isomer is intermediate between the two. A comparison of the activities of the dioximes XV–XVII with that of the monooximes XVIII–XX and XXI–XXIII, in the case of the enzyme inhibited by TEPP, shows that the α-hydroxyiminoketones XXI–XXIII have an activity like that of the dioximes, while the β-pyridyl-β-hydroxyiminopropioanilides XVIII–XX are about 10 times less active. This fall of activity may be due to the existence of a hydrogen bond between the hydroxyimino group in the β position and the amide group, as occurs in the *syn* α,β-dihydroxyimino-butyranilide and the *syn* β-phenyl-α,β-dihydroxyimino-propioanilide (6–8). No sound conclusion can be drawn from a comparison of the mono- and dioximes activities with the values of their dissociation constants; however, the fact that the reactivating properties of the oximes depends both on their degree of dissociation and the good nucleophilicity of the dissociated form appears to be confirmed.

REFERENCES

(1) R. J. Ellin and J. H. Wills, *J. Pharm. Sci.*, **53,** 995(1964).

(2) Y. Ashani and Sasson Cohen, *Israel J. Chem.*, **5,** 59(1967).

(3) R. J. Kitz, S. Ginsburg, and I. B. Wilson, *Biochem. Pharm.*, **14,** 1471(1965).

(4) M. Pitman and P. W. Sadler, *J. Chem. Soc.*, **1961,** 759.

(5) I. B. Wilson, S. Ginsburg, and C. Quan, *Arch. Biochem. Biophys.*, **77,** 286(1958).

(6) G. Alimenti, M. Grifantini, F. Gualtieri, and M. L. Stein, *Tetrahedron*, **24,** 395(1968).

(7) M. Grifantini, F. Gualtieri, and M. L. Stein, *Ann. Chim. (Rome)*, **58,** 189(1968).

(8) M. Grifantini, F. Gualtieri, S. Martelli, and M. L. Stein, *ibid.*, **58,** 200(1968).

(9) E. G. Vassian and R. K. Murmann, *J. Org. Chem.*, **27,** 4309(1962).

(10) D. H. Barton and J. M. Beaton, *J. Am. Chem. Soc.*, **83,** 4083(1961).

(11) R. M. Desai, M. R. Patel, and B. N. Mankad, *Current Sci. (India)*, **33,** 613(1964).

(12) Y. Ashani, H. Edery, J. Zahavy, W. Künberg, and Sasson Cohen, *Israel J. Chem.*, **3,** 133(1965).

(13) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Methuen and Co. Ltd., London, England, 1962, p. 1, 51.

(14) C. Belzecki and T. Urbanski, *Roczniki Chem.*, **32,** 779 (1958).

(15) C. Caradonna, M. L. Stein, and M. Ikram, *Ann. Chim. (Rome)*, **49,** 2083(1959).

(16) N. S. Vul'fson, V. E. Kolchin, and L. K. Artemchik, *Zh. Obschch. Khim.*, **32,** 3382(1962).

(17) N. S. Vul'fson and V. E. Kolchin, *ibid.*, **34,** 2387(1964).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 14, 1968, from the *Institute of Pharmaceutica and Organic Chemistry, University of Camerino, Camerino, Italy.*

Accepted for publication November 21, 1968.

This investigation was supported by the Italian National Research Council.

Studies on Mechanism of Action of Salicylates V: Effect of Salicylic Acid on Enzymes Involved in Mucopolysaccharides Synthesis

K. H. LEE and MICHAEL R. SPENCER*

Abstract □ Salicylic acid (SA) and acetylsalicylic acid (ASA), unlike cortisone, promote the release of lysosomal enzymes rather than protecting rat liver lysosomal membrane. Salicylic acid inhibits the oxidation of uridine-5-diphosphoglucose (UDPG) competitively with nicotinamide adenosine dinucleotide (NAD) and noncompetitively with UDPG. It also competitively inhibits the transferring of glucuronyl group of uridine-5-phosphoglucuronic acid (UDPGA) to the phenolic acceptor. The wound-healing retardation action of salicylates is probably due mainly to its inhibitory action on mucopolysaccharide synthesis.

Keyphrases □ Salicylates—action mechanism □ Mucopolysaccharides synthesis—salicylate effect □ Lysosome stability—salicylate effect □ UV spectrophotometry—analysis

Recently the authors have shown that aspirin retards skin wound healing in rats (1). Inflammation and acid mucopolysaccharide synthesis are two essential features in the early stage of wound healing (2). Aspirin inhibits both features. Two possible mechanisms of action have been proposed (3).

