ucts of the initial hemolysis were removed from the corpuscles remaining before these were incubated showed that the products of hemolysis had little or no influence on the development of incubation hemolysis in these series. As there was no initial osmotic hemolysis in all of the series including and above 0.70% saline direct comparisons can be made between those series. The possible effect of the phosphate buffer on hemolysis was eliminated by substituting a carbonate buffer in some of the tests. The results were essentially the same with the two buffers. Ham and Castle(1) infer that the increases in erythrocytic volume and fragility during incubation are due to metabolic processes which produce an increase in the osmotically active constituents of the erythrocytes. If this explanation be accepted it would seem that the salines from 0.41% to 1.28% are progressively more favorable to these postulated metabolic changes. The nature of these metabolic changes has not been determined, but Harris(8) followed the losses and gains in potassium and sodium by human erythrocytes with and without dextrose and concluded that metabolic activity was responsible for the movement of these cations.

In the present experiments it was found that 95% or more of the expected hemolysis in 0.70% saline resulting from 22 hours incubation at 37°C could be prevented by the addition of 32 mg of dextrose per 100 ml of the 1:100 saline dilutions of blood, and that as little as 1 mg of dextrose per 100 ml appreciably reduced 22 hour incubation hemolysis. On the other hand, hemolysis could be speeded up greatly, although the relative action of the several salines remained the same, by incubation at 42°C or at 45°C. Both the reaction to dextrose and to higher incubation temperatures might suggest metabolic changes as factors in incubation hemolysis.

Summary. In 1:100 dilutions of dog blood with buffered salines ranging from 0.41% to 1.28% the hemolysis resulting from incubation for 22 hours or less at 37°, 42° and 45°C varied directly although not proportionately with the osmotic tonicity of the saline.

Ninety-five percent or more of the expected hemolysis resulting from incubation in 0.70% saline at 37°C could be prevented by the addition of 32 mg of dextrose to 100 ml of the 1:100 saline dilution of blood, and as little as 1 mg of dextrose per 100 ml appreciably reduced 22 hour incubation hemolysis.

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8. Harris, J. E., *J. Biol. Chem.*, 1941, v141, 579.

Effects on Anaphylactic Shock of Salicylates, Aminopyrine and Other Chemically and Pharmacologically Related Compounds.* (17870)

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The effect of salicylates on antibody formation and antigen-antibody reactions has been studied sporadically for many years(1). Recently, Campbell(2) prevented anaphylaxis in a small number of animals by the administration of aspirin. Because salicylates have had a long, useful history in medical therapy, it seemed highly desirable to extend Campbell's results to learn more about the phenomenon and also to search for related

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1. Smith, P. K., *J. Pharmacol.*, Part 2, 1949, v97, 353.

2. Campbell, B., *Science*, 1948, v108, 478.

TABLE I.
Effect of Various Compounds on Number of Deaths from Anaphylactic Shock.

Compound	Control			With compound		
	Dose	D/T*	% deaths	D/T*	% deaths	Chi²
Acetylsalicylic acid	.64 g	39/74	52.8	8/57	14	21.3
Sodium salicylate	.57	32/60	53.3	9/43	20.9	11.0
Aminopyrine	.82	19/34	55.9	1/22	4.5	14.5
Acetanilid	.48	27/52	51.9	15/42	35.7	2.4
Neocinchophen	1.04	15/35	43	10/28	35.7	0.4
Sodium para-aminobenzoate	.56	18/43	41.8	9/34	26.4	1.9
Para-aminosalicylic acid	.54	19/42	45.2	9/31	29	2.0
Ortho-aminobenzoic acid	.49	8/17	47	4/18	22.2	2.4
Para-hydroxybenzoic acid	.49	8/17	47	4/18	22.2	2.4
Sulfanilamide	.61	5/15	33.3	2/16	12.5	2.0
Sodium gentisate	.63	8/28	28.5	8/19	42	1.1
" bicarbonate	.32	13/32	40.6	16/32	50	0.6
Nembutal	30 mg	14/28	50	11/30	36.7	1.1
Codeine	5	5/9	55.5	4/7	57.2	—

* Ratio deaths to total number of animals.

compounds with perhaps greater activity. As a first step, we have attempted to ascertain whether or not the inhibition by salicylates is related to (1) a particular type of chemical configuration, or (2) a specific type of pharmacological activity.

