

TABLE 2. Oxidation of isocitrate by *C. burnetii**

	TPNH formed	Specific activity
	μmoles	
<i>C. burnetii</i>	94	0.109
NYS + heated <i>C. burnetii</i>	3	0.0063

* Isocitrate, 8.5 mM; MnCl₂, 0.11 mM; TPN⁺, 0.54 mM; tris buffer, pH 7.5, 0.05 M; disrupted *C. burnetii* (LD₅₀ 10^{-4.8}), 0.72 mg N/ml; NYS + heated *C. burnetii*, 0.38 mg N/ml; final vol, 1.45 ml, incubated 15 min, 25 C.

J. Biol. Chem. **193**:405, 1951), employing a known reference sample of 6PG. The intermediate formation of 6-phospho-δ-gluconolactone was shown by trapping the lactone in hydroxy-la-

mine (Cori and Lipmann, J. Biol. Chem. **194**:417, 1951). In two experiments 0.10 and 0.20 μmoles of lactone were formed from 7 μmoles of G6P. Disrupted *C. burnetii* preparations also oxidized isocitrate in the presence of TPN (Table 2). The reaction was TPN-specific; diphosphopyridine nucleotide was not reduced when it replaced TPN in the incubation mixture. TPN reduction was linear, with Δ OD = 0.04/min.

Rickettsial ability to oxidize G6P identifies an important energy source for the organisms. The oxidation of isocitrate is consistent with the demonstration of citrate synthesis by *C. burnetii* and with reports of rickettsial oxidation of other tricarboxylic acid cycle intermediates.

SOME CLINICAL LABORATORY BRIEFS ON STAPHYLOCOCCI

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In several recent studies on staphylococci, a number of personal observations were made which may prove useful in the clinical laboratory.

In the performance of the coagulase test, fresh rabbit plasma gave higher positive results than dehydrated plasmas. The substitution of human plasma should be considered with caution, for Streitfeld, Sallman, and Shoelson (Nature **184**:1665, 1959) found that pooled gamma globulin inhibited coagulase production by staphylococci. Any new plasma, fresh or reconstituted, should be checked with known controls. One of the controls should be a weak coagulase producer or a strain which takes at least 24 hr to give a positive test.

Approximately 80% of *Staphylococcus aureus* strains isolated from patients reduced triphenyltetrazolium chloride in 30 to 40 min. *S. epidermidis* took about twice as long. The medium used was tryptose agar (Difco), pH 7.2. After autoclaving, it was cooled to 45 to 50 C and the following added: Seitz-filtered 10% D-mannitol,

final concentration 1%, and a sterile (autoclaved) solution of triphenyltetrazolium chloride 1:350, final solution having a concentration of 1:35,000.

S. aureus grew well on nutrient agar with a pH of 8.5, whereas other staphylococci were either completely inhibited or showed little growth.

In correlating production of coagulase with fermentation of mannitol, a number of discrepancies were apparent. A small percentage of staphylococci, approximately 1%, were coagulase positive and did not ferment mannitol. About 8% were coagulase negative and fermented mannitol.

When the coagulase test was used as the standard, most of the media currently available for the isolation and identification of staphylococci showed more "false positives" (coagulase negative) than "false negatives" (coagulase positive).

A small percentage of coagulase-negative staphylococci, about 0.5 to 1.0%, gave positive results when tested with all of the following media: (i) mannitol broth; (ii) deoxyribonucleic acid agar (DiSalvo, Med. Technicians Bull., U.S. Armed Forces Med. J. **9**:191, 1958); (iii) phe-

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nolphthalein diphosphate agar (Barber and Kuper, *J. Pathol. Bacteriol.* **63**:65, 1951); and (iv) fluorescein amine agar.

As a fermentation medium, mannitol broth showed significantly greater agreement with the coagulase test than did mannitol salt agar.

The following medium proved excellent in differentiating pigment production in staphylococci: Tryptose agar (Difco) was dissolved

according to package instructions, the pH adjusted to 7.2, and 1% CaCO₃ (ppt chalk, Baker and Adams) added. After routine autoclaving and cooling to 45 to 50 C, the medium was supplemented with Seitz-filtered 10% D-mannitol to give a concentration of 0.5%. As plates were being poured, sufficient agitation kept the chalk in suspension. The uniformity of the white background permitted more critical color contrast.

FLUORESCENT ANTIBODY STAINING OF PR8 INFLUENZA VIRUS ADSORBED ON CHICKEN ERYTHROCYTES

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Adsorption of PR8 influenza virus on chicken erythrocytes was visualized by means of fluorescent antibody staining. The fluorescent antibody staining titer was compared with the hemagglutination (HA) titer.

45 min. HA titer was determined by the pattern method (Salk, *J. Immunol.* **49**:87, 1944), using 1% chicken erythrocyte suspension. A two-plus agglutination was taken as the end point. Fluorescent antibody staining titer was determined as

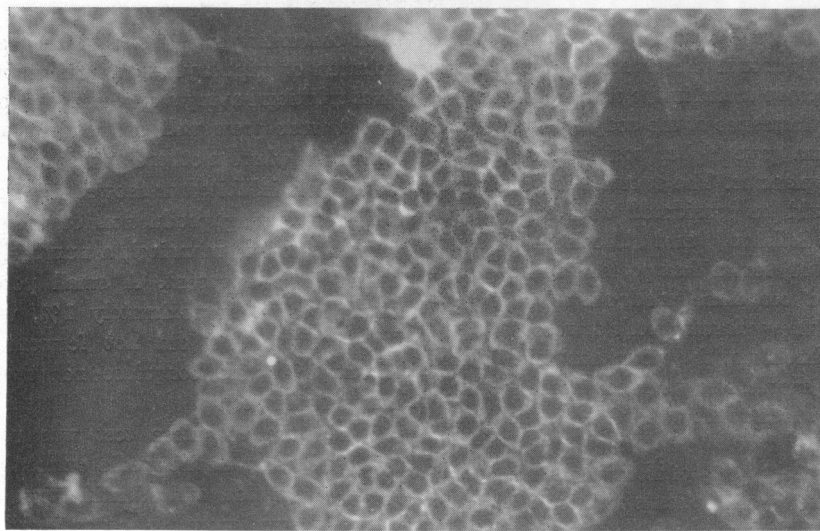


FIG. 1. *Fluorescent antibody staining of chicken erythrocytes with adsorbed PR8 influenza virus. Note the brilliant fluorescence at the periphery of each red cell (×560).*

PR8 virus was inoculated into the allantoic sac of 11-day-old chick embryos. The allantoic fluids were harvested after 72 hr incubation at 35 C. In a few experiments the allantoic fluids were concentrated by centrifugation at 18,000 rev/min for

follows: A constant amount of chicken erythrocytes was added to various twofold dilutions of virus suspension prepared from the same samples as used for HA determination. After about 10 min of mixing of the virus and erythrocyte suspen-