specific precipitation, in the absence of cellular extracts.

The effects recorded with artificial antigens, as described here, indicate the need for control of many conditions before either precipitins or specific precipitins can be related to biological causes. Furthermore, the well-known variability in immune response, even from the same organism at different times(12), may stem in part from the operation of such factors. With increasing knowledge of protein precipitation(13), it may be expected that the variability which has so often been noted in the immunochemical literature (*e.g.*, 2,12) may also be greatly diminished.

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Summary. Conditions have been studied for the simulation of specific precipitins. The factors which have been shown to operate may explain earlier reports of antibody production from yeast. It is suggested that some of the variability observed in conventional immunological test may have, at least in part, a similar basis in the effect of conditions of the tests upon the precipitability of the proteinaceous antigens.

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Nutritional Requirements of Clostridium Tetanomorphum. (20657)

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This study was undertaken to determine the nutritional requirements and general metabolic activities of an anaerobic bacterium listed in the Stanford Bacteriology Department collection as strain E-42 of *Clostridium cochlearium*. It was received from Dr. I. C. Hall in 1930 and at that time was designated by him as *Clostridium putrificum*, strain No. 3379. This strain was later identified as *C. cochlearium* by Hartsell and Rettger(1). During the course of our studies on the metabolism of E-42 it became evident that this species is capable of fermenting glucose (or maltose) and produces butyric, acetic, and lactic acids, carbon dioxide, and hydrogen from this sugar. In addition we observed that this strain ferments pyruvic and glutamic acids with the production of the products listed above, together with ammonia from glutamic acid, in approximately the same amounts as reported by Woods and Clifton(2) for *Clostridium tetanomorphum*. Threonine and ser-

ine were the only other amino acids fermented at an appreciable rate as evidenced by gas production. These observations indicate that strain E-42 as it is now constituted is a strain of *C. tetanomorphum* rather than of the closely related species, *C. cochlearium.*

Materials and methods. In the growth studies a salt solution consisting of 11.5 g of $Na_2HPO_4 \cdot 12\ H_2O$, 1.0 of KH_2PO_4 and 0.1 of $MgSO_4 \cdot 7\ H_2O$ per l of distilled water plus 2 ml of a trace element solution (Snell, *et al.,* 3) served to supply the inorganic requirements of E-42. Growth occurred in this solution at pH 7.4-7.6 when growth factor and amino acid requirements were supplied by 1% yeast extract and casein hydrolysate respectively, and reducing conditions were developed with 0.1% sodium thioglycollate. The addition of glucose or maltose supported heavier growth, and 0.5% glucose was employed in the test media. In the preliminary studies various growth factors were added in appropriate amounts to the medium described above from which yeast extract was omitted and to which 10 mg each of L-trptophan and L-cystine were added per l to make up for any possible deficiency of these amino acids. The various mixtures were autoclaved and 9.5 ml amounts were distributed aseptically into sterile test tubes. The fluid in each tube was covered with a 2 to 3 mm layer of sterile mineral oil. Before inoculation the tubes were heated in a boiling water bath for 15 min. to expel any dissolved oxygen and then rapidly cooled. Five-tenths ml of an inoculum comprising well-washed cells from 17 to 20 hr cultures was introduced into each tube and the development of turbidity on incubation at 37°C was observed. The amino acid requirements were determined in the same manner, one amino acid in turn being omitted from the basic medium to which no casein hydrolysate had been added. Finally growth experiments were conducted in the basal salt-glucose-thioglycollate medium to which only known amino acids and growth factors were added.

Results and discussion. Little or no growth occurred when either nicotinic acid (or its amide) or pantothenic acid was omitted from the medium and the crop yield was greatly decreased in the absence of biotin. This latter behavior was studied further and it was found that the sample of casein hydrolysate employed contained a trace of biotin. No growth occurred in the absence of biotin when a biotin-free casein hydrolysate was employed as the amino acid source. Choline, *p*-aminobenzoic acid, and thiamine appeared to have a slight stimulatory action on growth but had little or no influence on crop yield.

Little or no growth occurred when either L-glutamic acid or L-tryptophan was omitted from the basal medium devoid of casein hydrolysate. Tyrosine, leucine, and phenylalanine appeared to promote growth to a slight extent but were not essential for growth of E-42.

In further studies it was observed that fair growth of *C. tetanomorphum* occurred in the basal salt-thioglycollate solution containing 0.5% of glucose, maltose, or sodium pyruvate and, in amounts per 100 ml, 75 mg L-glutamic acid, 25 mg L-tryptophan, 20 μg each of nicotinic acid and calcium pantothenate, and 1 μg of biotin.

Summary. Strain E-42, presently identified as a strain of *C. tetanomorphum,* is exacting in its nutritional requirements. Growth will occur in a basal salt-glucose-thioglycollate medium only following the addition of pantothenic and nicotinic acids, biotin, L-glutamic acid, and L-tryptophan. *p*-Aminobenzoic acid, choline, thiamine, leucine, tyrosine, and phenylalanine may exert slight stimulatory action on growth but are not essential for growth of this organism.

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