In addition to the higher percentage of positive nasopharyngeal cultures obtained, the difference in the number of colonies which developed on the plates was striking. In repeated instances, there were only a few colonies of *Hemophilus pertussis* on a cough-plate or throat-plate culture, while the nasal culture revealed numerous colonies. Sometimes the growth was practically pure. The nasal culture often showed but a minimal growth of secondary invading organisms, while a heavy growth, on the other hand, usually occurred on the media inoculated from the throat. We believe that this difference in the occurrence of secondary invading organisms largely explains the difference in the percentage of positive cultures of *Hemophilus pertussis* isolated from the two sources. It may be, however, that the pertussis bacillus occurs in greater numbers in the nasopharynx during the disease and remains there for a longer period of time during convalescence. Further study will be necessary to decide this point.

A comparative study of the cough-plate, throat and nasopharyngeal cultures in pertussis indicates that the nasopharyngeal method is definitely the best, particularly for use in infants.

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Anticatalase Activity of Sulfanilamide and Related Compounds. V. Bacteriostatic Activity of some Sulfonhydroxamides.

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This is a report of a study in which the ability of sulfanilamide to produce catalase inhibition is compared with that exhibited by acyl aminobenzenesulfonhydroxamides. Since the latter compounds contain a hydroxylamino group, they are analogous in structure to the intermediate assumed to be responsible for the anti-catalase activity of sulfanilamide in bacterial cultures.[1, 2, 3] Possibly because of the presence of this group, the sulfonhydroxamides are capable of exerting a more rapid and more intense bacteriostatic effect than sulfanilamide.

[1] Locke, A., Main, E. R., and Mellon, R. R., *Science*, 1938, **88**, 620.

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

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

Bacteriostatic Action. Fig. 1 compares the speed and intensity of the bacteriostatic action of sulfanilamide and caproylaminobenzenesulfonhydroxamide, the latter being chosen as typically representative of the group. Cultures, made by inoculating 10 cc quantities of veal infusion broth, contained in 50 cc Erlenmeyer flasks, with 0.0001 cc of a highly virulent, 18-hr broth culture of Type I pneumococcus, were incubated at 39.5°C. After 1.5 hrs, plate counts were made and the compounds added as freshly prepared solutions in 60% alcohol, the concentrations of which were so adjusted that an addition of 0.1 cc would give the desired final concentrations. The same volume of 60% alcohol was added to control cultures. Incubation was continued and samples were withdrawn for plate counts 0.75, 1.75, and 3.5 hrs after addition of the drugs.

The rate of generation was estimated from the counts by the formula:

$$\frac{\text{count at the end of the observed interval}}{\text{count at the beginning of the interval}} = 2^n$$

The degree of retardation in rate of multiplication was obtained as the difference between the rate of generation in the control culture and in the culture containing the added drugs.

Example:

Count 1.75 hrs after addition of compound = 1220
Count 3.5 hrs later = 6100

$\frac{6100}{1220} = 5.0$ and therefore represents a 5.0-fold increase in population.

From the equation, $5.0 = 2^n$, $n = 2.3$ (total number of generations produced).

Number of generations produced per hr $= 2.3/1.75 = 1.3$.

Since the number of generations per hr in control cultures for the corresponding time interval was 1.8, the difference, 0.5, represents the inhibition in rate of multiplication.

The results show a distinct difference in rate and intensity of bacteriostatic action of the two compounds. In the cultures containing sulfanilamide (shaded columns, Fig. 1), a slight stimulation in rate of multiplication was observed at the end of the first interval. This was followed by inhibition which continued to increase during the remainder of the observation period. Inhibition by the sulfonhydroxamide (solid columns) was detectable by the end of the first interval and rapidly increased in magnitude, presumably as a result of increasing peroxide accumulation. By the end of the second period of observation, the inhibition was 2.5 times greater than that of sulfanilamide although the concentration was less than one-third as great.

Peroxide Accumulation and Growth Inhibition. Table I indicates

the comparative capacities of sulfanilamide and the sulfonhydroxamides for producing peroxide accumulation and growth inhibition. The ratios of peroxide concentration to amount of growth were determined under the experimental conditions previously described.[4] Alcoholic solutions of the drugs were used as in the experiments described above, but were added before inoculation. The inoculum, 0.1 cc of an 18-hr culture, was larger than that used in the above experiments in order that growth would be sufficiently large to be measured turbidimetrically from the fourth to the seventh hour of growth. Only the ratios found at the seventh hour are reported in the table. Under the conditions of the experiment, peroxide was usually detectable by the peroxidase test* by the third hour.