Method. Young rabbits weighing approximately 2 kg were sensitized by repeated injections of the undiluted white of hens' eggs. In the first 2 experiments, one with aspirin and one with nembutal, 3 injections of 1.0 cc were given on alternate days. The first 2 injections were given intravenously and the third, intramuscularly. In an attempt to increase the percentage death rate from anaphylactic shock, the animals in all later experiments received 2 additional subcutaneous injections of 1.0 cc on alternate days, a total of 5 injections over a 10-day period. Two weeks were allowed to elapse after the last injection for the development of sensitivity to the egg white. The animals were then shocked by 2 cc of undiluted egg white, administered intravenously.

For each new experiment, a separate group of control animals was studied. This was necessary because of the rather large variation in death rate among the control groups. An attempt was made to grade the degree of shock in the animals, but this proved to be too subjective. Consequently, in the preparation of our data, *only death rates were employed.*

The protective effect of a compound against anaphylactic shock was investigated as follows: sensitized animals were divided into an experimental and a control group. Approximately one hour before the shocking dose of egg white, the experimental group received the calculated amount of the compound suspended in water, and the control group, an equivalent amount of water, by intrapharyngeal tube. Thus, the 2 groups received equivalent handling and fluid intake. One hour after medication, the animals were shocked by intravenous administration of egg white. Most of the drugs were administered orally in equimolar doses but the nembutal and codeine were given subcutaneously in smaller doses. Neocincophen was difficult to administer by the tube method because of its insolubility; therefore, we are not sure of the exact dosage in this group of animals. Also, because this compound is absorbed less readily than the others, an extra dose was given 10 hours before injection of the shocking dose of egg albumin. As an added precaution, 2 to 3 hours were allowed to elapse between administration of the final dose of drug and the shocking dose of egg albumin.

Blood salicylate levels were determined by the method of Brodie *et al.*(3) in rabbits of the same weight one hour after administration of doses similar to those given above.

Several experiments were conducted to de-

3. Brodie, B. B., Udenfriend, S., and Coburn, A. F., *J. Pharmacol.*, 1944, v80, 114.

TABLE II.
Number of Experiments with Each Compound and Reproducibility of Results.

Drug	No. of experiments: Treated animals showed fewer deaths than simultaneous controls	Controls showed fewer deaths than treated animals	Treated = control	Total
Acetylsalicylic acid	7	0	1	8
Sodium salicylate	7	0	0	7
Aminopyrine	4	0	0	4
Acetanilid	3	3	0	6
Neocinchophen	2	1	1	4
Sodium para-aminobenzoate	2	1	2	5
Para-aminosalicylic acid	3	1	1	5
Ortho-aminobenzoic acid	2	0	1	3
Para-hydroxybenzoic acid	2	0	1	3
Sulfanilamide	2	1	0	3
Sodium gentisate	0	2	0	2
" bicarbonate	1	4	0	5
Nembutal	2	3	0	5
Codeine	0	1	0	1

termine the antihistaminic effect of the active compounds. Four mg of histamine hydrochloride in 1 cc 0.85 percent NaCl injected intravenously, killed about 80% of a group of rabbits. This dose was therefore used as a standard shocking dose. Animals were divided into 2 groups: the control group received water by intrapharyngeal tube, and the experimental group, the compound, suspended in water, one hour before the histamine shock dose.

Results. Both acetylsalicylic acid and sodium salicylate prevented death under the conditions of the experiment (Table I). Aminopyrine was also effective. We were not able to demonstrate a definite effect with any of the other compounds.