Lysosomal enzymes have been suggested to be involved in inflammation (4). Since aspirin has been reported by Miller and Smith (5) to protect lysosomal membrane, it was proposed that aspirin probably retards healing by a mechanism that prevents the release of lysosomal enzymes (1). However, this laboratory was not able to confirm the results reported by Miller and Smith. Acetylsalicylic acid and salicylic acid, in this laboratory, did not protect rat liver lysosomal membrane; instead, they accelerated the release of lysosomal enzymes. These findings were verified by using purified rat liver lysosome. The results are reported and discussed in this paper.

Mucopolysaccharide formation in the early phase of wound healing was noticed in many laboratories (6). The role of mucopolysaccharide in the formation of connective tissue or collagen has been of interest for many years (7, 8). Salicylates inhibit the biosynthesis of acid mucopolysaccharide and sulfate uptake for chondroitin sulfate synthesis by rat rib cartilage (9). It was found that aspirin also inhibits mucopoly-

saccharide synthesis in the granulation tissue of healing wounds in rats (10). Bollet reported that sodium salicylate inhibited glucosamine-6-phosphate synthesis (11). The enzyme involved in L-glutamine: D-fructose-6-phosphate aminotransferase (2.6.1.16). Glucosamine-6-phosphate formation is the first step of the sequence of reactions involved in mucopolysaccharide synthesis. Bollet's work was later confirmed by Schönhöfer (12). Recently, Kalbhen *et al.* (13), using fibroblast tissue culture, demonstrated that salicylate and a few other antiphlogistic drugs inhibited the incorporation of glucosamine-1-^{14}C into mucopolysaccharides. Their findings indicate that these drugs also inhibit mucopolysaccharide formation other than glucosamine synthesis.

It is well known that salicylate exerts a general inhibitory action on dehydrogenase enzymes by a mechanism involving reversible competition with the nicotinamide adenosine dinucleotide (NAD) required as cofactor (14). In the sequence of reactions of mucopolysaccharide synthesis, only the formation of uridine-5′-diphosphoglucuronic acid (UDPGA) involves dehydrogenation reaction. In the present study it was found that sodium salicylate inhibits uridine-5′-diphosphoglucose (UDPG) dehydrogenation reaction, and this inhibitory action can be reversed by NAD. The enzyme involved is UDPG dehydrogenase (1.1.1.22). The authors further found that salicylate also inhibits UDPG dehydrogenation reaction noncompetitively with UDPG.

The next step in mucopolysaccharide synthesis is the transferring of glucuronyl group to glucosamine or an acceptor. The enzyme involved is UDPglucuronyl-transferase (2.4.1.17). Since salicylic acid is also a glucuronyl acceptor, it is expected that it inhibits UDPglucuronyl-transferase reaction by competing with the acceptor. In this report the authors have shown that salicylic acid competes with aminophenol in glucuronyl-transferase reaction.

EXPERIMENTAL

Materials and Chemicals—UDPG (sodium salt, yeast origin), Sigma grade, UDPG-dehydrogenase (bovine liver origin), Type III, NAD, Grade III, and glycylglycine[1]; sodium salicylate, reagent grade[2]; β-glyceryl phosphate disodium and *N*-1-naphthylene diamine dihydrochloride.[3] *o*-Aminophenol[4] was sublimated and kept under nitrogen in a dark place.

Preparation of Purified Lysosome—The purified lysosome from rat liver was isolated according to the method described by Savant *et al.* (15). Dounce homogenizer (18) was used for preparing the liver homogenate.

Lysosomal Enzyme Assay—Lysosomal suspension was preincubated in the presence or absence of drugs so that the influence of these drugs on the amount of enzyme liberated under these conditions could be assayed. Portions of 3.2 ml. of the purified lysosomal suspension were introduced into 25-ml. conical flasks containing either one of the following compounds: acetylsalicylic acid (ASA), salicylic acid (SA), hydrocortisone-21-sodium succinate (HC-21-SS), or 0.5% surfactant[5] in 0.8 ml of sucrose acetate buffer pH 5.0. The surfactant was used to disrupt intact lysosomal membrane so that the full activity of the lysosomal enzyme could be estimated. For the controls, 0.8 ml. of sucrose acetate buffer was used. All of the reaction mixtures were in duplicate. The flasks containing reaction mixtures, except the samples for zero-time preincubation control, were incubated at 37° in a metabolic shaking incubator (Dubnoff) for 40 min. Zero-time control represents the activity of lysosomal enzymes present in the purified lysosome preparation before preincubation.

