wt., 363); TNM and Br_2 tests negative; no absorption at 205–300 mμ; no infrared hydroxyl or carbonyl bands; characteristic bands (CS_2) at 8.90, 9.22, 9.85, 10.22, 10.65, 11.53, and 12.54 μ; proton resonance peaks at 82 and 94 cps. in $CDCl_3$ (referred to benzene resonance peak; no other peaks in the range 0–160 cps.) indicating two hydrogens attached to different oxygen-linked carbons. The oxide is stable to hydrochloric acid in refluxing methanol–dioxane. That cyclization is not attended with rearrangement of the ring system was established as follows. When heated with *p*-toluenesulfonic acid in acetic anhydride the oxide afforded an oily unsaturated acetate which on hydrogenation and hydrolysis yielded B-norcoprostane-6α-ol, m.p. 102.5–103.5°, $[\alpha]_D$ −20° (C, 83.32; H, 12.30). Oxidation gave the 6-ketone, m.p. 98–98.5°, $[\alpha]_D$ +34°, λ^{CS_2} 5.77 μ (C, 83.59; H, 11.79). An identical ketone was obtained from B-norcoprostane-3,6-dione by partial reduction with sodium trimethoxyborohydride to an oil containing predominantly the 3-ol-6-one. Conversion to an ethyleneketal mixture, oxidation, and chromatography on alumina gave B-norcoprostane-3,6-dione 6-ethyleneketal, m.p. 120–121°; $[\alpha]_D$ +50°, λ^{CS_2} 5.83 μ (C, 77.76; H, 10.83). Wolff–Kishner reduction of the ketol and hydrolysis gave B-norcoprostane-6-one, identical with the above sample (m.p., $[\alpha]_D$, infrared).

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A CRYSTALLINE DECARBOXYLASE WITHOUT BIOTIN

Sir:

During an investigation of the mechanism of decarboxylation[1] we have crystallized the decarboxylase for acetoacetic acid from *Clostridium acetobutylicum*. The microörganism (American Type Culture Collection No. 862) was grown and harvested and the crude enzyme prepared by a modification of the procedure developed by Davies[2] and Seeley.[3] The partially purified enzyme was comparable in activity to the best samples obtained by Davies. Approximately 25 mg. of this preparation in 3 ml. of solution was dialyzed against 0.05 *M* phosphate buffer at *p*H 5.9 and then placed on a column of diethylaminoethyl cellulose.[4] On gradient elution to 0.05 *M* phosphate (*p*H 5.9)–0.1 *M* ammonium sulfate, the enzyme was spread over several fractions; these were combined and precipitated with 60% saturated ammonium sulfate. The precipitate was suspended in 1 ml. of 0.05 *M* phosphate (*p*H 5.9) and, on standing at room temperature, microscopic hexagonal plates of the enzyme crystallized out. The enzyme can be recrystallized from a solution in 0.05 *M* phosphate (*p*H 5.9) by adding ammonium sulfate to about 50% saturation. The crystalline enzyme is 2 to 3 times as active as Davies' best preparation. In a Spinco model E ultracentrifuge, the protein proved monodisperse, and showed a sedimentation constant (extrapolated to zero concentration) of about 15. Its molecular weight is then presumably of the order of 300,000.

Samples of the enzyme were hydrolyzed with 6 *N* sulfuric acid or 4.5 *N* potassium hydroxide solution at 120° for two hours, and the hydrolysate assayed microbiologically[5] for biotin, using *Saccharomyces cerevisiae* (American Type Culture Collection No. 7754). Control experiments showed that the vitamin, as measured by this assay, was not extensively destroyed under the experimental conditions employed; the method gave results approximating those in the literature for biotin-containing tissues.[6] The assay with hydrolyzed enzyme gave results which did not differ significantly from "blanks." Assuming that the concentration of the enzyme could be determined from the absorption in the 260–280 mμ region and that the enzyme was hydrolyzed under the above conditions, the data show that the enzyme contains less than 0.01 molecule of biotin per 300,000 molecular weight units. This vitamin has been implicated, and is presumably present, in another beta ketoacid decarboxylase,[7] and in the carboxylating enzyme in fatty acid synthesis.[8] Biotin may prove essential for the fixation of carbon dioxide by certain enzymes, but it is not present in the pure crystalline decarboxylase for acetoacetic acid.