In cultures containing 0.00014 to 0.00020 *M* caproylaminobenzenesulfonhydroxamide, the peroxide concentration per unit of growth was from 2 to 4 times greater than in control cultures. With a 0.00052 *M* concentration there was no visible growth during the 7 hours. In cultures containing 0.00052 *M* sulfanilamide, peroxide concentration per unit of growth was, on the average, 4 times greater than that of the control. In the presence of 0.00052 *M* acetamidobenzenesulfonhydroxamide the ratio was from 2 to 3 times greater and therefore this compound was only slightly less effective than sulfanilamide in the same concentration. Toluene- and benzenesulfonhydroxamides were approximately equal in effect to the acetamido compound.

Anti-Catalase Activity. The hydroxamides listed in Table I showed anti-catalase activity equal to or greater than that of irradiated sulfanilamide. The activity was not, however, constant. Upon standing in solution the sulfonhydroxamides undergo a change, shown by the development of a yellow color and an altered reaction with *o*-tolidine.

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

* A slight modification of the method previously described (Main and Shinn, *J. Biol. Chem.*, 1939, **128**, 417) was used. An aqueous extract of dried horseradish was used in place of the potato extract as the source of peroxidase, as suggested by Fuller and Maxted (*Brit. J. Exp. Path.*, 1939, **20**, 177). Determinations can be made over the range, 2 to 20γ per cc, while with potato extract, the range is 6 to 30γ.

It was necessary to use an additional blank in the determination of peroxide in the presence of sulfonhydroxamides, since, after standing at a pH of 7.0 or above for one-half hour or more, solutions of the sulfonhydroxamides give a yellow to orange color upon addition of the *o*-tolidine reagent. To make color comparisons with the peroxide standards, a blank consisting of sterile broth which contains the same concentration of compound as the culture is placed back of the standard tube in the comparator.

TABLE I.

Effect of Sulfonhydroxamides on Growth and Peroxide Accumulation in Broth Cultures of Type I Pneumococcus.

Ten cc quantities of veal infusion broth containing 0.2% glucose and the compounds in the concentrations given below were inoculated with 0.1 cc of an 18-hr broth culture of Type I pneumococcus. Samples were removed at intervals for determination of growth and peroxide content. The ratios, A and B, were calculated from determinations made after 7 hr growth.

Compound added	Millimols per l†	H_2O_2 concentration per unit of growth‡ in A Control cultures	H_2O_2 concentration per unit of growth‡ in B Cultures contg. compds.	Ratio, B : A
4-*n*-Caproylaminobenzenesulfonhydroxamide*	0.52	0.3	—§	—
	.20	.3	1.1	3.7
	.20	.4	.8	2.0
	.17	.4	.8	2.0
	.14	.2	.5	2.5
	.14	.4	.7	1.8
4-Acetamidobenzenesulfonhydroxamide	.52	.2	.6	3.0
	.52	.2	.4	2.0
	.52	.3	1.0	3.3
p-Toluenesulfonhydroxamide	.52	.2	.5	2.5
	.52	.2	.5	2.5
	.52	.3	.4	1.3
Benzenesulfonhydroxamide	.52	.2	.6	3.0
	.52	.3	.7	2.3
	.20	.3	.7	2.3
	.17	.4	.5	1.3
Sulfanilamide	.52	.3	1.2‖	4.0

* The sulfonhydroxamides were furnished to us through the courtesy of Sharp and Dohme, Inc.

† Stock solutions of the compounds in 60% alcohol were prepared in such concentrations that 0.1 cc contained the amount needed to give the final concentration. The control cultures contained 0.1 cc alcohol.

‡ Growth was estimated by comparisons with $BaSO_4$ standards which had been calibrated against a culture arbitrarily selected to represent 100% growth. The growth units are therefore percentages relative to the standard culture. H_2O_2 concentration is expressed in micrograms per cc.

§ There was no visible growth in cultures containing this concentration.

‖ This figure represents the average of all determinations made with sulfanilamide-containing cultures, which were included regularly as controls.

Discussion. Sulfonamide derivatives studied heretofore have contained a para amino group which is convertible into a hydroxylamino group, giving the compound anti-catalase properties. In bacterial cultures, these compounds check the action of catalase and thereby cause increased accumulation of peroxide. The amount of active intermediate present may be very small, its effect depending on its continuous release in the vicinity of the cell. A compound which contains a preformed hydroxylamino group should act more rapidly

