in or absorption from this portion of the gut. Our results cannot give a conclusive answer as to which of these factors is more important. Administration of a 5% urea solution produced as marked a rise in blood urea nitrogen when introduced into the stoma of one of the low ileostomy dogs as when introduced into one of the jejunostomy dogs. This would indicate the importance of the element of digestion but is not conclusive because urea is such a readily diffusible substance.

Conclusions. Alimentary azotemia in the dog occurs after the intragastric or intrajejunal administration of whole blood or red blood cells. On the other hand, similar amounts introduced low into the ileum or into the peritoneal cavity produced practically no rise in blood urea nitrogen. Alimentary azotemia results, then, only from the presence of blood in the upper intestinal tract.

13021

Detection of Minute Amounts of Serum Antibody by Agglutination of Antigen-Coated Bacterial Cells.

EUGENE C. ROBERTS AND LLOYD R. JONES.

From the Department of Bacteriology, St. Louis University School of Medicine.

The authors recently described a local serum reaction[1] occurring upon the ear of a rabbit (at the site of a prior injection of serum antigen) when a very small quantity of homologous antiserum was injected intravenously elsewhere in the body. The amount of antiserum injected was so small that it was not possible to detect antibody in the animal's serum by the use of the conventional method of testing, *i. e.*, the precipitation test employing the antigen dilution method. In order to determine such minute amounts of circulating antibody in the blood of injected animals it became necessary to devise a technic which would be both accurate and extremely sensitive. This was accomplished by developing a method which involves coating the cells of a suspension of *Serratia marcesens* with serum antigen and observing the agglutination of such cells upon addition of the animal serum being tested. That bacterial cells can be coated with serum antigen and subsequently agglutinated with an immune serum (homologous to the serum antigen) was reported by Jones.[2]

[1] Jones, L. R., and Roberts, E. C., *J. Immunol.*, 1941, **40**, 107.

[2] Jones, F. S., *J. Exp. Med.*, 1927, **46**, 303.

However, to our knowledge, no practical application of this type of antigen-antibody reaction has been made heretofore.

It became evident in preliminary experiments, that agar grown cells (washed twice with saline solution, suspended in distilled water and killed by flowing steam) could be satisfactorily coated by suspending them in horse or beef serum (antigen) and incubating the mixture at 37°C for not less than 9 hours. With such sera in order to achieve satisfactory coating it was necessary that the quantitative ratio of serum to cells be of the order of 5 to 1, as based upon the nitrogen contents of these materials. Electrolyte content of serum does not interfere with satisfactory adsorption of antigen upon the cells. This is a particular advantage of using bacteria rather than collodion particles as the particulate agents for antigen adsorption, as Cannon[3] has reported that in order to secure satisfactory coating upon collodion particles, the antigen solution should be dialyzed as free as possible from any dissolved salts.

The suspension, above referred to, is centrifuged to remove the antigen coated cells, these in turn are washed twice with saline solution and finally diluted in saline to the desired opacity (equivalent to a reference standard containing 0.25 mg of ground Pyrex glass per ml). By centrifuging gently (200 r.p.m.) for 5 minutes, the larger clumps or aggregates of bacteria are removed. Whereupon 0.5 ml of the supernatant suspension of coated bacterial cells is added to each of a series of small tubes to make the agglutination test. One ml quantities of serial dilutions of antiserum (fresh or inactivated) are added to the tubes. All tubes, including suitable controls are then incubated for 2 hours at 40°C, following which they may be read at once or placed in the refrigerator over night before making the final readings. Agglutination is of a fine granular type and corresponds to that which is ordinarily observed with 'O' type suspensions of bacteria. Employing this technic we have not observed agglutination with any of the sera of a group of normal rabbits.

In Table I is recorded the antibody titers, as determined by the conventional precipitation test employing the antigen dilution method and this newly devised technic which involves bacterial agglutination (B.A. method) of antigen coated cells at various time intervals after the intravenous injection of a rabbit with a single dose of rabbit anti-horse serum.