The authors wish to thank the National Institutes of Health for financial support, and Mr. J. Kucera for his assistance in the determination of the sedimentation constant.

(1) For previous studies of decarboxylation, see F. H. Westheimer and W. A. Jones, THIS JOURNAL, **63**, 3283 (1941); R. Steinberger and F. H. Westheimer, *ibid.*, **71**, 4158 (1949); **73**, 429 (1951); S. Seltzer, G. A. Hamilton and F. H. Westheimer, *ibid.*, in press.

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A NEW AND GENERAL METHOD FOR THE ISOLATION OF ANTI-PROTEIN ANTIBODIES

Sir:

Although a few general methods for the isolation of pure antibodies (Ab) directed against protein antigens (Ag) have been described,[1] the need exists for a convenient, reproducible and gentle method capable of good yields. We have developed such a method with which we have so far isolated pure

(1) H. C. Isliker, *Advances Protein Chem.*, **12**, 388 (1957).

rabbit Ab to hen ovalbumin (OA), bovine serum albumin (BSA), and bovine ribonuclease (RNase). In outline, the method is as follows. A protein Ag is treated with N-acetyl *d,l*-homocysteine thiolactone (AHT),[2,3] which places a number of SH groups on its surface without seriously affecting its capacity to precipitate with Ab directed to the original protein. A specific precipitate is then prepared with the thiolated protein (T–Ag) and the Ab. After the precipitate is freed from nonspecific proteins, it is dissolved in a glycine–sulfate buffer at *p*H 2.4, in which the Ag–Ab bonds are largely dissociated,[4,5] and the appropriate amount of the bifunctional organic mercurial[6] 3,6-bis-(acetoxymercurimethyl)-dioxane (MMD) is added. The T–Ag is cross-linked by –S–Hg– bonds and precipitates, leaving much of the Ab in solution. The detailed aspects of the method are illustrated by the preparation of pure rabbit anti-OA Ab.

Thiolation of OA.[7]—To 4.5 ml. of an 8.8% solution of OA in water was added 3.0 ml. of carbonate buffer, *p*H 10.7 (170 g. anhyd. K_2CO_3 and 15 g. anhyd. $NaHCO_3$ per liter), and 0.30 g. AHT (Schwarz Laboratories) in 1.5 ml. H_2O. After 2 hr. at 0°, the mixture was diluted with 35 ml. of phosphate buffer, *p*H 6.8, $\Gamma/2$ 0.4, to stop the reaction, and was dialyzed with agitation against phosphate buffer, *p*H 7.0, $\Gamma/2$ 0.05 for 24 hr. at 6°. This dialysis left only an insignificant amount of hydrolyzed AHT in the protein solution, and SH analysis[8] indicated that about 6.5 SH were coupled per OA molecule. T–OA was indistinguishable from OA in the ultracentrifuge, and specifically precipitated about 90% of the Ab precipitable by OA.

Purification of Anti-OA Ab.—To 1.0 g. of a γ-globulin fraction, containing 290 mg. of Ab precipitable by T–OA, was added 30 mg. of T–OA in a total volume of 240 ml. of phosphate buffer, *p*H 7.0, $\Gamma/2$ 0.05. After 2 hr. the specific precipitate was centrifuged and washed thoroughly with the phosphate buffer. It then was dissolved in 25 ml. of cold glycine–H_2SO_4 buffer, *p*H 2.4 (32 g. glycine and 240 ml. 1 *N* H_2SO_4 in 1 liter) and 4 ml. of 1.0×10^{-3} *M* MMD then was added. A precipitate formed almost immediately, and was centrifuged after 1 hr. at 6°. The supernatant was brought to neutral *p*H by the addition of 30 ml. of phosphate buffer, *p*H 7.22, $\Gamma/2$ 1.0. Overnight, a small amount of precipitate formed (13 mg.) and was removed. The supernatant, constituting the purified Ab preparation, contained 146 mg. of protein. Ag–Ab titration experiments showed that at least 90% of the Ab protein was precipitable by OA after a period of 40 hr. at 6°. The yield was 50%, based on the amount precipitable by T–OA.