Table II indicates the degree of reproducibility observed in separate experiments with the various compounds. Again it can be seen that the death rates among animals receiving salicylates and aminopyrine were consistently lower than in the corresponding control groups. Thus, in 7 of 8 experiments with acetylsalicylic acid, in 7 of 7 experiments with sodium salicylate, and in 4 out of 4 experiments with aminopyrine, the animals were protected. No such clear-cut effects were observed with any of the other compounds. In the doses studied, the serum salicylate levels in rabbit plasma following administration of aspirin ranged from 11.5 to 20.0 mg % and those for sodium salicylate, 18.0 to 26.3 mg %.

An attempt was made to determine whether the antipyretic effect of the compounds was related to the anti-anaphylactic activity. Aminopyrine was the most effective antipyretic compound, reducing the temperature 3 to 4°F. Acetanilid and neocinchophen were almost as effective. Salicylates were less effective, lowering the temperature only 1 to 2°F. Even in individual animals, there was no correlation between the degree of antipyresis and anti-anaphylaxis. Thus 2 salicylate animals with the most marked temperature response died, whereas others with a minimal response tolerated the shocking dose of albumin.

Both aspirin and aminopyrine were tested for antihistaminic activity. Of 10 animals receiving aspirin, 9 died in histamine shock, compared with 7 deaths among 10 controls. With aminopyrine, there were 6 deaths among 9 animals receiving the compound, and 7 deaths in 9 control animals.

Discussion. Some previous studies, in addition to those of Campbell(2), indicate that salicylates may suppress the union of antigen and antibodies. Thus Coburn and Kapp (4) found that salicylates inhibited the pre-

4. Coburn, A. F., and Kapp, E. M., *J. Exp. Med.*, 1943, v77, 173.

cipitation of horse serum euglobulin by rabbit antiserum, and also decreased the amount of precipitate in a system composed of crystalline egg albumin and its antibody. Moreover, precipitates previously formed could be partially dissolved. The effect was proportional to the salicylate concentration. They attribute the effect to "inactivation of the antibody" rather than to suppression of antigen-antibody combination. It seems likely that salicylates also inhibit the formation of antibodies. Swift showed that the production of antibodies in response to various streptococcic and pneumococcic antigens could be suppressed by the administration of sodium salicylate during the period of antibody development(5). Derick and co-workers(6) showed that when salicylates and neocinchophen were given within 24 to 48 hours and continued for 10 to 14 days after serum administration, they suppressed the arthritis as well as the antibody response in man. In both studies the sera tested for antibodies were not free from salicylates at the time of the determination.

The production of anti rH agglutinins in guinea pigs and rabbits was reduced by sodium salicylate administration, according to Homburger(7). At the time of the test, no free salicylates were present in the serum. Scherer, however, was unable to confirm this finding in rabbits(8). It was shown by Jager (9) that a salicylate serum level of 30 to 40 mg % reduced the antibody response to typhoid antigen. Salicylate effects in streptococcal infections are not so clear-cut. Thus, Rantz, *et al.*(10) found no effect on the antistreptolysin and antifibrinolysin titers; according to Perry(11), however, antifibrinolysin titers are lower after salicylate therapy. Salicylates can frequently prevent or mitigate the endocardial lesions which follow hypersensitization of rabbits, according to Smull and co-workers(12) and Roberts *et al.* (13). Sullivan(14) and Smull *et al.*(12) reported decreased occurrence of arterial lesions in animals similarly treated, but this observation was not confirmed by Roberts *et al.* (13) and Forman and co-workers(15). Good, Campbell and Good(16) studied the encephalitis produced in guinea pigs by administration of guinea pig brain plus special adjuvants. They were able to prevent the encephalitis either by a combination of moderate amounts of salicylates and para-aminobenzoic acid, or by large amounts of salicylates alone. Aminopyrine and neocinchophen also have been said to inhibit antibody formation(6,17).

