CERTAIN ASPECTS OF THE PHARMACOLOGY OF THE SALICYLATES*

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Few drugs have enjoyed an uninterrupted popularity comparable to that of the salicylates. Introduced as plant extracts centuries ago, the salicylates were among the first drugs to be synthesized and their use has increased enormously with time, the present annual production being approximately 6000 tons in the United States alone (206). Although many salicylates have been introduced into therapy, those most widely employed are acetyl-salicylic acid, sodium salicylate and methyl salicylate. It is fortunate for the present reviewer that a summary of the earlier studies on salicylates was published in 1927 by Hanzlik (126, 127) and more recently a complete bibliography with more than 4000 references was compiled by Gross and Greenberg (113). Accordingly, this review will omit most observations of primarily historical interest, as well as many studies that seem at this time to be of minor importance, and will concentrate on selected features of the pharmacology of the three most widely used salicylates.

Terminology: The word "salicylate" is generally employed as a generic term for the entire class of compounds containing the salicyl radical $C_6H_4(OH)\cdot CO\cdot O—$, and will be used for this purpose, in application to the free acid, its salts, ethers and esters. The anion $C_6H_4(OH)\cdot CO\cdot O—$ will be called salicylate ion, and its salts will be designated as salicylic salts. Occasionally the term "aspirin" is used instead of acetylsalicylic acid. The term salicyl is sometimes employed to designate the salicylic acid radical.

Methods of Determination: Early methods for determining concentrations of salicylate in biological fluids usually involved preliminary procedures for concentrating the drug, such as distillation (262) and sometimes bromination of the compound (40), but these require relatively large samples. At present, methods determining the compounds without preceding separation are preferred. The color which develops when a dilute solution of salicylate reacts with ferric ion (43, 98, 175, 262, 272) is still the basis for the most common method employed. Recently a very sensitive fluorophotometric procedure (238) has been described.

PHYSIOLOGICAL DISPOSITION

Absorption: The free acid, salts and esters are readily absorbed after cutaneous application (200). Methyl salicylate, which is frequently applied to the skin by rubbing, is so rapidly absorbed that measurable concentrations may be detected in the urine within 15 minutes (26); absorption is increased by dissolving the ester in alcohol, in liquid petrolatum or in anhydrous lanolin (44). Normal

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human subjects, to whom 0.2 gm. is applied locally, excrete about one fourth of the methyl salicylate in the urine (26). The absorption of various salicylic acid esters is rapid but the rate decreases as the length of the carbon chain of the ester increases (45). Salts of salicylic acid with organic bases may be absorbed three to five times as rapidly as sodium salicylate, when applied to the human skin (123).

Absorption of sodium salicylate from the oral cavity is relatively slow. No measurable concentrations appear in the urine of rabbits for 25 to 56 minutes after the drug is introduced in the mouth (31). In dogs with ligated pylorus, salicylates are fairly rapidly absorbed from the stomach; approximately two thirds of the dose are absorbed within one hour after an aspirin solution of pH 2.5 is administered, and somewhat less when sodium salicylate solution of pH 6.8 (41) is given. The absorption of both salicylic and acetylsalicylic acid from the stomach is slower when the solution is near neutrality than when the pH is low. In comparable experiments, acetylsalicylic acid solution of pH 2.9 is absorbed to the extent of 49 per cent in one hour whereas a salicylic acid solution of pH 2.5 is absorbed to the extent of 64 per cent in one hour. When salicylates are given orally to human subjects, the rate of absorption depends to an appreciable extent upon the emptying time of the stomach. Effervescent mixtures containing salicylate decrease the emptying time of the stomach (177), owing partly to the weak alkalinity of the solution and partly to the carbon dioxide produced. When salicylic acid is perfused through loops of the upper ileum in dogs, approximately two thirds of the amount perfused are absorbed in 30 minutes regardless of whether the solution is introduced at pH 2.5 or at pH 6.8 (41). When acetylsalicylic acid is introduced the absorption is approximately 50 per cent complete in 30 minutes. Absorption is slower in the stomach at high than at low pH values but the difference is less when the solutions are introduced into the intestine. Sodium salicylate, calcium salicylate and free salicylic acid are absorbed from the intestine of dogs more rapidly than acetylsalicylic acid (41). However, even in the case of acetylsalicylic acid, approximately two thirds of the dose are absorbed in one hour. When the absorption technic of Cori was employed in rats, it was found that sodium bicarbonate does not influence the rate of absorption from the gut (275). In studies on dogs designed to eliminate the effect of gastric emptying time and possible effects of anesthesia, solutions of salicylic acid made isotonic with sodium chloride and buffered to various pH values from 2.5 to 7.8 were passed through Thiry-Vella jejunal loops in dogs (171). The absorption rate was greatest at approximately pH 5.5 to 5.8 and approximately half as great at pH 7.8.

In contrast to the rapid absorption from the upper gastrointestinal tract, salicylates are rather slowly absorbed when given rectally, although measurable concentrations may be detected in the urine 15 minutes after administration (136). Some investigators have found that rectal administration of salicylate is feasible in patients (136). Others, using rabbits, have even concluded that absorption is more rapid from the large intestine than from the upper gastrointestinal tract; for example, one hour after the oral administration of 0.25 gram of

salicylate, 6.0 mgm. per cent is found in the blood, compared to 8.5 mgm. per cent after the rectal administration of the same dose (33). In general, however, those who have used rectal administration find low plasma levels, or at least that a given blood level is reached more slowly (143). Sodium bicarbonate diminishes the rate of absorption of salicylate from the rectum of man (212).

When sodium salicylate is given subcutaneously to rabbits the maximum concentration in the blood is attained between 30 and 60 minutes after administration; more than 40 per cent is excreted in six hours (30). After intramuscular administration of sodium salicylate to rabbits appreciable amounts are found in the urine after 30 minutes (32).

In rabbits, measurable concentrations of salicylate appear in the urine 40 minutes after intrapleural and 26 minutes after intraperitoneal administration; maximum urinary levels are obtained approximately 30 minutes after intraperitoneal and approximately 60 minutes after intrapleural administration (34). Intratracheal injection results in rapid absorption, the drug appearing in the urine two to 12 minutes after administration (30). A 2.5 per cent solution of sodium salicylate is not absorbed from the bladder of normal rabbits but is readily absorbed in animals in which the bladder mucosa is inflamed, the rapidity of absorption depending upon the degree of inflammation (32).

The evidence indicates that the salicylates are among the most rapidly absorbed drugs, regardless of the route of administration. Although the rate of absorption depends somewhat upon the pH, it is nevertheless rapid at all hydrogen ion concentrations within the physiological range.

Plasma Levels: The plasma levels of salicylate are a more precise measure of the amount of drug in the body than are whole blood levels, since the concentrations in the erythrocytes and plasma are not the same (168, 246). After a single oral dose of two grams of sodium salicylate in normal young men, the average peak salicylate level in the plasma is approximately 15 mgm. per cent and is reached in 100 to 120 minutes (246). Apparently acetylsalicylic acid is somewhat more slowly absorbed than sodium salicylate; two gram oral doses in normal young adults give peak levels of approximately 10 to 12 mgm. per cent after two to four hours (246). The levels attained are approximately proportional to the dose. Doses of 100 mgm. of sodium salicylate per kgm. give approximately 24 mgm. per cent peak concentration in the plasma (212); 150 mgm. per kgm. give 28 mgm. per cent, and 200 mgm. per kgm. give 40 mgm. per cent.

