Conclusions. The administration of p-amino benzoic acid, the calcium double salt of benzyl succinic and p-amino benzoic acids, or sodium p-amino benzoate prior to a test dose of epinephrine during cyclopropane anesthesia reduced the incidence of ventricular fibrillation. The intracardiac injection of procaine at the time when ventricular fibrillation developed effected a return to normal in a number of cases. Ventricular fibrillation was not ameliorated by the intracardiac injection of the other three p-amino benzoic acid derivatives.

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Anti-Catalase Activity of Sulfanilamide and Related Compounds. VI. Further Studies on Sulfonhydroxamides.

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In previous studies on the anti-enzymatic concept of the mode of action of sulfanilamide,[1-4] attention has been focused on catalase as one of the enzymes of importance. A time factor was postulated for the conversion of the inactive sulfanilamide to an active anti-catalase, which was presumed to result through oxidation to the hydroxylamino derivative. This furnishes an explanation of the characteristic lag period preliminary to the bacteriostatic action of sulfanilamide. It was therefore expected that p-hydroxylamino sulfanilamide, or a similar substance, would exert a bacteriostatic effect without this period of lag and that in addition the action would be more intensive. The sulfonhydroxamides contain a hydroxylamino group which, although located differently in the molecule, contributes anti-catalase activity. Hence they might

[1] Main, E. R., Shinn, L. E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 272.

[2] Shinn, L. E., Main, E. R., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 591.

[3] Main, E. R., Shinn, L. E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 115.

[4] Mellon, R. R., Locke, A. P., and Shinn, L. E., *Publication No.* 11, *A. A. A. S.*, 1939, pp. 98-113.

behave as preformed active derivatives. It was demonstrated[5] that *p*-caproylaminobenzenesulfonhydroxamide was capable of initiating bacteriostasis in broth cultures of the pneumococcus without the degree of lag manifested by sulfanilamide and that the bacteriostatic power per mole was more than 4 times as great as that of sulfanilamide. All of the sulfonhydroxamides examined were capable of effecting the accumulation of hydrogen peroxide in pneumococcus cultures.

These results were obtained in the absence of blood. The known reactivity of hydroxylamine and its derivatives for hemoglobin would lead to the expectation that in the presence of blood the hydroxylamino group would be destroyed before reaching the bacterial cell* with a resulting diminution of the bacteriostatic effect. It would not be expected that the immediate nature of the action would be impaired. This bacteriostasis should, however, prove to be transient. There are thus 3 points which can be tested experimentally.

Cultures of a virulent pneumococcus (Neufeld Type I) were established by seeding 1.5 cc samples of defibrinated rabbit blood contained in 1x11 cm tubes with 0.1 cc of a 1/100,000 broth dilution of a 16-hr. blood broth culture (300 organisms) and incubating at 39.5°C for 1.5 hr with intermittent shaking. Plate counts were made at the end of this period and the required compounds added as 0.1 cc of a blood dilution of an alcoholic stock solution. Controls received corresponding amounts of alcohol.† Incubation and shaking were continued and further counts made at 0.75, 1.75, 3.5, and 8.5 hr after the additions. Inhibition was calculated as in the preceding paper.[5]

Fig. 1 A, B, C, F, gives the results of such experiments for *p*-caproylaminobenzenesulfonhydroxamide and sulfanilamide. In order to attain a degree of bacteriostasis comparable with that pro-

[5] Main, E. R., Shinn, L. E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 593.

* Blood containing the sulfonhydroxamides took on a brownish hue during incubation. While no experimental proof of the point is available, this was in all probability the result of methemoglobin formation and an indication of the lability of the hydroxamide group. It has been found in the Sharp and Dohme Laboratories that these sulfonhydroxamides decompose in contact with moisture to yield oxides of nitrogen. Obviously these oxides would tend to produce methemoglobin. They also appear to be responsible for the yellow color produced when broth containing the sulfonhydroxamides was treated with *o*-tolidine.[5]

† The concentration of alcohol in the cultures was 1-2%. It appeared to have no effect on growth.