Table II similarly indicates the titers observed (2 hours post-injection) in rabbits receiving varying quantities of the same antiserum.

---

[3] Cannon, P. R., and Marshall, C. E., *J. Immunol.*, 1940, **38**, 265.

TABLE I.
Antibody Titers in the Serum of a Rabbit Passively Immunized with a Single Dose of Rabbit Anti-Horse Serum.*

Time interval after antiserum administration	Precipitation test	B.A. method
2 hr	+ undil.	1:512
24 "	+ "	1:512
4 days	—	1:128
7 "	—	1:32
9 "	—	1:8
11 "	—	1:16
12 "	—	1:16

*Intravenous injection of 1 ml per kg. (Precipitin titer 1:60,000).

TABLE II.
Antibody Titers in Serum of Rabbits Two Hours After Passive Immunization with a Single Dose of Rabbit Anti-Horse Serum.

Antiserum amt inj. per kg, ml	Precipitation test	B.A. method
1.0	+ undil.	1:512
0.5	—	1:64
0.25	—	1:16
0.125	—	1:2

These tables indicate that the B.A. method possesses greater sensitivity in the detection of serum antibody than the conventional precipitation test. This greater sensitivity is again depicted in Table III, wherein the 2 tests were employed to detect the presence of antibody in an animal actively immunized with a single, small dose of horse serum.

TABLE III.
Antibody Titers in the Serum of a Rabbit Actively Immunized with a Single Dose of Horse Serum.*

	Precipitation test	B.A. test
Before antigen inj.	—	—
24 hr after "	—	1:4
2 days " "	—	1:64
3 " " "	—	1:256
4 " " "	+ undil.	1:512
5 " " "	—	1:64
6 " " "	—	1:64

*Subcutaneous injection of 0.15 ml per kg.

It is significant that antibody was observed not only much earlier with the B.A. method, but in addition antibody could be detected 24 hours after antigen administration. The early appearance of antibody, detectable by this sensitive technic, has been confirmed in other similarly injected animals.

During the course of this study, the blood sera of a number of

human beings who were receiving relatively large amounts of an antigenic foreign protein, were made available to us at intervals during the course of their therapeutic programs.* These sera were tested for specific antibody content by the conventional precipitation test and by the bacterial agglutination (B.A.) technic herein described. A comparison of the results obtained by the 2 methods of antibody determination indicates that the B.A. method when also applied to the serum of man serves to detect antibody earlier and that significant titers persist longer than is observed with the conventional precipitation test.

It seems likely, therefore, that the B.A. method of antibody detection possesses certain advantages over the conventional precipitin test as made by the antigen dilution method. While the B.A. method may not be more sensitive than the collodion particle agglutinative method as described by Freund,[4] by Cannon and Marshall,[3] and others, we have found, however, that it is more convenient and that the bacteria employed exhibit greater suspension stability than collodion particles.

Since the B.A. method is capable of detecting extremely small quantities of antibody in serum it is conceivable that it may be advantageously applied to various immunological problems, *e. g.,* allergic states, virus diseases and other conditions wherein very small amounts of circulating antibody may be involved in the mechanism of the disorder. Our current studies involving the use of this newly devised technic in a virus disease (encephalitis of the St. Louis type) will be reported subsequently, wherein it will be shown that the sera of convalescent patients agglutinate bacterial cells treated with mouse brain virus antigen.

13022 P

Paradoxical Blood Concentrating Effect of Intravenous Four Normal Plasma Injections.

HENRY N. HARKINS, ROBERT T. BOALS AND BROCK BRUSH.

From the Division of General Surgery, Henry Ford Hospital, Detroit.

If plasma is injected intravenously, one would expect the blood to become diluted. If concentrated plasma is similarly injected, be-

* These sera were supplied by Dr. R. O. Muether and the precipitation tests were made by Miss Ruth Kettler.

4 Freund, J., *Am. Rev. Tbc.*, 1925, **12**, 124.