (HSA) by a slight modification of the method described by Mandell and Hershey(2). Antisera to MeHSA and to HSA were obtained from mice immunized with 2 subcutaneous injections of 0.25 mg quantities of one or the other protein given a week apart in 0.1 ml volumes of water-in-oil emulsion(3). Bleedings were made several weeks after immunization: precipitins to HSA appeared within 3 to 4 weeks and to the MeHSA within 5 to 8 weeks. Gelling agents included Difco Bacto agar, SeaKem agarose, agarose donated by Hyland Laboratories, and Difco Bacto gelatin. Both types of albumin were used as 1% solutions in distilled water for electrophoresis and at 0.05% for immunodiffusion, and both types of test were set up on microscope slides. During immunodiffusion tests, which require several hours of standing, these slides were humidified by enclosure within Petri dishes, each containing a disk of wet filter paper(1). All operations except those of preparing slides, adding reactants, and observing results were executed at 4°C.

Experiments and results. Preliminary double diffusion tests using 1% agar and agarose in pH 7.4 physiologic phosphate buffer indicated that antiserum to HSA and hyperimmune antiserum to MeHSA could precipitate HSA, though the reaction was diffuse and weak for the latter antiserum; but neither antiserum appeared to react with MeHSA. To determine whether this apparent lack of reactivity might be due to nondiffusibility of MeHSA through such gels, we tested both types of albumin for electrophoretic migration through 1% agarose at pH values of 8.6, 7.0, 6.0, 5.5, and 5.0 in buffers made with barbiturate, phosphate, citrate, or acetate at ionic strengths of between 0.01 and 0.05 M. We used weak solutions of methylene blue and of thiazine red, made up in whatever buffer was being employed, as visible indicators of cathodic and anodic migration, respectively, electrophoresis being conducted at 75 volts and usually for about 1 hour until the thiazine red had migrated from its origin near the cathodic end of the slide 5 cm toward the anode(1).

Regardless of experimental conditions, our results were uniform in two respects: HSA migrated toward the anode and MeHSA toward the cathode; HSA remained in solution and migrated as a circular spot, whereas MeHSA formed a trail of precipitate as it moved from its origin. But when we electrophoresed the two albumins in 2% gelatin made with pH 5.5, 0.01 M citrate buffer, neither precipitated. These differences in electrophoresis between agarose and gelatin are illustrated in Fig. 1. They indicated that the apparent lack of reactivity between antisera and MeHSA could have been due to nondiffusibility of this antigen, and that if gelatin were substituted for agar such a problem might be avoided

This possibility was investigated using

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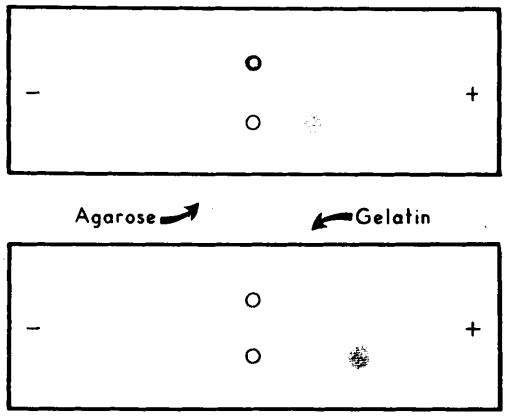

FIG. 1. Comparative electrophoretic migrations of HSA (upper walls) and MeHSA (lower walls) in pH 5.5, 0.01 M citrate buffer solutions of 1% agarose and 2% gelatin. Electrophoresis performed for 1 hr at 75 volts.

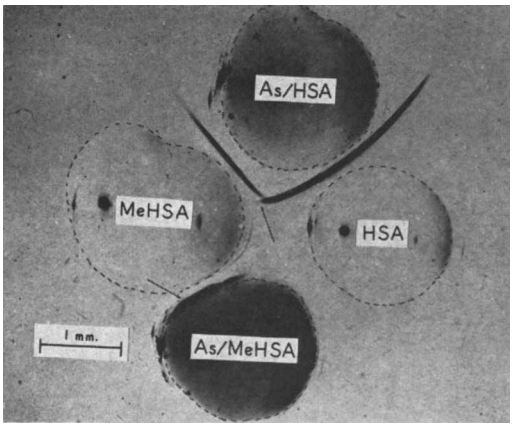

FIG. 2. Microdouble-diffusion test in 2% gelatin of native and methylated human serum albumin (HS A and MeHSA, respectively) against corresponding antisera. Upper arrow points to reaction spur, lower arrow to precipitin band formed by MeHSA reacting with its antiserum. The antiserum to MeHSA used in this test did not contain precipitins to native HSA.