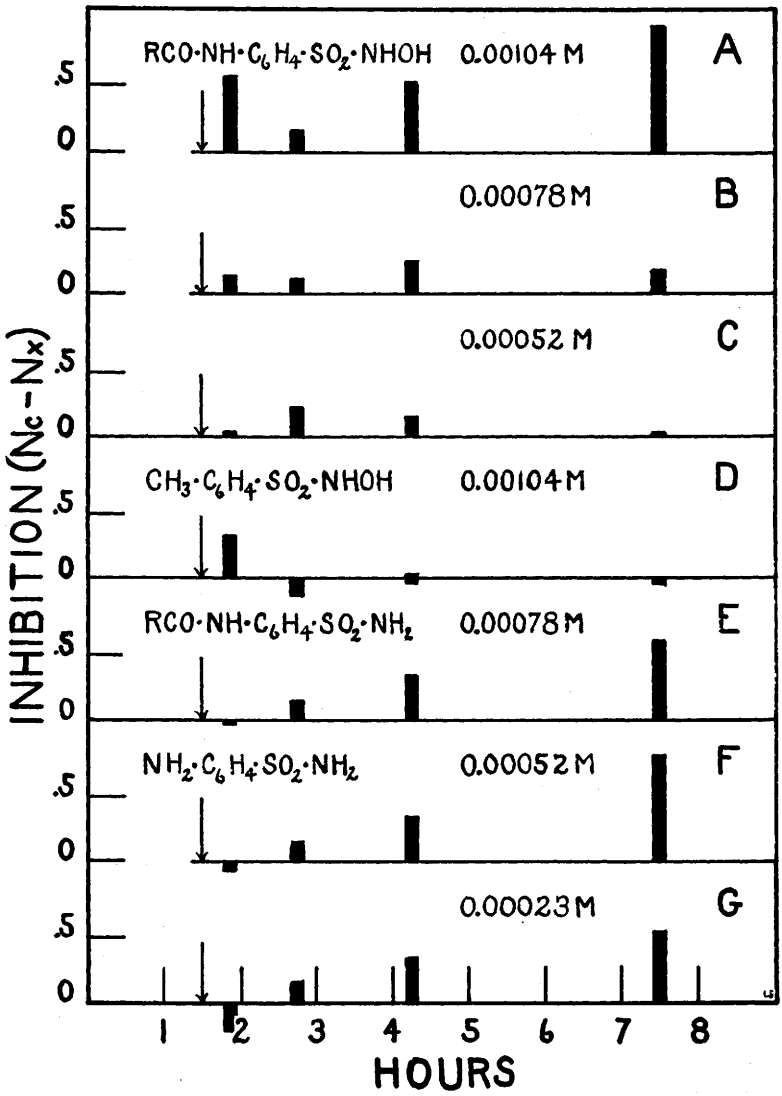

FIG. 1.

Inhibition of growth of Type I pneumococcus in defibrinated rabbit blood at 39.5°C by: (A) *p*-caproylaminobenzenesulfonhydroxamide,‡ 0.00104 M; (B) the same, 0.00078 M; (C) the same, 0.00052 M; (D) *p*-toluenesulfonhydroxamide,‡ 0.00104 M; (E) *p*-caproylaminobenzenesulfonamide,‡ 0.00078 M; (F) sulfanilamide, 0.00052 M; (G) the same, 0.00023 M. Inhibition is expressed as the difference between the number of generations produced per hour in a control culture (Nc) and in a culture containing the compound added (Nx) over the intervals 1.5-2.25, 2.25-3.25, 3.25-5.0, and 5.0-10.0 hr. The values are plotted at the mid-point of the interval concerned. Cultures were inoculated at 0 hours and the compounds added at 1.5 hr (arrow).

