loss of fish became apparent, numbers of dead fish being observed around the edge of the affected areas.

HARVEY L. SWEETMAN
H. E. WARFEL
MASSACHUSETTS STATE COLLEGE

PATHOGENICITY AND VIRULENCE OF CERTAIN BACTERIA

INCIDENTAL to studies on the egg-propagation of certain filtrable viruses as previously reported[1, 2] opportunity was afforded for observing the effect of introducing several species of bacteria into developing eggs of the chicken and other domesticated fowl. At the outset the sensitivity of the embryo and its membranes to various filtrable viruses and to numerous toxic influences[3, 4, 5, 6] was reflected in the response to the injection of different concentrations of *Salmonella pullorum*. With given strains of freshly isolated *S. pullorum* the extent and severity of the lesion produced as well as the survival time of the embryo were quite definitely and uniformly correlated with the quantity of the inoculum and the virulence of the culture for baby chicks.

These results suggested the possible adaptability of the method of egg inoculation for determining the pathogenicity and for ascertaining the virulence of various strains and species of bacteria. In investigating this hypothesis the preliminary observations here recorded were confined to inoculations upon the chorio-allantoic membrane of eggs incubated 10 to 15 days prior to treatment. The cultures used represented 4 strains of *Brucella abortus,* var. *bovis* and *suis,* 3 strains of diplococci of equine origin, 3 strains of hemophilic bacteria isolated from the upper respiratory tract of young chickens, 8 species of Salmonella and 3 species of Pasteurella. Eggs employed as controls were injected with the sterile suspending medium or with suspensions of the various bacteria killed by heat. Proof of infection of the embryo and/or its membranes was established by the production of gross lesions and direct pure culture isolation of the organism inoculated.

The cultures of diplococci and hemophiles appeared virtually devoid of pathogenicity for the developing egg even in the relatively large quantities employed (as much as 0.2 cc of the undiluted 15 to 24-hour broth cultures). These 2 groups of organisms in other trials were not proved to possess specific pathogenic properties for the homologous host. Brucella, Pasteurella and Salmonella cultures were lethal to the embryo in very dilute concentrations, while the control suspensions of dead organisms produced no more than slight local injury to the extra-embryonic tissues and were seldom associated with the death of the embryo.

Marked differences in virulence for developing eggs were manifested between smooth stock and freshly isolated cultures of Salmonella and Pasteurella. Simultaneous comparative titrations with Pasteurella cultures on 2 to 10-day old chicks revealed a correlation in results, although much less definite and uniform than in the egg-inoculation method. The *P. avicida* culture, when inoculated subcutaneously, killed chicks in dosages 10^7 times smaller than were required with the *P. equiseptica,* while *P. cuniculicida* required larger doses than *P. equiseptica.* For eggs the *P. equiseptica* and *P. cuniculicida* required dosages approximately 10^4 times greater than did *P. avicida* to kill chicken embryos within 48 hours. Intracranial inoculations of the *P. avicida* and *P. equiseptia* strains into a group of 9 horses gave results which could be interpreted as validating the titrations upon eggs and chicks. However, the more uniform and accurate measurements of virulence obtained by egg inoculation as compared to animal inoculation emphasizes the superiority of the new method.

The delicacy with which differences of pathogenicity and/or of virulence among strains of certain bacteria may be determined by inoculating the developing avian egg suggest that this method may also be utilized to detect alterations in these characters among variants of a certain strain.

The potential value and adaptability of the developing avian egg for other phases of purely bacteriological investigation and experimentation is suggested by the findings here reported and in consequence of the simplicity of application and economy of the method.

C. A. BRANDLY
ROBERT GRAHAM
UNIVERSITY OF ILLINOIS

DOWNWARD SHIFT OF pH CAUSED BY ADDITION OF GLUCOSE TO BORIC ACID BUFFER SOLUTIONS

THE accompanying table was prepared for use in a study of O_2 consumption by yeast. It may be useful in other studies. In each test, to 20 ml of boric acid buffer solution (prepared according to Clark: "Determination of H ions," 2d ed., 1928; table 35) was added a known weight of glucose, as shown, and the resultant pH value (at 25°) was measured potentiometrically (quinhydrone electrode).

[1] C. A. Brandly, *Jour. Inf. Diseases* 57: 201–206, 1935.
[2] C. A. Brandly, *Jour. Am. Vet. Med. Asn.* N. S. 41: 5, 587–599, 1935.
[3] F. N. Marcellus, R. Gwatkin and J. S. Glover, *Proc.* of Section on Diseases and Its Control, 4th World's Poultry Cong., pp. 401–408, 1930.
[4] G. Schmid, *Arch. für Geflugk.,* 4: 5, 177–182, 1930.
[5] Alan Deakin and Geo. Robertson, *Poultry Science,* 12: 6, 378–381, 1933.
[6] A. Bauman and E. Witebsky, *Ann. de L'Inst. Past.,* 54: 3, 282-289, 1934.
[7] Robert Graham and V. M. Michael, *Poultry Science,* 13: 1, 40–43, 1934.

