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Research Article

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The Influence of Pyrogen-Induced Fever on Salicylamide Metabolism in Man

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ABSTRACT Salicylamide is metabolized in man by biotransformation to salicylamide glucuronide, salicylamide sulfate, and gentisamide glucuronide. The metabolites are quantitatively and rapidly excreted in urine. Study of the metabolism of this drug in volunteers during episodes of pyrogen-induced fever shows a significant reduction in the half-life ($t_{\frac{1}{2}}$) of the excretion of the drug metabolites. The proportion of the drug transformed to its major metabolite, salicylamide glucuronide, is significantly reduced by fever, with concomitant increase in the proportion of one or both of the other metabolites. Thus, the pattern of urinary metabolites of salicylamide is altered. The shortened $t_{\frac{1}{2}}$ of the metabolite excretion is probably due to increased hepatic and renal blood flow known to accompany pyrogen-induced fever. This concept was supported by the observation that when two subjects were placed in a high-temperature environmental chamber, a condition in which hepatic and renal blood flows are known to diminish, the $t_{\frac{1}{2}}$ of salicylamide metabolite excretion actually increased. No simple explanation exists to explain the changed metabolite pattern noted during febrile periods. It is most likely to be due to complex interactions between the direct or indirect effects of the pyrogens and the factors affecting the hepatic biotransformation of drugs.

INTRODUCTION

Despite the widespread use of pharmacological agents such as analgesics, antipyretics, antibiotics, and antimetabolites in diseases associated with fever, little is known of the effect of fever on the disposal of drugs in man. We have studied the metabolism of salicylamide in normal volunteers before and after induction of artificial fever by administration of etiocholanolone or endotoxin (1–3). Salicylamide (*o*-hydroxybenzamide) is an amide derivative of salicylic acid that has mild analgesic and antipyretic properties. It is rapidly absorbed from the gastrointestinal tract after oral ingestion (4, 5, 6) and undergoes biotransformation primarily to its ether glucuronide and ester sulfate (7, 8). A small proportion of the drug is metabolized to gentisamide glucuronide (7, 9). All metabolites (Fig. 1) are rapidly eliminated by renal excretion (7). The metabolites are stable in urine and their assay is relatively simple (7, 9). These properties, plus the absence of significant binding of the free drug to plasma proteins (10), make salicylamide a convenient agent in the investigation of the effect of short-term physiologic perturbations such as artificial fever on drug metabolism.

The method used in these studies is based on a procedure developed and extensively employed by Vesell and his coworkers (11–15) in which each normal volunteer serves as his own control so that the rate of decay of a test drug is determined in each individual both before and after the introduction of some form of treatment. For example, using antipyrine half-lives in the plasma of identical and fraternal twins before and after treatment for 2 wk with phenobarbital, Vesell and Page (11) demonstrated that large differences among normal subjects in phenobarbital-induced reduction of plasma antipyrine half-life were under very rigid genetic control. The method was later used to establish that the following drugs, administered in commonly employed doses for a given time, prolonged the metabolism of other therapeutic agents: nortriptyline (12), allopurinol (12), disulfiram (13), and L-dopa (14). Ethanol was shown to accelerate the metabolism of antipyrine (15).

METHODS

Pharmacological agents. Salicylamide was purchased from ICN Nutritional Biochemicals Div., Cleveland, Ohio. Its homogeneity was checked by the Pharmaceutical Development Service, Pharmacy Department, NIH. The drug was judged to be 98.5% pure (16, 17). It was administered

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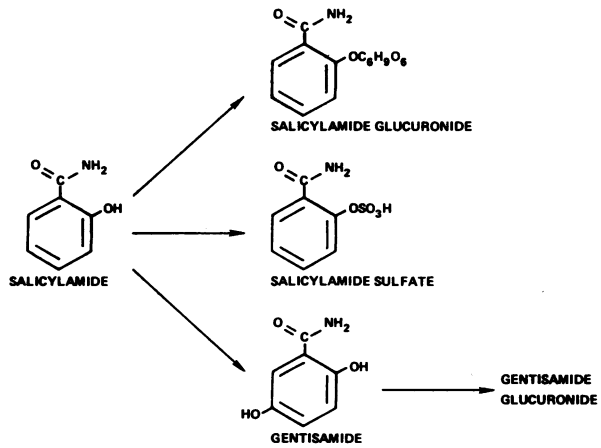