Essentially the same procedure was used to isolate anti-BSA and anti-RNase Ab. About 90% and 70% of the Ab precipitable by BSA and RNase, respectively, were precipitated by the corresponding thiolated proteins. Of this Ab, 83% and 35%, respectively, were obtained in essentially pure condition. A more detailed account of these and related results will be published.

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(3) R. Benesch and R. E. Benesch, *Proc. Nat. Acad. Sci. U. S.*, **44**, 848 (1958).

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(5) S. J. Singer, L. Eggman and D. H. Campbell, *ibid.*, **77**, 4855 (1955).

(6) J. T. Edsall, R. H. Maybury, R. B. Simpson and R. Straessle, *ibid.*, **76**, 3131 (1954).

(7) The thiolation procedure herein described was developed before the method of Benesch and Benesch[3] was available. The latter method involves milder thiolation conditions, but we have not yet determined whether such preparations are satisfactory for our Ab purification procedure.

(8) F. A. Pepe and S. J. Singer, *ibid.*, **78**, 4583 (1956).

(9) Contribution No. 1538 from Sterling Chemistry Laboratory. This work was supported in part by grant E-1204 to Professor S. J. Singer from the U. S. Public Health Service, and grant G-2855 to Professor J. M. Sturtevant from the National Science Foundation.

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SOLUTION OF METALS IN PEROXYTRIFLUOROACETIC ACID

Sir:

A solution prepared by the addition of 90% hydrogen peroxide to concentrated trifluoroacetic acid has been found to dissolve very readily several metals which fall below hydrogen in the electromotive series: Cu, Bi, Ag, Hg, Tl. In general, the rate of solution increases with an increase in temperature, in concentration of the trifluoroacetic acid and hydrogen peroxide, and in the surface area of metal exposed. In the case of bulk silver, the reaction with anhydrous or very concentrated acid and 90% hydrogen peroxide produces a dark film on the surface of the silver metal. Conceivably, the unusual dissolving property of the trifluoroacetic acid–hydrogen peroxide mixture is due to the *in situ* formation of peroxytrifluoroacetic acid which has been found[1] to have remarkable oxidizing properties in the case of certain organic substances. Silver metal also dissolves in perfluorobutyric and perfluoroöctanic acids upon addition of hydrogen peroxide, the rate of solution in these mixtures appearing to increase with the number of carbon atoms in the acid.

An attempt has been made to exploit the reaction of silver with the hydrogen peroxide–trifluoroacetic acid mixture as a possible method for determining ratios of interest to atomic weight problems. Only carefully purified materials were used in these experiments. As the first step, a weighed sample (approximately 1 g.) of fused silver was converted to silver trifluoroacetate by the action of concentrated trifluoroacetic acid and 90% hydrogen peroxide. The reaction system was warmed during the dissolution of the silver metal. The product trifluoroacetate was dried and weighed to yield the ratio $AgO_2C_2F_3$:Ag. Next, by treatment with dry hydrogen chloride gas at 150°, the trifluoroacetate was transformed to AgCl which, after fusion, also was weighed. From the latter weight and the weight of silver metal, one can calculate the ratio AgCl:Ag. All of these operations were carried out in the same reaction vessel of fused quartz.

(1) W. D. Emmons and A. F. Ferris, THIS JOURNAL, **75**, 4623 (1953).