The residues were dissolved in a very small amount of methanol for TLC studies. An amount of 0.5 mcg. of 3,3′-dihydroxyazoxybenzene dissolved in methanol was used for spotting. For Samples A, B, C, and D, sufficient amounts to make the spot visually distinct were applied. Thin-layer plates were developed in various solvent systems, and the spots were located by visible or UV light. The results are given in Table I.

RESULTS AND DISCUSSION

Preliminary studies indicated that air oxidation of an aqueous solution of *m*-aminophenol was much faster than that of *p*-aminosalicylic acid and its sodium salt. It is thus postulated that some of the chromogens which occurred in aged *p*-aminosalicylic acid dosage forms might be contributed from oxidation of *m*-aminophenol, a common impurity of *p*-aminosalicylic acid and its sodium salt (5, 6).

To effect mild oxidation of *m*-aminophenol, hydrogen peroxide was used as the oxidizing agent in this study. The reaction conditions chosen were room temperature and about neutral pH. A dark cherry-red compound was isolated and identified as 3,3′-dihydroxyazoxybenzene. Sunlight irradiation of *m*-aminophenol in ethanol also yielded the same azoxy compound.

Commercially available tablets of *p*-aminosalicylic acid, *p*-aminosalicylic acid–isoniazid, and sodium *p*-aminosalicylate were tested for the presence of this specific chromogen. TLC studies of samples in various solvent systems clearly indicated that 3,3′-dihydroxyazoxybenzene is a common chromogen which occurred in those samples being tested (Table I), although this compound was not detected in the reference material of sodium *p*-aminosalicylate.

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Inhibition of Nicotinate Phosphoribosyl Transferase by Nonsteroidal Anti-Inflammatory Drugs: A Possible Mechanism of Action

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Abstract □ When incubated with ^{14}C-nicotinic acid and phosphoryl-ribose-1-pyrophosphate, human platelet lysate incorporates radioactivity into nicotinic acid mononucleotide. Under these conditions, the apparent K_m of nicotinic acid for nicotinate phosphoribosyl transferase is 2.4×10^{-5} *M*. Along with 2-hydroxynicotinic acid ($K_i = 2.3 \times 10^{-4}$ *M*), the following nonsteroidal anti-inflammatory compounds competed reversibly with nicotinic acid for the enzyme: flufenamic acid ($K_i = 4.6 \times 10^{-5}$ *M*), mefenamic acid ($K_i = 7.6 \times 10^{-5}$ *M*), salicylic acid ($K_i = 1.6 \times 10^{-4}$ *M*), phenylbutazone ($K_i = 1.6 \times 10^{-4}$ *M*), and indomethacin ($K_i = 4.2 \times 10^{-4}$ *M*). Such inhibition may explain the decreases in nicotinamide adenine dinucleotide phosphate content of rat liver after administration of salicylate. Furthermore, suppression of nicotinamide adenine dinucleotide biosynthesis by anti-inflammatory drugs may restrain mucopolysaccharide biosynthesis and, thereby, reduce inflammatory responsiveness. Hence, this antagonism of niacin may be involved in the pharmacologic activity and, particularly, the toxicity of anti-inflammatory drugs.

Keyphrases □ Nicotinate phosphoribosyl transferase—inhibition by nonsteroidal anti-inflammatory drugs, mechanism of action, using radiolabeled nicotinic acid □ Human platelet lysate—evaluation of influence of nonsteroidal anti-inflammatory drugs on nicotinate phosphoribosyl transferase, mechanism of action □ Nicotinic acid, radiolabeled—used to evaluate influence of nonsteroidal anti-inflammatory drugs on nicotinate phosphoribosyl transferase

The human platelet incorporates 7-^{14}C-nicotinic acid into nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate, and other compounds (1) consistent with intermediates of the pathway of Preiss and Handler (2, 3). Such incorporation is hindered by analogs of nicotinic acid, by salicylic acid, and by other anti-inflammatory drugs (4–6).

Data concerning the intact platelet suggested inhibition of nicotinamide adenine dinucleotide biosynthesis early in the pathway, probably at the step catalyzed by nicotinate phosphoribosyl transferase (5). To evaluate this possibility further, the influence of various nonsteroidal anti-inflammatory drugs on nicotinate phosphoribosyl transferase in human platelet lysate was studied.

METHODS

The following compounds were obtained from commercial sources: 7-^{14}C-nicotinic acid (sp. act. 59.1 mc./mmole)[1], phosphoryl-ribose-1-pyrophosphate[2], nicotinamide mononucleotide[3], salicylic

[1] Amersham Searle.
[2] Mann Research Lab.
[3] Sigma.