Distribution in the Body: A large part of the salicylate in the blood is bound to plasma proteins. As long ago as 1923 it was demonstrated that plasma salicylate is not completely diffusible through a semi-permeable membrane (54), and it was concluded from studies on the renal clearance of salicylate that most of it is bound to serum proteins (55). In *in vitro* studies, as much as 50 per cent of sodium salicylate was found to be bound by serum proteins at salicylate concentrations of 200 mgm. per cent (102). Most of the salicylate in the sera of rheumatic fever patients is associated with non-diffusible solids of the plasma (246), and at levels of 200 mgm. per cent only about 30 mgm. per cent is diffusible; at

levels of 400 mgm. per cent, only 125 mgm. per cent is diffusible; at levels of 500 mgm. per cent, about 250 mgm. per cent is diffusible. Exactly equal amounts of protein-bound salicylate were found in the plasma of patients who had been receiving the drug, and in normal plasma to which comparable amounts of the drug were added. It has been suggested that some salicylate is bound to cholesterol (258).

The salicylate content is usually greater in the serum than in the whole blood of animals that have been given sodium salicylate. When either single or repeated doses of salicylate are administered orally to rats the concentration of salicylate is usually slightly higher in the serum than in the whole blood (246); but when the concentrations are estimated on the basis of water content, the amount in whole blood is usually slightly higher than that in the serum, which would indicate a slight accumulation of the drug in the erythrocytes. The concentration of salicylates in the erythrocytes of normal subjects (168) and of rheumatic fever patients during treatment (246) is quite low and of the same order of magnitude as would be expected if the concentration in the red cells were similar to that found in the plasma ultrafiltrate. This suggests that the human red cell membrane is freely permeable to the ultrafiltrable portion of the plasma salicylate and that the drug is not bound by the proteins of the erythrocytes.

Early studies (242) demonstrate that the concentrations of salicylate in blood and joint fluid are similar; but the concentration of salicylate in parotid saliva is approximately 0.3 to 0.6 as high as in the serum (169), which suggests that in secretory glands protein binding may influence concentration gradients.

Apparently salicylates cross the placental barrier fairly readily. When pregnant rabbits are given large doses of sodium salicylate the concentration of salicylate in the fetal serum is approximately two thirds that in the maternal serum (147).

The salicylates are distributed through a volume of body water which appears much greater than that of the extracellular fluid. Studies on rats showed that the concentrations in the liver, kidney, and lung are similar to those in the serum (246). When the salicylate concentrations are calculated on the basis of water content, the liver contains about two thirds as much as serum, muscle approximately one fifth as much, the kidneys a similar amount, brain one third to one half as much and lungs contain one half to two thirds as much. In the case of a suicide committed with acetylsalicylic acid, the concentration of salicylate in the kidneys was approximately twice that in the liver; that in the spleen and muscle was somewhat smaller, and that in the brain only about half that in the liver (124). The binding of salicylates by proteins other than those of the plasma has not been especially studied, but the above results suggest that they are bound by proteins of most tissues.

The Degradation and Conjugation of Salicylates: It has been known for a long time that a fraction of the administered salicylic acid combines with glycine to form salicyluric acid, a substance analogous to hippuric acid (9, 10). The conjugation product has no therapeutic effect in rheumatic fever patients (253) and

is excreted unchanged after administration to rabbits. In earlier studies in the dog (221) it was found ortho-hydroxyl substituted derivatives of benzoic acid were conjugated with glycine to a smaller extent than the isomeric meta- and para-hydroxyl derivatives; later, the same investigator (223) reported the isolation of salicyluric acid from the urine of men receiving salicylic acid. This suggests that dogs metabolize the drug in a manner different from man, and later observations (246) suggest also that smaller amounts of salicyluric acid are formed in the dog. Rabbits apparently are unable to form salicyluric acid when salicylate is administered (42, 69). Despite the failure of some investigators (125), salicyluric acid can be recovered from the urine of persons who have been given salicylates (9, 10, 11, 12, 138). Holmes found that approximately 60 per cent of the salicylate is excreted in man as salicyluric acid (138).

The thorough studies of Kapp (153) and Kapp and Coburn (154), who found that children excrete 55 to 60 per cent of salicylate as salicyluric acid, leave no doubt that this conjugation product is an important metabolite. Studies on rheumatic fever patients (246) demonstrate that, whereas an appreciable amount of salicyluric acid is excreted in the urine, the amount therein may be no more than one third of the total amount excreted. This is in accord with the observations (153, 176) that rheumatic fever patients apparently metabolize appreciable amounts of salicylic acid in a different manner.

It has been known for a long time that when salicylates are administered to human patients the non-glucose reducing substances of the urine are increased (163). Others measured this increase in reducing substances and ascribed it to glucuronic acid (101). The glucuronic acid appearing in the urine after oral acetylsalicylic acid has been measured quantitatively (193). Some salicylate is excreted with one mole of glucuronic acid and some with two moles (101). In dogs, salicylate is bound to twice its molecular equivalent of glucuronic acid (222). In rabbits, small amounts of both ester glucuronide and ether glucuronide are excreted (42). There is evidence (153) that two different glucuronates appear in human urine, although they have not been isolated in pure form. The amount of salicylate excreted as glucuronate in about 20 to 25 per cent of the total in nonfebrile rheumatic fever patients (246), but the percentage may be smaller in febrile patients (153), partly because a greater amount is metabolized by oxidation.

There is at least one other well-established metabolite of salicylate. It is gentisic acid or 2,5-dihydroxybenzoic acid (6, 9). This substance can be identified by its transient blue color reaction with iron salts in dilute solution. It has been isolated from the urine of rats after the administration of sodium salicylate, acetylsalicylic acid and methyl salicylate (183). It fails to be formed in animals whose livers have been damaged by the administration of phosphorus or carbon tetrachloride (183). Normally from four to eight per cent of the salicylate administered is converted to gentisic acid and related compounds in non-febrile patients, but appreciably more is converted in rheumatic fever patients (153).

Baldoni's isolation (9) of a compound of the general formula $C_{16}H_{15}NO_8$, and named "acidoduraminsalicilico", has been verified by Kapp (153). It is closely

related to gentisic acid and may be a combination of gentisic acid and salicyluric acid, having the formula $HO \cdot C_6H_4 \cdot CO \cdot N(CH_2COOH) \cdot C_6H_2(OH)_2 \cdot COOH$.

In studies designed to determine the possible precursors of viramin C, Longenecker *et al.* (178) administered acetylsalicylic acid to rats but were unable to find any marked increase in vitamin C excretion and concluded that the salicylate is probably not utilized for ascorbic acid formation.

When methyl salicylate is administered to patients, some of it is probably absorbed unchanged. Normal subjects eliminate 45 to 57 per cent of it as salicyl, but rheumatic fever patients excrete a more variable percentage (130). In dogs and cats the urine contains much less salicyl after the administration of methyl salicylate than after the administration of sodium salicylate (133). The metabolism of methyl salicylate has not been studied as thoroughly as that of salicylic acid, but its excretion may extend over a longer period than that of sodium salicylate (128).

