Table 2. Comparison of the activities of antihuman properdin rabbit serum in different systems that require properdin.

Method	Investigators	Units of properdin inactivated by 1 ml of anti-HP(A)
Inactivation of C'3 in zymosan assay (*1*)	This laboratory	80–120
Inactivation of C'3 in zymosan assay (*9*)	H. Isliker	60– 80
Hemolysis of PNH erythrocytes (*6*)	C. F. Hinz, Jr.	80–120
Bactericidal activity against *Sh. dysenteriae* (*4*)	R. J. Wedgwood	100–200
Inactivation of coliphage, T2 (*3*)	H. Van Vunakis, J. L. Barlow, and L. Levine	100–200

fied human properdin, under appropriate conditions, results in the appearance in their serum of antibodies which inactivate properdin. This suggests that the use of heterologous properdin in experimental animals or patients may be undesirable because of its antigenicity.

Antihuman properdin rabbit serum is stable to heating at 56°C for 1 hour; it inactivates properdin almost instantaneously at either 37°C or 1°C; and its antiproperdin activity is removed by adsorption with purified properdin or properdin-zymosan complexes (PZ) but not by human serum free of properdin (RP) or by zymosan. The antiserum after adsorption with RP appears to inactivate properdin specifically. Such adsorbed serums do not inactivate complement or its components, nor do they react visibly when added to untreated human serum or purified properdin. Either the antibody content of the serums is too low to give a visible precipitate with properdin or antiproperdin is a nonprecipitating antibody. Isliker (*8*) was unable to detect the formation of any bands in the Ouchterlony agar diffusion technique with mixtures of anti-HP(A) and human serum. However, work being conducted with E. R. Arquilla shows that human red cells which have been coupled to purified properdin by diazotization are agglutinated by antiproperdin rabbit serum but not by normal rabbit serum or by antihuman gamma globulin or antihuman serum rabbit serums. Hemagglutination is inhibited by properdin and serum reagents containing properdin. This suggests that hemagglutination inhibition reactions may eventually be employed for the detection or quantitative measurement of properdin.

It is encouraging that five different laboratories employing four different means of measuring properdin were in agreement on the antiproperdin activity of the serum [anti-HP(A)] furnished them (Table 2). Thus, antiproperdin antibodies inhibited the bactericidal, virus-inactivating, hemolytic, and C'3 inactivating properties of normal serum or human properdin in a similar manner.

The availability of antiproperdin serums should help in the characterization and measurement of properdin and in the clarification of the nature of the interactions of the properdin system with various infectious agents, red cells, and polysaccharides.

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References and Notes

1. L. Pillemer *et al.*, *Science* **120**, 279 (1954); L. Pillemer *et al.*, *J. Exptl. Med.* **103**, 1 (1956).
2. R. J. Wedgwood, H. S. Ginsberg, L. Pillemer, *J. Exptl. Med.* **104**, 707 (1956).
3. H. Van Vunakis, J. L. Barlow, L. Levine, *Proc. Natl. Acad. Sci.* **42**, 391 (1956).
4. A. C. Wardlaw and L. Pillemer, *J. Exptl. Med.* **103**, 533 (1956).
5. P. Gronroos, *Ann. Med. Exptl. et Biol. Fenniae (Helsinki)* **33**, 310 (1955); H. A. Feldman, L. Pillemer, L. T. Miller, *Federation Proc.* **15**, 587 (1956).
6. C. F. Hinz, Jr., W. S. Jordan, Jr., L. Pillemer, *J. Clin. Invest.* **35**, 453 (1956); C. F. Hinz, Jr., J. Abraham, L. Pillemer, *Proc. Soc. Exptl. Biol. Med.* **94**, 230 (1957).
7. This investigation was conducted under the auspices of the Commission on Immunization, Armed Forces Epidemiological Board, and was supported in part by the Office of the Surgeon-General, Department of the Army, Washington, D.C.
8. H. Isliker, personal communication.
9. H. Isliker, *Vox Sanguinis* **1**, 8 (1956).

15 May 1957

Graded Collodion Membranes for Separation of Small Molecules

In the course of doing work on a problem in protein chemistry, it became apparent to us that it would be useful to have membranes that could make separations between molecules that are smaller in molecular weight than 30,000. The preparation of membranes of graded porosity in the range of pore sizes above those we were interested in had already been worked out in detail (*1*). However, there has been no detailed study of the preparation of membranes with graded porosity in the lower range of pore sizes.