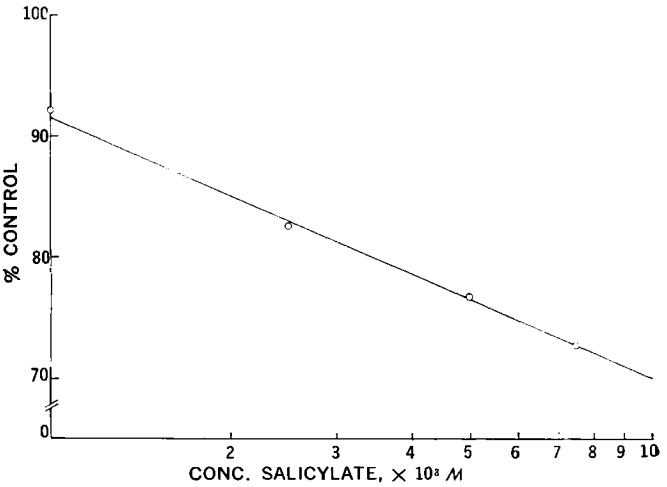

Figure 1—*Effect of salicylic acid on UDPG-dehydrogenase activity.*

Free acid phosphatase (3.1.3.2) assay was used to assess the degree of lysosomal disruption (17). At the end of the preincubation period, a 1-ml. portion of substrate solution, containing 0.25 m*M* β-glyceryl-phosphate in sucrose acetate buffer pH 5.0, was added to each of the preincubated and zero-time preincubation control samples. The incubation was continued at 37° in an incubator (Dubnoff) for 10 min. At the end of the incubation period the reaction was stopped by the addition of 1 ml. of 48% trichloroacetic acid. The protein precipitated was separated by centrifugation. Aliquots of the supernatant were used for the determination of inorganic phosphate content by the method described by Fisk and Subbarow (18).

The results of three experiments in duplicate are shown in Fig. 1. Student *t* test was used for testing significance.

UDPG-dehydrogenase Assay—UDPG-dehydrogenase (1.1.1.22) activity was measured according to the method described by Strominger *et al.* with some modifications (19). The activity of the enzyme was estimated from the initial rate of reduction of NAD recorded by a recording spectrophotometer[6] at 340 mμ in silica cells of 1.0-cm. light path at room temperature after the addition of the enzyme. The system contained, in addition to enzyme, 0.1 μ*M* UDPG and 0.5 μ*M* NAD in a total volume of 4.0 ml. of 0.1 *M* glycine buffer, pH 8.7. A blank without UDPG was used. Sodium salicylate was first dissolved in the glycine buffer and graded concentrations were used for the studies on mechanism of action, graded concentrations of either NAD or UDPG were used as indicated in Figs. 2 and 3.

Isolation of Aminophenylglucuronide—*o*-Aminophenylglucuronide used for preparing the standard curve in UDPGA transferase study was produced in the animal body in response to the administration of *o*-aminophenyl according to the method described by Williams (20) with some modifications. Two rabbits (3 kg.) under halothane anesthesia were each given 1 g. of freshly sublimated *o*-aminophenol suspended in 10 ml. of water by a syringe through a stomach tube, followed by 5 ml. more of water in order to rinse the syringe and stomach tube. The rabbits were kept in a metabolic cage for urine collection. Rabbit pellets and water were fed *ad libitum*. No urine was collected at the end of 6 hr. after the administration of the drug. Next morning, 20 hr. after drug administration, 210 ml. of dark but relatively clear urine was collected. The urine gave an intense naphthoresorcinol reaction, a red color with nitrous acid, and dimethyl-α-naphthylamine. The urine was acidified by the addition of four drops of glacial acetic acid and then treated with saturated normal lead acetate solution until no further precipitation was observed. A total volume of 20 ml. of saturated normal lead acetate

[1] Sigma Chemical Co., St. Louis, Mo.
[2] J. T. Baker Chemical Co., Phillipsburg, N. J.
[3] Eastman Organic Chemicals, Rochester, N. Y.
[4] Aldrich Chemical Co., Milwaukee, Wis.
[5] Triton X-100, Rohm & Haas, Philadelphia, Pa.
[6] Beckman DB, Beckman Instruments, Inc., Fullerton, Calif.