The metabolism of acetylsalicylic acid differs somewhat from that of sodium salicylate. Some of it appears to be absorbed unchanged, since unhydrolyzed acetylsalicylic acid has been found in the plasma of human subjects two hours after they received the drug (168). When isolated intestinal loops of dogs are perfused with acetylsalicylic acid solution, the outflowing solution contains no more free salicylate than the solution introduced, which suggests that the ester is not hydrolyzed in the intestinal lumen but only during or after absorption (171). Hanzlik and Presho (129) found unchanged acetylsalicylic acid in the urine of patients given the drug but their analyses depended upon the difference in intensity of the color reaction with iron before and after hydrolysis of the urine. It is known from later work (153, 246) that the glucuronates are hydrolyzed by the preparatory procedures they employed. Therefore, their conclusion that 25 per cent of the salicylate was unchanged acetylsalicylic acid is probably erroneous. The metabolites are almost equal in amount after administration to human subjects of equal molar amounts of sodium salicylate and of acetylsalicylic acid (246). Normal human plasma hydrolyzed acetylsalicylic acid fairly readily, a dilute solution being almost completely hydrolyzed within three hours (247). The drug is rapidly hydrolyzed to salicylic acid, when administered intravenously to dogs (246), and the tissues of several species of animals are capable of hydrolyzing it readily (247, 265). The liver and kidney of guinea pigs and rats are richest in the hydrolyzing enzyme, each of these tissues containing at least five times as much per gram as the plasma (247, 265); guinea pig brain contains less and muscle none (265). The crude enzyme is a very water-soluble substance not precipitable even by saturated sodium sulfate solution. It has its maximum activity at pH 6.0 to 6.5, is readily destroyed by heat, does not hydrolyze acetamide linkages such as in acetyl-para-aminobenzoic acid or acetanilid, but rapidly hydrolyzes ethyl butyrate (247). By concentrates of a crude enzyme prepared by procedures described for liver esterase, both ethyl butyrate and acetylsalicylic acid are hydrolyzed at rates proportional to those observed with the crude tissue extract. This indicates that the enzyme is similar to liver esterase (8, 90).

After administration of either sodium salicylate or acetylsalicylic acid, the

serum contains salicylate ion, and after administration of acetylsalicylic acid it also contains a small amount of the unchanged ester (168); but no other metabolite of the two drugs has ever been demonstrated in the serum. The studies of Brodie *et al.* (43) show that the plasma contains no salicyluric acid.

Excretion: Although a small part of administered salicylate may be excreted in other body fluids (156), most of it is excreted in the urine. The quantity as well as the form in which salicylates are excreted varies with the complex of factors which govern the formation of degradation and conjugation products in the tissues and the excretion of the absorbed substances and their metabolites. Of the total dose administered, about 80 per cent is excreted in normal human subjects in the form of compounds containing the salicyl group, and about four to eight per cent is converted to gentisic acid and related compounds (154, 246).

It has been known for a long time that patients tolerate salicylates better when sodium bicarbonate is given simultaneously (264); sodium bicarbonate lessens the tendency to nausea and vomiting. This beneficial effect of sodium bicarbonate has often been attributed to reduction of a local irritant effect in the gastrointestinal tract, but the origin of the late nausea and vomiting is largely central and many observations show that the main effect of sodium bicarbonate is an increased salicylate excretion. Smull *et al.* (248) were the first to point out that the administration of sodium bicarbonate definitely lowers the serum salicylate level during the therapy of patients with acute rheumatic fever. They ascribe this reduction to one or more of the following factors: interference with absorption, increased extracellular fluid volume, and increased renal excretion. It was later demonstrated that the last factor is of primary importance. When patients are given sodium bicarbonate in addition to their usual dose of sodium salicylate, the plasma levels promptly fall by 33 per cent, and the amount of free salicylate in the urine increases rapidly and almost in proportion to the diminution of plasma level (246). Moreover, when patients with a fairly constant plasma level of about 45 mgm. per cent receive the same dose of salicylate with an equal amount of sodium bicarbonate, the average plasma level falls rapidly to 20 mgm. per cent (246). As soon as sodium bicarbonate is discontinued the plasma salicylate concentration rises rapidly to the prior level.

Sodium bicarbonate increases the urinary excretion of salicylate in children so rapidly that the blood level four hours after its administration may be only half of that attained without sodium bicarbonate (187). It has been suggested (53) that alkali should not be given with salicylate except when it is desirable to reduce the plasma level. In dogs, sodium bicarbonate decreases the serum level and increases the output in the urine (275). In both dogs and human subjects, the administration of either sodium bicarbonate or potassium citrate diminishes the plasma level by 10 to 25 per cent by increasing the amount of free salicylate in the urine (212). Ammonium chloride and para-aminobenzoic acid produce the opposite effect. When the urinary pH is increased from 5.2 to 8.0, the free salicylate excreted increases from 10 per cent to about 60 per cent of the total excreted.

The renal clearance of salicylate showed a close relationship to the urinary

pH. The relationship is not linear; the clearance rises slowly with an increase in the urinary pH from 5.5 to 7.0 and rapidly with further increasing pH; at pH 7.7 clearance is approximately 10 times that at pH 6.0 (246).

Perhaps the rise in renal clearance with increase in urinary pH can be explained by considering that the ratio of non-ionized to ionized salicylate increases with the hydrogen ion concentration. The ionized form is highly water-soluble and relatively insoluble in lipids; the reverse is true for the undissociated acid (65). If the renal tubules are more permeable to the lipid-soluble form, then reabsorption of salicylate would be more complete at a low urinary pH. Conversely, at a high pH, the preponderance of the lipid-soluble form would result in high clearance values. If this hypothesis is true then the change in clearance with pH should be independent of the nature of the alkalinizing agent and should also be observed in the excretion of other weak acids. Additional evidence comes from the studies on the excretion of nicotine (121) and of quinacrine in man (150). In both cases these basic substances are excreted much less rapidly when the urine is alkaline; that is, the excretion rate varies with urinary pH in a manner opposite to that of salicylate. Similar conditions have been shown to govern the permeation of weak electrolytes through the cornea (65). In these studies it was shown that permeability is a function of pK. Regardless of whether the active principle is a weak acid such as salicylic acid, or a weak base such as atropine, penetration of the salt is slower than that of the free lipid-soluble principle.

Oral administration of para-aminobenzoic acid has been shown to cause an increase in the plasma level of salicylates (78). This has been amply confirmed by investigators who found that the salicylate levels can be increased two to five times by the administration of two to 24 grams of para-aminobenzoic acid per day (23, 203). In a preliminary report (78) it was not clear whether the para-aminobenzoic acid was administered as the free acid or as the sodium salt, but from a later report (237) it is evident that, in man, in either case the plasma levels are higher. The investigators suggested that para-aminobenzoic acid depresses the formation of salicyluric acid, that the pH of the urine is lower and that both these factors decrease the amount excreted as free salicylate. It was also shown that para-aminobenzoic acid depresses the formation of hippuric acid in man. It has been demonstrated that para-aminohippuric acid is synthesized by the liver (66). This observation coupled with the observation that para-aminobenzoic acid interferes with the formation of salicyluric acid suggests that the enzyme system responsible for the conjugation of the two acids with glycine may be the same.

It seems probable that, in addition to the depression of salicyluric acid formation and the lowering of the urinary pH, para-aminobenzoic acid depresses the excretion of salicylate by depleting the body of base. Para-aminobenzoic acid when given as the free acid is rapidly conjugated to para-aminohippuric acid (245) which is then excreted by the renal tubules (27), probably taking precedence over the excretion of free salicylate or salicyluric acid.

PHARMACOLOGICAL ACTIONS

Effects on the Gastrointestinal Tract: Whether salicylates severely irritate the mucous membranes of the enteric tract is controversial. In some cases of poisoning, the gastrointestinal tract showed hemorrhage and ulceration (28), but in other cases of severe poisoning gastrointestinal alterations were absent (82). Even in methyl salicylate poisoning the gastrointestinal symptoms may persist for some time with no permanent damage to the mucosa (219). In dogs, it was possible to produce petechial hemorrhages of the gastric mucosa by intravenous administration of sodium salicylate, oral ingestion of acetylsalicylic acid or subcutaneous administration of methyl salicylate (76). The doses necessary are of the order of 300 mgm. per kgm. per day for three days or more. Gastric ulceration in rats can be produced by the oral ingestion of 300 mgm. of acetylsalicylic acid for 10 days (17). It can also be produced by subcutaneous administration. Patients receiving acetylsalicylic acid for some time gave no evidence of hyperemia or hemorrhage upon gastroscopic examination. Chronic doses produced no gastritis; the epigastric distress observed was ascribed to increased acid production or to pylorospasm (215).

