4. Fernell, W. R., and King, H. K., *Analyst,* 1953, v78, 80.
5. Dische, Z., and Shettles, L. B., *J. Biol. Chem.,* 1948, v175, 595.
6. Randall, R., Wetmore, P. W., and Warner, A. R., *J. Lab. and Clin. Med.,* 1949, v34, 1411.
7. Unpublished data from this laboratory.
8. Hiatt, C. W., Rothstein, N., and Yager, R. H., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 703.

Received November 30, 1953. P.S.E.B.M., 1954, v85.

Adrenal Response of Rats to Salicylamide and Sodium Salicylate with and Without Para-Aminobenzoic Acid.*† (20777)

J. C. FORBES, J. A. BOARD, AND G. M. DUNCAN.

From the Department of Biochemistry, Medical College of Virginia, Richmond, and Department of Chemistry, Randolph-Macon College, Ashland, Va.

The administration of either acetylsalicylic acid or sodium salicylate to rats has been shown to cause a marked drop in the content of ascorbic acid and to a lesser extent of cholesterol in the adrenals(1-4), an effect which is not found in the hypophysectomized animal(1,2). Cronheim *et al.*(1) gave salicylamide to a few animals and found its effect on the adrenals to be somewhat less than that of sodium salicylate. In later experiments these investigators administered para-aminobenzoic acid (PABA) along with sodium salicylate but failed to get an augmented effect(5). They suggested that their inability to demonstrate an additional effect with PABA was probably due to the fact that the animals were sacrificed too soon after the administration of the drug, namely, 2 hours.

Since the analgesic effect of salicylamide in the experimental animal has been shown to be greater than that of acetylsalicylic acid (6-8), it was decided to carry out a more extensive investigation on its adrenal effects, studying not only the intensity of its action but also the time required for the adrenal cholesterol and ascorbic acid to return to normal concentrations. For comparative purposes experiments were carried out with both salicylamide and sodium salicylate, alone or with an equivalent amount of PABA.

Methods. Albino rats were used throughout. All animals purchased from a commercial dealer were placed on our stock diet of Rockland rat pellets for at least a week before they were used in the experimental work. No food was allowed for 16 hours prior to the ad-

TABLE I. Effect of Oral Salicylamide with and without Para-Aminobenzoic Acid on Adrenal Cholesterol and Ascorbic Acid of Male Rats.

Concentration following drug administration, %				
Ascorbic acid		Cholesterol		
2 hr	4 hr	2 hr	4 hr	Remarks
.324 ± .053* (3)	.310 ± .037* (10)	3.25 ± .29* (3)	2.91 ± .37* (9)	Controls receiving gelatin
.164 ± .021 (4)	.274 ± .088 (14)	2.70 ± .61 (4)	2.51 ± .65 (14)	Salicylamide, 300 mg/kg
.180 ± .029 (4)	.272 ± .097 (15)	2.93 ± .43 (4)	2.51 ± .72 (15)	*Idem.* + equimolar quantity of PABA

* Stand. dev.
No. of animals in parentheses.

* We gratefully acknowledge the financial assistance given by Charles C. Haskell & Co., Richmond, Va., in support of this work.

† Part of this work was submitted by one of us, J. A. B., in partial fulfillment of requirements for the degree of Bachelor of Science, Randolph-Macon College.

TABLE II. Effect of Intraperitoneal Administration of Salicylamide or Sodium Salicylate with and without Sodium Para-Aminobenzoate on Cholesterol and Ascorbic Acid Content of Adrenals of Male Rats.

Exp.	Concentration following drug administration, %										Supplement
	Ascorbic acid				Cholesterol						
	2 hr	4 hr	6 hr	12 hr	24 hr	2 hr	4 hr	6 hr	12 hr	24 hr	
1	.422±.077* (10)	—	—	—	—	3.02±.44* (10)	—	—	—	—	Controls
	.202±.023 (4)	.238±.055 (8)	.254±.039 (8)	.385±.063 (11)	—	2.45±.23 (4)	2.08±.44 (8)	1.77±.59 (8)	3.11±.98 (11)	—	Salicylamide 300 mg, Na PABA 350 mg/kg
	.170±.024 (3)	.282±.034 (4)	.272±.083 (4)	.377±.062 (11)	—	2.28±.83 (3)	2.10±.39 (4)	1.80±.39 (4)	3.22±.81 (11)	—	Salicylamide 300 mg
2	.322±.077 (9)	—	—	—	—	3.36±.92 (9)	—	—	—	—	Controls
	.187±.024 (9)	.165±.030 (8)	.181±.020 (5)	.239±.024 (8)	.406±.031 (5)	2.53±1.55 (9)	2.46±.87 (8)	1.67±.34 (5)	2.03±.72 (8)	4.46±.40 (5)	Na salicylate 350 mg, Na PABA 350 mg/kg
	.178±.027 (9)	.158±.024 (7)	.191±.013 (5)	.254±.044 (8)	.351±.052 (5)	2.76±.56 (9)	2.15±1.03 (7)	1.67±.34 (5)	2.33±.43 (8)	3.64±.43 (5)	Na salicylate 350 mg

* Stand. dev.

