with constant levels established, following a peak during age of sexual maturity. There is a significant rise in total HUFA content of liver tissue, associated with emergency of birth. Lack of catalytic activity of ascorbic acid upon *in vitro* oxidative reaction involved in embryonic tissue has been demonstrated.

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Influence on Circulating 17-Hydroxycorticosteroid Concentrations of Compounds Structurally Related to Salicylate.* (22736)

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Earlier publications(1,2) reported the finding of marked elevations in circulating 17-hydroxycorticosteroid (17-OHCS) concentrations in guinea pigs following intraperitoneal or oral administration of large doses of sodium salicylate. Hypophysectomy, as well as adrenalectomy, abolished this effect(2). In the present investigation, various other derivatives of benzoic acid were studied with regard to their effects on circulating 17-OHCS concentrations in guinea pigs in an attempt to ascertain whether these effects are related to the structural configuration of the administered compound.

Methods. Male guinea pigs weighing 450 to 750 g were used in these experiments. Sodium salts‡ of the compounds listed in Table I were prepared by dissolving the acids in an equivalent amount of 1 N NaOH; the pH of each solution then was adjusted to neutrality using the Beckman glass electrode. Solutions were diluted with distilled water to contain the equivalent of 100 mg of the acid per ml. Compounds tested were injected intraperitoneally in doses equivalent (on the basis of molecular weight) to 200 mg salicylic acid per kilo body weight. Four hours later the animals were rapidly anesthetized in an ethyl ether atmosphere and bled from the abdominal aorta into heparinized syringes. Additional groups of animals treated with the monohydroxy derivatives of benzoic acid were sacrificed at 1 and 2 hours following injection. Plasmas were separated by centrifugation immediately and stored in deep-freeze until used for analyses. Plasma concentrations of 17-OHCS were determined by the method of Nelson and Samuels(3) as modified by Eik-Nes, Nelson and Samuels(4).

Observations. Data concerning the influence on plasma 17-OHCS concentrations of the various compounds tested are shown in Table I. Benzoic acid had no significant effect. For purposes of statistical analysis the

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‡ For convenience, compounds will be referred to as acids.

TABLE I. Influence of Benzoic Acid Derivatives on Circulating 17-OHCS Concentrations in Guinea Pigs. (Doses equivalent to 200 mg/kg salicylic acid.)

Compound†	No. determinations	17-OHCS* (μg/100 ml)	P vs. benzoic acid	P vs. salicylic acid
Untreated	51	37 ± 2.7‡		
Benzoic acid	4	43 ± 5.2		
Monohydroxy derivatives:				
o-OH (salicylic)	5	81 ± 5.6	<.01	
m-OH[1,2]	4	58 ± 9.3	>.05	>.05
p-OH[1,2]	5	57 ± 3.0	>.05	.01–.02
Ether derivatives:				
o-Methoxy[1,2]	7	76 ± 6.7	<.01	
o-Ethoxy[2]	4	119 ± 11.2	<.01	.02
m-Methoxy[2,3]	5	75 ± 11.3	.02–.05	
p-Methoxy[1,2]	5	77 ± 16.7	>.05	
p-Ethoxy[1,2,4]	3	50 ± 2.4	>.05	<.01
3,4,5-Trimethoxy[2,4]	4	119 ± 11.1	<.01	.02
Amino derivatives:				
o-Amino[5]	5	62 ± 4.7	.02–.05	.02–.05
p-Amino (PABA)	3	71 ± 6.9	.02–.05	
Dihydroxy derivatives:				
2,3-OH (o-pyrocatechuic)[4]	3	127 ± 16.6	<.01	.02–.05
2,4-OH (β-resorcylic)[1–4]	9	89 ± 18.6	.02–.05	
2,5-OH (gentisic)[1,4,6]	7	86 ± 11.5	<.01	
2,6-OH (γ-resorcylic)[1,2]	5	123 ± 10.2	<.01	<.01
3,4-OH (protocatechuic)[3]	2	(26, 67)		
3,5-OH (α-resorcylic)[1,4]	4	81 ± 11.6	.02–.05	
Mixed derivatives:				
2-OH, 3-Methyl[1,2,4]	2	(132, 77)		
2-OH, 4-Methyl[2,4]	4	117 ± 14.2	<.01	.05
2-OH, 3-Methoxy[3,6]	7	110 ± 6.8	<.01	<.01
2-OH, 5-Methoxy[2,4]	7	117 ± 18.9	<.01	>.05
2-OH, 4-Amino (PAS)[2]	5	64 ± 16.1	>.05	>.05
Acetylsalicylic acid	7	132 ± 15.2	<.01	.01
Salicyluric acid[2]	4	36 ± 5.9		<.01

* Four hr after intraper. inj. † Each administered as the sodium salt. ‡ Mean ± S.E.