In contrast to the observations on the irritant effects of salicylate on the gastrointestinal tract, it was shown that the administration of aspirin to rats inhibits gastric ulceration. This occurred whether the drug was given intraperitoneally, intravenously or subcutaneously. Aspirin was slightly less effective than sodium salicylate (214). Sodium salicylate in a concentration of 1:2500 does not alter the response of rabbit duodenal or uterine muscle to autonomic drugs (261).

Aspirin given in doses of 70 to 90 mgm. per kgm. daily for five days to Cope pouch dogs caused no increase in gastric secretion but twice this dose was effective (57).

Effects on the Liver: Liver damage does not occur frequently even after large salicylate doses. However, in several cases of salicylate poisoning hepatic abnormalities of various degrees have been described (56, 82). A 26-year old woman who took 29 grams of acetylsalicylic acid exhibited hepatic damage but no gastrointestinal symptoms (82). In a fatal case of acetylsalicylic acid poisoning (159) in a 54-year old man who probably took 5.0 to 6.6 grams of the drug, the autopsy disclosed petechial hemorrhages in the liver. In children, swelling and fatty degeneration of the liver from methyl salicylate have repeatedly been described (81, 86, 162). Similarly, fatty degeneration of the liver in rabbits has been produced experimentally with sodium salicylate (186). Definite liver damage occurs in rats given 400 mgm. of sodium salicylate per kgm. per day, but in dogs 300 mgm. per kgm. are not enough to produce significant bromsulfonphthalein retention (18). *In vitro* studies have demonstrated that high concentrations of salicylate are necessary to depress rat liver respiration (182). In rabbits receiving large doses of sodium salicylate (147) liver glycogen may be greatly diminished. Sodium salicylate had no constant effect upon the nitrogen metabolism of dogs (241), and the excretion of bile salts was not affected (250).

Relation to Prothrombin Concentration: Since prothrombin is formed primarily in the liver and since salicylates can induce hypoprothrombinemia, the question of the effect of salicylates on this function of the liver has engaged a great deal of attention. It has been suggested (227) that the prolongation in prothrombin time sometimes observed after salicylate reflects liver damage. It has been postulated (151) that salicylates are converted to dicumarol or some similar substance by bacterial action in the intestinal tract, since oral administration of sodium salicylate to rabbits prolongs the prothrombin time but intravenous administration does not. After oral administration of sodium sulfasuxidine to rabbits salicylates do not prolong the prothrombin time, a fact which suggests that bacteria are responsible for the conversion. Hypoprothrombinemia can be produced in suckling rats (93) by feeding dicumarol or large doses of acetylsalicylic acid to the mothers. The hypoprothrombinemia is less marked in the young if the mother has been given vitamin K (172). Some investigators have found that sodium salicylate, acetylsalicylic acid and methyl salicylate all cause a decrease in the prothrombin in rats (58), but it is also produced by certain other drugs such as acetanilid and antipyrine. Others found no appreciable change in prothrombin time and no dicumarol in the urine after the administration of salicylates to rats (167). The prothrombin levels may be 10 to 60 per cent below normal in acute rheumatic fever (211) at salicylate levels of 35 mgm. per cent; but these cases showed no liver damage and the prothrombin time returned to normal despite continued salicylate therapy. Of 24 patients receiving salicylate for 15 to 35 days, prothrombin time was prolonged in six cases by the second to fifth day, but it was always normal by the ninth day (110). A fatal case of sodium salicylate poisoning exhibited a severe hypoprothrombinemia but insignificant hemorrhages (59). Children with rheumatic fever who are on salicylate therapy may develop hypoprothrombinemia but no hemorrhage (92). Although the prothrombin level may return to normal spontaneously, vitamin K is effective in hastening the return. It has been suggested that one mgm. of vitamin K be given per gram of salicylate (243) if liver function is adequate, but others believe its use is unwarranted (143). Much larger doses of vitamin K would be required in case of liver damage.

In a rather large group of patients, sodium salicylate produced a moderate but definite decrease in the prothrombin level, unrelated to the dose; no hemorrhagic tendency was observed (49). Others (67) correlate low prothrombin levels with high plasma salicylate levels but find only insignificant hypoprothrombinemia at plasma salicylate concentrations of 35 to 60 mgm. per cent. Patients receiving 1.3 to 5.3 grams of sodium salicylate or aspirin a day consistently develop low prothrombin values and exhibit prolonged clotting time (49). In rheumatic fever studies in military personnel (49) the prothrombin level was low in the early stages of treatment but it soon rose. The usual dose in these studies was 10 grams of acetylsalicylic acid per day combined with eight grams of sodium bicarbonate. The prothrombin level after salicylates varies in different species depending on the ease with which vitamin K deficiency can be produced (210). The electrophoretic patterns of the plasma of patients receiving salicylates and

of normal men ingesting four grams of aspirin a day for seven days showed no evidence of a prothrombin deficiency (79).

In summarizing the evidence Quick (224) expresses the opinion that the salicylates resemble dicumarol in their action on prothrombin but are less potent. Agreement exists that salicylates produce a moderate decrease in prothrombin but the doses required are usually large. Even then the tendency to spontaneous hemorrhage is not marked.

Sodium gentisate, a metabolic product of salicylate, when administered to patients in doses up to 10 grams per day, produces no significant increase in prothrombin time (196).

Effect on Sedimentation Rate: In *in vitro* experiments the sedimentation rate, especially when high, is markedly reduced by sodium salicylate (139). Sodium benzoate and sodium bicarbonate are ineffective. *In vivo* salicylates also decrease the sedimentation rate (140). Plasma fibrinogen is reduced as much as 50 per cent and the reduction is proportional to the total dose given and not to the plasma salicylate level; the sedimentation rates parallel the fibrinogen concentration (226). Patients with rheumatic fever and with carcinoma show similar changes in sedimentation rate and plasma fibrinogen. The alteration of the fibrinogen content of plasma by salicylates (140) is attributed to a direct action both on the plasma and on liver function (226). If salicylates are stopped as soon as the elevated sedimentation rate becomes normal a secondary rise may be observed later (170). Since changes in sedimentation rate are believed to be associated with the activity of the rheumatic fever process, the effect of salicylates on the sedimentation rate cannot be differentiated clearly from the effects of salicylates on the rheumatic process itself.

Effects on Uric Acid Excretion: In two normal men given 6.6 grams of salicylate per day, excretion of nitrogen, inorganic phosphorus and uric acid was increased (74). It has been concluded that salicylates lower the renal threshold for uric acid (73); when the urinary uric acid concentration rises, the blood level falls (95). Whereas salicylates increase the uric acid in the urine of human subjects (232), the meta and para isomers have no influence. Recent studies (99) of the effects of sodium salicylate upon the excretion of uric acid in the rat show that, at first, the urinary uric acid is increased without a change in the blood uric acid in the renal blood flow or in the glomerular filtration rate, but that with the administration of more salicylate the blood level of uric acid falls. The urinary excretion of uric acid decreases after salicylate administration is discontinued (233). It is concluded that salicylates interfere with the tubular reabsorption of uric acid (99, 103). Earlier studies in the Dalmatian coach hound (111) suggested that salicylates increase uric acid excretion in the urine but decrease it after denervation of the kidneys. This was attributed to alteration of tubular reabsorption. More recently observations indicate that sodium salicylate has no effect on the clearance of uric acid in the Dalmatian dog (100). Gentisic acid in the urine will interfere seriously with the usual determination of uric acid (284).