FIGURE 1 Salicylamide and its metabolites in man. Salicylamide glucuronide is the major metabolite.

as aqueous solution. Etiocholanolone (Merck, Sharp, and Dohme, West Point, Pa.), prepared in propylene glycol to a concentration of 10 mg/ml, was given intramuscularly. The endotoxin, Lipexal (Dorsey Laboratories, Inc., Lincoln, Nebr.), derived from *Salmonella abortus equi,* was prepared and stored at −20°C in sterile isotonic NaCl solution at a concentration of 0.5 μg/ml.

Normal volunteers. 18 volunteers consisting of 14 men and 4 women ranging in age from 19 to 41 yr were admitted to the Clinical Center, NIH for the present studies. All volunteers were in good health as determined by history, physical examination, and routine laboratory tests. All had normal renal function as evidenced by normal urinalyses and blood urea nitrogen measurements. All had normal hepatic function as judged by routine liver function tests. None of the volunteers had a history of habitual ingestion of drugs and none had received medications during the period of at least 1 wk before each study.

The experimental details of studies on salicylamide metabolism in normal human beings were first described by Levy and Matsuzawa (7). They consist essentially of administration of the drug in a rapidly absorbed form and serial collections of urine thereafter for quantitative analyses of the drug metabolites. The experimental design which we employed in the present study is a modification of this method and is as follows: the volunteers remained in bed fasting from midnight before the day of the salicylamide studies. Starting at 6 a.m., central body temperature was monitored every 30 min by means of electrical rectal probes (Yellow Springs Instrument Co., Yellow Springs, Ohio.). At 9–10 a.m., 1 g of salicylamide dissolved in 500–600 ml of water was administered by mouth. Urine was collected every 30 min for 4 hr and 50–100 ml of water was given after each urine collection to ensure urine outputs of more than 1,000 ml during the 4-hr periods. Each urine specimen was measured and 10-ml samples were frozen in sealed glass ampules and stored at −20°C. In some volunteers, simultaneous specimens of heparinized venous blood were collected through indwelling needles. 5-ml samples of plasma were similarly stored. The study during the afebrile period was designated as control study. Control studies were carried out only if the subjects' temperatures were below 37.0°C at 6 a.m. The results from these studies were used as the base line in the evaluation of febrile responses during all other studies.

14 subjects underwent, in addition to the control studies, identical investigations during a period of fever induced by etiocholanolone. The details of the latter studies, designated as etiocholanolone study, were the same as the control studies except for the intramuscular injection of etiocholanolone (0.3 mg/kg) at 8–10 p.m. the evening before the experiment and the administration of larger amounts of water (200–500 ml every 30 min) during urine collections to obtain urine flows comparable to those noted during control studies.

Six volunteers underwent, in addition to control and etiocholanolone studies, the salicylamide studies during febrile periods induced by endotoxin. The endotoxin, Lipexal, was injected intravenously (5 mμg/kg) at 9–10 a.m., and salicylamide was administered 3–4 hr later at or near the time of peak febrile response. The amounts of water ingested during these lipexal studies was adjusted as in etiocholanolone studies.

During periods of fever induced by both pyrogens, blood pressure, pulse, and respirations were checked every 30 min until temperatures returned to normal levels. In volunteers undergoing more than one experiment, an interval of at least 1 wk was allowed between each study.

Salicylamide metabolism was studied in two volunteers in a metabolic chamber maintained at a temperature of 48.9°C (120°F) and a relative humidity of 12%. The drug was administered 1 hr after volunteers entered the chamber. They were loosely covered with blankets during their 5 hr stay in the chamber. Urine collections and monitoring of rectal temperatures were carried out as in other studies.

