Although no experiments were performed to demonstrate the character of this exchange, the authors feel that it must certainly be heterogeneous, probably through a mechanism involving the simultaneous transfer of hydrogens in hydrogen bonds within groups of N_2H_4–ND_3 molecules. This explanation must not be too hastily accepted, however, because the absence of any pronounced super-conductivity of the hydrogen ion in hydrazine systems,[2] and the lack of tendency for ammonia and hydrazine to form mixed crystals[3] argue against such a mechanism. The effect of small amounts of water on the formation of mixed crystals[3] suggests that the observed exchange may have been due to water catalysis, although considerable care was taken to ensure the absence of water in the above experiments. Further experiments are desirable before more definite conclusions concerning the mechanism are attempted.

These observations were made during the course of a study of exchange reactions with deuterium which was supported by a grant from the Carnegie Institution of Washington.

(2) P. Walden and H. Hilgert, *Z. physik. Chem.*, **A165**, 241 (1933).
(3) F. Friedrichs, *Z. anorg. allgem. Chem.*, **127**, 221 (1923).

DEPARTMENT OF CHEMISTRY
STANFORD UNIVERSITY
STANFORD UNIVERSITY, CALIF.
RECEIVED FEBRUARY 18, 1938

Preparation of Barium Chlorite and Solubility of Silver Chlorite

BY W. V. SMITH, K. S. PITZER AND W. M. LATIMER

Our attention has been called to the omission of two references which might properly have been included in our paper on silver chlorite.[1]

Bruni and Levi[2] prepared pure barium chlorite and Levi[3] reported values for the solubility of silver chlorite, which are in close agreement with our value at 25°.

(1) Smith, Pitzer and Latimer, THIS JOURNAL, **59**, 2640 (1937).
(2) Bruni and Levi, *Gazz. chim. ital.*, **45**, II, 169 (1915).
(3) Levi, *ibid.*, **53**, 525 (1923).

DEPARTMENT OF CHEMISTRY
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RECEIVED FEBRUARY 10, 1938

COMMUNICATIONS TO THE EDITOR

BENTONITE AS AN ADSORBENT IN THE PURIFICATION OF INVERTASE[1]

Sir:

In the course of investigations on invertase we have developed, in the preparation of this enzyme, certain procedures which may be of value not only in connection with invertase but also in regard to other problems in biochemistry. Bentonite, a colloidal clay already well-known commercially, has been found to be an excellent adsorbent for invertase. Bentonite can be used, without preliminary treatment, in the undiluted autolysates from yeast. Both adsorption and elution can be carried out under conditions more favorable for the stability of invertase than those generally used with other clays. The optimal *p*H for adsorption is 4.1–4.3 while an acetate or phosphate solution of *p*H 5.3 or greater produces satisfactory elution. The amount of bentonite required for complete adsorption is relatively small and the five different samples of bentonite so far investigated have all proved excellent adsorbents for invertase, yielding preparations of similar time values.

Invertase solutions with time values of 0.20–0.27 minute as expressed in the customary units[2] have been obtained by dialysis following a single bentonite treatment of various types of autolysates from bakers' yeast. Similar solutions have been prepared from unenriched brewers' yeast by a slight modification involving fractional adsorption on bentonite with 10–20% adsorption and loss in the first fraction. From enriched brewers' yeast (*i. e.*, yeast which has been allowed to ferment a sucrose solution) preparations have been obtained with time values of 0.15–0.18 minute. The invertase solutions thus prepared do not lose activity during dialysis or subsequent storage over a period of several months in the refrigerator.

The following describes a typical procedure. A fractional autolysate of bakers' yeast was prepared by treating 430 g. of yeast (time value, 34.3) at 30° with 43 cc. of ether, adding 43 cc. of toluene,

(1) Publication authorized by the Surgeon General, U. S. Public Health Service.

(2) C. Oppenheimer, "Die Fermente und ihre Wirkungen," fifth edition, 1928, Vol. III, pp. 770–774.