Effect on Ascorbic Acid Excretion: Hemorrhages of small vessels attributed to

salicylates have been related to a deficiency of vitamin C (229). The scorbutic guinea pig with bacterial infection exhibits a pathological picture resembling that of rheumatic fever. In earlier studies it was found that administration of acetylsalicylic acid (71) or of sodium salicylate (156) increased the excretion of substances reacting with the indophenol reagent used for the detection of vitamin C. Later it turned out that the reagent reacts with many other reducing substances and, thus, may react with some other component in the urine, formed as a result of the administration of salicylate. By the use of more specific analytical methods, it was found that daily administration of acetylsalicylic acid to normal human subjects in doses varying from 0.6 to 2.6 grams did not affect the excretion of ascorbic acid in the urine (266, 282).

Effects on the Central Nervous System: Cases of salicylate intoxication often exhibit evidence of central nervous system disturbances such as delirium (1 189), and occasionally a psychosis may develop (38). In rheumatic fever patients receiving large doses of salicylates, evidence of mental confusion and dizziness is fairly common (68), and children receiving large doses often exhibit confusion and irritability. Occasionally delirium occurs in rheumatic fever patients after the administration of sodium salicylate (68), but possibly only after intravenous and not after oral administration (189). Cerebral irritability, dullness, stupor and coma may slowly develop after the administration of methyl salicylate (219). Evidence of alterations in the brain has been found after poisoning with sodium salicylate (7). An infant dying from methyl salicylate poisoning exhibited marked hyperemia of the brain and meninges (142).

Antipyretic Action: One of the most characteristic actions of salicylate is the fall in body temperature which it produces in animals with fever. This is striking in patients with rheumatic fever (13, 14, 61). In monkeys with fever produced by the subcutaneous administration of yeast, aspirin reduces the temperature, principally by causing increased sweating and vasodilatation (117); in contrast, the drug causes only a slight decrease in the temperature of normal monkeys. In both patients and monkeys, sodium salicylate prevents the rise in temperature produced by the administration of xanthine or caffeine (190). Barbour (15) conducted extensive studies on the mechanism of antipyresis by salicylate and showed that in fever the heat loss increases from a basic value of 37.7 to 52.1 Cal. per sq. meter. In normal individuals one gram doses of aspirin produce an increase in carbon dioxide output, and heat production rises from 37.8 to 40.3 Cal. per sq. meter (16). The maximum effect occurs 90 to 120 minutes after drug administration. Respiratory quotient and pulse rate are unchanged. Lesions in the anterior and anterolateral hypothalamus of monkeys cause marked temperature lability but no changes in response to acetylsalicylic acid (118). Barbour (15), as evidence for his idea that antipyretics act by causing hydremia, reported that aspirin was active in this manner, but it was not clear whether a central mechanism was involved. Large doses of salicylates raise the body temperature of dogs (39, 194). The rise is not prevented by nicotine, curare, atropine or decapitation and is therefore peripheral. It is prevented or lessened by potassium cyanide.

Analgetic Action: The most important therapeutic action of the salicylates is to reduce pain of moderate severity, but it is difficult to obtain unequivocal evidence of this action either in animals or normal human subjects. When a light is focussed on the tail to produce a pain stimulus, no analgetic effect can be detected in rats even after doses ranging from 0.5 to 2.5 gm. per kgm. (87). Similarly, the threshold to electrical stimulation in mice (160) is not altered by aspirin in doses up to 500 mgm. per kgm. In guinea pigs, acetylsalicylic acid in juxta-lethal dosage abolishes the skin twitch response to pain, but the dose required is 270 mgm. per kgm. (278). Dogs given two grams of aspirin exhibit some analgesia when a twitch of the musculature of the back is taken as an indicator of pain produced by a locally applied stimulus; but the rise in threshold is smaller and of shorter duration than in man (4).

The most common method used for producing pain in human studies of analgetic drugs is that of focussing radiant energy from a bright light source on a blackened area of the skin (134). The heat intensity required to elicit pain is used to measure analgesia. In three trained subjects 1.8 gram of aspirin was reported to raise the pain threshold by 35 per cent (134). The peak rise in threshold is proportional to the dose and the onset of action occurs ten to 15 minutes after ingestion. The effect persists for at least five hours with doses of 0.6 to 1.8 gram. A maximum analgetic effect is maintained with doses of 0.3 gram every two hours but not with 0.6 gram every three hours. Single doses larger than 0.3 gram do not further increase the pain threshold but their effect lasts longer.

These studies have been criticized because of the paucity of subjects and because placebos were not employed (113); also elevation of the pain threshold probably is not an adequate measure of analgetic action (106). Moreover, the sharp twinge of pain produced by superficially applied radiant heat is scarcely comparable to the deep, dull persistent type of pain for whicb the salicylates are so commonly employed. Much of the rise in pain threshold attributed to salicylates has been ascribed to psychological factors such as suggestion (106).

When pain is induced in human subjects (107) by an alternating current applied to metal fillings in the teeth, aspirin does not elevate significantly the pain threshold (108, 213). When intense pain of an aching character is produced by the aid of a sphygmomanometer cuff, the threshold for pain induced by the alternating current is raised (106, 107); this rise does not occur if aspirin, 0.65 gram, is administered 30 to 40 minutes prior to the test (108, 213).

Nausea and Vomiting: Since the work of Eggleston and Hatcher (85) in 1912 evidence has been increasing that the salicylates produce emesis by central stimulation. They found that the minimal emetic dose of sodium salicylate in dogs is 200 to 300 mgm. per kgm when the drug is given intravenously, but 750 mgm. per kgm. when it is administered orally. They obtained evidence of emetic effect even in eviscerated dogs. In rheumatic fever patients in whom high plasma levels of salicylate are maintained, nausea is common as a side effect (51, 52); but nausea is almost as frequent after intravenous as after oral administration of the drug (267) and occurs even when the gastric contents contain no salicylate (57, 112). Nausea is said to occur at salicylate plasma levels of about 37 mgm. per

cent regardless of whether the drug is given orally or intravenously. However, some workers find that the vomiting is not necessarily related to the plasma level, and Graham and Parker (112) report that, although vomiting occurs at an average plasma salicylate contentration of 28 mgm. per cent, there is some evidence that local irritation is a factor. In children with rheumatic fever vomiting is not necessarily related to the blood salicylate level, and therefore may be a local effect of the drug (188).

Effect on Respiration: Agreement exists that the increased respiratory rate observed after large doses of salicylate is the result of a direct central stimulating effect of the drug. The evidence is increasing that an acidosis, if it occurs, is secondary to the renal adjustment to the respiratory changes (3, 20, 68, 91). In most cases of salicylate poisoning that have been reported, respiration is deep and rapid and the rate may be as high as 38 per minute (21, 81, 91, 201). Occasionally the hyperpnea is of the Kussmaul type and difficult to distinguish from that in diabetic acidosis (37). After methyl salicylate poisoning it is common to find hyperpnea and dyspnea (86, 219) and a marked increase in respiratory rate (162). In normal human subjects 12 grams of acetylsalicylic acid given over a period of eight hours produce a respiratory alkalosis with an increase in respiratory volume of four liters per minute (91). There is frequently an alkalosis with a rise in blood pH, and numbness and tingling of the extremities similar to that observed in tetany (68). Some believe that in patients there is primarily an acidosis which should be treated with sodium bicarbonate (76, 209, 219). Others find a primary hyperventilation due to central stimulation, which then leads to a carbon dioxide-deficit type of alkalosis with an alkaline urine and a low blood bicarbonate (3, 37, 68, 119, 120, 137, 208, 225). Later a ketosis develops with a bicarbonate-deficit type of acidosis (137). The ketosis probably is not related directly to the administration of salicylate since it occurs after voluntary hyperventilation (218) and may result from the administration of alkali (185). It may be related to the inability to synthesize glycogen from protein (185).