[1] Kindly supplied by Eli Lilly & Co., Indianapolis, Ind.
[2] " " " S. E. Massengill Co., Bristol, Tenn.
[3] " " " The Squibb Institute for Medical Research, New Brunswick, N. J.
[4] " " " Parke-Davis & Co., Detroit, Mich.
[5] " " " Dow Chemical Co., Midland, Mich.
[6] " " " Abbott Laboratories, North Chicago, Ill.

group of animals given benzoic acid were considered the control group. The reasons for this were that the number of animals corresponded more closely with those in the other experimental groups and the animals were treated similarly in that they received an intraperitoneal injection of a potentially irritating solution which in each case contained an equivalent amount of sodium and was of identical pH. *Monohydroxy derivatives of benzoic acid.* The animals given o-hydroxybenzoic (salicylic) acid had highly significant elevations of plasma 17-OHCS concentrations. In contrast, the groups given m- or p-hydroxybenzoic acid did not have significant elevations of 17-OHCS concentrations at the 4 hour interval. *Ether derivatives of benzoic acid.* The o-substituted methoxy and ethoxy derivatives likewise were active in producing elevated 17-OHCS concentrations, the latter appearing perhaps to be more active than either the o-methoxy compound or salicylic acid. The elevations of plasma 17-OHCS produced by the m- and p-methoxy derivatives are of questionable significance because of wide variations in values among the animals tested; however, no significant effect was observed with p-ethoxybenzoic acid. On the other hand, 3,4,5-trimethoxybenzoic acid produced highly significant elevations of

plasma 17-OHCS concentrations, possibly greater than those produced by salicylic acid ($p = .02$). *Amino derivatives of benzoic acid.* Two mono-amino benzoic acids, the o- and p- derivatives, were studied. Both of these compounds produced steroid elevations of borderline significance ($.02 < p < .05$ vs. benzoic acid). *Dihydroxy derivatives of benzoic acid.* Among the dihydroxybenzoic acids, the 2,3; 2,5 and 2,6-substituted derivatives resulted in highly significant plasma 17-OHCS elevations. Elevations of borderline significance ($.02 < p < .05$ vs. benzoic acid) occurred with the 2,4 and 3,5-substituted compounds. Insufficient data are available for the 3,4-substituted compound to permit conclusions. *Mixed derivatives of benzoic acid.* Derivatives of o-hydroxybenzoic (salicylic) acid with substitution of a methyl group at the 3 or 4 position or a methoxy group at the 3 or 5 position produced elevations of plasma 17-OHCS concentrations which were highly significant. With the small numbers of animals studied there appeared to be no significant difference between p-aminosalicylic acid and benzoic acid in their effects on 17-OHCS concentrations.

The greatest mean elevation of plasma 17-OHCS concentrations in any group of animals occurred in that group given acetylsalicylic acid (aspirin), the response being significantly greater than that obtained with salicylic acid. Salicyluric acid exerted no effect on plasma 17-OHCS concentrations. *Time relationship of steroid response to monohydroxybenzoic acids.* Table II concerns plasma 17-OHCS concentrations at varying intervals following treatment with the monohydroxybenzoic acids. Animals treated with o-hydroxybenzoic (salicylic) acid had no significant elevation of plasma 17-OHCS concentrations at 2 hours, but had highly significant elevations at 4 hours. Those treated with m-hydroxybenzoic acid showed a suggestive ($.02 < p < .05$ vs. benzoic acid) elevation of 17-OHCS concentrations at 2 hours. p-Hydroxybenzoic acid did not significantly affect 17-OHCS concentrations at any of the 3 sampling intervals (1, 2 or 4 hours).