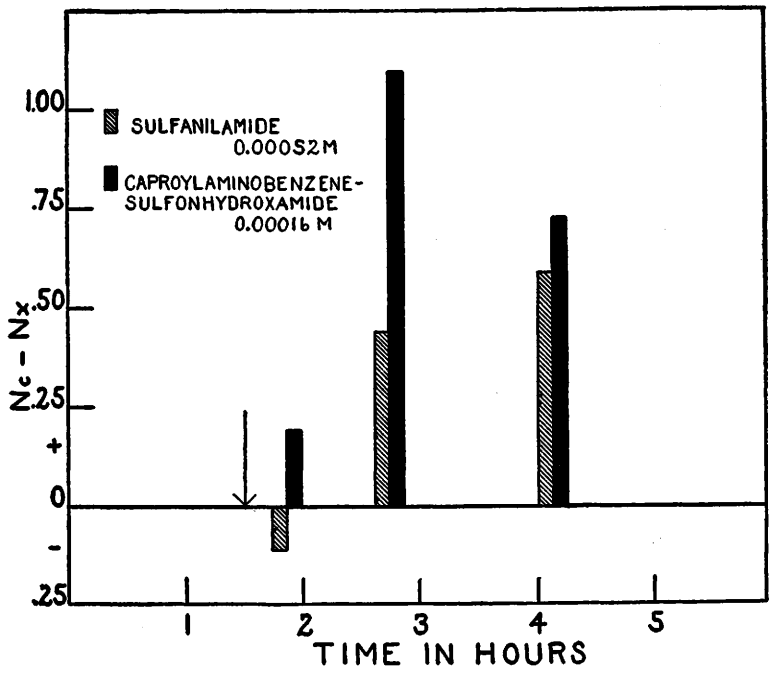

FIG. 1.

Comparison of the rates of development of bacteriostasis in the presence of sulfanilamide and 4-*n*-caproylaminobenzenesulfonhydroxamide.

N_c is the number of generations produced per hour in the control cultures and N_x the number produced in the cultures containing compound added. The additions were made at the time indicated by the arrow. Each column represents the average of results from 10 experiments.

and, because of the presence of active substance in high concentration, should have a more intense effect. The experiments reported here indicate that this is the case with the acyl aminobenzenesulfonhydroxamides, compounds of the structure, $RNH\text{-}C_6H_4\text{-}SO_2NHOH$, where R is the caproyl, valeryl, heptanoyl or acetyl group. When compared mole for mole, caproylaminobenzenesulfonhydroxamide is a much stronger bacteriostatic agent than sulfanilamide. Bacteriostatic activity is demonstrable almost immediately and increases rapidly while peroxide is accumulating. Since the toluene and benzenesulfonhydroxamides, compounds which contain no para amino group, caused marked stasis and peroxide accumulation, the activity of the acyl compounds *in vitro* is apparently not due to a deacylated amino group.

The results given here apply only to stasis in broth cultures. The concentration of the hydroxamide required to produce the same amount of stasis in blood culture is appreciably greater. Results of *in*

vivo experimentation[5] indicate that when given daily in doses of 1 mg per g for 6 days, the acyl aminobenzenesulfonhydroxamides and sulfanilamide possess approximately the same therapeutic activity against pneumococcus infection in mice.

Summary. The acyl aminobenzenesulfonhydroxamides have strong anti-catalase activity and, when present in broth cultures of the pneumococcus, cause inhibition of growth associated with increased accumulation of hydrogen peroxide. When the caproyl compound is added to growing cultures, inhibition of growth is detectable almost immediately but reaches a maximum only after time for accumulation of peroxide has elapsed. Inhibition by sulfanilamide, on the other hand, is detectable somewhat later and approaches a maximum more slowly.

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Gonadotropic Potency of Gonadectomized Rats' Pituitary after Tryptic Digestion.

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The evaluation of the amount of the follicle-stimulating and luteinizing hormones of the pituitary of normal as well as gonadectomized animals has interested a number of investigators. The recent reports of McShan and Meyer[1] and Chen and van Dyke[2] on tryptic digestion of pituitary extract appear to afford a convenient means of estimating the relative amounts of these fractions, as the luteinizing activity is largely destroyed by trypsin to which the follicle-stimulating activity is resistant. In the following experiments the gonadotropic activity of castrate rats' pituitary following tryptic digestion was compared with that of the castrates' pituitary not subjected to such treatment.

The donors of the pituitary consisted of 24 male and 38 female albino rats which were gonadectomized at the age of 1-3 months. Three to 6 months (usually 3 months) after gonadectomy, the animals were sacrificed. The anterior pituitary was obtained, weighed, and ground up fresh in an appropriate quantity of 0.02%

5 Cooper, F. B., Gross, P., and Lewis, M., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 491.

1 McShan, W. H., and Meyer, R. K., *J. Biol. Chem.*, 1938, **126**, 361.

2 Chen, G., and van Dyke, H. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 172.