dietary supplement of PGA. Brain and kidney tissue slices from rats receiving a high dietary level of PGA respired at the same rate as corresponding tissues from control rats, with or without added glutamic acid.

ADDENDUM: After this manuscript was submitted, an article appeared (Grossowicz, N., *J. Biol. Chem.*, 1948, **173**, 729) which reported that glutamine inhibited the growth-promoting effect of glutamic acid for *Staphylococcus aureus*, and and that this inhibitory effect of glutamine could be overcome by pteroylglutamic acid, by glutathione, or by extra glutamic acid. These results apparently indicate that under certain circumstances pteroylglutamic acid and glutamic acid may function interchangeably in the nutrition of micro-organisms.

16362 P

Effect of Tween 80 on Certain Strains of *C. diphtheriae.*

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In attempts to prepare a fluid synthetic medium for general use in diphtheriology, difficulty was encountered in that recently discovered minimus strains[1,2,3] and a few, closely related, minute-colony strains, failed to grow in a medium suitable for most other strains. The synthetic medium used consists of amino acids, inorganic salts, carbohydrates and growth factors in as pure form as could be obtained. It has no peptone, casein or other constituents of variable or unknown composition. Details of the medium will be published elsewhere.

The minimus strains have also uniformly failed to grow well in infusion broth. The granular nature of growth of the minimus strains in infusion broth suggested the possibility that a surface tension reducent might facilitate growth, following the line of thought suggested by Dubos *et al.*[4,5,6] in their studies of the tubercle bacillus. Accordingly, Tween 80 was added aseptically from a freshly prepared, sterile, aqueous solution, in a concentration of about 0.05%, to the synthetic medium just before inoculation. Immediate and striking stimulation of growth of the minimus-type strains was observed. Concentrations of Tween up to about 0.5% have since been used with no apparent inhibition of the minimus type. No optimum or inhibitory range has yet been determined.

With other types of diphtheria bacilli, the results of adding Tween 80 to the medium are somewhat variable. Some strains, particularly of the mitis type, appear slightly inhibited; others show little or no difference in growth response to Tween. Gravis and gravis-like strains which normally grow with pellicle formation, lose their pellicle and grow diffusely throughout the medium. No types other than minimus are stimulated to any great degree, with the exception of a few minute-colony forms sent by Dr. McLeod from Leeds sometime ago. These strains were designated by McLeod as the intermedius type. It is of interest that the 4 strains of this group which Frobisher found to be identical with minimus type[3] were more markedly stimulated by addition of Tween than were the other 6.

Similar stimulation of growth of the mini-

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1 Eller, C. H., and Frobisher, M., Jr., *Am. J. Hygiene*, 1945, **42**, 179.

2 Frobisher, M., Jr., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 330.

3 Frobisher, M., Jr., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 304.

4 Dubos, R. J., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 361.

5 Dubos, R. J., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 56.

6 Dubos, R. J., and Davis, B. D., *J. Exp. Med.*, 1946, **83**, 409.

mus-type strains was observed when Tween 80 was added to tubes of heart-infusion broth. The difference in growth resulting from addition of Tween 80 to this medium was not so striking as in the synthetic medium, since growth of the minimus strains is normally better in heart-infusion than in synthetic medium. Again, the results with strains of other types were variable. None, other than minimus type, was definitely stimulated, with the exception of the minute-colony forms noted above.

Plates of McLeod's chocolate agar were prepared; half with about 0.3% Tween, and half without. A small loopful of 24-hour broth culture of various types of diphtheria organisms was streaked on both media. Results of this experiment paralleled those obtained with liquid media. Minimus colonies were markedly increased in size, from less than 0.1 mm diameter on plates without Tween to diameters of 2.0 to 3.0 mm on the plates with Tween added. Colony size of other types was variable, the intermedius strains of McLeod being the only ones which showed any significant increase in diameter (0.1 mm to 1.5 mm).

It has been repeatedly observed in this laboratory[7] that at least 1 ml of 48-hour culture of the minimus type organisms is required to kill guinea pigs and that even that dose kills irregularly; whereas, the lethal dose of the gravis and mitis types approximates 0.2 ml or less. Though the minimus strains grow more sparsely than do the other types, after seeing the broth cultures it is a little difficult to accept the idea that this difference in lethal dose is due to *numbers* of organisms alone. Therefore, 48-hour broth cultures of a minimus strain, grown with 0.05% Tween, and in the same medium without Tween, were injected intradermally into duplicate guinea pigs in amounts of 0.2, 0.5, and 1.0 ml. All animals receiving the Tween culture were dead within 72 hours. Autopsy showed typical signs of diphtheritic deaths. Only one pig with the culture without Tween died, and that was one of the two which had received 1.0 ml of culture. All other experimental and control animals survived without evidence of disease for more than two weeks. This experiment had been repeated in part with a second minimus strain, with the same results.

In alcohol-ether and acetone fractionation of the gravis, mitis and minimus types, Parsons[8] found that the minimus strains contained more lipid material than did gravis or mitis strains. This suggests a relationship of wetting agent (Tween 80) to the lipid-rich minimus type, analogous to the relationship of the same wetting agent to the lipid-rich tubercle bacilli, shown to exist by Dubos *et al.* The surface tension of the synthetic medium was found to be relatively high. Tween 80 reduces this surface tension markedly. That the surface tension of the heart-infusion without Tween is considerably lower than that of the synthetic medium might partially account for the normally better growth of the minimus strains in heart-infusion broth.

The role of Tween as a non-toxic source of oleic acid must also be considered in view of the work of Cohen *et al.*,[9] who found oleic acid to be one of two growth factors, present in serum and milk, essential for a gravis strain when very minute inocula were used on solid medium. No statement was made by these authors as to the effect of these factors in liquid media.

Summary. It has been observed that the addition of Tween 80 to solid and liquid media markedly stimulates the growth of minimus type diphtheria bacilli. This suggests a relationship between this wetting agent and the lipid-rich minimus organisms analogous to the relationship of the same agent to the lipid-rich tubercle bacilli, demonstrated by Dubos *et al.* There is also evidence that Tween 80 enhances the virulence of the minimus strains.

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[7] Frobisher, M., Jr., and Parsons, E. I., unpublished data.

[8] Parsons, E. I., unpublished data.

[9] Cohen, S., Snyder, J. C., and Mueller, J. H., *J. Bact.*, 1941, **41**, 581.