No. of animals in parentheses.

ministration of the drug. All animals were sacrificed by rapid decapitation. The adrenals were removed at once and analyzed for ascorbic acid by the method of Roe and Kuether(9) and for cholesterol by the method of Outhouse and Forbes(10). Animals raised in our own colony tended to have a higher concentration of both cholesterol and ascorbic acid in their adrenals than those purchased from commercial dealers. On account of this variation both test and control animals used in any one experiment were always of the same sex and of comparable age and stock. Data recorded in Tables I and II were obtained on rats purchased from the same dealer and weighing around 170 g at the time of sacrifice.

Table I shows the results obtained following the administration by stomach tube of 300 mg/kg of salicylamide with and without an equimolar amount of PABA. The salicylamide was suspended in .125% gelatin. Control animals received an equivalent volume of the gelatin solution. Previous work(11) showed that the insertion of the stomach tube was in itself a sufficient stress to cause a slight drop in the ascorbic acid content of the adrenals but that it did not exert a demonstrable effect upon the cholesterol concentration. The controls were sacrificed 2 and 4 hours after administration of the gelatin solution. It will be seen that the adrenal ascorbic acid concentration showed a marked drop 2 hours after the administration of the salicylamide, as compared with the controls, but was well on the way back to normal in 4 hours. Addition of PABA did not augment the salicylamide effect. The drop in adrenal cholesterol was much less definite than in ascorbic acid. The general trend, however, was distinctly downward. In other experiments in which the salicylamide dose was 500 mg/kg, the degree of reduction of ascorbic acid was about the same as was obtained with the 300 mg/kg dose but the effect on the cholesterol concentration was somewhat more definite.

In an attempt to obtain more definite information on the adrenal response to salicylamide and the possible influence of PABA, it was decided to study their effect when given intraperitoneally, using the sodium salt of

PABA instead of the free acid. Salicylamide was given at a level of 300 mg/kg and Na-PABA at a level of 350 mg/kg. Each animal received 2 ml of the appropriate solution per 100 g of body weight. When salicylamide was used it was suspended in .125% gelatin-.64% NaCl solution. The NaCl was omitted when Na-PABA was used. It was also omitted in the accompanying experiments in which Na-salicylate was used. Control animals received 2 ml of the gelatin-NaCl solution per 100 g of body weight. A few were given 350 mg/kg of Na-PABA only, and since the results on these animals did not differ from those obtained in the other controls, they have been included in the control values.

The experimental results which are given in Table II show that the addition of the Na-PABA did not affect the adrenal changes, either from the standpoint of intensity of effect or duration of action. Although the drop in both the ascorbic acid and cholesterol was very much the same with both salicylamide and Na-salicylate, the duration of the effect was somewhat longer with Na-salicylate. Both compounds showed the lowest average concentration of adrenal cholesterol 6 hours after their administration, but the return to normal was more rapid in the salicylamide-treated animals. The lowest concentration of adrenal ascorbic acid was found in those animals sacrificed 2 hours after salicylamide, but 4 hours following Na-salicylate administration. The ascorbic acid content of the adrenals of the salicylamide-injected animals was showing a distinct return towards normal by the 4th hour, the time at which the Na-salicylate-injected animals showed the lowest values.

Discussion. The concentration of both ascorbic acid and cholesterol in the adrenals at a given time must represent a balance between their rates of utilization or removal on the one hand, and their rates of formation or deposition on the other. Consequently, the more prolonged effect of Na-salicylate on the concentration of these substances in the adrenal does not necessarily signify a longer period of increased adrenal activity. Further studies along other lines would be necessary in order to establish a definite answer.

Summary. Both oral and intraperitoneal administration of salicylamide to rats was found to cause a drop in the ascorbic acid and cholesterol content of the adrenal glands. The simultaneous administration of PABA or its sodium salt along with either salicylamide or Na-salicylate had no influence on the intensity or duration of the adrenal effect.

1. Cronheim, G., King, J. S., and Hyder, N., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 51.

2. Hetzel, B. S., and Hine, D. C., *Lancet,* 1951, v261, 94.

3. Van Cauwenberge, H., *ibid.,* 1951, v261, 374.

4. Blanchard, K. C., Dearborn, E. H., Maren, T. H., and Marshall, E. K., Jr. *Bull. Johns Hopkins Hosp.,* 1950, v86, 83.

5. Cronheim, G., King, J. S., and Hyder, N., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 109.

6. Hart, E. R., *J. Pharm. Exp. Therap.,* 1947, v89, 205.

7. Ichniowski, C. T., and Hueper, W. C., *J. Am. Pharm. Assoc., Sci. Ed.,* 1946, v35, 225.

8. Litter, M., Moreno, A. R., and Donin, L., *J. Pharm. Exp. Therap.,* 1951, v101, 119.

9. Roe, J. H., and Kuether, C. A., *J. Biol. Chem.,* 1943, v147, 399.

10. Outhouse, E. L., and Forbes, J. C., *J. Lab. Clin. Med.,* 1940, v25, 1157.

11. Forbes, J. C., and Duncan, G. M., *Quart. J. Stud. Alc.,* 1951, v12, 355.

Received November 30, 1953. P.S.E.B.M., 1954, v85.