pigs should result in a more rapid diagnosis. It might be noted that the strain of *M. phlei* mentioned earlier produced a yellow-orange pigment in these media, in contrast to the white, nearly colorless growth produced by the human and bovine strains of tubercle bacilli.

Conclusion. The new synthetic media recently described by Dubos for the rapid cultivation of Mycobacteria can be successfully employed to isolate tubercle bacilli from various pathologic material. A combination of rapid culture with guinea pig confirmation where indicated should result in a marked reduction of the time required for the laboratory diagnosis of tuberculosis.

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Significance of Thromboplastic Activity of Antigens Used in Complement-Fixation Tests.

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Most antigens employed in serologic tests, particularly crude preparations, cause nonspecific reactions in varying degree. They are also usually anticomplementary. The underlying cause of these effects has not been understood. In practice, antigens are diluted to avoid the anticomplementary reaction as far as possible and sera are either used in amounts beyond the range where nonspecific reactions occur or the specificity is evaluated on the basis of the reacting dilution. In some instances nonspecific reactions have been avoided by heating sera above their inactivation temperature, to 60° or 65°C.[1,2] In general this is considered poor serologic practice because of the destructive effect of higher temperatures upon antibodies and of the reactivating effect of such heated sera upon the hemolytic activity of complement.

Relationships between the coagulative and complement activity of serum have been previously noted.[3,4] Cephalin, which is highly thromboplastic, inhibits complement to a degree closely parallel to its clotting activity. This thromboplastic activity of cephalin may be markedly enhanced by the addition of normal mammalian sera, even after inactivation at 56°C for 30 minutes.[5] Since many antigens possess thromboplastic qualities it seems likely that their inhibitory action on complement may be due to the enhancing effect of inactivated serum upon these thromboplastic qualities. It has been our experience that reduction in the thromboplastic quality of the tissue extract used in the complement-fixation test for syphilis diminished the number of nonspecific reactions obtained. The purified antigen, cardiolipin, now widely used, does not possess thromboplastic activity.[6]

The interdependence of these phenomena may have practical importance in the serologic study of sera in neoplastic and virus diseases in which tissue extracts are frequently used as antigens. Normal rabbit sera and fresh tissue extracts have been found to fix complement, which led to the conclusion that normal tissue antibodies were present in such animals.[7] Since fresh tissue extracts are highly

[1] Casals, J., and Palacios, R., *Science*, 1941, **93**, 162; *J. Exp. Med.*, 1941, **74**, 409.

[2] Thomas, L., and others, PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 121.

[3] Wadsworth, Augustus, Maltaner, Frank, and Maltaner, Elizabeth, *J. Immunol.*, 1936, **30**, 417.

[4] Wadsworth, Augustus, Maltaner, Frank, and Maltaner, Elizabeth, *J. Immunol.*, 1937, **33**, 297.

[5] Maltaner, Frank, and Maltaner, Elizabeth, *Arch. Biochem.*, 1943, **2**, 37.

[6] Maltaner, Frank. Unpublished data: Report, June 8, 1942.

[7] Kidd, J. G., and Friedewald, W. F., *J. Exp. Med.*, 1942, **76**, 543.

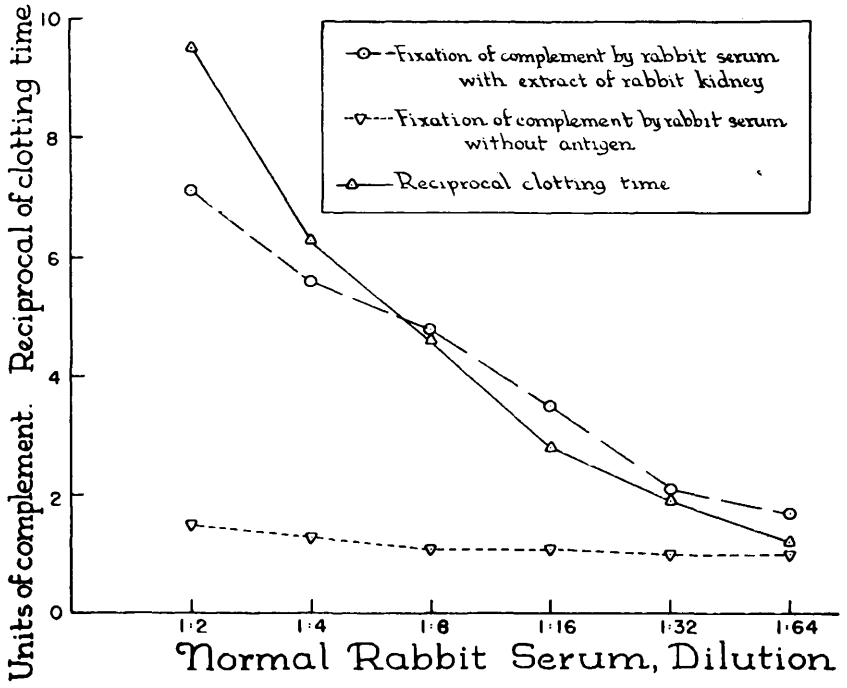

Fig. 1.
Comparative tests of the complement-fixing and clotting activities of normal rabbit sera inactivated at 56°C for 30 minutes and fresh extracts of rabbit kidney.

thromboplastic, a comparative study was made of the enhancement of their thromboplastic and complement-fixing activities by normal rabbit sera; also of the effect upon both activities of heating the normal sera beyond their reactivation temperature.