Severe dyspnea occurs in patients at an average plasma salicylate level of 50 mgm. per cent. Graham and Parker reported that all patients exhibit hyperventilation and some exhibit severe dyspnea when the plasma levels are above 35 mgm. per cent (112).

Experimental studies on dogs (39) show that the intravenous administration of 0.19 to 0.6 gram of sodium salicylate per kgm. causes hyperventilation. At first, the blood carbon dioxide content, oxygen content or red cell volume of arterial blood are not significantly changed. Later body temperature rises sharply, respiratory rate increases, arterial carbon dioxide falls, and blood pH and hematocrit values increase. Others (119) have confirmed that in dogs there is central respiratory stimulation; in rabbits and cats there is marked respiratory stimulation with increased pulmonary ventilation (152). The intravenous administration of salicylates in dogs and guinea pigs produces an acceleration of the respiratory rate (174). Wright (281), in studies on cats, found that salicylate stimulates both the respiratory center and the sino-aortic nerve endings. Thus the locus of action is both central and peripheral. The peripheral reflex effect

predominates in anesthetized animals and the central stimulatory effect in decerebrate animals. In rabbits stimulation of respiration is obtained by salicylate administered intravenously (112). This effect is immediate and the carbon dioxide capacity changes only secondarily. The salicylate is equally effective when given intravenously with sodium bicarbonate (112). The respiratory effect is not depressed by morphine or phenobarbital. In cats and rabbits the respiratory stimulation is not abolished by removal of the carotid bodies or by atropine (112). Bilateral vagotomy invariably alters the respiratory rate and subsequent injections of salicylate fail to stimulate respiration significantly. It is concluded that the mechanism of stimulation is reflex via vagal afferent nerve fibers (112).

Effect on Acid-Base Balance: The changes in electrolyte pattern are probably related to respiratory stimulation. In almost every case in which it has been measured, the carbon dioxide combining power was found reduced, and the carbon dioxide dissociation curve was of the hypocapnic type owing either to overproduction of acid or to respiratory stimulation (201). The values reported for the carbon dioxide content of the venous blood range between 14 and 38 volumes per cent (7, 76, 209). A value of 57 volumes per cent, well within the normal range, was reported in one case of methyl salicylate poisoning, (86), and in other cases a moderate average fall of 12 volumes per cent has been observed (92). The carbon dioxide content of arterial blood has been found to be as low as 15 volumes per cent (236). In some cases of sodium salicylate or acetylsalicylic acid poisoning the carbon dioxide combining capacity may be 50 volumes per cent (37), but it may later fall to 29 volumes per cent (276). Other investigators, however, have found no correlation between the salicylate level of the plasma and the alkali reserve (110).

Evidence that the diminished carbon dioxide capacity and the rise in pH are due to respiratory stimulation with a subsequent loss of carbon dioxide has been presented by several investigators (3, 68, 91, 137, 208). Normal subjects receiving 12 grams of aspirin exhibit an average rise of 0.06 in blood pH and a fall in plasma bicarbonate of 3.0 millimoles per liter (91). This is associated with an average increase of four liters per minute in respiratory volume. In 27 patients receiving large doses of salicylate the carbon dioxide capacity was decreased to as little as 32 volumes per cent, but the average pH rose from 7.42 to 7.51 (208). Andersen *et al.* (3) studied 7 patients who had received 19 to 32 grams of acetylsalicylic acid in two days and noted a reduction of 20 to 30 per cent in alkali reserve. Similar results were obtained with sodium salicylate but larger doses were believed necessary. The investigators estimated that if hydrochloric acid instead of acetylsalicylic acid were given it would require three to four times as much to produce a comparable fall in alkali reserve. They believe that the hyperventilation is primarily due to central stimulation and that the favorable effect of sodium bicarbonate is probably due to the increased urinary excretion of salicylate.

In advanced salicylate intoxication, particularly in children, both serum carbon dioxide content and pH may be distinctly decreased, but this is usually preceded by a ketosis and follows an initial period of hyperventilation and uncompensated respiratory alkalosis (88).

Other Central Nervous System Effects: Several other symptoms presumably associated with stimulation or depression of the central nervous system have been described after the administration of salicylates. In cases of poisoning some patients are confused and incoherent (159, 252), and dizziness and irresponsibility have been noted. Tinnitus has long been found associated with large doses of salicylate. It begins in a range of 10 to 39 mgm. per cent plasma concentration of salicylate (112). Diminished high-tone acuity may develop in a range of 10 to 46 mgm. per cent; it occurred in all of 33 patients in the first 7 days at levels above 35 mgm. per cent. Jager and Alway (148) observed tinnitus in 34 of 38 patients, but no serious toxic effects occurred below 40 mgm. per cent. Prenatal medication with salicylates has been suggested as a factor in deafness of the newborn (259), but in four cases cited all the mothers had received quinine *ante partum* and none had received salicylates. Acetylsalicylic acid antagonizes the hypnotic action of phenobarbital in rats (105) whereas acetophenetidin does not; from studies on dogs it was concluded (120) that hypnotics should not be used in salicylate poisoning because the central nervous system is unusually susceptible to their action. Acetylsalicylic acid in doses comparable to therapeutic doses in man has little effect on spontaneous activity, maze learning or relearning of albino rats even when given throughout their life cycle (36). The thresholds of perception for touch, vibration, smell and hearing are not raised by therapeutic doses of acetylsalicylic acid (274). However, the two-point discrimination threshold was slightly raised in two subjects by 1.8 grams of acetylsalicylic acid. In doses of five to 40 mgm. per kgm. aspirin produces no change in the threshold for electrical convulsions in rabbits (256). The vasopressor effect of epinephrine is markedly increased by salicylates (194).

Allergic Effects of Salicylate: Severe reactions from the administration of relatively small amounts of salicylates have repeatedly been reported. An asthmatic patient who took 0.3 gram of acetylsalicylic acid died within 10 minutes (83). She had had two previous severe reactions to the drug and knew that she was hypersensitive to it. The case was complicated by the fact that the patient had sarcoma of the dura. In another asthmatic patient death occurred after 0.6 gram of aspirin (97). This patient was also known to be hypersensitive to the drug. Hypersensitiveness to acetylsalicylic acid manifested by an angina pectoris syndrome has been described in two patients; one of them also had urticaria (244). A small "granule" of acetylsalicylic acid applied to the tongue produced a violent attack of coughing, asthma and itching within one minute in individuals hypersensitive to the drug (80).

The incidence of hypersensitivity to acetylsalicylic acid is probably no greater than two per 1000 of the general population (104). Hypersensitivity to acetylsalicylic acid is much less frequent than that to quinine (104), but the total number of cases is relatively large because of the large number of people who take acetylsalicylic acid. Buchstein and Prickman (48, 220) reviewed 62 allergic reactions to acetylsalicylic acid at the Mayo Clinic and 33 from the literature. Their survey did not include cases resulting from overdosage. They concluded that the skin test is dangerous and unreliable. The hypersensitivity may be remarkably

specific, with no cross-sensitivity to salicylic acid. Sensitivity is more frequent in females and in patients with other allergies, especially asthmatics.

Effects on Enzyme Systems: High concentrations of salicylate are necessary to depress the respiration of rat liver *in vitro* (182). They produce some effect on xanthine oxidase, on carboxylase and the dismutation between hexosediphosphate and pyruvate (182). The salicylates inhibit catalase activity (277) and this has been suggested as the mechanism by which antipyretic drugs lower body temperature. However, other analgesics such as acetanilid, acetophenetidin, antipyrine and quinine do not inhibit catalase activity. It has been demonstrated *in vitro* that sodium salicylate partially inhibits the digestion of egg albumin by pepsin (2).

