vitro. Preliminary examination has shown the induced tumors to have a histological structure considerably more uniform than that of most crown galls, with extensive hyperplasia but relatively little disorganization. This last feature is quite marked and may possibly be due to the uniform distribution of the mechanism of hyperplasia in contrast to the scattered and localized centers of stimulus characteristic of bacterial galls. Fusion with the host tissue was excellent, although growth was mostly if not entirely a function of the transplant, as is the case with transplanted animal neoplasms. It is clear from these results that the affected tissues have undergone a drastic change which is indicated, first, by their capacity to produce galls, a quality not found in normal tissue, and, second, by their behavior *in vitro*, where their growth habits differ markedly from those of normal tissues under identical conditions. That this change was originally brought about by some stimulus from the crown gall organism seems clear. That its maintenance is not dependent on the continued presence of the bacteria is equally clear. Further details will be published elsewhere.

PHILIP R. WHITE
ARMIN C. BRAUN

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, PRINCETON, N. J.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

COLLODION FIXATION: A NEW IMMUNOLOGICAL REACTION

THE study of filterable viruses and of diseases caused by them is handicapped by the relative lack of *in vitro* reactions which can detect very small amounts of virus substance. The present dependence on pathological processes for the identification of viruses involves a considerable time factor. As an approach to the solution of these difficulties experiments were undertaken with a well-known immunological system in an effort to increase sensitivity, since this would appear to be the first requirement of any new method. The system employed was the reaction between antipneumococcus serum and the specific capsular polysaccharide of the pneumococcus. Under optimum conditions, specific precipitin reactions can be obtained with polysaccharide dilutions as high as 1–5,000,000; with complement fixation to 1–20,000,000.

The first series of experiments involved the adsorption of *antibody* on the surface of collodion particles, thereby artificially increasing the size of the antibody. Many workers have adsorbed *antigen* on collodion or other particles (*cf.* review by Cannon and Marshall[1]) and have demonstrated increased sensitivity as regards detection of antibody. Cannon and Marshall[1] sensitized collodion pellets with egg albumin; Weir[2] used tuberculin sensitized pellets for the study of antibody formation in animals. Our experiments in the adsorption of antibody were successful under very limited conditions; the most fortunate results were obtained with the use of purified horse and rabbit antipneumococcus sera. Particles so sensitized were agglutinated in the presence of the specific capsular polysaccharide in dilutions of approximately 10^{-10}. The details of these experiments will be published elsewhere. It may be said, however, that these systems are very sensitive to non-specific factors, such as broth, proteins, changes in electrolyte concentration, etc., and hence are not suitable for the general purposes of the method most desired.

In an approach to a more satisfactory method it was recalled that although both antigens and antibodies are not remarkable for adsorptive phenomena, the antigen-antibody complex is extraordinary in this respect: the complement fixation reaction is an example of this property.[3] Experiments were therefore undertaken to learn whether the antigen-antibody complex would adsorb collodion particles, thus, as it were, magnifying immunological reactions.

Collodion particles were prepared after the method of Cannon and Marshall,[1] the stock suspension being adjusted to a density such that a 1–10 dilution would match number 3 on the McFarland scale.[4] Suitable and constant quantities of collodion suspension are added to varying amounts of antigen in agglutination tubes. To these mixtures are then added appropriate amounts of immune sera; the total volume is then brought to 1.0 cc by the addition of physiological saline. With systems of some refinement, such as that with antipneumococcus serum, the tubes remain at room temperature for one hour and are then centrifuged for 5 minutes at 500 r.p.m. Each tube is "flipped" and the amount of particulate agglutination estimated. Control tubes, not containing antigen, give free and smooth resuspension. With less refined systems, such as the viruses, the tubes are placed in the icebox overnight, then centrifuged and read. An example of results obtained with the antipneumococcus system is given in Table I.

These results present two important points: (a) The

[1] P. R. Cannon and C. E. Marshall, *Jour. Immunol.*, 38: 365, 1940.

[2] J. M. Weir, *Proc. Soc. Exper. Biol. and Med.*, 46: 47, 1941.

[3] K. Goodner and F. L. Horsfall, Jr., *Jour. Exper. Med.*, 64: 201, 1936.

[4] J. McFarland, *Jour. Am. Med. Assn.*, 49: 1176, 1907.

