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-

cipitated by phosphotungstic acid. In continuing this research, Peters' eluate [*Biochem. J.*, **27**, 225 (1933)] was used as a potent concentrate of B_6, in batches usually containing 10 to 50 thousand B_6 units. As the first step toward purification, vitamin B_6 was adsorbed on fuller's earth at *p*H 1.4, using 1 g. of earth to 10 units of B_6. Adsorption was repeated three times. The combined adsorbates were washed by grinding in a mortar with 2 ml. of 0.1 *N* hydrochloric acid per gram of fuller's earth used. The adsorbate was then eluted twice with 0.1 *N* barium hydroxide (12 ml. per gram of earth) by grinding in a mortar and letting stand overnight in the refrigerator. The filtrates were precipitated immediately with sulfuric acid and filtered. This filtrate was adjusted to *p*H 6.8 to 7 with 10% sodium hydroxide and evaporated to dryness. The residue was extracted five times with 95% ethyl alcohol, using 100 ml. each time, and the filtrate was evaporated to 50 ml. and treated with 450 ml. of ethyl acetate. After standing overnight in the refrigerator, the precipitate was filtered off and the filtrate evaporated to dryness. The residue was then taken up in 200 ml. of water, filtered, and the filtrate subsequently precipitated (1) with platinum chloride (activity remaining in the filtrate), then (2) with phosphotungstic acid (20% in 1 *N* sulfuric acid). The phosphotungstate was decomposed with barium hydroxide. The combined filtrates were precipitated with sulfuric acid, filtered, and the filtrate (neutralized with sodium hydroxide) evaporated to dryness. The residue was extracted several times with 95% ethyl alcohol to make 100 ml., filtered, and the extract precipitated with 400 ml. of ether and let stand overnight in the refrigerator. The ether was then filtered, evaporated, and the residue taken up in water. Activity of this concentrate corresponded to 20 to 100 γ of solids for one "rat day dose," with a total yield of 10 to 30% of the original strength. Repeated precipitation with phosphotungstic acid followed by regeneration gave aqueous solutions from which crystalline preparations were obtained having an activity of 5 γ per "rat day dose." Daily administration of 15 γ cured rat acrodynia in two weeks, of 5 to 10 γ in three to four weeks. Crystals were colorless rods of varying size, with rounded ends. They seemed to have a tendency to coalesce in rosets or fan-shaped formations.

The curative influence of these crystalline preparations was confined to disappearance of the specific skin symptoms. Growth was not promoted. Even the skin effect was not regularly attained unless a further supplement, corresponding to the so-called "filtrate factor" [*J. Biol. Chem.*, **114**, 109 (1936)], was added.

BABIES AND CHILDRENS HOSPITAL AND DEPARTMENT OF PEDIATRICS OF WESTERN RESERVE UNIVERSITY SCHOOL OF MEDICINE CLEVELAND, OHIO

PAUL GYÖRGY

RECEIVED FEBRUARY 21, 1938

THE PRODUCTION OF AN ANTIRACHITIC PROVITAMIN FROM CHOLESTEROL

Sir:

It has already been reported [F. C. Koch, M. E. Koch and Ragins, *J. Biol. Chem.*, **85**, 141 (1929); Waddell, *ibid.*, **105**, 711 (1934); Hathaway and Lobb, *ibid.*, **113**, 105 (1936); Haman and Steenbock, *ibid.*, **114**, 505 (1936)] that the natural antirachitic provitamin D may be related to cholesterol rather than to ergosterol, and Windaus, Lettre and Schenck [*Ann.*, **520**, 98 (1935)] actually prepared from cholesterol by a number of difficult steps 7-dehydrocholesterol which upon irradiation with ultraviolet light acquired strong antirachitic properties.

If the precursor of the animal antirachitic provitamin is cholesterol, then its formation in the body may result from the partial dehydrogenation of the latter under the influence of dehydrogenating enzymes or under that of light in the presence of hydrogen acceptors. Some time ago we decided to test this view chemically by allowing cholesterol acetate to react with equimolecular proportions of hydrogen acceptors in the presence or absence of dehydrogenating catalysts and of light.

When cholesterol acetate, spectroscopically free from the antirachitic provitamin, was allowed to react with benzoquinone in a sealed tube at 120–130° for about two hours and the product subsequently freed from quinhydrone, unconverted quinone, etc., it was found to contain substantial quantities of 7-dehydrocholesterol. The crude mixture was then irradiated in pure ethyl ether with a quartz mercury lamp for four hours and the resulting product assayed biologically for us by Professors Robert S. Harris and J. W. M. Bunker of this Institute. They reported an antirachitic potency of considerably more than 6500 U. S. P. vitamin D units per gram, whereas a blank with our purified cholesterol acetate had a