human plasma and to dilutions of plasma made in 0.01 M oxalated 0.9% sodium chloride solution. The coagulation time for each dilution of plasma was then determined after addition of active rabbit serum. It will be observed (Fig. 2) that the activity of the "prothrombin-converting factor" in rabbit serum is dependent upon prothrombin concentration of the plasma. A curve can therefore be constructed (Fig. 2) which resembles the reaction curve obtained in the one stage prothrobin test of Quick.[6] A similar curve can be obtained by substitution of rabbit for human plasma. This suggests that rabbit and human plasma contain nearly identical amounts of prothrombin.

It will be observed furthermore (Fig. 2), that plasma diluted in 0.9% sodium chloride solution rather than 0.01 M oxalated sodium chloride solution, produced faster coagulation in the diluted fractions when the active serum was added. This suggests that the "prothrombin-converting factor" may be partially inhibited by the oxalate ion (calcium effect?) or that "prothrombin-converting factor" may be auto-catalytically activated from plasma.

Discussion. The residual coagulating power of serum, after complete coagulation of whole blood has taken place, is not related to thrombin but rather to a "prothrombin converting factor". It is assumed that this factor is initially activated through the action of plasma thromboplastin and platelets. The "prothrombin-converting factor" then causes thrombin production by reacting with prothrombin. The thrombin quickly disappears after fibrin is formed, whereas the "prothrombin-converting factor" can be easily measured in the serum for at least 100 minutes from the time of venipuncture. In sera of patients who receive dicumarol, the "prothrombin-converting factor" effect is greatly prolonged over that observed in normal serum.

An accurate analysis of plasma prothrombin concentration can be made by use of a relatively stable "prothrombin-converting factor" obtained from rabbits receiving dicumarol. This technic of assay reproduced results obtained with the one stage determination of prothrombin by Quick's method.[6]

Owren,[7] who described factor VI (which we believe to be the same as "prothrombin-converting factor"), has reasonably credited Bordet and Gengou[4] with the first demonstration of this forgotten concept in blood coagulation.

[6] Quick, A. J., *The Hemorrhagic Diseases and Physiology of Hemostasis* (Thomas, 1942).

[7] Owren, P., *Acta Med. Scand.*, 1947, Supp. 194.

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Evaluation of Dubos' Solid Medium Containing Penicillin in the Isolation of Tubercle Bacilli.

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Following the observation by Dubos and Davis that a liquid medium containing Tween 80 would allow rapid, submerged growth of mammalian tubercle bacilli,[1] the diagnostic potentialities of this medium have been explored in many laboratories.[2,3] The results have in general been favorable, but two disadvantages have become apparent. First, no

[1] Dubos, R. J., and Davis, B. D., *J. Exp. Med.*, 1946, **83**, 409.

[2] Foley, G. E., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 298.

[3] Foley, G. E., *J. Lab. and Clin. Med.*, 1947, **32**, 842.

satisfactory agent has been found to restrain contaminating organisms which are not destroyed by treatment with alkali. Penicillin is not suitable for this purpose, since in the presence of Tween it causes inhibition of tubercle bacilli in concentrations greater than 1 or 2 units per cc.[4] The other disadvantage is the necessity of staining the organisms growing in the medium to be certain they are acid-fast bacilli. If only a few tubercle bacilli are present, and particularly when there are contaminants, it is often tedious and difficult to demonstrate their presence.

More recently Dubos and his associates have described a solid medium containing oleic acid, albumin, and agar, which is relatively clear and transparent, and supports the growth of small numbers of tubercle bacilli.[5] In contrast to the liquid Tween medium, penicillin can be added in concentrations of 100 units per cc without inhibiting the growth of tubercle bacilli.[4] Virulent tubercle bacilli grow on this medium in a characteristic cord-like manner, easily observed under the low power of the microscope, which distinguishes them from avirulent tubercle and from the colonial forms of other bacteria.[6]

Because of these apparent advantages, a study was undertaken to compare the solid oleic acid-albumin medium with a standard egg-potato medium in the isolation of tubercle bacilli from clinical specimens, and to determine the effectiveness of penicillin in restraining contaminants.

Methods and Materials. Each specimen was digested with an equal volume of 4% NaOH for one hour at 37°C, with a 10 minute period of agitation in a shaking machine. Smears were made of the neutralized, centrifuged sediment, which was suspended in 0.5 cc of saline, and 2 loopfuls of the suspensions were inoculated on each medium.

The media employed were: (1) solid oleic acid-albumin, (2) solid oleic acid-albumin containing 100 units per cc of penicillin, (3) a widely used egg-potato medium containing 0.02% malachite green.[7] The media were allowed to solidify in one ounce prescription bottles, or small (1.5 by 5 cm) Petri dishes sealed with scotch tape. Only one sample of each medium was inoculated from any one specimen.