TABLE I

COLLODION FIXATION IN ANTIPNEUMOCOCCUS SYSTEM

0.1 cc antiserum 0.3 cc saline 0.1 cc collodion suspension 0.5 cc capsular polysaccharide dilution	One hour at room temperature Centrifuged "Flipped" and read in terms of agglutinated particles

Antisera:	Horse antipneumococcus Type I		Rabbit antipneumococcus Type I
Pneumococcus capsular polysaccharide:	Type I	Type III	Type I
Antigen dilution			
10^{-5}	++++	–	+++
10^{-6}	+++	–	++
10^{-7}	+++		–
10^{-8}	++		–
10^{-9}	+		–
10^{-10}	+		–
Saline control	–		–

great delicacy of the reaction with antipneumococcus horse serum—in other experiments the limiting dilution of capsular polysaccharide was determined to be greater than 10^{-10}. (b) Antipneumococcus rabbit serum does not give this effect. In polysaccharide concentrations which produce a visible precipitate the visibility of the reaction is sharpened by the enmeshing of collodion particles, but there is no enhancement of limiting dilution. This result is precisely opposite that obtained with complement fixation, for with this reaction antipneumococcus rabbit serum gives positive results whereas horse serum fails. In so far as can now be determined the paradoxical results follow precisely a classification of species reported earlier.[5]

Although paradoxical in a species sense this reaction of collodion fixation bears many analogies to complement fixation. Thus, if the collodion particles are present at the time of antigen-antibody interaction an excellent result is obtained, whereas if they are added one hour after admixture of antigen and antibody the result is usually entirely negative.

That this method is applicable to work with filterable viruses is demonstrated by the results shown in Table II.

There is evidence to indicate that in virus systems the species derivation of the immune serum may be very important. Thus, in various experiments, human and goat immune sera have given positive results, whereas experiences with monkey and rabbit sera have thus far proven negative. It is probable that much will have to be learned of variables such as this before any general application to virus work can be carried out. Work already completed shows that the method can be applied successfully to the identification and typing of influenza virus in throat washings, to the identification of yellow fever virus, to the determination of the presence of antiviral antibodies in the sera of persons recovered from yellow fever, to the study of antibodies reactive with malarial parasites in both human and animal sera, to the reaction between poliomyelitis virus and specific antisera. The possibilities of application appear to be extraordinary in scope. This subject will be discussed at length at another place.

[5] F. L. Horsfall, Jr. and K. Goodner, *Jour. Immunol.*, 31: 135, 1936.

TABLE II

COLLODION FIXATION IN VIRUS-ANTIVIRUS SYSTEMS

0.1 cc antiserum 0.3 cc saline 0.1 cc collodion suspension 0.5 cc virus solution (1–100 dilution of original material)	Overnight in ice box Centrifuged "Flipped" and read in terms of agglutinated particles

Antiserum	Virus	Result
Normal goat serum	Fluid from chick embryos infected with "Influenza A" virus	–
Serum of goat immunized with "Influenza A" mouse lung preparation	" " " "	+++
" " " "	As above but infected with "Influenza B"	–
Normal human serum	Fluid from chick embryo infected with yellow fever virus	–
Human serum from convalescent yellow fever	" " " "	++

SUMMARY

Collodion fixation by immunological complexes presents a method of great delicacy—about 1,000 times that of any heretofore described reaction. This delicacy is of an order which may permit the *in vitro* identification of filterable viruses.

KENNETH GOODNER

LABORATORIES OF THE INTERNATIONAL HEALTH DIVISION,
THE ROCKEFELLER FOUNDATION

BOOKS RECEIVED

ALLEN, PAUL W., D. FRANK HOLTMAN and LOUISE A. MCBEE. *Microbes Which Help or Destroy Us.* Pp. 540. 102 figures. Mosby.

ANDERSON, H. V. and T. H. HAZLEHURST. *Qualitative Analysis.* Third edition, revised. Pp. xi + 266. Illustrated. Prentice-Hall. $3.70.

BABOR, JOSEPH A. and ALEXANDER LEHRMAN. *Laboratory Manual for Introductory College Chemistry.* Pp. 276. 56 figures. Crowell. $1.75.

FOSTER, WILLIAM and HUBERT N. ALYEA. *An Introduction to General Chemistry.* Second edition. Pp. xi + 790. 226 figures. Van Nostrand. $3.75.

GAMOW, GEORGE. *Biography of the Earth; Its Past, Present and Future.* Pp. xiii + 242. 58 figures. Viking Press. $3.00.

KELLOGG, CHARLES E. *The Soils That Support Us.* Pp. xi + 370. 80 figures. Macmillan. $3.50.

RUGH, ROBERTS. *Experimental Embryology; A Manual of Techniques and Procedures.* Pp. ix + 302. Illustrated. New York University Bookstore. $3.00.

TOP, FRANKLIN H. *Handbook of Communicable Diseases.* Pp. 682. 73 figures. 10 plates. Mosby.

TULEEN, LAWRENCE F. and others. *Test It Yourself!.* Pp. vi + 290. Illustrated. Scott, Foresman. $0.96.

VON LAUE, M. *Physik und Chemie in Einzeldarstellungen. Band VI, Röntgenstrahl-Interferenzen.* Pp. viii + 367. 120 figures. Akademische Verlagsgesellschaft, Leipzig. RM. 32.