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

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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-