Quantitation of febrile responses. In quantitating the fever responses to Lipexal, the temperatures obtained during the hour before the administration of the endotoxin (i.e., 8–9 a.m.) were used as base line values. For etiocholanolone studies, the temperatures noted during comparable periods of the day of control studies served as base line values. Febrile responses were quantitated by three parameters (2, 3): T_{max},[1] the maximum or peak temperature obtained with the pyrogens; ΔT, the maximum change in temperature from base line; and fever index (FI), the area in square centimeters obtained from planimetry of a 5 hr fever curve above base line plotted on a graph paper where 1 hr and 1°C each equaled 2 cm. The 5 hr fever curve included the period of 1 hr before the administration of salicylamide and the 4 hr test period after the drug administration.

Measurements of salicylamide and its metabolites. Urine levels of salicylamide and its metabolites were determined by a modification of the methods described by Levy and Matsuzawa (7): frozen urine or plasma specimens in glass ampules were thawed by immersion in luke-warm water after 1–6 wk of storage. Free salicylamide was determined by adding 1 ml of 0.2 M sodium phosphate buffer (pH 7.0) to 2 ml of undiluted urine or plasma, and by shaking this mixture with 15 ml of ethylene dichloride (Fisher Scientific Co., Pittsburgh, Pa.) for 1 min. Salicylamide extracted into the organic layer was then measured by shaking 10 ml of the ethylene dichloride layer with 5 ml of $Fe(NO_3)_3$ reagent[2] for 1 min. The absorbance of the aqueous phase at 530 mμ was determined in a Cary 15 spectrophotometer, Cary Instruments, a Varian Subsidiary, Monrovia, Calif.

[1] *Abbreviations used in this paper:* FI, fever index; ΔT, the maximum change in temperature from base line; T_{max}, peak temperature obtained with the pyrogens.

[2] Prepared fresh by diluting 5 ml stock solution of 1% (w/v) $Fe(NO_3)_3$ solution to 9 ml with distilled water.

The millimolar extinction coefficient of salicylamide standard was 0.220 under these assay conditions. Distilled water carried through in an identical manner served as a reagent blank.

Salicylamide glucuronide in the urine samples was measured as free salicylamide after enzymatic cleavage of the β-glucosidic bond with β-glucuronidase. In a 25 ml Erlenmeyer flask, 1 ml of purified beef liver β-glucuronidase (Ketodase, Warner-Chilcott Laboratories, Morris Plains, N. J.) containing 5,000 U[3] of the enzyme, 2 ml of 0.4 M sodium acetate buffer (pH 4.5), and 5 ml of urine suitably diluted with 0.04 M sodium acetate buffer (pH 4.5) were added. The reagent blank was prepared in an identical manner with distilled water instead of urine. The flasks were sealed with rubber stoppers and incubated in a shaking water bath at 38°C for 15 hr. Free salicylamide in the incubation mixture was then determined as described above. For salicylamide glucuronide in plasma, the incubation mixture was as follows: 1 ml of 0.4 M sodium acetate buffer (pH 4.5), 1 ml of β-glucuronidase, and 1 ml of plasma. The mixture was incubated for 16 hr in a similar manner.

Salicylamide sulfate in the urine samples was determined as free salicylamide after enzymatic hydrolysis with aryl sulfatase partially purified from limpets (Sigma Chemical Co., St. Louis, Mo., Type III Sulfatase). Since all commercially available sulfatases are contaminated with trace amounts of β-glucuronidase activity, known amounts of purified beef liver β-glucuronidase were added to the reaction mixtures: in 25-ml Erlenmeyer flasks, 1 ml of aryl sulfatase dissolved in 0.1 M sodium acetate buffer (pH 5.2) containing 10,000 U[4] of the enzyme activity, 0.5 ml of β-glucuronidase, and 5 ml of urine diluted with 0.1 M sodium acetate buffer (pH 5.2) were added. The sealed flasks were incubated for 96 hr at 38°C in a shaking water bath and free salicylamide determined. The amount of salicylamide sulfate was calculated by substracting from this value the result of the salicylamide glucuronide assay. Nonenzymatic hydrolysis of salicylamide glucuronide, as well as sulfate, under identical incubation conditions in the absence of the enzymes was negligible.