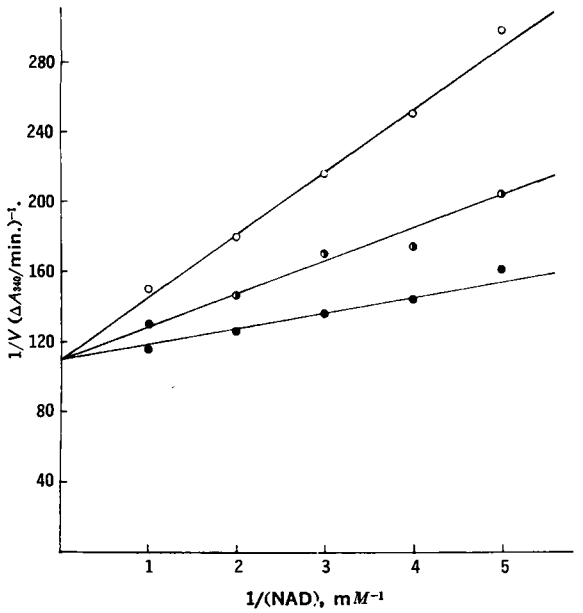

Figure 2—*Competitive inhibition of UDPG-dehydrogenase by salicylate: effect of various concentrations of NAD and salicylate. Key: ●, control; ◑, 2.5 mM salicylate; ○, 5 mM salicylate.*

solution was added. A light tan-colored precipitate was formed which settled down easily upon standing. The precipitate was separated by centrifuge and discarded. The clear supernatant was neutralized with a few drops of concentrated ammonium hydroxide to pH 7.0, and treated with saturated basic lead acetate solution until no further precipitate was found. A total volume of 275 ml. of saturated basic lead acetate was added. An almost white-colored fine precipitate was formed. The suspension was centrifuged and the precipitate was washed twice with small amounts of water. The lead salt was suspended in 150 ml. of water and H_2S gas was bubbled into the suspension to decompose the lead salt until no more lead sulfide was formed. The precipitate of lead sulfide was filtered off by suction. The filtrate was concentrated *in vacuo* at 45–50°, when the *o*-aminophenylglucuronide began to separate as a light yellow crystalline solid, and finally taken to dryness. The residue was shaken with about 40 ml. of 95% ethanol and the whole filtered in a Buchner filter. The yellow color was removed by the ethanol and off-white crystals remained on the filter. The crystals were washed with ethanol and anhydrous ethyl ether on the filter. They were then dried by suction. Yield was 0.55 g. The crude *o*-aminophenylglucuronide was dissolved and decolorized with charcoal in 150 ml. of water at 95°. The charcoal was filtered through a Buchner filter. The filtrate was

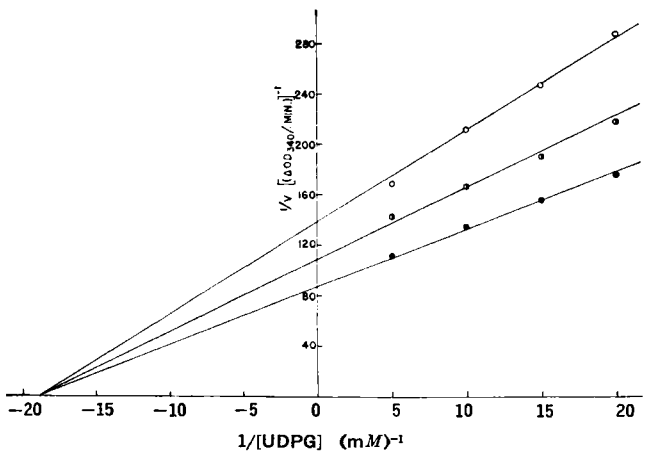

Figure 3—*Noncompetitive inhibition of UDPG-dehydrogenase by salicylate: effect of various concentrations of UDPG and salicylate. Key: ●, control; ◑, 2.5 mM salicylate; ○, 5 mM salicylate.*

Table I—Influence of ASA, SA and HC-21-SS on Acid Phosphatase Release from Liver Lysosomes