Table I shows the results obtained with two buffer solutions, having initial pH values of 7.68 and 8.80,

TABLE I

Glucose (g. per 20 ml.)	pH	Glucose (g. per 20 ml.)	pH
No glucose	7.68	No glucose	8.80
0.104	7.21		
0.282	6.78	0.363	7.79
0.736	6.26		
1.159	5.83	1.186	6.95
1.987	5.47	2.353	6.42
3.434	4.96		
4.242	4.79	4.900	5.76
5.553	4.56		
6.412	4.45	7.152	5.47
10.627	4.03	10.868	5.20
13.834	3.82	14.170	4.91
16.613	3.66		
19.101	3.54	19.884	4.60
19.833	3.52		
21.298	3.46	21.127	4.56

respectively. With increasing glucose concentration the glucose effect is seen to have been relatively less pronounced; the effect of adding about 2 g. of glucose produced about half as much depression of pH value as was produced by adding about 21 g., and the difference between the pH depression caused by addition of about 10 g. and that caused by addition of about 21 g. was only about 0.60. The maximal decrease in pH value was the same for both solutions, namely 4.22 and 4.24. The effect was shown to be reversible; after glucose had been added, pH was increased when boric acid or borax was added to the solution. A number of writers have given evidence that glucose may form a union or complex with such substances as boric acid, but the exact nature of the union is still uncertain.

P. S. TANG
C. Y. LIN

NATIONAL WU-HAN UNIVERSITY
WUCHANG, CHINA

ISOLATION OF IMMUNOLOGICALLY PURE ANTIBODY

IT is now well known that the polysaccharide prepared from Type I pneumococcus precipitates specifically the antibody from Type I anti-pneumococcus serum. The precipitate so obtained can be washed free from inert proteins. If the washed precipitate is dissolved in dilute alkali, allowed to stand over night and then neutralized, about 50 per cent. of the protein in the precipitate is recovered in a soluble form. This soluble protein can be precipitated from the solution by dialysis (to remove the salts) and adjustment of the pH to 7.6 which may be regarded as its isoelectric point.

The protein so obtained agglutinates, and protects mice from an otherwise fatal dose of, Type I Pneumococci, as does the original serum, but the titer of these reactions is increased 15 to 20 fold. From a 0.2 per cent. solution the protein is 90 per cent. precipitable by the homologous polysaccharide, the remaining 10 per cent. can be accounted for by the solubility of the immune precipitate. If the immune precipitate obtained with the recovered protein is again dissolved in alkali, allowed to stand and neutralized, the protein recovered for the second time has quantitatively the same activity as before. The protein may therefore be regarded as a pure antibody, at least immunologically.

We have made essentially the same observations with the precipitate obtained from Type I Pneumococcus antisera of horse and rabbit and with Type III Pneumococcus antiserum of rabbit. We have found it also possible to recover the antibodies from the Type I Pneumococcus agglutinate by essentially the same method used for the precipitate. The method described above for the isolation of antibody thus appears to have a general application.

The isolation of immunologically pure antibody is theoretically and practically significant. It has been a debatable question whether antibody is itself a protein or something else carried by the protein. Our present findings leave little doubt that the antibody itself is a protein. The mechanism of immunological reactions is not yet clear. Now that pure antibody is available, we can advantageously restudy the mechanism of immune reactions, especially the precipitin reaction. On the practical side, the preparation of pure antibody places in the hands of the clinicians therapeutic agents where serum therapy was not practical before, *e.g.*, in Type III pneumonia the antiserum for which has a very low antibody content.

The details of our observations will be reported in the *Chinese Journal of Physiology.*

BACON F. CHOW
HSIEN WU

PEIPING UNION MEDICAL COLLEGE
PEIPING, CHINA

BOOKS RECEIVED

ANREP, G. V. *Lane Medical Lectures: Studies in Cardiovascular Regulation.* Pp. 118. 38 figures. Stanford University Press. $1.50.

BAYLEY, P. L., and C. C. BIDWELL. *An Advanced Course in General College Physics.* Pp. xv + 340. 235 figures. Macmillan. $3.50.

CANNON, WALTER B. *Digestion and Health.* Pp. ix + 160. 14 figures. Norton. $2.00.

DAVID, SIR T. W. EDGEWORTH, and R. J. TILLYARD. *Memoir on Fossils of the Late Pre-Cambrian (Newer Proterozoic); From the Adelaide Series, South Australia.* Pp. xi + 122. 13 plates. Angus and Robertson, Sydney. 7/6.

GAGE, SIMON H. *The Microscope.* Sixteenth edition, revised and enlarged. Pp. viii + 617. 313 figures. Comstock. $4.00.

MAYOR, JAMES W., and LEONARD B. CLARK. *A Laboratory Manual in General Biology.* Pp. v + 201. 44 figures. Macmillan. $1.75.

PRESTON, CARLETON E. *The High School Science Teacher and His Work.* Pp. xvii + 272. McGraw-Hill. $2.00.