TABLE II. Plasma 17-OHCS in Guinea Pigs at Varying Intervals following Treatment with Monohydroxy-Benzoic Acids. (Doses, 200 mg/kg by intraperitoneal injection.)

Compound*	Sampling interval 1-hr	2-hr	4-hr
o-Hydroxybenzoic (salicylic) acid		44 ± 10.9 (3)	81 ± 5.6 (5)
m-Hydroxybenzoic acid	63 ± 7.3† (3)	71 ± 8.5 (4)	58 ± 9.3 (4)
p-Hydroxybenzoic acid	49 ± 8.8 (4)	58 ± 6.6 (2)	57 ± 3.0 (5)

* Each administered as the sodium salt.
† Mean ± S.E.
No. in parentheses = No. of determinations.

Discussion. Equimolar doses of each of the compounds studied were administered in order to permit estimations of relative potency of these compounds in producing elevations of circulating 17-OHCS concentrations. However, certain variables preclude strict comparisons. Substantial differences may exist, among the compounds tested, in absorbability from the peritoneal cavity. The sampling interval of 4 hours was chosen because the maximum steroid response to salicylate was found to occur at that time(1). However, maxima for the other benzoic acid derivatives may not have coincided with the time of sampling used in these studies. Moreover, although the dose of each compound was equivalent on a molar basis there was wide variance in the ratio of administered to toxic dose. A non-specific toxic effect therefore is more likely with some of these substances than with others. None of the compounds studied was lethal in the dose employed. The majority of the compounds used in the present investigation have been studied only to a limited degree, or not at all, with respect to antiphlogistic potency. Moreover, where such data are available, categorical conclusions as to whether or not the particular compound has antiphlogistic properties cannot be drawn in most instances since results have differed from one type of experiment to another. Therefore, any comparison of this effect with influence on 17-OHCS concentrations necessarily must be incomplete and will be attempted only insofar as available data permit.

Among the monohydroxy derivatives of

benzoic acid, only the o-substituted compound (salicylic acid) exerted a pronounced effect on 17-OHCS concentrations at the 4 hour post-injection interval. Blanchard, *et al.* (5) and Cronheim, *et al.*(6) found the m-compound to have no effect on adrenal ascorbic acid, but others(7-10) have found that it produces significant depletion. Smith(9) reported significant adrenal ascorbic acid depletion with both m- and p-hydroxybenzoate. However, the doses employed (330 and 400 mg/kg, respectively) were much higher than those used in the present study and produced less depletion of adrenal ascorbic acid than did comparable doses of salicylate or even benzoate. The author attributed the differences in response to the lower circulating concentrations of the m- and p-hydroxybenzoates. He found that the peak plasma concentration of p-hydroxybenzoate occurred 30 minutes after intraperitoneal injection; none could be detected 1½ hours later. The greatest depletion of adrenal ascorbic acid was noted after the former, and no significant depletion after the latter, interval. This finding was suggestive that the interval between injection and removal of the adrenals may have been too long in the experiments of other workers(5,6) who failed to demonstrate an effect of this compound on adrenal ascorbic acid content. The failure in the present study to demonstrate a significant effect on circulating 17-OHCS at a time when the salicylate effect is maximum (4 hours after injection) might be explained similarly. For this reason, further studies of animals treated with m- and p-hydroxybenzoic acid were made at one- and 2-hour intervals. In the case of p-hydroxybenzoic acid, no effect on 17-OHCS concentrations was demonstrable. With m-hydroxybenzoic acid there was a mean 17-OHCS concentration at 2 hours which was elevated by comparison with benzoic acid-treated ($.02<p<.05$), or untreated ($p<.01$), animals; no significant effect was demonstrable at 4 hours following injection. Of the 3 monohydroxy derivatives of benzoic acid, only o-hydroxybenzoic (salicylic) acid has been reported to have antirheumatic properties(11) and to inhibit the development of "anaphylactic arthritis"(12).

