seconds is a suitable time of flow, but this varied from 50 to over 500. Both inner and outer surfaces of the capillary must be very clean and dry. If a tube was to be used a second time it was cleaned with strong chromic acid solution. Fluids to be used were left over night close to the apparatus in a room where the temperature seldom varied more than one degree.

Several runs with a sulfur-free crude oil gave a time of flow of 110 seconds with and 103 seconds without water jacket, the ratio $110:103=1.068$, of which the fourth root is 1.0166. The tube radius 0.4 mm divided by 1.0166 is .3935; or the radius is decreased by .0065 mm = 6.5 microns or 6.5×10^{-4} cm. If the adsorbed portion of the oil consists of the larger polar molecules, 6×10^{-6} cm is a reasonable estimate of their length. A layer of these 6.5×10^{-4} cm thick would therefore be about 100 molecules deep. The adsorptive force of silica for water is known to die out after the adsorbed layer becomes about 100 molecules deep (4×10^{-6} cm). This is of course far too small to be observed by flow methods.

Of over thirty combinations tested, about half showed no observable increase in time of flow due to an exterior liquid and none showed an effect exceeding that of the crude oil-water pair. Fresh soap solution gives an effect nearly as large, but sineresis (and aging) soon bring it below an observable limit. Apparently the long molecules lie parallel to the glass surface. Stearic acid dissolved in chlorex gave 5.8 microns, myristic acid 4.0. With sodium carbonate solution outside, the latter showed the same adsorption (4.0) as with pure water in the water jacket. Apparently the strongly basic sodium ions did not add to the effect of the water. A benzene extract of used fuller's earth showed no observable effect of a water envelope, but an ethylene glycol extract did, 2.1 microns decrease. Triethanolamine and 1 per cent. gelatine solution gave no effect, but crepe rubber in benzene gave 2.8 microns decrease.

In flowing crude oil through a fresh tube, the time of flow became constant only after about five minutes, the first run being about 3 per cent. faster than in the steady state as though several minutes were required to complete the adsorbed film. After application of the water outside, there was a similar delay in coming to an equilibrium. The observed effect is not due to compression for many liquids failed to show it.

There seems to be no question that the adsorption of hydroxyl or other anions on one side of a thin glass wall can enhance the adsorption of electropositive material on the opposite side by a measurable amount through at least 0.1 mm of glass. The action of the glass electrode in measuring pH appears to be of a similar nature. Although cell walls are not impermeable, attractive effects through them may well alter the Donnan equilibrium.

P. G. NUTTING

U. S. GEOLOGICAL SURVEY

SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE SEPARATION AND CONCENTRATION OF THE ISOHEMAGGLUTININS FROM HUMAN SERUMS[1]

THE difficulty of obtaining donors who possess high-titred isoagglutinins in their serums made it desirable at the Army Medical School to prepare from random A and B donors human isohemagglutinating serums of uniformly high titer and constant potency. During the course of this study, it was discovered that proteins containing isoagglutinins can be separated from both Group A and B serums with little or no loss of hemagglutinin activity by precipitation with methanol and that after this separation, they can be concentrated to the desired isoagglutinin titer.

The method is simple enough for general use and may be easily applied to large-scale production. The conditions under which separation and concentration is obtained follow.

Ten volumes of pooled group specific human serum obtained from fasting donors are added to 5 volumes of an acetate buffer of ionic strength 0.4[2] and pH 5.4 and to 7.5 volumes of distilled water. The solution is mixed and chilled to 1° C., and this temperature is maintained during the preparation of the concentrated isoagglutinins. Seven and five tenths (7.5) volumes of C.P. methanol (previously chilled to 1° C.) is slowly added with gentle stirring through a capillary tube at the rate of 0.25 volumes per minute to the protein buffer mixture. The final concentration of methanol is 25 per cent., the pH near 6.5 and the final ionic strength about 0.13. The mixture is allowed to stand at 1° C. for one hour. The proteins separating under these conditions are removed by centrifugation in the cold room or in a refrigerated centrifuge at 2,500 r.p.m. for 30 minutes. The precipitated proteins are washed once with 25 per cent. methanol (optional) and as much as possible of the excess methanol solution allowed to drain off by inverting the centrifuge tubes on filter paper.[3]

The separated material is then dissolved in the desired amount of M/15 phosphate buffer of pH 7.8[4]

[1] From the Blood Research Division, Army Medical School, Washington, D. C.

