mechanism of the reaction or of the specificity of the enzyme, except that the presence of unsaturated fat acids is apparently required. The results reported in the present paper show that vitamin A alcohol is destroyed to a considerably greater extent than vitamin A acetate. The work of Hickman,[13] showing that vitamin A esters are more stable than free vitamin A when fish oils are aerated, is of interest in connection with these results. The effect of xanthophyll can probably be explained on the basis of the nonspecificity of the enzyme that results in the gastro-intestinal destruction of considerable quantities of the ingested xanthophyll, thereby sparing corresponding amounts of vitamin A or carotene. It is possible that, with certain human dietaries and animal feeds supplying vitamins A and E in suboptimum quantity, the protective effect of xanthophyll may be of practical significance.

Summary. Vitamin A-deficient rats receiving a daily supplement of 1 μg of vitamin A alcohol with fresh lard in the absence of added α-tocopherol lost weight rapidly and 100% of the animals died. Carotene fed under the same conditions at a level of 2 μg/rat/day produced fair growth and a mortality rate of only 14.3%. Intermediate results were obtained with vitamin A acetate. The addition of xanthophyll apparently decreased the gastro-intestinal destruction of carotene, vitamin A alcohol, and vitamin A acetate and, thus, enhanced the response to the 3 vitamin A sources at the levels fed.

[13] Hickman, K. C. D., *Ind. and Eng. Chem.*, 1937, **29**, 1107.

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Use of Dubos Medium for Culture of *M. tuberculosis* from Sputum.

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The liquid culture medium described by Dubos[1] can initiate rapid growth of *M. tuberculosis* from small inocula and maintain this growth in diffuse form. These media may offer, therefore, a great advantage for detection of *M. tuberculosis* in pathological material. Dubos tried his culture method on sputum (liquefied by sodium hydroxide), but concluded that "there is no indication that in its present form, the medium can be utilized with advantage for diagnostic work." At about the same time, Foley[2] reported successful culture of *M. tuberculosis* on Dubos medium from 165 pathological specimens (among them 31 sputa); thus demonstrating the suitability of the new medium for routine diagnosis. However, Foley emphasized the handicaps of some routine culture methods as applied in the use of Dubos media; "the digestion even for as short a period as 15 minutes with hydrochloric acid is liable to sterilize a specimen which contains only a few bacilli." "Little advantage results from the use of sulfadiazine to eliminate contaminating organisms in the specimens inoculated without digestion." It should be remembered that other authors, for instance Blacklock,[3] have studied the sterilizing effect of the alkaline digestion of sputa. Thus, the "digestion" (concentration) and the considerable elimination of contaminating bacteria without damage for acid-fast organisms, seem to be the essential condition for successful use of Dubos media in routine diagnosis.

Experimental. We have found in our pre-

[1] Dubos, R. J., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 361; Dubos, R. J., *J. Exp. Med.*, 1946, **83**, 409.

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

[3] Blacklock, J. W. C., *Med. Res. Council Rep.*, 1932, p. 5.

liminary experience with sputa mixed with a small number of *M. tuberculosis* organisms that the treatment of these sputa for 4 hours at 37° with a 2.5% solution of ammonium carbonate (1) does not sterilize even very small inocula (10^{-5} mg of an avian strain), (2) destroys many Gram-negative organisms, (3) liquefies the sputum readily (with few exceptions which require longer incubation and frequent shaking) and after centrifugation leaves a small volume of sediment, (4) promotes the rapid decomposition (with formation of ammonia) of ammonium carbonate and its neutralization by mucus thus rendering unnecessary its neutralization with acid which is required after digestion with sodium hydroxide.

To supplement by some other factor the relatively weak effect of ammonium carbonate on contaminating bacteria a solution of sodium penicillin in water was added to a concentration of 0.5 to 2 units per ml. Such amounts do not influence the growth of *M. tuberculosis* as was established by Abraham *et al.*[4] and confirmed in our comparative experiments on Dubos media with and without penicillin, inoculated with human or avian *M. tuberculosis* organisms, (laboratory strains). The addition of penicillin suppressed more or less completely the growth of contaminating organisms in cultures.

Methods. Cubes of ammonium carbonate ($NH_4HCO_3NH_2CO_2$) are used for preparation of 2.5% solution every 2 or 3 days. The solution is not stable.

The Dubos medium is prepared according to the formula: KH_2PO_4—1.0 g, $Na_2HPO_4 \cdot 12\ H_2O$—6.25 g; Na citrate—1.5 g, Mg $SO_4 \cdot 7\ H_2O$—0.6 g; Tween 80 5 ml of 10% solution; casein hydrolysate (N. Z Amine, type B, Sheffield) 20 ml of 10% solution; water to 1000 ml. The medium is adjusted to pH 6.8 and autoclaved for 15 minutes at 15 lb pressure. It becomes later slightly more alkaline by traces of ammonium carbonate in the inoculum.