Gentisamide glucuronide was subjected to acid hydrolysis and determined as gentisic acid (9). 1 ml of 6 N HCl and 5 ml of urine diluted with distilled water (so that the final concentration of gentisic acid in the hydrolysate was 0.05–0.08 mg/ml) were sealed in 10-ml glass ampules and heated in an oven at 100°C for 3.5 hr. 1 ml of the hydrolyzed urine was mixed with 0.5 ml of 7 N H_2SO_4, 9.5 ml of distilled water, and 20 ml of diethyl ether and shaken for 5 min. After centrifugation at 1,500 rpm for 5 min in a refrigerated centrifuge, 10 ml of the ether layer was transferred and shaken with 6 ml of 5% (w/v) $NaHCO_3$ for 5 min. After centrifugation, 5 ml of the $NaHCO_3$ extract was transferred to a graduated centrifuge tube and 1 ml of concentrated HCl and 1 ml of N phenol reagent (Fisher Scientific Co.) were added. The volume of the mixture was brought to 10 ml with distilled water. It was allowed to stand at room temperature for 20 min and absorbance at 750 mμ was read on a Cary 15 spectrophotometer. The concentration of gentisic acid was calculated by comparison with a gentisic acid standard (Fisher Scientific Co.). The amounts of all salicylamide metabolites excreted in urine were expressed as equivalent amounts of free salicylamide.

[3] 1 U of β-glucuronidase is defined as the activity of the enzyme that hydrolyzed 1 μg of phenolphthalein glucuronide per hr at pH 4.5 and at 38°C.

[4] 1 U of sulfatase is defined as the activity of the enzyme that hydrolyzed 0.1 μmole of nitrocatechol sulfate per hr at pH 5.0 and at 37°C.

TABLE I

*Salicylamide Metabolism in Normal Volunteers during Etiocholanolone-Induced Fever**

Subjects	T_{max}‡	ΔT‡	FI‡	Salicylamide metabolites§ SG	SS	GG	Recovery	$t_{\frac{1}{2}}$
	°C	°C	cm²	mg	mg	mg	%	min
L. L. (21, F)‖	—	—	—	577	276	123	98	77
	37.7	1.2	20.7	547	246	159	95	69
W. H. (22, M)	—	—	—	581	142	146	87	79
	39.0	2.6	30.4	562	182	159	90	73
W. R. (20, M)	—	—	—	493	240	137	87	86
	37.4	0.9	15.6	447	259	219	93	98
H. L. (21, M)	—	—	—	513	251	160	92	71
	38.4	1.9	32.3	476	297	215	99	67
S. J. (19, F)	—	—	—	564	175	180	92	66
	37.8	0.7	6.4	504	200	195	90	58
J. O. (19, F)	—	—	—	543	239	155	94	75
	37.9	1.3	16.9	506	235	160	90	63
E. H. (19, F)	—	—	—	521	302	91	91	74
	37.6	1.1	11.6	497	348	126	97	58
M. S. (41, M)	—	—	—	492	271	131	89	75
	38.9	2.4	26.3	486	288	146	92	53
C. B. (19, M)	—	—	—	576	241	106	92	72
	38.8	2.3	21.2	486	282	182	95	51
S. L. (19, M)	—	—	—	593	176	142	91	69
	39.5	3.0	51.0	570	214	200	98	47
M. H. (19, M)	—	—	—	540	225	111	88	81
	38.4	1.4	12.0	474	265	193	93	65
A. J. (19, M)	—	—	—	542	261	128	93	70
	39.6	2.8	31.6	434	406	124	96	64
W. T. (20, M)	—	—	—	548	251	156	95	63
	39.5	2.9	35.0	467	282	176	93	71
S. B. (20, M)	—	—	—	580	201	148	93	66
	37.7	0.9	14.1	583	221	163	97	56
T. C. (19, M)	—	—	—	559	272	114	94	70
	39.4	3.1	41.3	516	336	172	102	54

* Values indicated on the first row corresponding to each volunteer are the results of control studies. The results of etiocholanolone studies are indicated on the second row.

‡ For definitions of T_{max}, ΔT, and FI, see text.

§ SG, salicylamide glucuronide; SS, salicylamide sulfate; GG, gentisamide glucuronide.

‖ Number in parentheses are the ages of each subject. F, female; M, male.