As early as 1915, Brown (*2*) showed that dried collodion membranes swelled when they were placed in solutions of ethanol and water: the higher the concentration of ethanol, the greater the degree of swelling, and, hence, the greater the porosity of the membrane. However, this idea has never been followed up to any extent, and very little use has been made of it. The purpose of the present work (*3*) has been to make a detailed study of the method of Brown in an effort to make the desired graded collodion membranes under carefully controlled conditions. The procedure that we have found suitable for the reproducible preparation of such membranes is as follows.

Collodion membranes are cast according to the procedure described by Gregor and Sollner (*4*). Collodion solution is poured in succession over six rotating test tubes that have been specially prepared for this purpose (*5*). The tubes each have a 2-mm hole in the bottom which has been sealed by allowing a drop of caramelized sugar to harden in the hole.

Three minutes after the first layer is placed on the tubes, a second layer is put on in exactly the same way as the first. The resulting two-layer films are allowed to dry on the rotating tubes for at least 30 minutes. The tubes can then be removed from the casting apparatus and allowed to stand at room temperature for about 6 hours or until they are completely free of solvent. They can stand longer at this point; however, it is best to remove the membranes from the tubes within 1 day after casting.

To remove the membranes from the tubes, the tubes are placed in water at room temperature for 2 hours. This allows the caramelized sugar in the hole at the bottom of the tubes to dissolve completely and also allows the membrane to become saturated with water. If flat membranes are desired, they may be cut off the tubes with a sharp knife. If bag-shaped membranes are desired, they can sometimes be obtained by slipping the membranes off the tubes after the soaking in water. When the membranes do not loosen and come off after this treatment, the tubes with the membranes still on them are placed in 70 percent ethanol for 1 to 2 hours. They are then put back in water and, after 2 or 3 minutes, the membranes may be slipped off the tubes. After the membranes have been removed from the tubes, they are allowed to dry again at room temperature. Drying is continued until no water droplets are visible.

The next step, which results in the grading of the porosity, is to place the dried films in varying concentrations of ethanol. Our ethanol solutions have been prepared as follows: An 85-percent

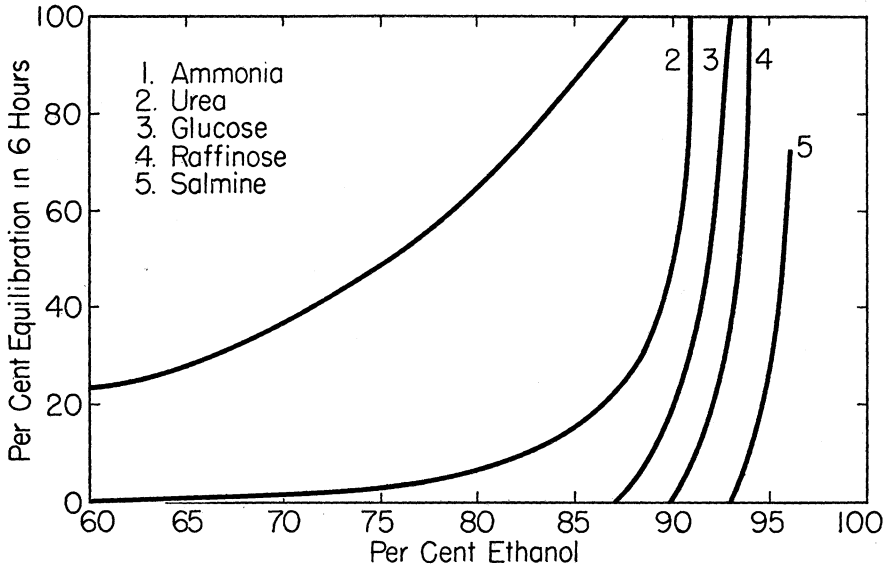

Fig. 1. Permeation of solutes through collodion membranes previously swelled in ethanol-water solutions.

ethanol solution is made by mixing 85 ml of absolute ethanol (water-free) with 15 ml of water; a 95-percent ethanol solution consists of 95 ml of ethanol and 5 ml of water, and so forth. The membranes are kept in the ethanol solutions for 3 hours, preliminary experiments showing that the degree of swelling changes very little after this time. They are then placed in water until they are used.

The determination of the degree of porosity of the membranes was carried out, for the most part, by measuring the rate at which solutes of various sizes diffused through the membranes. In this way, we would know directly whether or not a certain degree of swelling was suitable for a particular separation.

A solution containing the solute to be tested was placed inside a bag-shaped membrane (12 by 100 mm), and water was placed outside. The membrane with solution was placed in a 25- by 100-mm test tube containing 20 ml of water. It was then placed in a thermostat at 25.1°C, and the concentration of the solute which appeared in the outside solution was determined after 6 hours. From the initial volumes and concentrations in the two compartments, the equilibrium concentration was known. From the concentration in the outside solution at 6 hours, the percentage of the equilibrium value was calculated. A summary of results we have obtained using this procedure is shown in Fig. 1.

