ment of the complement complex in the course of specific inactivation by the recommended procedures.

It is pertinent to report that the corresponding components of rabbit and human complements have also been found to be mutually substitutive.[8]

Since submitting this article we have become acquainted with an excellent paper by O. G. Bier, G. Leyton, M. M. Mayer, and M. Heidelberger which appears in the May, 1945, issue of the *J. Exp. Med.* These authors demonstrate that corresponding components of guinea pig and human complements are mutually substitutive; and emphasize that testing reagents for complement components must contain an excess of the desired components and must be used in dilutions which ensure absence of anti-complementary effects. Further, though it came to our attention too late for incorporation in the present paper, the suggestion of Bier *et al.* that C′ component titers be expressed solely in units is constructive, and will be observed in our subsequent publications.

[8] Dozois, T. F., Seifter, S., and Ecker, E. E., in preparation.

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Rapid and Submerged Growth of Mycobacteria in Liquid Media.

RENE J. DUBOS.

From the Rockefeller Institute for Medical Research, New York City.

Attempts to obtain rapid and diffuse growth of virulent tubercle bacilli have led us to recognize that certain complex lipids exert a remarkable stimulatory effect on the multiplication of mycobacteria. Particularly striking results have been obtained with the following materials (a) phosphatide fractions prepared from egg yolk, cattle brain, human erythrocytes and soya bean.* (b) synthetic non-ionic surface active agents consisting of esters of long chain fatty acids and of polyhydric alcohols.†

The growth promoting effect of these substances has been tested with 5 strains of saprophytic mycobacteria, 5 virulent avian strains, 1 bovine strain (Ravenel) and 2 human strains (H37 RV and Jamaica No. 22). The results indicate that the different groups of mycobacteria (saprophytic, avian, bovine, and human) differ greatly in their optimal requirements with reference to the lipids mentioned above; on the other hand, within one given group, the different strains tested have exhibited a striking similarity of behavior. The avian strains are only little affected by the phosphatide preparations but are greatly stimulated by the fatty acid esters; on the contrary the bovine and human strains respond remarkably well to the different phosphatides but are inhibited by minute concentrations of the non-ionic surface active agents. The following table illustrates the composition of satisfactory media prepared by adding to Long's medium different concentrations of soya bean phosphatide and of a polyoxyalkylene derivative of sorbitan monostearate (Tween 60).‡

Mycobacteria can be made to grow in the lipid media without the necessity of floating

* The brain and red cell phosphatides were prepared by Dr. Jordi Folch-Pi. The soya bean phosphatide was obtained through the courtesy of Dr. A. Scharf of Associated Concentrates, Inc., Atlanta, Georgia.

† A variety of these synthetic non-ionic surface active agents are produced by the Atlas Powder Company, Wilmington, Delaware, and by the Glyco Products Co., Inc., Brooklyn, N. Y. Although the present report deals only with the product marketed under the name of Tween 60 by the former company, similar results have been obtained with a number of other long chain fatty acid esters of polyhydric alcohols.

‡ Tween 60 is directly dispersible in water. The soya bean phosphatide was dissolved in ether and dispersed from ether solution into water. Both these products can be autoclaved with Long's medium.

TABLE I.

Strains of Mycobacteria	Long's medium (2% glycerine or 0.5% glucose). +									
Saprophytic	0	-0.05%	Soya	bean	phosphatide	+	0.02-0.2	%	Tween	60
Avian	0	-0.05%	"	"	"	+	0.02-0.1	%	"	"
Bovine and Human	0.01-0.05%		"	"	"	+	0	-0.002%	"	"

the inoculum on the surface of the fluid as is the usual practice with these organisms. The cultures grow readily throughout the fluid and can be transferred repeatedly into the same media. Addition of 0.1 cc of culture to 10 cc of new medium is sufficient to secure growth of the saprophytic and avian strains within 24 hr, and of the bovine and human strains within 72 hr; growth is slower with smaller inocula.

We have also established that the addition of purified serum albumin (0.1% or less) to these liquid media further enhances the growth of tubercle bacilli and permits in particular more rapid multiplication of small inocula.

The cultures obtained under these conditions (with and without serum albumin) are typical in morphology and staining characteristics. When returned to the classical Long's medium (in the absence of the lipid fractions), they manifest again their typical mode of growth, developing slowly, in the form of heaped masses, on the surface of the medium.

Summary. Addition to Long's synthetic medium of small amounts of phosphatides and of long chain fatty acids esters of polyhydric alcohols permits submerged and rapid growth of tubercle bacilli; the different groups of mycobacteria appear to exhibit differential optima with reference to these two types of substances.

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The Microtechnical Demonstration of Sites of Lipase Activity.*

GEORGE GOMORI.

From the Department of Medicine, the University of Chicago.

It is known that lipase is not destroyed by acetone dehydration; in fact, the enzyme is usually prepared from acetone-dried tissues. Nor is it too sensitive to heat; in preliminary experiments no appreciable decrease in activity was observed when acetone-dried pancreas powder was kept at 62°C for 2 hr. On the basis of these facts it appeared feasible to demonstrate lipase in paraffin sections by following the principles of the microtechnical demonstration of phosphatases.[1-3] These include the incubation of the slides with an ester, known to be split by the enzyme, in the presence of a salt the metal ions of which form an insoluble salt with the liberated acid and in this way trap the ions of the acid *in situ*. However, when it was attempted to put these principles into practice, great difficulties were encountered. The substrate must meet three requirements which, for a long time, seemed to be mutually exclusive. First, it must be hydrolyzed by lipase; second, it must be water-soluble since lipase is greatly inhibited even by traces of non-aqueous solvents such as alcohols or ketones;[4,5] third, it must form insoluble salts with some metal, non-toxic to the enzyme, such as Ca or Mg. Monocarboxylic acids which have water-soluble esters (up to

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