Glassware. The medium is distributed in amounts of 5 ml in test tubes, 25 mm diameter. A 5% solution of bovine albumin (serum fraction V. Armour Laboratories) in 2% NaCl is sterilized by filtration, heated for 30 minutes at 56° and added in amounts of 0.2 or 0.3 ml to each tube.

The diluting fluid of Dubos consists of 2% glucose and 0.2% Vegex (we used up to 1% solution of Vegex) and is sterilized by autoclaving.

The penicillin solution is prepared by dissolving 100,000 units of sodium penicillin in 0.85% NaCl solution and preparing a 1:1000 dilution. Since the potency of penicillin in high dilutions decreases rapidly during storage, it is advisable to prepare frequently fresh dilutions from the stock solution.

Technic. 5 to 10 ml of the sputum were transferred from its container with some warm solution of ammonium carbonate (warmed to 60° in order to start production of ammonia) into a 50 ml centrifuge tube to which more solution was added so as to nearly fill the tube. The tightly plugged tubes were incubated at 37° for 4 hours, shaken every hour and centrifuged. The sediment was mixed with an equal volume of diluting fluid and inoculated in amounts of 1 to 3 ml into tubes containing each 5 ml of Dubos synthetic medium with 0.2 or 0.3 cc of albumin solution. Penicillin solution was added at the rate of 0.5 to 2 units per ml (the smaller amount was used when a solution was freshly prepared).

The inoculated tubes were incubated at 37°, smears from the cultures were prepared and examined in most series on the 8th and on the 15th day of incubation while in other series, on 10th to 12th days. Transplants of positive cultures to Loeffler's medium were made for eventual diagnosis of saprophytic bacteria in about half of the cultures. No saprophytic acid-fast bacilli were found.

Results. We have cultured 400 specimens of sputa from clinical patients sent for routine smear examination to the Diagnosis Laboratory of the New York City Department of Health. Only specimens containing an excess over that sufficient for the routine examinations were available for our experiments. Thus, the selection of the material

[4] Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. J., *et al.*, *Lancet*, 1940, **2**, 176.

was not based on any clinical criteria. We have obtained 116 positive cultures, 81 within 8 days and 35 within 9 to 15 days.

(a) Microscopic appearance: The beginning of the growth manifested itself by the presence either of "nests" of 2 or 3 short acid-resistant slender bacilli bunched closely together or of very long slightly S-shaped, thick acid-resistant organisms. In the latter shape, the organisms formed large clusters and differed in appearance from the bacilli scattered diffusely all over the microscopic field.

(b) Contamination: If sufficient penicillin had been added to the medium the microscopic preparation presented a diffuse light blue background with a few contaminants scattered through the field; after transfer of such cultures to nutrient broth the contaminants grew sparingly or not at all. In several cultures, the staphylococci, streptococci and other cocci appeared intact, but in very small numbers and showed little or no growth after transfer of the cultures into broth. Some Gram-negative bacilli and molds were quite abundant in cultures but never obscured the presence of acid-fast organisms.

(c) Comparison of the results of sputum culture and routine microscopic examination of sputum concentrate. A large portion of each sputum used for comparison in these experiments had been concentrated in the routine laboratory treatment with an equal volume of tergitol and sodium hypoclorite and the sediment after centrifugation examined microscopically. We have compared the results of this method with our culture experiments. In no case was a sputum positive by microscopic examination (82 specimens) and negative in culture. On the other hand, somewhat more than 8% of all sputa were found negative on microscopic examination and positive by the culture method. Nearly all sputa positive on microscopic examination (70 out of 82 or 85.3%) yielded positive cultures within 8 days and only in 12 cultures (14.7%) were the organisms detected on further incubation. On the contrary of 34 sputa positive by culture alone only 11 (32.5%) produced detectable growth within 8 days and 23 (67.5%) within 10 to 15 days. These data suggest that the specimens positive on microscopic examination contain relatively large numbers of organisms and that this method is inefficient for sputa with small numbers of organisms which may be, however, detected by culture in Dubos medium. In our final experiments we have mixed known numbers (10^{-4} mg) recently isolated human strains with negative sputa and treated them by the above described method; in all cases positive cultures were obtained in Dubos medium with penicillin.

Summary. The use of a mild reagent (2.5% solution of ammonium carbonate) for homogenization and concentration of tuberculous sputa and the addition of penicillin (0.05 to 2 units per ml) to the culture medium permits the successful application of Dubos medium to the routine culture of *M. tuberculosis* from sputum. Of 400 examined sputa, 34 (8.5%) were positive by culture method and negative by microscopic examination. The advantage of the culture method is obvious. As compared with other culture methods, the use of Dubos media is inexpensive, easily learned by an average technician, suitable for daily examination of a large number of specimens, and what is more, offers the advantage of a rapid (within 8 to 15 days) culture diagnosis of sputa containing relatively small numbers of *M. tuberculosis.*