Group	Drugs	Acid Phosphatase Activity % of Control[a]
I	Control	100
II	$1 \times 10^{-3}M$ HC-21-SS	74.5
III	$2 \times 10^{-3}M$ ASA	117.5
IV	$2 \times 10^{-3}M$ SA	120.0

[a] Average of three experiments in duplicates. The difference between II and I ($p < 0.001$), III and I ($p < 0.005$), and IV and I ($p < 0.001$) are significant. Student *t* test.

concentrated *in vacuo* to about 50 ml. and was left in the refrigerator overnight in a small crystallizing dish. The white crystals were separated by centrifugation and washed twice with 95% ethanol and twice with anhydrous ether. The crystals were kept in a desiccator over P_2O_5 and a piece of paraffin wax. The recrystallized *o*-aminophenylglucuronide was used for preparing the standard curve.

Isolation of UDPglucuronyl Transferase (2.4.1.17)—This enzyme is not commercially available. The authors first tried to isolate this enzyme from rat liver according to the method described by Pogell and Krisman (21) with some modifications. The activity of rat liver preparations was rather low. The authors then tried to use guinea pig liver. A guinea pig, weighing 400–500 g. was stunned by a blow to the head, the blood was drained off by decapitation, and the liver removed and placed in an ice cold 0.15 *M* KCl solution within 1 min. The liver, after weighing, was homogenized in an ice cold Dounce homogenizer (16) with four volumes (v/w) of ice cold 0.15 *M* KCl solution. The homogenate was centrifuged at $2,000 \times g$ in a refrigerated centrifuge.[7] The precipitate was discarded and the supernatant fluid was centrifuged 90 min. at $18,000 \times g$. The precipitate was suspended in an equal volume of KCl solution and centrifuged 60 min. at $18,000 \times g$. The washing was repeated once and the precipitate was resuspended to the original volume in KCl solution. The activity of such microsomal preparation was stable for several weeks when stored at −15°. The preparations were usually divided into small portions, frozen in liquid nitrogen, and stored in a freezer.

UDPglucuronyl Transferase Assay—For UDPglucuronic transferase assay *o*-aminophenol was used as glucuronic acid acceptor since *o*-aminophenylglucuronide can be estimated in microquantities in the presence of free *o*-aminophenol (22). The procedure described by Mills and Smith (23) was followed for UDPglucuronyl transferase assay.

Standard Curve for *o*-Aminophenylglucuronide—The standard curve for *o*-aminophenylglucuronide can be prepared as follows: pipet various amounts of purified *o*-aminophenylglucuronide solution into 0.5 ml. of 0.2 *M* glycylglycine buffer, pH 7.7. Add distilled water to a final volume of 2 ml. Add to each tube 2 ml. of glycine-trichloroacetic acid buffer. Mix and pipet 3 ml. of the mixture into another tube.

Add $NaNO_2$, ammonium sulfamate, *N*-(1-naphthyl)-ethylenediamine solutions and develop color as described above. Read the absorbance at 535 mμ. Plot the absorbance against μ*M* of *o*-aminophenylglucuronide per tube. The curve is linear with concentration range of *o*-aminophenylglucuronide from 20 to 200 μ*M*.

Preparation of UDPGA—UDPGA was isolated from guinea pig liver by the method described by Dutton and Storey (24) and Smith and Mills (25). Guinea pig-liver homogenate was used for the preparation.

RESULTS

The results of three independent experiments in duplicates on the effect of various compounds on lysosome stability studies are shown in Table I. In the presence of hydrocortisone-21-sodium succinate at $1 \times 10^{-3}M$ concentration, lysosomal acid phosphatase released was only 74.5% of that of the control. This lysosomal membrane protection effect by hydrocortisone compares favorably with the results obtained by others (17, 26). Salicylic acid and acetylsalicylic acid at

[7] Servall.

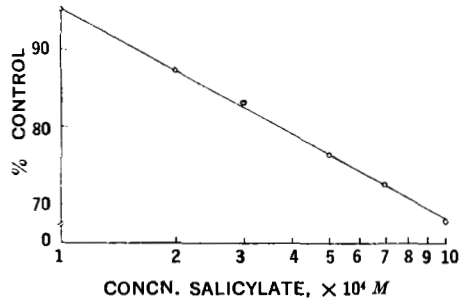

Figure 4—*Effect of salicylate on UDPGA-transferase.*

$2 \times 10^{-3}M$ significantly released lysosomal acid phosphatase to 120% and 117.5% of the control, respectively These results differ from those reported by Miller and Smith (5). Student *t* test was used for evaluation.