RESULTS

Salicylamide metabolism during afebrile periods. The distribution of urinary metabolites of salicylamide in 18 volunteers undergoing control studies during afebrile periods is tabulated in Table I. There was considerable individual variation in the amount of each of the metabolites excreted. Of the 1 g salicylamide administered, the volunteers excreted mean amounts of 548 mg as salicylamide glucuronide, 240 mg as salicylamide sulfate, and 127 mg as gentisamide glucuronide during the 4-hr periods of study. Thus, the mean recovery of the drug as metabolites was 92%. The recovery was virtually 100% with further prolongation of urine collections up to 24

hr. Urinary excretion of unmetabolized salicylamide was negligible and amounted to 0–2% of the administered drug. Such trace amounts of free salicylamide were demonstrable in urine only during the initial 60–90 min after oral ingestions.

The plasma concentration of free salicylamide was nearly undetectable during all phases of the study. Trace amounts of salicylamide glucuronide, averaging 0.015 mg/ml, were found in plasma during the initial 60 min of each study.

The time course of excretion of combined urinary metabolites showed that the excretion rate followed approximate first-order kinetics during the initial 2–3 hr of study. Fig. 2 shows the representative excretion rates of salicylamide metabolites observed in one of the volunteers. From such representation of excretion rates of combined metabolites, the initial half-lives of excretion ($t_{\frac{1}{2}}$) can be obtained. The mean value of $t_{\frac{1}{2}}$ in the present series of control studies was 72 min.

Because of the reports of sex differences in the rate of metabolism of certain drugs in experimental animals (18–20), the data from the control studies were analyzed according to sex (14 males and 4 females). No significant differences in the pattern of distribution of urinary metabolites, the amounts of drug recovered during the investigations, or the apparent half-lives of metabolite excretion were noted between the two sexes.

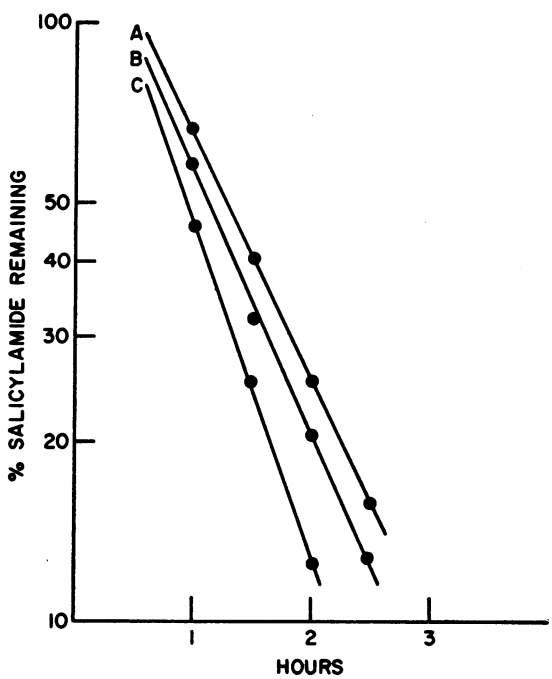

FIGURE 2 Kinetics of excretion of total urinary metabolites of salicylamide in a volunteer (L. L.). Cumulative excretion of combined salicylamide metabolites at 60, 90, 120, and 150 min after the oral ingestion of 1 g of salicylamide during control study (A), etiocholanolone study (B), and Lipexal study (C). The rate of excretion is indicated as the proportion of the drug remaining unexcreted at each point.

Febrile responses to pyrogens. After the administration of etiocholanolone, the fever peak was noted in 9–12 hr. The mean ΔT was 2.2°C. The febrile responses in the four female volunteers during etiocholanolone studies was significantly less than those of males (2). The peak of endotoxin-induced fever occurred about 4 hr after the administration of the pyrogen. The mean ΔT was 1.4°C. These findings concerning the febrile responses are consistent with previous reports on the pyrogenic properties of endotoxin and etiocholanolone in man (1–3). Salicylamide, in the amount employed during the present studies, had no effect on the fever induced by Lipexal or etiocholanolone.