The effects of certain other derivatives of benzoic acid on circulating 17-OHCS concentrations appear not to parallel their effects on adrenal ascorbic acid concentrations. Although o-methoxy and o-ethoxybenzoic acid both caused definite elevations of 17-OHCS concentrations, these compounds have been found not to deplete adrenal ascorbic acid (6,7). Plasma 17-OHCS concentrations following m- or p-methoxybenzoic acid were so variable that although the mean 17-OHCS values in these groups were comparable with those of salicylate-treated animals, these elevations were not statistically significant with the small numbers of animals used. Of these compounds, only p-methoxybenzoic acid has been found to deplete adrenal ascorbic acid significantly(7). p-Ethoxybenzoate has no effect either on 17-OHCS concentrations or adrenal ascorbic acid(6).

Administration of the o- and p-amino derivatives of benzoic acid resulted in mean 17-OHCS concentrations which were intermediate between those produced by benzoic acid and salicylate. p-Aminobenzoic acid has been reported to have no effect on adrenal ascorbic acid concentration(6) and to have no(13), or suggestive(14), antirheumatic properties. Neither compound was reported to inhibit anaphylaxis(15).

The addition of a second hydroxyl or an ether group to the salicylate structure appeared, in certain instances, to enhance the effect on circulating 17-OHCS. Thus, treatment with 2,3-dihydroxybenzoic acid resulted in suggestively, ($.02<p<.05$), and 2-hydroxy, 3-methoxy- or 2,6-dihydroxy-benzoic acid in definitely ($p<.01$), greater elevations of 17-OHCS concentrations than did salicylic acid. 2,6-Dihydroxy benzoate (γ-resorcylate) has considerably greater antirheumatic potency than salicylate(16). Gentisic acid had essentially the same magnitude of effect on 17-OHCS concentrations as salicylic acid and has been reported to have comparable antirheumatic potency, despite observations that it produces a somewhat lesser depletion of adrenal ascorbic acid(6,7,10).

A somewhat surprising finding was the sig-

nificantly greater effect on circulating 17-OHCS concentrations of aspirin than of salicylic acid when the 2 compounds were given in equimolar amounts. Indeed, aspirin produced the greatest effect on 17-OHCS concentrations of any of the compounds tested. The increment in steroid response to aspirin over salicylic acid is proportional to the increment in milligram dose, suggesting that the 2 compounds may have similar potency when administered in equal quantities.

Salicyluric acid, the principal metabolite of salicylate, exerted no effect on 17-OHCS concentrations. This substance has been reported to cause a questionable depletion ($p = .02$) of adrenal ascorbic acid(6) but no inhibitory effect on "anaphylactic arthritis" (12).

Since relatively few of the compounds investigated in the present study have been evaluated with regard to antirheumatic potency, it is difficult to assess the relationship of this property to ability to produce elevated circulating corticosteroid concentrations. However, insofar as available data permit such comparison, these two effects appear to correlate well. On the other hand, there appear to be numerous discrepancies between adrenal ascorbic acid-depleting ability of these drugs and either their antirheumatic effects or their influence on plasma 17-OHCS concentrations.

Summary. 1. Twenty-five compounds representing structural derivatives of benzoic acid were studied with regard to their effects on circulating 17-hydroxycorticosteroid (17-OHCS) concentrations in guinea pigs. 2. Benzoic acid had no effect on these concentrations. 3. Among the monohydroxybenzoic acids, the o- and m-substituted derivatives produced elevated plasma 17-OHCS concentrations. The latter compound exerted its effect earlier following administration than did salicylate. p-Hydroxybenzoate had no significant effect. 4. Among the mono-ether derivatives of benzoic acid only the o-substituted compounds produced significant elevations of plasma 17-OHCS concentrations. Significant elevations were produced by 3,4, 5-trimethoxybenzoic acid. 5. Both o- and p-aminobenzoic acid produced steroid elevations of borderline significance. 6. The addition to the salicylate structure of a second hydroxyl group in the 3 or 6 position or of an ether group in the 3 position appeared to augment the effect on 17-OHCS concentrations. 7. Aspirin exerted the greatest effect on plasma 17-OHCS concentrations of any compound tested. 8. The salicylate metabolite, salicyluric acid, had no effect. 9. A comparison of the influence of these compounds on 17-OHCS concentrations with antirheumatic and anti-inflammatory properties and with effects on adrenal ascorbic acid concentrations, as reported by others, was attempted.

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