dystrophic from antisterility activity is discussed with relation to the function of vit. E in muscular dystrophy.

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The Precipitability of Two Modified Proteins.* (20656)

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Precipitin-type reactions, exhibiting specificity between iodinated proteins and arsonobenzeneazoproteins, have been reported for extracts of *Saccharomyces cerevisiae* repeatedly cultured in the presence of such modified proteins(1). From extended attempts to extrapolate this work, it has been learned that such precipitates may be obtained from complex combinations of experimental conditions, in the absence of the yeast. The specificity which was originally observed is believed, therefore, to be non-biological in all or most of the cases studied. That a major factor contributing to the observed precipitin-like reactions and their variation may have been pH was suggested by the fact that precipitin-like behavior was frequent during some periods of experimentation, yet infrequent in others. After many such periods, the former type appeared to be correlative with higher acid content in the samples of ether employed in cytolysis. The finding that some cultures yielded more reactive extracts was probably due to fortuitous acid content rather than to a parallelism between behavior of yeast and the long-known erratic antibody-producing power of animals(2).

Haurowitz(3) has shown that reported instances of precipitin reactions from *in vitro* incubation of serum globulin with acid azoovalbumin(4) is ascribable predominantly to charge effects. In the case of the yeast cultures, however, the more rigorous test of specificity of reaction appeared to be met. The results reported in this paper suggest that such results may appear when the interplay of conditions leads to overlapping, yet in part discrete, pH regions of precipitability for two or more antigens. In the course of these studies, attention was particularly focussed upon the pH factor by the related finding that evaluation of specificity of protease-substrate interaction was dependent upon pH to a degree much greater than that earlier recognized (5-9). In both types of situation, other factors than pH contribute to the effects observed.

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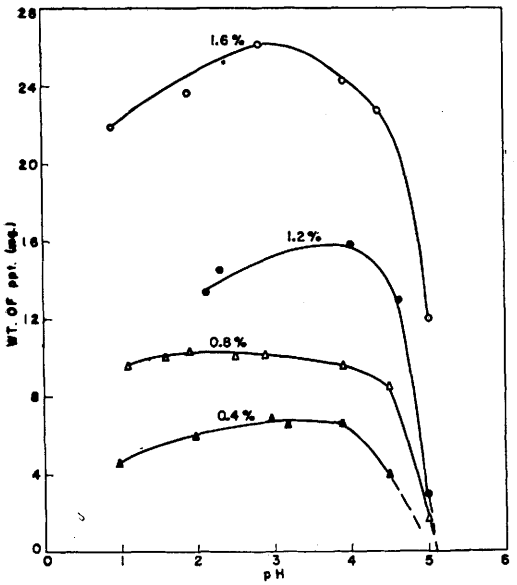

FIG. 1. Precipitation range for iodocasein solutions of different concentrations.

Materials and methods. The iodocasein was prepared by adding a solution of 2.0 g of powdered iodine in 50 ml of 10% potassium iodide solution to a solution of 25 g of Smaco casein in 1 l. of 1 N sodium carbonate solution, allowing the mixture to stand overnight, neutralizing with hydrochloric acid to maximum precipitation, and washing with water. Found: 1.04% I, calculated to a dry weight basis. For testing, the iodocasein was dissolved in 1 N sodium hydroxide solution and the liquid was neutralized with 1 N hydrochloric acid. The arsonobenzeneazocasein was prepared by the method of Boyd and Mover (10) in sodium carbonate solution. Found: 2.0% As, calculated to a dry weight basis. Solutions were prepared in the same manner as for the iodocaseins.

For the *results* of Fig. 1 and 2, concentrations of the iodocasein and arsonoprotein solutions were varied as indicated. In each test, 1 ml of solution of modified protein was diluted with 5 ml of 5% sodium chloride solution and the pH was adjusted with concentrated sulfuric acid added from a micropipet. Precipitates were washed with water, allowed to dry in air, and weighed.

In Fig. 3 the effect of hydrochloric acid may be observed, and is seen to result in curves different from those obtained with sulfuric acid. Although variation in shape of the curve has been observed with each acid, each is characteristic in its behavior.

Discussion. Aside from the effects of protein concentration, pH, and acid type; salt concentration, salt type, and other proteins in the system (3) may be expected to influence the response. Any added protein, such as yeast globulin, might therefore modify the results observed. Indications that conditions of storage and of incubation affect such precipitations have also been noticed.

The exact conditions of Fig. 3 would suggest more frequent precipitation of iodoprotein than of arsonoprotein, whereas the opposite has been recorded (11). The results presented, however, indicate that the phenomena described permit essentially the simulation of

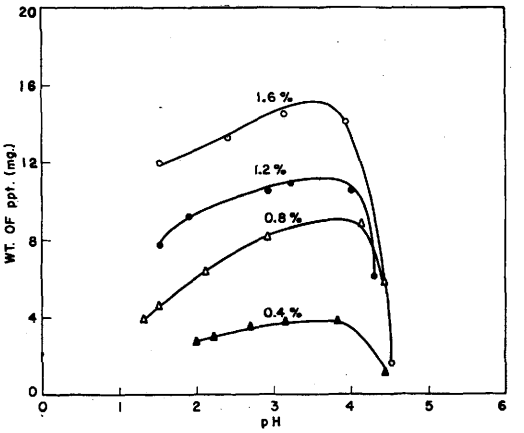

FIG. 2. Precipitation range for arsonobenzeneazocasein solutions of different concentrations.

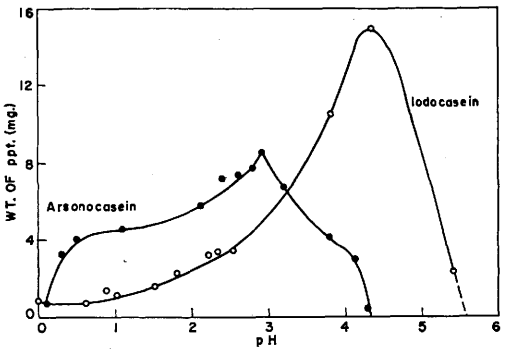

FIG. 3. Relatively discrete pH ranges of precipitation for iodocasein (1.0%) and arsonobenzeneazocasein (1.2%).

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.

Marrack, J. R., and Grant, R. A., (*Brit. J. Exptl. Path.*, 1953, v34, 263) record and discuss effects of salt concentration and pH on antigen-antibody reaction.

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-