Salicylamide metabolism during etiocholanolone-induced fever. Table I summarizes the experimental results in each of the 15 volunteers during control and etiocholanolone studies. 6 of the 15 volunteers underwent control studies first and then had etiocholanolone studies 5–7 days later. In the remaining nine volunteers, etiocholanolone studies were carired out first, to be followed by control studies 5–7 days later. Results of etiocholanolone studies, when statistically analyzed, did not depend on whether they preceded or followed the control studies. The mean recovery of salicylamide as metabolites during the 4 hr period of etiocholanolone studies was 95% and was slightly higher than the mean recovery during control studies.

The mean value of salicylamide excreted as glucuronide decreased from 548 mg to 504 mg, representing a 13% decline in the proportion of the administered drug excreted as its glucuronide. The conversion of salicylamide glucuronide was significantly reduced ($P < 0.001$, t test) during etiocholanolone studies.

On the other hand, the amounts as well as the proportion of the drug excreted as salicylamide sulfate and gentisamide glucuronide were significantly increased during etiocholanolone-induced fever in comparison to results obtained during control studies. Mean amounts of these metabolites excreted were 271 mg and 172 mg, respectively, for salicylamide sulfate and gentisamide glucuronide. These changes represent increases of 26–29% ($P < 0.01$) and 14–18% ($P < 0.001$) in the proportion of salicylamide excreted as salicylamide sulfate and gentisamide glucuronide, respectively.

The initial rates of excretion of combined urinary metabolites of salicylamide were significantly more rapid during etiocholanolone studies (Fig. 2). The mean $t_{\frac{1}{2}}$ for metabolite excretion was 63 min in comparison to 72 min observed during control studies ($P < 0.01$).

Considerable individual variations in the degree of fever-induced responses in all the parameters tested

TABLE II

*Salicylamide Metabolism in Normal Volunteers during Endotoxin-Induced Fever**

Subjects	T_{max}‡	ΔT‡	FI‡	Salicylamide metabolites§			Recovery	$t_{\frac{1}{2}}$
				SG	SS	GG		
	C	*C*	*cm²*	*mg*	*mg*	*mg*	%	*min*
L. L. (21, F)‖	—	—	—	577	276	123	98	77
	37.5	1.0	14.7	487	420	119	103	56
W. R. (20, M)‖	—	—	—	493	240	137	87	86
	37.5	1.0	16,1	494	282	136	91	73
S. L. (19, M)‖	—	—	—	593	176	142	91	69
	38.6	1.8	27.0	576	201	127	90	62
M. H. (19, M)‖	—	—	—	540	225	111	88	81
	38.3	1.3	16.9	494	283	112	89	67
A. J. (19, M)‖	—	—	—	542	261	128	93	70
	38.6	1.4	16.0	387	276	142	81	66
T. C. (19, M)‖	—	—	—	559	272	114	94	70
	38.2	1.8	27.1	525	350	96	97	58

* Values indicated on the first row corresponding to each volunteer are the results of control studies. The results of endotoxin studies are indicated on the second row.

‡ For definition of T_{max}, ΔT, and FI, see text.

§ SG, salicylamide glucuronide; SS, salicylamide sulfate; GG, gentisamide glucuronide.

‖ Number in parentheses are the ages of each subject. F, female; M, male.

should be emphasized (Table I). This was particularly true of the changes in $t_{\frac{1}{2}}$ values induced by etiocholanolone. In two volunteers (W. R. and W. T.), the $t_{\frac{1}{2}}$ values actually increased in contrast to the remainder of the group in whom decreases in $t_{\frac{1}{2}}$ values of variable degrees were induced by etiocholanolone.

Salicylamide metabolism during endotoxin-induced fever. Experimental results in each of the six volunteers undergoing Lipexal studies are summarized in Table II. In three volunteers, control studies preceded the Lipexal studies by 5–7 days, and in the remaining three volunteers, the order was reversed. Results of the Lipexal studies, upon inspection, did not appear to depend on whether they were preceded or followed by control studies, but the number of studies were too few for statistical analysis. The mean recovery of salicylamide as metabolites during Lipexal studies was 92% and did not differ significantly from that observed during control studies.