The effect of salicylic acid on UDPG-dehydrogenase is shown in Fig. 1. The plot of inhibition *versus* the log of drug concentration is linear within the range of drug concentrations studied. Salicylic acid inhibits UDPG-dehydrogenase through two mechanisms of action. It inhibits competitively with NAD and, at the same time, noncompetitively with UDPG. The Lineweaver and Burk plots (27) of these two inhibitions are shown in Figs. 2 and 3. The K_m value, averaged from 16 independent experiments, for this reaction is $9.6 \times 10^{-5}M$. The K_i value for the competitive inhibition is $2.1 \times 10^{-3}M$ and the K_i value for the noncompetitive inhibition is $8.7 \times 10^{-3}M$. Salicylic acid also inhibits UDPGA-transferase reaction. This is shown in Fig. 4. The competitive inhibition of salicylic acid on UDPGA-transferase with *o*-aminophenol is shown in Fig. 5. Judging from the Lineweaver and Burk plot, it is obvious that SA is competing with the phenolic compound. The K_m value for this reaction, averaged from six independent experiments in duplicates, is $2.44 \times 10^{-4}M$. The K_i value for competitive inhibition is $1.47 \times 10^{-4}M$.

DISCUSSION

Duthie has hypothesized that salicylate might stabilize lysosomes (28). Miller and Smith have reported that acetylsalicylic acid prevented the liberation of liver lysosomal enzymes (5). Lysosomes are particularly plentiful in inflammatory sites (29), and inflammation is an important feature in healing (2). Based on these findings, the authors suggested that the lysosomal membrane protection activity of ASA was one of the possible mechanisms of action of ASA in retarding wound healing. Contrary to the results obtained by Miller and Smith (5), it was found that both ASA and SA actually release lysosomal enzymes.

De Duve *et al.* have shown that cortisone stabilized lysosomal membrane (17). Subsequently, Weissmann and Thomas found a few other anti-inflammatory steroids: prednisone, prednisolone, betamethasone, but not corticosterone, also protected lysosomal membranes (30). There is reasonable evidence to support the hypothesis that anti-inflammatory steroids exert their pharmacologic action, at least in part, upon the membranes of lysosomes (17). However, De Duve, in the same report (19), also showed that dexamethasone, a potent anti-inflammatory agent and a close analog of cortisone, was ineffective as a lysosome protector, whereas another product,[8] which has deoxycorticosterone-like properties, was effective. Acetylsalicylic acid is an inflammatory agent and inhibits wound healing, but releases lysosomal enzymes. It is interesting to know that vitamin E has anti-inflammatory activity (3), but it also releases lysosomal enzymes (17). Vitamin E also reverses the wound-healing retardation action of aspirin (3). Recently, the role of lysosomal enzymes in wound healing was questioned by Raekallio, who studied enzymatic response to injury in experimental skin wounds by using histochemical methods (32).

Acid mucopolysaccharides, such as chondroitin sulfate, are important in the formation of connective tissue or collagen in wound healing (7, 8). Salicylates are known to inhibit the biosynthesis of acid mucopolysaccharides and the incorporation of sulfate chondroitin synthesis (9). The authors found that acetylsalicylic acid also

[8] SKF 3150.

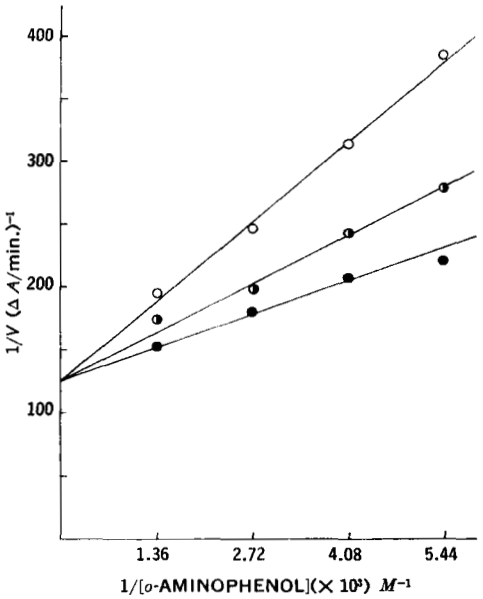

Figure 5—*Competitive inhibition of UDPGA-transferase by salicylate: effect of various concentrations of aminophenol and salicylate. Key:* ●, *control;* ◑, 1×10^{-4} M *salicylate;* ○, 5×10^{-4} M *salicylate.*