430 cc. of water and 3.2 g. of sodium carbonate after the yeast had liquefied, and four hours after the addition of the ether filtering through filtercel with 80 g. of filtercel added to the mixture before filtration. The filtrate containing 7.7% of the invertase was discarded. To the residue was added 43 cc. of toluene and 430 cc. of water and autolysis was continued for five days at 20°. After filtration this autolysate was dialyzed immediately in Visking sausage casings. To a mixture of 80 cc. of 0.5% bentonite suspension and 27 cc. of a solution of *p*H 4.1 prepared by mixing 1 *N* acetic acid and 1 *N* sodium hydroxide, was added 265 cc. of this dialyzed autolysate which contained 7.53 units[2] of invertase per 100 cc. and had a time value of 2.24 minutes. The bentonite was separated by centrifuging, washed by stirring with 200 cc. of distilled water and again centrifuged. Ninety-two per cent. of the invertase was adsorbed. Elution was effected by shaking gently with three portions, 40, 30, and 20 cc., respectively, of an acetate solution of *p*H 5.7 prepared from mixtures of 0.1 *N* acetic acid and 0.1 *N* sodium hydroxide solutions. The three extracts represented 57.8, 13.2, and 3.6% of the invertase in the original autolysate and after dialysis had time values of 0.216, 0.215, and 0.278 minute and contained 10.5, 2.26, and 0.64 units, respectively.

NATIONAL INSTITUTE OF HEALTH MILDRED ADAMS
WASHINGTON, D. C. C. S. HUDSON

RECEIVED MARCH 17, 1938

ZINC SULFIDE AS AN ADSORBENT IN THE PURIFICATION OF INVERTASE[1]

Sir:

In the preceding communication from this Laboratory, by Mildred Adams and C. S. Hudson, was described a method for purifying invertase solutions by adsorption on and subsequent elution from the colloidal clay "bentonite." A second excellent adsorbent has been found in zinc sulfide when precipitated directly in a solution of invertase under certain conditions; the resulting eluted and dialyzed enzyme solutions are of the same purity and stability as those obtained with bentonite. Adsorption of the invertase is carried out in an acetate buffer at about *p*H 4.4, and elution is effected with an ammonium phosphate buffer of *p*H 6.1; the solutions contain 1% sodium chloride to prevent the zinc sulfide from becoming colloidal. A typical preparation is recorded.

(1) Publication authorized by the Surgeon General, U. S. Public Health Service.

A bakers' yeast of relatively high invertase content was allowed to autolyze fractionally in the manner described in the preceding communication, and the first fraction discarded. The main autolysate was dialyzed in Visking sausage casings, and then represented 60% of the original invertase in the yeast. To 1940 cc. of this solution, containing 110.2 invertase units, was added 1940 cc. of water, 43.5 cc. of a 10% zinc acetate solution, 160 cc. of a buffer solution of *p*H 4.5 (made by mixing 2 *N* sodium hydroxide and 2 *N* acetic acid), and 450 cc. of a 10% sodium chloride solution. Hydrogen sulfide was bubbled through the solution, and the zinc sulfide separated by centrifuging; the supernatant liquid had a *p*H of 4.4, and contained only 6% of the invertase. The zinc sulfide was washed by shaking with 1500 cc. of a 1% sodium chloride solution and again centrifuged. The invertase was eluted by shaking with 400, 200, and 100 cc. portions, respectively, of a solution containing 1% sodium chloride and 1% mono- and dibasic ammonium phosphates such that it had a *p*H of 6.1. The combined extracts, after dialysis, contained 77.6 invertase units, and had a time value of 0.20 minute.

Zinc sulfide has been used in similar fashion in purifying the dialyzed autolysates of brewers' yeast of relatively low invertase content. With these solutions a fractional adsorption with zinc sulfide is necessary, 15–25% of the invertase being discarded in the first portion; adsorption and elution as described then produced invertase solutions of time value 0.21–0.22 minute.

These communications represent only a portion of the studies we have been making on invertase, but the use of the adsorbents may be of sufficient interest in the general field of biochemical purifications to warrant their earlier publication.

NATIONAL INSTITUTE OF HEALTH NELSON K. RICHTMYER
WASHINGTON, D. C. C. S. HUDSON

RECEIVED MARCH 17, 1938

CRYSTALLINE VITAMIN B_6

Sir:

Vitamin B_6 is that part of the vitamin B_2 complex [*Nature*, **133**, 498 (1934); *Biochem. J.*, **29**, 741 (1935)] responsible for cure of the "rat acrodynia" observed in young rats fed a vitamin B free diet supplemented with vitamin B_1 and riboflavin.

It has been shown [*Biochem. J.*, **30**, 304 (1936)] that vitamin B_6 can be adsorbed by fuller's earth from acid solution, eluted with $Ba(OH)_2$ and pre-