The mean value of the drug excreted as glucuronide was again noted to be decreased during Lipexal studies: from 548 mg to 494 mg ($P = 0.05$). 54% of the drug was excreted as glucuronide during the Lipexal studies in contrast to the 60% excretion noted during the control studies. As in the etiocholanolone studies, the amount of salicylamide excreted as its sulfate was significantly increased during Lipexal studies. The mean value of this metabolite excreted was 302 mg during the Lipexal studies as compared to 240 mg excreted during the control studies ($P < 0.05$). On the other hand, there was no signficant change in the amount of the drug excreted as gentisamide glucuronide. Analyses of plasma levels of salicylamide glucuronide during the etiocholanolone as well as the Lipexal studies showed only trace amounts of this metabolite (averaging less than 0.015 mg/ml), which was no different from the results observed during control studies.

The $t_{\frac{1}{2}}$ for excretion of combined metabolites was significantly shorter during the Lipexal studies than that observed during the control studies. The mean $t_{\frac{1}{2}}$ was 64 min during this fever period in contrast to 72 min noted during the control studies ($P < 0.01$). In both etiocholanolone and Lipexal studies, statistical analyses showed no correlation between any of the three parameters of febrile responses and the degree of changes in the metabolism or excretion of salicylamide.

Salicylamide metabolism in the high-temperature metabolic chamber. No fever was produced in the two volunteers placed in the high-temperature metabolic chamber. Due to efficient thermoregulation, their rectal temperatures were virtually identical to those observed during comparable periods of respective control studies. Despite this absence of fever, the amount of salicylamide excreted as glucuronide was substantially decreased in both volunteers (Table III). Of interest was the fact that the $t_{\frac{1}{2}}$ of combined metabolites showed considerable prolongation in both volunteers (Table III) in contrast to the results noted during the pyrogen studies. The amount of salicylamide sulfate excreted was slightly increased during the studies in the metabolic chamber.

TABLE III

*Salicylamide Metabolism in Two Subjects Placed in a Metabolic Chamber**

Subjects	Study	Salicylamide metabolites‡ SG	SS	GG	Recovery	$t_{\frac{1}{2}}$
			mg		%	*min*
A. J.	Control	542	261	128	93	70
	Metabolic chamber	411	274	104	79	88
E. K.	Control	532	260	124	92	75
	Metabolic chamber	494	296	118	91	88

* Temperature in chamber, 48.9°C at a relative humidity of 12%.
‡ SG, salicyamide glucuronide; SS, salicylamide sulfate; GG, gentisamide glucuromide.

DISCUSSION

The study of factors influencing drug metabolism in man, although rapidly advancing at present (21), is still in its infancy, and extrapolation from the available results of animal studies is not always possible (22). This is particularly true of the present investigations. For, despite the pharmacological and clinical implications of possible alterations in drug metabolism by fever, there are virtually no published data describing the relationship between the physiological changes produced by fever on the one hand and the various metabolic functions that underlie drug metabolism on the other. Simple explanations for the fever-induced changes in the metabolism of salicylamide noted in the present study are not available, and plausable explanations have to be synthesized from the fragmentary and often anecdotal studies available in the litearture. Therefore, the speculative nature of the arguments presented below is to be emphasized.

The liver and kidney play a central role in the metabolism and excretion of salicylamide, respectively. All three of the biotransformation reactions involving the drug, viz. glucuronidation, sulfation and hydroxylation, are known to take place primarily in the liver (23, 24), and all the reaction products are quantitatively recoverable in the urine. The virtually undetectable plasma levels of free salicylamide and the trace amounts of salicylamide glucuronide found in plasma during the present studies, together with the rapid recovery of the drug metabolites in the urine, indicate that salicylamide is metabolized and excreted rapidly after absorption, with a short, if any, interaction with hypothetical tissue binding sites.

The elevation of central body temperature by endotoxin or by extracorporeal heating of blood produces significant increases in hepatic as well as renal blood flows. Bradley (25) showed that typhoid vaccine-induced fever in man was associated with marked increase in hepatic and renal blood flows, which largely accounted for the augmented cardiac output associated with fever. The elevation of body temperature by heating the blood in the pump in cardio-pulmonary bypass (26, 27) in rabbits and dogs results in increases in renal blood flow and in glomerular filtration rates.