Our experiments indicate that both salicylates and aminopyrine are effective in preventing death from anaphylactic shock in rabbits. The drug was administered only once, one hour before the animal was shocked. Therefore, the effect cannot be attributed to inhibition of antibody formation. The results we have obtained confirm those of Campbell's(2) for salicylate but are based on a larger number of animals, and a more rigorous criterion of activity, *i.e.* death of the animal *versus* survival, instead of the grading of symptoms. We present a new observation, namely, that aminopyrine also inhibits anaphylaxis. Our results suggest that salicylates and aminopyrine affect the immune mechanism by interfering with the specific interaction of antigen and antibody. For example, death in animals receiving his-

5. Swift, H. F., *J. Exp. Med.*, 1922, v36, 735.

6. Derick, C. L., Hitchcock, C. H., and Swift, H. F., *J. Clin. Invest.*, 1928, v5, 427.

7. Homburger, F., PROC. SOC. EXP. BIOL. AND MED., 1946, v61, 101.

8. Scherer, W. F., *Am. J. Med. Sci.*, 1947, v215, 33.

9. Jager, B. V., and Nickerson, M., *Am. J. Med.*, 1947, v3, 408.

10. Rantz, L. A., Boisvert, P. J., and Spink, W. W., *Science*, 1946, v103, 352.

11. Perry, C. B., *Arch. Dis. Child.*, 1939, v14, 32.

12. Smull, K., Wissler, R. W., and Watson, J. M., *J. Lab. Clin. Med.*, 1948, v33, 936.

13. Roberts, R. C., Crockett, K. A., and Laipply, E. C., *Arch. Int. Med.*, 1949, v83, 48.

14. Sullivan, C. E. J., Parker, T. W., and Herbert, R. W., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 508.

15. Forman, C., Seifter, J., and Ehrich, W. E., *J. Allergy*, 1949, v20, 273.

16. Good, R. A., Campbell, B., and Good, I. A., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 341.

17. Hueper, W. C., *Med.*, 1948, v27, 43.

tamine is quite similar to death from anaphylactic shock. But one would have expected a reduction in the number of histamine deaths if the compounds under study produced merely a non-specific increase in resistance. Furthermore, no correlation could be found between anti-pyretic and anti-anaphylactic activity. Also, a different type of analgesic, codeine, and the hypnotic compound, nembutal, were ineffective. However, it is impossible to state with certainty that pharmacological or chemical effects other than interference with the immune reaction may not be important.

The "negative" results obtained with the other compounds indicate only that they are not as effective as salicylate and aminopyrine. One cannot be sure that more tests with this or other methods will find them devoid of activity. The results with neocinchophen may have been influenced by difficulty in administration and absorption. However, because this compound produced a definite anti-pyretic effect, it appears certain that a fairly large percentage of the dose must have been absorbed.

The failure of sodium gentisate to prevent anaphylactic shock is interesting because of the suggestion of Meyer and Ragan(18) that salicylate acts in rheumatic fever only after conversion to gentisate.

It is hoped that further studies with salicylates, aminopyrine, and related compounds will elucidate the site and mechanism of action of the inhibitory effect. Their influence on other protein, as well as non-protein, sensitizations should be of interest. A simpler technic for screening compounds for activity is desirable because the present method is too cumbersome and expensive.

Summary. 1. Oral administration of salicylates and aminopyrine in rabbits decreased the incidence of death from anaphylactic shock. 2. A number of compounds closely related, chemically or pharmacologically, did not produce a similar inhibitory effect. 3. The mechanism of action is not antihistaminic, nor does it appear to be related to characteristic pharmacological effects, *e.g.* antipyresis. It may represent a direct effect on the specific combination of antigen and antibody.

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18. Meyer, K., and Ragan, C., *Science*, 1949, v108, 281.

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Effect of Metal-Combining Globulin (Fraction IV-7) in Severe Mediterranean Anemia.* (17871)

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Iron is transported in the serum only in attachment to a B_1-globulin, called the "metal-combining globulin", which resides in Fraction IV-7 of the plasma(1). Except under certain conditions this globulin is not completely saturated with iron, and the excess in reserve is measurable. The determination of the serum iron level measures the degree of saturation of this protein. The method of Rath and Finch(2) employed for the determination

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1. Surgenor, D. M., Koechlin, B. A., and Strong, L. E., *J. Clin. Invest.*, 1949, v28, 73.

2. Rath, C. E., and Finch, C. A., *J. Clin. Invest.*, 1949, v28, 79.