Salicylates have been found to inhibit the spreading effect of hyaluronidase in rheumatic fever patients (115, 116). Sodium salicylate inhibits the effect of hyaluronidase from bull testis and from *Cl. perfringens in vivo* and *in vitro*, but a higher concentration of salicylate is needed for the *in vitro* inhibition (77). Salicylates do not inhibit the effect of hyaluronidase on an elevated sedimentation rate produced in rabbits by the injection of sodium hyaluronate (283). This is evidence that salicylate does not exert a direct action of hyaluronidase. It has been suggested that the inhibition of hyaluronidase *in vivo* may be due to the gentisate formed from salicylate (197), but there is conflicting evidence concerning the inhibition of the enzyme by gentisic acid (180, 234). Others confirm that neither salicylate nor gentisic acid inhibits hyaluronidase *in vitro* but that carboxyparabenzoquinone does inhibit slightly at 0.001 molar and higher concentrations (180). This appears of no particular significance in therapy with salicylates since there is no evidence that carboxyparabenzoquinone is formed in appreciable amounts.

Effects of Micro-organisms: It has been known for some time that salicylates have antibacterial properties. For example, salicylate was therefore suggested for the preservation of urine samples (22). Potassium salicylate, 0.2 molar, inactivates tomato spotted wilt virus and tobacco mosaic virus (25), and acetylsalicylic acid inactivates yellow fever virus (94), but the concentrations required are extremely high and the action is probably non-specific. Sodium salicylate inhibits the growth of *Rickettsia typhi* in embryonated hen eggs and is comparable in effectiveness to para-aminobenzoic acid (230). Sodium salicylate markedly increases the oxygen consumption of tubercle bacilli (24), perhaps by serving as a substrate; acetylsalicylic acid is similarly effective, but only after hydrolysis. Para-hydroxybenzoic acid has little and methyl salicylate and meta-hydroxybenzoic acid have no effect. Sodium salicylate, 60 mgm. per cent, inhibits the growth of the tubercle bacillus in a glycerin egg culture (199); the effect is additive with diaminodiphenylsulfone. Whitehead, however, found that the growth of the tubercle bacilli *in vitro* is slightly enhanced by the addition of low concentrations of salicylate (273), and the observed inhibiting effect at higher concentrations may be nonspecific.

The stimulating effect of salicylic acid on the tubercle bacillus is inhibited by p-aminosalicylic acid (184). Salicylic acid has some inhibitory effect on fungal

infections when applied to the skin, and 36 of 54 patients were symptomatically cured (260). Of several salicylates tested some were effective in destroying the fungi associated with mildew diseases (60). In studies with *Streptococcus aureus* and *Escherichia coli*, evidence has been obtained that the antiseptic action is due to inhibition of the synthesis of pantothenic acid (144, 145). Pantothenic acid, pantolactone and pantoyltaurine antagonize the inhibiting effect of salicylate on *E. coli* (251) and pantothenic acid antagonizes the effect on *Myco. tuberculosis* (146).

Effects on Immunological Phenomena: Because of the favorable effect of salicylates in rheumatic fever, many investigators have been concerned with the effect of salicylates on immunological phenomena. A saturated solution of sodium salicylate neutralizes diphtheria and tetanus toxins without destroying their antitoxinogenic capacity (29). This may be due to protein denaturation, a known effect of high salicylate concentrations (6, 240). Toxic filtrates of hemolytic or non-hemolytic streptococci are not neutralized (72). Salicylates prevent precipitate formation when added to a system of egg albumin and its antibody (63). The inhibition is proportional to the concentration of salicylate. The effect is reversible and appears to be due to inactivation of the antibody.

Administration of two to three grams of acetylsalicylic acid per day has no appreciable effect on the development of agglutinins following typhoid inoculation (217). Guinea pigs and rabbits injected with rhesus monkey erythrocytes form less anti-Rh agglutinins when treated with sodium salicylate (141). Sodium salicylate given to pregnant mothers in doses of 8 to 10 grams a day for 20 weeks failed to prevent the development of erythroblastosis fetalis (195). Salicylates suppress antibody formation to typhoid H and O antigens in rheumatic fever patients injected with typhoid vaccine; the changes in leucocytes, plasma fibrinogen, erythrocytes, and sedimentation rate, which were observed in the controls, were absent (149).

Salicylates do not prevent experimental streptococcal arthritis in rabbits, but the animals manifest less joint inflammation (35). Sodium salicylate injected into rabbits suppresses the allergic dermal reaction to hemolytic streptococcal filtrates, but the subsequent proliferation of vascular lesions is the same as in the control animals (122).

In studies on immune body production in serum disease in rabbits, serum disease arthritis is usually prevented if salicylate treatment is started immediately after the serum injection and continued through the usual period of incubation (75). In similar experiments, others (263) found that salicylates do not prevent the histological changes. When sodium salicylate is injected into rabbits daily for eight days before administration of horse serum antigen, the formation of arterial lesions is prevented even though circulating antibody is present (254). It is postulated that salicylate prevents the fixation of antigen in the tissue cells. Some investigators found that sodium salicylate decreases the severity of the arterial and valvular lesions produced by the injection of horse serum into rabbits (249); others (231) found no prevention of either the vascular or the myocardial lesions but some prevention of the valvular lesions.

Rabbits sensitized to egg albumin are protected by acetylsalicylic acid from shock produced by the injection of a challenging dose of egg albumin (50). Nine of 10 sensitized animals given acetylsalicylic acid showed no shock, whereas all 10 controls responded with shock. This observation has been confirmed; the administration of sodium gentisate does not prevent the shock but aminopyrine does (166). Salicylates have no effect on histamine shock (50).

If guinea pigs are injected with guinea pig brain and certain adjuvants, a progressive encephalomyelitis develops that cannot be prevented by salicylates; but it is usually prevented by a combination of salicylate and p-aminobenzoic acid (109).

Acute Toxicity of Salicylates: As is true for many old and widely used drugs, less information on the acute toxicity in animals exists for salicylates than for more recently introduced drugs. The fatal dose in guinea pigs (route of administration not stated) is approximately 1.5 gm. per kgm. (96). The LD_{50} for acetylsalicylic acid orally in mice is 1.36 gm. per kgm. (46). In white mice, the minimal lethal dose (route of administration not stated) of sodium salicylate is 5 millimoles per kgm. (800 mgm. per kgm.), as compared with 2 millimoles per kgm. for methyl salicylate and 0.5 millimoles per kgm. for phenol (155). In rats, the oral LD_{50} for acetylsalicylic acid is 1.24 gm. per kgm. (47). The acute toxicity of sodium salicylate for rabbits is reduced by sodium bicarbonate and is possibly increased by magnesium trisilicate (69). Ascorbic acid, as the sodium or the calcium salt, reduces the toxicity of sodium salicylate (69, 114, 216). Magnesium salts reduced the toxicity of salicylates in mice (279), but a protective action of calcium gluconate in acetylsalicylic acid poisoning could not be demonstrated conclusively (19).

The single fatal dose of salicylates in man is not precisely known. The fatal cases of poisoning have been reviewed in detail by Gross and Greenberg (113). The fatal dose of salicylate in man may be as low as 0.3 gram in individuals who are particularly hypersensitive to it, but patients have survived 40 grams of acetylsalicylic acid (173), and an arthritic patient is on record who took eight grams a day for 17 years without apparent harmful effects (70).

In his survey Lowy (181) found 1.6 deaths per million hospital admissions in the United States, attributed to aspirin. This compares with 50 deaths per million admissions, attributed to barbiturates.

Therapeutic Effects: The inadequate demonstration that in normal human subjects acetylsalicylic acid raises the pain threshold for a circumscribed heat stimulus (134) provided tentative experimental confirmation for the long established use of salicylates for the relief of pain. The major use of salicylates is for analgesia, particularly for treatment of simple headache or neuralgic pain.