Saline extracts of liver and kidney of normal rabbits and guinea pigs were prepared as described by Kidd and Friedewald[7] and used as antigens. The complement-fixation tests were conducted in 0.3 ml volumes. Complement was titrated against a 5% suspension of sheep cells maximally sensitized with an equal amount of amboceptor to determine the unit (the amount required for 50% hemolysis). Dilutions were prepared so that the required number of units, 1—2 in the control tests of serum or antigen alone and 2, 3 and 6 units in the tests of serum and antigen, were contained in 0.1 ml.[8] Fixation was for 2 hours at room temperature. Sensitized cells were added in amounts of 0.2 ml and 15 minutes in the water bath at 37°C was allowed for hemolysis. No effort was made to determine the optimum dosage required for maximum reaction because of the labile character of the tissue extracts.

The clotting activity of the inactivated sera was determined by testing comparable dilutions of serum and antigen with recalcified oxalated rabbit plasma. Plasma was secured by the paraffin technic[4] and the amount of calcified saline used was sufficient to clot the plasma in 20 to 30 minutes in the presence of the test dose of tissue extract. The test was conducted in the water bath at 37°C and the interval between adding plasma and definite clot formation was measured.

Fig. 1 shows that parallelism existed between the units of complement fixed and the reciprocal of the clotting time when various dilutions of normal rabbit serum were tested with rabbit kidney extracts. Similar results were secured when guinea-pig kidney extracts and liver extracts of both guinea pig and rabbit were tested with several different rabbit sera. When sera were inactivated at 56°C,

[8] Wadsworth, A. B., *Standard Methods of the Division of Laboratories and Research of the New York State Department of Health*, 2d ed., Baltimore, Williams and Wilkins, 1939, p. 243.

60°C, and 65°C respectively the diminution in complement fixation was closely paralleled by the decrease in clotting activity.

Conclusions. These results give further evidence of the correlation between clotting and complement activity and indicate that antigens possessing thromboplastic activity may give nonspecific complement fixation.

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A Note on the Relationship Between the Intermedius and Minimus Types of *C. diphtheriae*.*

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Frobisher[1] and Eller and Frobisher[2] described bacteriologically and epidemiologically an outbreak of diphtheria in Baltimore and called attention to 2 hitherto undescribed types of diphtheria bacilli. One of these is a virulent saccharose-fermenting variety. The other, with which the present communication deals, is characterized by the following special properties: (a) the production, on the cystine-tellurite agar, used routinely in these laboratories and previously described,[3] of very small, often hardly visible colonies that are always flat, but which may be either rough or smooth; (b) failure to ferment dextrose promptly (*i.e.*, within 48 hours at 37°C), *when first isolated.* In the article referred to,[1] attention was also called to the fact that other small-colony strains of diphtheria bacilli were encountered during the epidemic described but that the term *minimus* type was reserved for the minute-colony strains which failed to ferment dextrose as do strains ordinarily encountered in Baltimore and tested under the same conditions.

It is well known that small-colony types of *C. diphtheriae* are not uncommon. Such forms have been described by Corbett and Phillips,[4] Morton,[5] and Mittag.[6] However, failure to ferment dextrose by such forms has not been described previously and it seemed desirable to designate so distinctive a variety by a special name, following the example of Anderson *et al.*[7] Since publishing this description several workers, in personal communications, have suggested that the *minimus* type is in reality identical with the *intermedius* type described by Anderson *et al.* It is the purpose of this note further to clarify the status of the *minimus* type among other small-colony types.

First, as to colony form, the original description of the *intermedius*-type colony states that the colonies are small as are those of the *mitis* type. In other respects the organisms of the *intermedius* type are closely similar to the *mitis* type except that in broth cultures the *intermedius* type produces a granular sediment, rather than a smooth turbidity like the typical *mitis* strains. In this respect the *intermedius* strains are also like the *minimus* strains. No pictures of the *intermedius* colonies were shown in the original article,[7] but colored plates were presented which illustrate the *gravis* and *mitis* colonies. In a monograph by Dudley[8] a similar colorplate shows *intermedius* colonies and these are not at all like colonies of the *minimus* type.

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5 Morton, H. E., *Am. J. Path.*, 1932, **8**, 605.

6 Mittag, G., *Zentr. Bakt. Parasitenk.*, 1937, I Orig., **138**, 426.

7 Anderson, J. S., Happold, F. C., McLeod, J. W., and Thompson, J. G., *J. Path. and Biol.*, 1931, **34**, 667.

8 Dudley, S. F., May, P. M., and O'Flynn, J. A., *Active Immunization Against Diphtheria*, His Majesty's Stationery Office, 1934.