‡ Furnished us through the courtesy of Dr. Maurice Moore of Sharp and Dohme, Inc.

duced by 0.00052 M (9 mg%) sulfanilamide, the concentration of the hydroxamide must be raised to 0.00104 M. This is in contrast to the results for the same compound in broth where less than 0.00016 M was required for the same result.[5] The expected action of blood in reducing the bacteriostatic efficacy of this type of compound is hence demonstrated. As was anticipated, the bacteriostatic power of the sulfonhydroxamide is established within the first observational period, whereas that of sulfanilamide shows a brief period of stimulation.

The dip in bacteriostatic power at the second interval in Fig. 1 A suggests two diverse actions. The second action appears at about the time that the first is vanishing and resembles that produced by sulfanilamide. This suggests that it can be attributed to a free *p*-amino group produced from the caproylamino group whereas the transient portion can be referred to the hydroxamide group.

This was tested by employing two other compounds: *p*-toluenesulfonhydroxamide and *p*-caproylaminobenzenesulfonamide in which the two groups are removed to separate molecules. The results are shown in Fig. 1 D and E. When the caproylamino group is absent (D) only the immediate transient action is obtained. When the hydroxamide group is replaced by an amide group (E) no immediate action is found but the second action of *p*-caproylaminobenzenesulfonhydroxamide is approximately reproduced. Hence it appears that the postulate regarding the prompt but transient nature of the action of the hydroxamide group is correct but that the complete acylaminobenzenesulfonhydroxamide molecule has a double activity. This result made it advisable to investigate the extent of deacylation of caproylamino derivatives in blood.

To whole defibrinated rabbit blood was added *p*-caproylaminobenzenesulfonhydroxamide or *p*-caproylaminobenzenesulfonamide to a concentration of 0.00078 M. The samples were incubated at 39.5° and portions withdrawn for analysis by the Marshall method. The results are given in Table I. Both compounds underwent substantial deacylation in blood. In broth no significant deacylation of the sulfonhydroxamide was noted; indicating that deacylation in blood was largely enzymic. That the degree of conversion to free amine in the case of the sulfonhydroxamide is sufficient to account for the bacteriostasis produced is not conclusively demonstrated but in Fig. 1 G is shown the result of experiments with a lower concentration of sulfanilamide (4 mg%) in which very substantial bacteriostasis was produced.

TABLE I.
Deacylation of *p*-Caproylaminobenzenesulfonhydroxamide and *p*-Caproylaminobenzenesulfonamide in Broth and Defibrinated Rabbit Blood at 39.5°C.

	A			B	
Hr	% deacylation in broth pH 7.4	% deacylation in blood	mg% sulfanilamide equivalent in blood	% deacylation in blood	mg% sulfanilamide equivalent in blood
0	1.3	0.8	0.1	0.4	0.05
0.75	—	3.6	0.5	4.1	0.5
1.75	—	5.8	0.8	7.5	1.0
3.50	1.3	10.8	1.5	18.0	2.7
6.75	—	15.7	2.1	32.0	4.3
24.00	—	29.8	4.0	76.0	10.2

A. *p*-caproylaminobenzenesulfonhydroxamide 0.00078 M.
B. *p*-caproylaminobenzenesulfonamide 0.00078 M.

Discussion. *p*-Caproylaminobenzenesulfonhydroxamide appears to represent an approach to that hypothetical compound which shall be "preformed" in the sense of possessing the group or groups necessary to bacteriostatic activity which are normally formed by the micro-organism itself. That the present compound is not ideal is demonstrated by the transient nature of the powerful early effect. The demonstration of two successive activities arising from the same molecule opens interesting fields of speculation and experiment regarding future objectives in the production of better therapeutic agents.

Summary. *p*-Caproylaminobenzenesulfonhydroxamide produces bacteriostasis of pneumococci in blood without the lag characteristic of sulfanilamide. The activity is only about one-eighth of the corresponding bacteriostatic power in broth. That the immediate nature of the effect is due to the hydroxamide group was demonstrated by the use of *p*-toluenesulfonhydroxamide in which the potentially active *p*-amino group is absent. The effect of the hydroxamide group is transient in nature. A second period of bacteriostatic activity manifested by *p*-caproylaminobenzenesulfonhydroxamide is probably due to the free amino group formed by deacylation of the caproylamino group.