inhibits mucopolysaccharide synthesis in the granulation tissue of healing wounds (10). Bollet found that sodium salicylate inhibited glucosamine-6-phosphate synthesis from glutamine and fructose-6-phosphate (11) which was verified recently by Schönhöfer (12). Glucosamine-6-phosphate is one of the components involved in mucopolysaccharide synthesis. The other component is UDPGA. UDPGA is formed from UDPG. UDPG formation requires adenosine triphosphate (ATP). Salicylic acid is a potent oxidative phosphorylation uncoupler (33) which then can inhibit UDPG formation. The formation of UDPGA from UDPG involves NAD requiring enzyme UDPG-dehydrogenase or UDPG:NAD-oxidoreductase (1.1.1.22). In the present study it has been shown that SA inhibits UDPGA formation by competitive mechanism with NAD. The authors also have shown that SA inhibits UDPGA formation by noncompetitive mechanism with UDPG. Salicylic acid is a quite potent inhibitor for UDPG-dehydrogenation reaction. The K_m value for this reaction is $9.6 \times 10^{-5}M$. The K_i values for the competitive inhibition with NAD is $2.1 \times 10^{-3}M$ and the K_i for the noncompetitive inhibition with UDPG is $8.7 \times 10^{-3}M$. Since the rate of reduction of NAD was employed as a measurement of enzyme activity, the concentration of NAD used was much higher than that normally present in the normal tissues. In the competitive inhibition studies the lowest concentration of NAD used was $2.0 \times 10^{-4}M$ (see the last point in Fig. 2), and the activity of the enzyme, in the presence of $1.25 \times 10^{-3}M$ of salicylic acid was only 73.7% of the control. In the noncompetitive inhibition studies the lowest concentration of UDPG tried was $5 \times 10^{-5}M$ (see the last point in Fig. 3), and the activity of the enzyme, in the presence of $2.5 \times 10^{-3}M$ of salicylic acid was only 80% of the control. In tissues, the concentration of NAD is only in the neighborhood of $10^{-7}M$. The concentration of salicylic acid required to reduce 30% of the activity of UDPG-dehydrogenase activity should be about $10^{-6}M$.

Salicylic acid inhibits several other NAD-requiring dehydrogenases by competitively inhibiting NAD (14). It is not known whether SA also competes with other substrates involved in dehydrogenation reactions. Salicylic acid also inhibits glutamate-dehydrogenase (1.4.1.2) activity noncompetitively *in vitro*. The mechanism of inhibition of glutamate-dehydrogenase is reversible but does not involve competition with either glutamate or NAD(34).

The next step involved in mucopolysaccharide synthesis is the condensation of glucuronic acid and glucosamine. The enzyme involved in transferring glucuronyl group from UDPGA is UDPglucuronate-glucuronyl-transferase or UDPglucuronyl-transferase. The acceptor of this reaction is nonspecific, which involves phenols, alcohols, amines, and fatty acids. Salicylate, itself, is also an acceptor, therefore, it is expected that SA inhibits this reaction competitively.

Salicylic acid is also a potent inhibitor of UDPglucuronyl-transferase reaction. The K_m value for this reaction is $2.44 \times 10^{-4}M$. The K_i value for the competitive inhibition of salicylic acid is $1.47 \times 10^{-4}M$. In addition, salicylates are known to inhibit the incorporation of sulfate in acid mucopolysaccharide synthesis (9).

Salicylic acid thus inhibits every known step in acid mucopolysaccharide synthesis. It is very likely that salicylic acid retards wound healing by a mechanism that involves acid mucopolysaccharide synthesis. The gastrointestinal irritation effect of salicylic acid is probably also due to its inhibitory action on mucopolysaccharide synthesis. Mucopolysaccharide is an important component of the protective lining of the gastrointestinal tract.

REFERENCES

(1) K. H. Lee, *J. Pharm. Sci.*, **57**, 1042(1968).

(2) J. E. Dunphy and D. S. Jackson, *Am. J. Surg.*, **104**, 273 (1962).