[2] A. A. Green, *Jour. Am. Chem. Soc.*, 55: 2331, 1933.

[3] The precipitate can also be freed of methanol by the application of vacuum.

[4] W. M. Clark, "Determination of Hydrogen Ions," Williams and Wilkins, Baltimore, 1928.

containing 1 mg of merthiolate per ml, the final pH being about 7.2. The proteins dissolve readily in the buffer solution, yielding clear solutions at protein concentrations greater than 10 per cent. It is the practice here to dissolve the precipitated proteins in a volume of buffer equal to one quarter of the original serum volume. By this means, sufficiently high concentrations have always been obtained. The solution may be further clarified by recentrifugation at high speed or by filtration through a fine sinter-glass filter. The proteins precipitated under the above conditions comprise about 10 to 15 per cent. of the total serum proteins and contains from 90 to 100 per cent. of the isoagglutinating activity originally present in the pooled serums.

The separated and concentrated material lends itself well for blood-grouping purposes. It is not as viscous as whole serum, but possesses sufficient surface tension to form well-rounded droplets on a glass slide. The addition of merthiolate to a final concentration of 1:1000 does not appear to interfere with the interactions of the isoagglutinins with the red cells and further eliminates the necessity for filtering out bacteria. The isoagglutinating activity of the separated globulines stored at room temperature (25° C) remained unimpaired for four weeks.

Employing the macroscopic slide technique, the concentration of this material can be adjusted so that agglutination with incompatible erythrocytes occurs visibly in 5 seconds time, with complete agglutination occurring in 60 seconds.

Studies are now in progress on the physico-chemical and immunological properties of the separated and concentrated proteins, and attempts are being made to further purify the isoagglutinins from this material. The details of this study will be reported later.

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A SIMPLE PROCEDURE FOR THE RECOVERY OF AGAR

RECENTLY, there was published a notice[1] of the War Production Board's request that agar be conserved. It was suggested that work be done on developing methods whereby agar may be recovered from culture media and re-used.

In this connection, therefore, attention is called to the common practice in the manufacture of agar of dissolving it in hot water and then removing the water by freezing.

A satisfactory procedure for the recovery of agar, based on the freezing method, has been employed in this laboratory. Discarded culture media containing agar is kept in separate waste pans from liquid media. This used media is autoclaved as usual for the purpose of sterilizing, and while still in the fluid state is filtered through cheese-cloth to remove coagulated proteins, *i.e.,* blood and serum. It is then poured into trays from the freezing compartment of a refrigerator and allowed to cool. The trays are returned to the freezing compartment and left overnight.

The following morning the frozen material is rapidly melted in warm alcohol. The aqueous alcohol, containing the particles of agar, is filtered, with cheese-cloth again being used instead of paper in order to speed the filtration. The agar thus collected is washed repeatedly with distilled water.

Dehydration of the agar is secured by washing with alcohol. We have found it convenient, following the washing with water, to gather the corners of the cheese-cloth together to form a sack. This sack is immersed in 95 per cent. alcohol and compressed to remove the water. The shreds of agar thus obtained are spread out in a porcelain evaporating dish and dried by placing in a 37° C. incubator or in a desiccator.

Agar recovered in this manner is as satisfactory as the fresh commercial product. The quantity recovered depends largely on the care taken in handling the material throughout the above procedure.

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[1] *News Letter of the Society of American Bacteriologists,* Vol. 8: No. 2, p. 3, Office of the Secretary-Treasurer, April, 1942.

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