TABLE I.
General Comparison of Positive Cultures Obtained on Three Media.

	No. of cultures positive	Avg. No. days required to obtain positive culture
Total No. of specimens positive by all methods	156	
Oleic acid-albumin medium	91	14
Oleic acid-albumin medium plus penicillin	134	14.6
Egg-potato medium	120	24.4

TABLE II.
Analysis of Results from the Standpoint of Positive and Negative Cultures Obtained with Each Medium.

	No. of cultures
Positive oleic acid plus penicillin Negative plain oleic acid	55
Positive plain oleic acid Negative oleic acid plus penicillin	8
Positive oleic acid plus penicillin Negative egg-potato	20
Positive egg-potato Negative oleic acid plus penicillin	14

Results. Tubercle bacilli grew on one or more of the media in 156 of 300 specimens cultured. Of the 156 positive specimens, 61 were sputa, 91 urines, 2 gastric washings, and 2 were pus from abscesses. Comparative results are presented in Table I. The most striking observation, evident from the second column, is that positive cultures were obtained 10 days sooner, on the average, with oleic acid-albumin medium than with the egg-potato. There was no evidence that growth was delayed by the addition of penicillin to the Dubos medium.

[4] Kirby, W. M. M., and Dubos, R. J., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 120.

[5] Dubos, R. J., and Middlebrook, G., *Am. Rev. Tuberc.*, 1947, **56**, 334.

[6] Middlebrook, G., Dubos, R. J., and Pierce, C., *J. Exp. Med.*, 1947, **86**, 175.

[7] American Trudeau Society, *Am. Rev. Tuberc.* (Abstracts), 1946, **54**, Nos. 4-5.

From the standpoint of total positive cultures, the solid oleic acid-albumin medium containing penicillin appeared to be slightly superior to the egg-potato, there being 134 positives with the former, and 120 with the latter. This slight superiority is also apparent in Table II, where it can be seen that the Dubos medium containing penicillin was positive in 20 instances when the egg-potato was negative, whereas the reverse situation, (positive egg-potato, negative Dubos plus penicillin) occurred with 14 specimens.

The Dubos medium was much more efficient with the addition of penicillin than without. In 32 instances in which recovery of tubercle bacilli was prevented by the overgrowth of contaminants on the plain Dubos medium, the contaminants were suppressed on the penicillin-containing medium, and positive cultures were obtained. In one of the 8 instances in which the plain Dubos plate was positive, while that containing penicillin was negative, the tubercle bacillus was found to be inhibited by a concentration of only 10 units per cc of penicillin.

Comment. The present study indicates that Dubos' solid oleic acid-albumin medium, containing 100 units per cc of penicillin, is slightly more sensitive than a standard egg-potato medium in detecting tubercle bacilli in specimens obtained from clinical sources, and has the advantage of giving positive results 10 days earlier, on the average.

Another desirable feature is that the cord-like growth of virulent tubercle bacilli is highly characteristic on the transparent oleic acid-albumin plates; the colonies can be recognized at a glance by focusing on the agar surface with the low power of the microscope. This ability to differentiate virulent from avirulent tubercle bacilli on simple morphological grounds will probably obviate the necessity of inoculating guinea pigs; to our knowledge, no avirulent strains have been discovered which produce typical cord-like colonies.

Penicillin appears to be a highly satisfactory agent for inhibiting the growth of contaminants on the oleic acid-albumin medium. It should be noted that tubercle bacilli isolated from the urine in one patient were inhibited by 10 units per cc of penicillin; in the 134 other instances growth of the tubercle bacilli was in no way affected by a concentration of 100 units per cc of penicillin.

The widespread notion that Dubos media provide isolation from clinical specimens in 10 days, as opposed to 4 to 8 weeks with egg-potato media, is incorrect. Actually, cultures usually become positive on Dubos media from 7 to 14 days earlier than on egg-potato, regardless of the length of time required for growth to appear. With small inocula, several weeks are required; with larger inocula, growth is often visible in 7 to 14 days.

Summary and Conclusions. Of 300 clinical specimens, 134 were positive for tubercle bacilli on Dubos' solid oleic acid-albumin medium containing 100 units per cc of penicillin, and 120 were positive on a standard egg-potato medium. Without the presence of penicillin to restrain contaminants, only 91 cultures were positive on the Dubos medium. The Dubos medium was also considered superior because of the ease with which growth could be observed on the transparent agar, and because of the characteristic cord-like appearance of virulent tubercle bacilli, which apparently distinguishes them from avirulent forms, and from other organisms.