N⁴-(5-Nitro-2-pyridyl)-4-homosulfanilamide—A mixture of 2-chloro-5-nitropyridine (0.25 g.), 4-homosulfanilamide hydrochloride (0.32 g.), and freshly fused sodium acetate (0.2 g.) was refluxed with 30 ml. absolute ethanol for 24 hr., after which the reaction mixture was diluted with water until complete separation. The product which was obtained in 60% yield (0.3 g.) melted at 190° after being crystallized from ethanol.

Anal.—Calcd. for $C_{12}H_{12}N_4O_4S$: N, 18.30. Found: N, 17.97.

N-(5-Nitro-2-pyridyl)-benzylsulfonamide—A mixture of 2-chloro-5-nitropyridine (0.8 g.), potassium benzyl sulfonamide (0.9 g.) and dimethyl formamide (30 ml.) was heated at 110–130° for 3 hr. The reaction mixture was then evaporated under diminished pressure nearly to dryness, and the residue (1 g., 62% yield) was crystallized from ethanol. It melted at 260° and the melting point was not depressed on admixture with a pure specimen prepared by Ullmann's method.

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COMMUNICATIONS

Simple Assay for Determination of Carbonic Anhydrase Activity

Keyphrases ☐ Carbonic anhydrase activity—analysis ☐ Carbon dioxide-^{14}C liberation, *in vitro*—carbonic anhydrase ☐ Scintillometry, liquid—radioactivity determination

Sir:

A method has been developed for the *in vitro* determination of carbonic anhydrase activity. The method is based on the principle that the reactions involved in attaining equilibrium between bicarbonate ion and carbon dioxide in solution are both catalyzed by carbonic anhydrase. Since the function of carbonic anhydrase *in vivo* is to maintain this equilibrium, the activity of the enzyme can be determined *in vitro* by shifting the equilibrium in the desired direction. Carbon dioxide-^{14}C liberated from labeled bicarbonate in a buffer system, containing carbonic anhydrase, is trapped on alkali-moistened filter paper strips. The radioactivity collected on the strips is subsequently determined by liquid scintillation counting. A preliminary report of this work has been presented (1).

Figure 1 illustrates the absorption tube used for collecting the labeled CO_2. A 1.59 × 3.81-cm. ($^5/_8$ × $1^1/_2$-in.) strip of Whatman No. 1 filter paper is impaled lengthwise on the wire of a tube stopper, and wetted with about 100 μl. of 0.5 *M* NaOH. The incubation mixture used consists of 1.2 ml. of phosphate buffer (two parts 0.1 *M* KH_2PO_4 and three parts 0.1 *M* Na_2HPO_4), pH 7.0, 0.2 ml. of enzyme preparation, and 1.0 ml. of $NaH^{14}CO_3$ solution (6.25×10^{-3} *M* containing 1.55 μc./m*M*). The substrate solution is dissolved in water and stored frozen. The enzyme preparations employed are listed below at the concentration routinely used and the useful range over which linearity is known to exist is recorded in parentheses, (*a*) 0.35 (0.07 to 0.70)% saline solutions of hemolyzed rat or dog

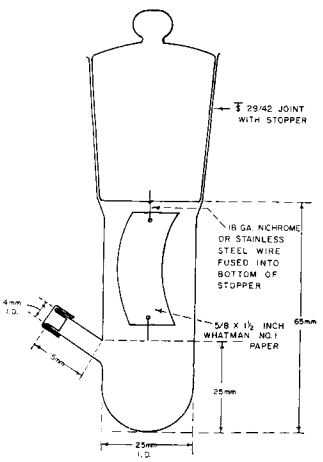

Figure 1—*Absorption tube used for collecting the labeled carbon dioxide.*

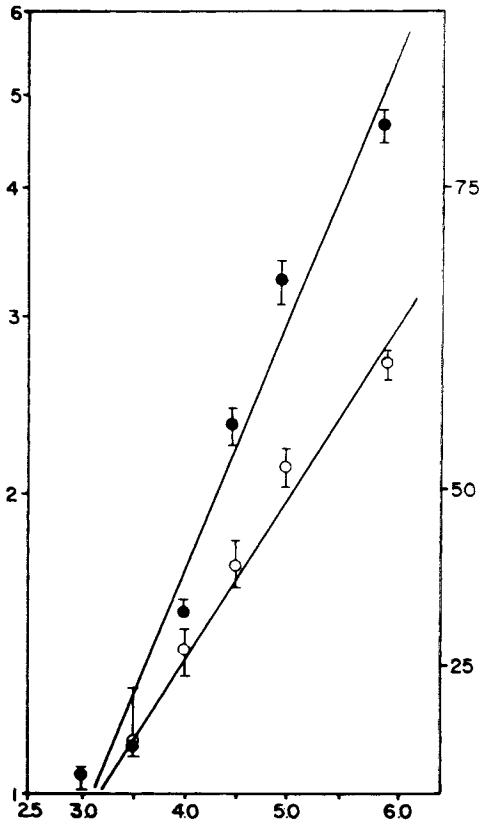

Figure 2—*Influence of dichlorphenamide* (●) *and acetazolamide* (○) *on carbonic anhydrase activity.*

erythrocytes; (*b*) tissue homogenates in 0.154 KCl containing the equivalent of 3.5 (2.5 to 45.0) mg. of rat kidney or 30 (15 to 200) mg. of rat brain; or (*c*) 750 (200 to 850) units of a commercial preparation (Nutritional Biochemicals, 1,000 units/mg.). The enzymatic reaction is linear with time for at least 12 min. In blank tubes for the determination of spontaneously evolved CO_2, enzyme is omitted and 1.4 ml. of phosphate buffer is used.

In a typical assay, stoppers with wetted paper strips attached are inserted in tubes containing enzyme and/or buffer. At zero time substrate is injected through the capped sidearms and the tubes are shaken in an ice bath on a reciprocating shaker at 75 excursions/min. Four minutes after the addition of substrate, the reaction is stopped by the introduction of 0.2 ml. of 10 *M* NaOH through the sidearm. The tubes are then shaken for an additional 10 min. at room temperature. Then the stoppers are removed and allowed to stand in an inverted position until the paper strips dry. When dry, the strips are removed, creased lightly lengthwise, and placed in glass counting vials that contain 17 ml. of 0.4% PPO (2,5-diphenyloxazole) and 0.1% dimethyl POPOP (*p*-bis-[2-(4-methyl-5-phenyloxazolyl)]benzene) in toluene. The count rates of the strips are measured in a liquid scintillation spectrometer.

The procedure is easily adapted to the screening of compounds which inhibit the activity of carbonic anhydrase. In inhibition studies either a portion of phosphate buffer is replaced with an equal volume of solution containing the suspected inhibitor, or a tissue preparation from an animal dosed with a suspected inhibitor can be used as the enzyme source. Figure 2 shows the influence of concentration of two reference inhibitors, dichlorphenamide and acetazolamide, on carbonic anhydrase activity where Po is product formed (as c.p.m.) in tubes containing no inhibitor and Pr is product formed in tubes containing inhibitor. In the illustrated experiments performed with 750 units of commercial enzyme, the I_{50} values obtained for dichlorphenamide and acetazolamide were 4.4×10^{-6} *M* and 5.0×10^{-6} *M*, respectively.

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