Whereas the salicylates are probably most frequently employed for the relief of headache and neuralgia, their use in the treatment of rheumatic fever is perhaps more important since few of the effective drugs are safe enough to be administered in sufficient dosage for the long periods of treatment required. In considering the status of the salicylates in the therapy of rheumatic fever, it is interesting to recall that Latham (161) published a paper in The Lancet in 1885

entitled "Why does salicylic acid cure rheumatism?" and that 63 years later Reid published a paper (228) entitled "Does sodium salicylate cure rheumatic fever?". The salicylates are very effective in the symptomatic treatment of rheumatic fever (164), but it has been known for a long time (131) that other analgesics will also give prompt and effective relief of symptoms and may even provide permanent relief. Opinions are at variance on whether rheumatic fever as such responds to salicylates. Master and Romanoff (192) compared a group of rheumatic fever patients given 12 grams of sodium salicylate a day with a control group of patients receiving no salicylate and found no essential difference in the duration of the attacks. Acute pericarditis and myocarditis occurred with similar frequency in the two groups. In an attempt to assess the value of salicylate in rheumatic fever, it was found that in 86 per cent of 139 cases not receiving salicylate the symptoms ceased within four weeks, whereas 77 per cent of the 59 patients receiving salicylates not only had symptoms for the same length of time but suffered subsequent relapses (205). Relapses were rare, however, when the patients received more than eight grams a day. A group of 67 children receiving 1.3 grams of acetylsalicylic acid a day for six months gained more weight than did control patients. This was attributed to the increased comfort, and it was concluded that the salicylates were primarily of value in preventing pain (164). Roskam (235), who usually treated his patients with either eight grams of sodium salicylate or six grams of acetylsalicylic acid, found that acetylsalicylic acid was sometimes effective in patients resistant to sodium salicylate.

In a study of military personnel, a minimum concentration of 25 mgm. per cent of salicylate had to be maintained in the plasma if the acute phase of the disease was to be suppressed (188). Ordinarily 10 grams of acetylsalicylic acid plus eight grams of sodium bicarbonate a day were given to maintain an average plasma level of 32 mgm. per cent. The salicylates exert only a slight effect on the sedimentation rate in polycyclic attacks of rheumatic fever and such attacks do not respond as well as does the initial attack (49).

Coburn (61, 62) has suggested that rheumatic fever patients receiving large doses of salicylate for a long enough period of time are less prone to develop valvular heart lesions. He gave the drug intravenously to raise the plasma level rapidly to 40 mgm. per cent. None of his 38 patients who received 10 grams of sodium salicylate daily and had plasma salicylate levels over 35 mgm. per cent developed cardiac lesions; but on smaller doses of salicylate, 21 of 63 patients did develop valvular lesions.

Others (84) have commented on the studies of Coburn and criticized them (239), claiming that his criteria of cardiac damage were not adequately described, that the follow-up period was too short, that the criteria for classifying cases as severe or mild were not precisely stated, and that in some patients the sedimentation rate did not return to normal.

Doses of 0.1 gram of salicylate per pound body weight, which produce plasma levels of 30 to 45 mgm. per cent, are effective in rheumatic polyarthritis and acute rheumatic carditis in children (257), but smaller doses are ineffective. Using the erythrocyte sedimentation rate as the criterion of effectiveness, Reid (228) found no change when plasma salicylate levels were less than 20 mgm. per cent, a slow

fall at plasma levels between 20 and 30 mgm. per cent and a rapid fall at plasma levels between 30 and 40 mgm. per cent. Usually two grams of sodium salicylate five times per day were given to maintain the high plasma levels. It was concluded that the drug effected a real cure in rheumatic fever. In similar studies on rheumatic fever patients, it was shown that large doses of salicylates do not reduce the relapse rate but that the patients are promptly relieved of pain and that the sedimentation rate usually returns to normal in a short time (269). The plasma salicylate levels were kept above 30 mgm. per cent in these patients. Wégria and Smull (270, 271) treated rheumatic fever patients by keeping the plasma levels at 35 to 50 mgm. per cent. They found that the course of the attack was ordinarily not shortened by the high doses of salicylates. Comparable results were obtained by Warren *et al.* (268). Murphy (204) treated 12 patients with large doses of salicylates, and his results did not support the view that salicylates cause the subsidence of rheumatic joint inflammation. Keith and Ross (157) found that large doses of salicylate were effective in relieving the acute symptoms, but that they were ineffective in preventing cardiac lesions.

Salicylates have been shown (64) to be effective in preventing the development of the clinical manifestations of rheumatic fever in patients with intercurrent streptococcal pharyngitis. Only one of 47 such patients receiving five gm. of sodium salicylate a day had a clinical attack of rheumatic fever whereas 57 of 139 control patients had clinical attacks.

Meyer and Ragan (196) studied the effects of the sodium salt of gentisic acid, a metabolic product of salicylate, in rheumatic fever patients. The compound had the same anti-rheumatic effect as the salicylates. Prothrombin time did not significantly decrease and the drug could not be detected in the blood. The doses used were of the order of 10 grams a day, which is probably far in excess of any amount originating from the salicylate doses ordinarily administered to rheumatic fever patients.

The spectacular results obtained with the adrenal cortical hormone, compound E, in rheumatic fever (135) and the possibility that adrenocorticotrophic hormone may have a favorable influence (135) raise the question of the future use of salicylates in this disease. It is too early to do more than speculate. Should these compounds prove to be effective consistently in rheumatic fever, then it remains to be seen whether they can be produced in adequate quantity and whether their long-continued administration will produce endocrine disturbances or other toxic effects. The inconvenience and pain of frequent parenteral administration will be factors in determining their use. While it is generally agreed that salicylates reduce the fever, relieve the pain, and arrest the inflammatory processes, there is insufficient evidence that they prevent cardiac complications; if compound E is effective in this respect, it will probably have a very important place in rheumatic fever therapy.

SUMMARY

The many intensive studies on the pharmacology of salicylates have revealed the principal features of their actions and physiological disposition. They are rapidly absorbed from all portions of the gastrointestinal tract, absorption being

most rapid in the small intestine from slightly acid solutions. Distribution is throughout the body, appreciable amounts being within the cells. Degradation includes partial oxidation or conjugation with glycine or glycuronic acid. Excretion is moderately rapid, the free salicylate being excreted more rapidly when the urinary pH is high.

The salicylates are among the least toxic of commonly used drugs; but rarely, even in moderate doses, they produce severe reactions, particularly in asthmatic patients. Large doses may produce a transient fall in plasma prothrombin, but there is seldom a hemorrhagic tendency. Nausea and vomiting are usually associated with high plasma levels and are primarily central in origin. Large doses of salicylates stimulate the respiration; this produces an alkalosis which may continue until a ketosis develops.

Several decades of research on the salicylates have left many problems still unsolved. One of the most important is whether the salicylates act *per se* or through the degradation product, gentisic acid. In favor of the latter hypothesis is the beneficial action of gentisic acid in rheumatic fever (196). But there is some question whether the compound inhibits hyaluronidase (180, 197, 234), and the dose required in therapy is larger than would be expected on the basis of the fraction of salicylate known to be converted to gentisate (154); also the drug fails to prevent anaphylactic shock (166) whereas the salicylates are effective (50, 166).

The metabolism of methyl salicylate has not been studied as intensively as that of salicylic acid and acetylsalicylic acid. It may be that it differs appreciably.

The failure to demonstrate consistently an analgesic effect of salicylates may be due to inadequate methods of studying analgesia.

The demonstration that salicylates prevent anaphylactic shock raises the question of the possible relation of this phenomenon to the mechanism of salicylate action in rheumatic fever.

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