(3) K. H. Lee, *J. Pharm. Sci.*, **57**, 1238(1968).

(4) J. W. Uhr, G. Weissmann, and L. Thomas, *Federation Proc.*, **20**, 303(1961).

(5) W. S. Miller and J. G. Smith, *Proc. Soc. Exptl. Biol. Med.*, **122**, 634(1966).

(6) J. P. Bentley, *Acta Chem. Scand.*, **19**, 15(1965).

(7) J. E. Dunphy and K. N. Udupa, *New Engl. J. Med.*, **253**, 847(1955).

(8) J. P. Bentley, *Ann. Surg.*, **165**, 186(1967).

(9) H. Bostrom, K. Bertsen, and H. W. Whitehouse, *Biochem. Pharmacol.*, **13**, 413(1964).

(10) K. H. Lee, unpublished data.

(11) A. J. Bollet, J. F. Goodwin, and D. V. Anderson, *Arthritis Rheumat.*, **4**, 624(1961).

(12) P. Von Schönhöfer, *Med. Pharmacol. Exptl.*, **15**, 491(1966).

(13) D. A. Kalbhen, K. Karzel, and R. Domenjoz, *Med. Pharmacol.*, **16**, 185(1967).

(14) W. J. W. Hines and M. J. H. Smith, *Nature*, **201**, 192(1964).

(15) P. L. Savant, S. Shibko, U. S. Kumta, and A. L. Tappel, *Biochim. Biophys. Acta*, **85**, 82(1964).

(16) A. L. Dounce, R. F. Witter, K. F. Monty, S. Pate, and M. A. Cottone, *J. Biophys. Biochem. Cytol.*, **1**, 139(1955).

(17) C. De Duve, R. Wattiaux, and M. Wibo, *Biochem. Pharmacol.*, **9**, 97(1962).

(18) C. H. Fiske and Y. Subbarow, *J. Biol. Chem.*, **66**, 375(1925).

(19) J. C. Strominger, E. S. Maxwell, J. Axelrod, and M. Kalchar. *J. Biol. Chem.*, **224**, 79(1957).

(20) R. T. Williams, *Biochem. J.*, **37**, 329(1943).

(21) B. N. Pogell and C. R. Krisman, *Biochim. Biophys. Acta*, **41**, 352(1960).

(22) G. A. T. Levvy and I. D. E. Storey, *Biochem. J.*, **44**, 295 (1949).

(23) G. T. Mills and E. E. B. Smith, in "Methods of Enzymatic Analysis," H. U. Bergmeyer, Ed., Academic Press, New York, N. Y., 1965.

(24) G. J. Dutton and I. D. E. Storey, *Biochem. J.*, **57**, 275(1954).

(25) E. E. B. Smith and G. T. Mills, *Biochim. Biophys. Acta*, **13**, 386(1954).

(26) J. T. Dingle, in "Ciba Foundation Symposium on Lysosomes," A. V. S. de Reuck and M. P. Cameron, Eds., Little, Brown, Boston, Mass., 1963.

(27) H. Lineweaver and O. Burk, *J. Gen. Physiol.*, **17**, 591(1934).

(28) J. J. R. Duthie in "Salicylates, an International Symposium," A. S. J. Dixon, B. K. Martin, M. J. H. Smith, and P. H. N. Wood, Eds., Little, Brown, Boston, Mass., 1963.

(29) Z. A. Cohn and J. G. Hirsch, *J. Exptl. Med.*, **112**, 983(1960).

(30) G. Weissmann and L. Thomas, *Recent Progr. Hormone Res.*, **20**, 215(1964).

(31) V. W. Stuyvesant and W. B. Jolley, *Nature*, **216**, 585(1967).

(32) J. Raekallio, *Exptl. Mol. Pathol.*, **4**, 303(1965).

(33) T. M. Brody, *J. Pharmacol. Exptl. Therap.*, **117**, 39(1956).

(34) B. J. Gould, A. K. Huggins, and M. J. H. Smith, *Biochem. J.*, **88**, 346(1963).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 2, 1968, from the *University of California School of Pharmacy, San Francisco Medical Center, San Francisco CA 94122*

Accepted for publication December 2, 1968.

This work was supported by research funds from the Academic Senate Committee on Research, University of California, San Francisco Medical Center, San Francisco, CA 94122

* Recipient of President's Undergraduate Fellowship.