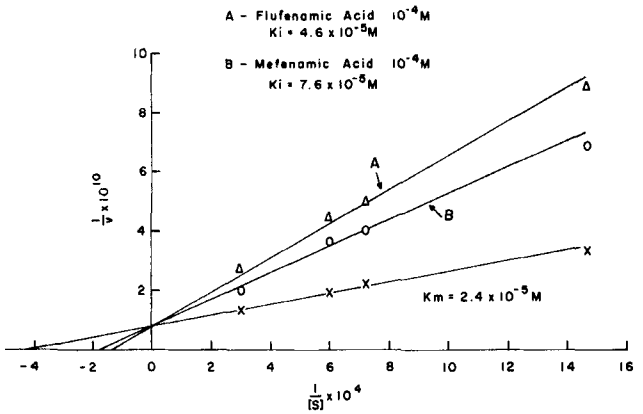

Figure 1—*Inhibition of nicotinate phosphoribosyl transferase in human platelet lysate by flufenamic and mefenamic acids. Velocity is expressed as moles nicotinic acid mononucleotide formed per milligram platelet per hour; substrate concentration is expressed as moles per liter. Results of representative experiments are shown.*

acid[4], phenylbutazone[5], indomethacin[6], and flufenamic and mefenamic acids[7]. 2-Hydroxynicotinic acid was synthesized[8]. 7-^{14}C-Nicotinic acid proved >98% pure on thin layers of cellulose in the solvent systems employed by Hagino *et al.* (7). Nicotinic acid and nicotinic acid mononucleotide exhibited the same R_f's on cellulose as were previously reported for paper chromatography (7). Nicotinic acid mononucleotide was prepared from nicotinamide mononucleotide by the method of Wagner (8).

Approximately 200 mg. (wet weight) of human platelets was lysed in 1.5 ml. of distilled water. Then 0.2-ml. aliquots were incubated in Ca^{+2}-free, Krebs–Ringer bicarbonate buffer modified to contain: Mg^{+2}, 3.3 m*M;* phosphorylribose-1-pyrophosphate, 1.5 m*M;* and various concentrations of 7-^{14}C-nicotinic acid. Solutions of anti-inflammatory agents were added to some incubates in parallel experiments. Incubations were carried out in total volumes of 0.5 ml. in a metabolic shaker under 95% O_2–5% CO_2 for 1 hr. at 37°. The reaction was stopped by immersing the tubes in solid CO_2–acetone mixture. Subsequently, "carrier" nicotinic acid and nicotinic acid mononucleotide were added to the incubates, and the compounds were separated on thin layers of cellulose in the solvent systems employed by Hagino *et al.* (7). More than 98% of the radioactivity migrated with these standard compounds.

RESULTS

7-^{14}C-Nicotinic acid incorporation into nicotinic acid mononucleotide was linear up to 3 hr. of incubation and proportional to substrate concentrations at 6.8, 14, 17, and 34 μM. Kinetics were evaluated according to the method of Lineweaver and Burke (9), in which velocity was expressed as moles of nicotinic acid mononucleotide formed per milligram platelet per hour (Fig. 1). The intercepts, for calculation of the apparent K_m and K_i's, were obtained by the method of least squares. The apparent K_m for nicotinic acid, with phosphorylribose-1-pyrophosphate and Mg^{+2} in excess, was 2.4×10^{-5} *M* and agreed closely with that of purified bovine liver nicotinate phosphoribosyl transferase (2.5×10^{-5} *M*) reported by Smith and Gholson (10). The formation of nicotinic acid mononucleotide was competitively inhibited by flufenamic acid ($K_i = 4.6 \times 10^{-5}$ *M*), mefenamic acid ($K_i = 7.6 \times 10^{-5}$ *M*), salicylic acid ($K_i = 1.6 \times 10^{-4}$ *M*), phenylbutazone ($K_i = 1.6 \times 10^{-4}$ *M*), 2-hydroxynicotinic acid ($K_i = 2.3 \times 10^{-4}$ *M*), and indomethacin ($K_i = 4.2 \times 10^{-4}$ *M*).

DISCUSSION

Platelet aggregation, a precursor of blood coagulation (11), is inhibited by various nonsteroidal anti-inflammatory drugs; hence, O'Brien (12) postulated a connection between inflammation and platelet function. The present study describes another common feature of these drugs in the platelet: inhibition of nicotinate phosphoribosyl transferase by reversible competition with nicotinic acid. Although anti-inflammatory agents inhibit the biosynthesis of nicotinamide adenine nucleotides, obligatory cofactors in a host of reactions supportive to metabolism in the platelet, the possible correlation between niacin antagonism and platelet aggregation remains undefined.

A more attractive hypothesis relates niacin antagonism by these drugs to their anti-inflammatory activity. Such drugs also inhibit the oxidation of uridine-5′-diphosphoglucose, competitively with nicotinamide adenine dinucleotide and noncompetitively with uridine-5′-diphosphoglucose (13). Therefore, Lee and Spencer (13) theorized that mucopolysaccharide biosynthesis is restrained and inflammatory responsiveness is reduced. It follows that inhibition of nicotinamide adenine dinucleotide biosynthesis by anti-inflammatory agents would exert the same restraint on this pathway since tissue concentrations of the cofactor would be diminished.

Imsande and Handler (14) suggested that the reaction catalyzed by nicotinate phosphoribosyl transferase was rate limiting for nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate formation. Therefore, such inhibition of this enzyme may explain the decreases in nicotinamide adenine dinucleotide phosphate content of rat liver after administration of salicylate, as reported by Slater and Sawyer (15).

The concentrations of nicotinic acid employed in the present study were greater than those in human serum and within the range of those in human whole blood (16); also, the apparent K_i's of the inhibitory drugs are within the range of therapeutic blood levels (17, 18). Hence, this antagonism of niacin at the cellular and enzyme level may be involved in the pharmacologic activity and, particularly, the toxicity of anti-inflammatory drugs.

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[5] Geigy Pharmaceuticals.
[6] Merck and Co.
[7] Parke-Davis and Co.
[8] By Dr. Alden Beaman, Hoffmann-La Roche.

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