On the other hand, if the central temperature is elevated by means of heating the body surface, e.g., by application of heat to the skin with water-perfused suits (28) or heating pads (29), hepatic and renal blood flow declines. This is probably due to the redistribution of cardiac output toward arterioles of the skin and muscles (28), where vasodilatation as a result of direct application of heat would be more pronounced.

These hemodynamic changes probably account for the accelerated rates of excretion of salicylamide metabolites observed in the volunteers during endotoxin- or etiocholanolone-induced febrile states. The rates of biotransformation of rapidly metabolized drugs may depend on hepatic blood flow (30, 31), and the renal excretion of polar drug metabolites such as the salicylamide metabolites takes place by means of glomerular filtration with little subsequent tubular reabsorption (32–34). The shortened $t_{\frac{1}{2}}$ values noted in our volunteers during pyrogen-induced febrile periods are probably related to increases in blood flow through the liver and kidney. These arguments are strengthened by our observation that in the volunteers subjected to external heating (Table III), a situation where blood flow to these organs decrease, the $t_{\frac{1}{2}}$ values actually increased, with slower urinary excretion of salicylamide metabolites. The possible changes in the rates of the intestinal absorption of salicylamide accompanying altered splanchnic blood flow during fever are not likely to contribute significantly to these alterations in $t_{\frac{1}{2}}$ values, because experimental studies (35, 36) have shown that it takes changes in the splanchnic blood flow of many orders of magnitude greater than those seen in the hepatic blood flows in febrile human beings (25, 28) to produce noticeable changes in the rates of drug absorption.

It is more difficult to explain the alterations in the pattern of salicylamide metabolites excreted during febrile periods, viz. the decrease in the amount of salicylamide glucuronide excreted and the increase in the amounts of one or both of the other two metabolites. Since the total recovery of the administered salicylamide as metabolites was 92–95% during all studies and was nearly complete, the altered excretion pattern cannot be due to a preferential excretion of salicylamide sulfate and gentisamide glucuronide over salicylamide glucuronide. Furthermore, the plasma concentrations of the latter metabolite during pyrogen-induced fever were not higher than those ob-

served during afebrile periods, which might have been the case had the other two metabolites been excreted more rapidly by the kidney. By exclusion, then, such alterations must be due to an alteration in the biotransformation of the drug, which, in the case of salicylamide, is primarily the function of the liver (23, 24). In addition, it should be noted that considerable individual variation in the fever-induced responses was observed. The explanation for these findings is presently unknown.

Such an influence on the hepatic handling of the drug could be due to an increase in the liver temperature itself, augmented hepatic blood flow, or direct or indirect metabolic effects of the pyrogens themselves. Very little is known of the effect of the liver temperature or blood flow on the metabolic functions of the human liver in general, and on its drug-metabolizing capacity in particular. Recently, there has been an increasing amount of interest in the direct and indirect effects of pyrogens, especially endotoxin, on various metabolic functions of the liver. Thus, endotoxin impairs the oxidative metabolism of a number of substrates including fatty acids, pyruvate, and citrate (37), increases plasma concentrations of cortisol and growth hormone (38–40), and inhibits glucocorticoid induction of various hepatic enzymes (41, 42).

One or more of these factors no doubt affect the complex biochemical pathways that eventually result in glucuronidation, sulfation, or hydroxylation of drugs in the liver. It is well known that each of these biotransformation reactions involves a series of enzyme-catalyzed reactions and that the over-all rate of each reaction is dependent on multiple factors such as the concentration of substrates or cofactors in the hepatocytes, the activity of enzymes catalyzing the key components of the reaction series, the endocrine status of the host, etc. (31, 43).

With such a diversity of possible interactions, it is likely that the altered metabolism of salicylamide observed during febrile periods is the result of multiple, complex interactions between the effects of pyrogens, fever, or certain physiological, or biochemical changes associated with them on the one hand, and the factors affecting the hepatic metabolism of salicylamide on the other. The exact nature of such interactions is not known at present. Such a complex relationship could also explain the lack of quantitative relationship between the febrile responses and the metabolism of the drug noted during the present investigations.

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