double-diffusion tests set up as follows. Clean microscope slides were flooded with 3.5 ml volumes of warm gelatin solution (pH 6.5, 0.1 M sodium phosphate-buffered) of which 2 ml immediately were withdrawn so as to leave only a thin layer on each slide constituted by the remaining 1.5 ml. The slides were placed in humidified Petri dishes and allowed to stand at 4°C for 20 to 30 minutes for gelling. Then, droplets of reactants were placed in patterns of 4 as indicated by Fig. 2, two patterns on each slide, the droplets being deposited in approximately equal size by disposable capillary pipets. This is a convenient technique for gelatin, which is too soft for sharp holes to be cut in it and too sticky for application of templates. Its surface characteristics allow one to place a droplet on it and to build this up, without having it spread laterally, to a sphere of approximately 1 mm in diameter. These slides with their reactants in place then were returned to the refrigerator for development of immunodiffusion patterns at 4°C.

The patterns began to appear within 3 hours, and were well-developed within 24 hours. Fig. 2, a negative photograph of a 24-hour pattern illuminated indirectly(1), shows that antigen diffusing from the MeHSA source reacted with antiserum to both native and methylated albumins and, by the spur formed over it by antiserum to HSA reacting with HSA, that this antigen is related to but not the same as HSA(4). It probably is the MeHSA itself, because no contaminants exist in our HSA preparations in quantities detectable by this type of test(5), and because electrophoresis of the MeHSA in gelatin revealed only one fraction.

Discussion. Most proteins which have been studied immunologically are anionic, bearing a net negative charge at neutral or alkaline pH. This is especially true for immunodiffusion analyses because agar gels, by far the most popular medium for these analyses, also are anionic and so permit unimpaired diffusion of anionic or neutral antigens while binding and sometimes precipitating cationic antigens like lysozyme(1). We have found in our experiments with cationic MeHSA that this binding may not be specially obvious and therefore easily can be overlooked Since it forestalls the appearance of precipitin bands, this can lead to one or both of 2 serious misinterpretations—that an antiserum contains no precipitins to a given cationic antigen, or that an antigen preparation seems purer than it truly is because its cationic constituents remain undetected. Therefore, using uncharged gels like gelatin is more than a novel technical variation in immunodiffusion analyses; it is a necessary contrasting complement to the use of agar gels for detecting antigens

and their corresponding precipitins.

Interestingly, although we expected agarose to serve as a suitable medium-gelling agent for our cationic antigen because it is supposed to be a neutral derivative of agar(6), we found it unsatisfactory since it too bound this antigen. Why this happened we do not know.

Summary. A cationic (basic) protein, methylated serum albumin, could not be analyzed by immunodiffusion in agar or agarose gels because it would not diffuse through them. Using gels of gelatin solved this problem. This finding may be applicable to gel-diffusion studies of other basic protein antigens, and it indicates that immunodiffusion tests in gelatin can be employed to complement those performed in agar to detect otherwise overlooked cationic antigens and corresponding precipitins.

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Localization of C-Type Virus Particles in Lymphoid Germinal Centers of C58 Mice.* (32556)

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Various aspects have been investigated of the distribution and ultrastructural properties of C-type virus particles in tissues of mice with spontaneous(1,2) or experimentally virus-induced(3-8) leukemia. Few studies have been reported for C58 mice, a strain with a high incidence of spontaneous lymphoid leukemia(9). Accordingly, a study was undertaken by electron microscopy to trace the frequency and distribution of virus particles in the hematopoietic and lymphoid tissues of C58 mice during the course of spontaneous leukemia. One paramount reason for conducting such a study was to obtain insight into the sequence of events at the preleukemic stage which lead to the evolution of fatal leukemia. Thus, mice were studied immediately after birth and at each succeeding month until they died of lymphocytic leukemia.

This study reports that C-type virus particles are readily detectable by electron microscopy in tissues throughout the preleukemic stage. The most significant finding was the localization of C-type virus particles in the germinal centers of the spleen and lymph nodes.

Materials and methods The lineage of the C58 mice used in this study has been described(10). In accordance with recommended nomenclature(11), they are designated the C58/Wm line. Starting with newborn mice, males and females were killed by cervical dislocation at approximately monthly intervals of age. Bone marrow, mesenteric lymph node, spleen and thymus were removed for histologic and electron microscopic study.

For light microscopy, tissues were fixed in Bouin's fixative and stained with hematoxylin and eosin. Tissues for electron microscopy were fixed in 2.5% phosphate-buffered glutaraldehyde, post-fixed in 1% OsO_4, and embedded in Epon 812. Thick sections (.5 to 1 μ) were cut and stained with toluidine blue for purposes of orientation and identification of germinal centers. Thin sections were

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