The curves in Fig. 1 are quite reproducible if such variables as membrane thickness and swelling conditions are controlled carefully. For example, in a given series, ten 90-membranes tested with urea gave an average value of 63.4 ± 2.5 percent equilibration. The variation from series to series, however, is not quite as good. When a particular point on one of the curves is checked with new collodion solutions, new reagents, and so forth, the variation may be as much as 15 to 20 percent from the original value.

For membranes swelled in 94-percent ethanol and higher concentrations, we have used ultrafiltration as a method of characterization. Ultrafiltration of solutions being tested was carried out with the 12- by 100-mm membranes according to the technique of Clegg (*6*). The membranes were placed in nylon bags which were then suspended in 15-ml centrifuge tubes. When centrifugation was carried out at 1000 to 1500 rev/min, liquid collected at the bottom of the tube, the rate depending on the porosity of the membrane. The resulting liquid was then tested for solute.

Using this procedure, we found that the 94-percent membranes just held back lysozyme, the 96-percent membranes just held back egg albumin, and the 97-percent membranes just held back serum albumin. This is about the upper limit in porosity that can be obtained with this method. Membranes swelled in 98-percent ethanol for 30 minutes are very weak, and 99-percent ethanol dissolves the film completely in a very short time.

To test these membranes further, separations of two different substances by dialysis have been attempted by placing a solution of the two substances inside a given membrane and water outside. With a membrane swelled in 90-percent ethanol and urea and sucrose as the solutes, it was found that in 36 hours the urea concentration was reduced to 10 percent of its original value, while the sucrose concentration was unchanged. With a membrane swelled in 87.5-percent ethanol and urea and glucose or urea and the tripeptide, glycylglycylglycine as the solutes, it was found that when the urea concentration was reduced to 5 percent of its original value, there was a 10 to 15 percent loss of the larger solute in the dialyzate. In these instances when the ratio of the molecular weights of two solutes is as low as 3/1, a complete separation is not obtained. Nevertheless, such separations could still be practical, especially if fractional dialysis were employed. Craig and King (*7*) have recently shown that fractional dialysis with cellophane membranes can be quite useful in estimating the size and homogeneity of unknown solutes. The availability of a series of graded membranes covering the smaller solute range should make this technique even more useful.

We have made practical use of these membranes in the separation of salts from low-molecular-weight proteins and polypeptides by means of electrodialysis. In addition, we have been able to make studies on the equilibrium dialysis of solutes which diffuse through the conventional dialyzing membranes (*8*).

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References and Notes

1. W. J. Elford, P. Grabar, J. D. Ferry, *Brit. J. Exptl. Pathol.* 16, 583 (1935); W. J. Elford, *Proc. Roy. Soc. (London)* B112, 384 (1933); J. H. Bauer and T. P. Hughes, *J. Gen. Physiol.* 18, 143 (1934–35).
2. W. Brown, *Biochem. J. (London)* 9, 591 (1915).
3. This investigation was reported in preliminary form before the American Society of Biological Chemists, San Francisco, 1955. It was supported in part by a research grant, H-1618, from the National Institutes of Health, U.S. Public Health Service, and in part by a grant from the Medical Research Fund of the Graduate School of the University of Minnesota.
4. H. P. Gregor and K. Sollner, *J. Phys. Chem.* 50, 53 (1946).
5. Most of our collodion solutions have been prepared by dissolving 40 g of collodion cotton (Pyroxylin, J. T. Baker) per liter of ether-ethanol (50/50). In one series of experiments in which Mallinckrodt Parlodion was used, the membranes showed the same degree of swelling as those prepared from the Baker collodion.
6. R. Clegg, *Chemist Analyst* 38, 87 (1949).
7. L. C. Craig and T. P. King, *J. Am. Chem. Soc.* 77, 6620 (1955); 78, 4171 (1956).
8. C. W. Carr and K. R. Woods, *Arch. Biochem. and Biophys.* 55, 1 (1955); H. B. Klevens and C. W. Carr, *J. Phys. Chem.* 60, 1245 (1956).

22 March 1957

Liver Catalase and Tumor Inhibition by Urinary Extracts

The influence of tumor growth on the liver catalase content has been noted by many workers; in particular, Adams (*1*) has reported on the changes in this enzyme that follow the injection into mice of homogenized sarcoma-37 tissue.