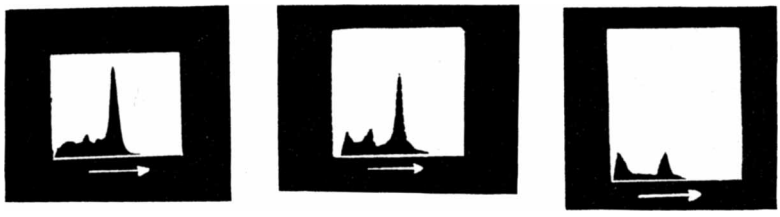

A B C

FIG. 1.

Electrophoretic patterns for cerebrospinal fluid (A) with high total protein content (case 3), (B) with normal total protein content (case 2), and (C) with abnormal colloidal gold curve (case 7).

Two cases of neurosyphilis whose spinal fluids showed abnormal colloidal gold curves, had a marked increase in the gamma globulin fraction. When the gamma globulin of case 7 (Fig. 1 C) was separated from the albumin electrophoretically, all of the colloidal gold reactivity was found in the gamma fraction and none in the albumin fraction. Although the colloidal gold reacting material is found in the gamma fraction, it may differ from the normal gamma component of spinal fluid since other fluids which contain equally large amounts of gamma globulin yield normal colloidal gold curves.

Summary. The electrophoretic pattern of the cerebrospinal fluid proteins resembles that of the plasma. Alterations in the composition of the serum proteins produce similar changes in the spinal fluid patterns. Colloidal gold activity in pathological fluids is associated with the gamma globulin.

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Inactivation of Sulfonamide Inhibitor by Azochloramid.

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The bactericidal effect *in vitro* of sulfonamide derivatives has been extensively investigated. Recently Neter[1] has reported that of a variety of bactericidal compounds which he studied only Azochloramid when used jointly with sulfonamides showed more than a mere additive effect upon the test organism.

1 Neter, E., PROC. SOC. EXP. BIOL. AND MED., 1941, 47, 303.

TABLE I.
Effect of Sulfanilamide and Azochloramid in a Synthetic Medium.
Inoculum 2,000,000 *E. coli*/ml.

Tube	Concentrations mg %		24-hr plate count × 000
	Azochloramid	Sulfanilamide	
1	0	0	182,000
2	0	5	185,000
3	0	15	163,000
4	.05	0	120,000
5	.10	0	93,000
6	.05	5	2
7	.05	15	0
8	.10	5	0
9	.10	15	0

We have examined this effect by quantitative methods. By plating out the contents of our mixed chemical-organism suspension we have obtained the following data: Two million *E. coli* per ml were inoculated into a synthetic medium and incubated for an hour at 37°C. The inoculated medium was then distributed in test tubes to which the drugs had been added in appropriate concentrations. The final drug concentrations and 24-hour counts are recorded in Table I.

A similar experiment with *Staph. aureus* with the exception that 20% sterile normal horse serum was added to increase the difficulty of killing, gave the results shown in Table II.

Similar effects upon the respiration of a growing culture were observed when using a Warburg respirometer as shown in Fig. 1.

The molecular ratio of the agents employed rules out any possibility that this synergism might be due to a chemical reaction between them, resulting in the formation of a more toxic compound. Neter's observations with other mixtures, particularly those obtained when examining the action of optochin and sulfapyridine on pneumococci, which two substances fail to show any signs of potentiation, make it extremely unlikely that the synergistic effect between Azochloramid

TABLE II.
Effect of Azochloramid and Sulfanilamide on *Staphylococcus aureus* in a Synthetic Medium + 20% Sterile Normal Horse Serum.

Inoculum of *Staph. aureus*, 2,000,000/ml			
Concentrations mg %		Plate counts	
Azochloramid	Sulfanilamide	24 hr × 000	48 hr × 000
.4		33,000	126,000
	20	93,000	34,000
.4	20	10	120
Inoculated control		120,000	140,000

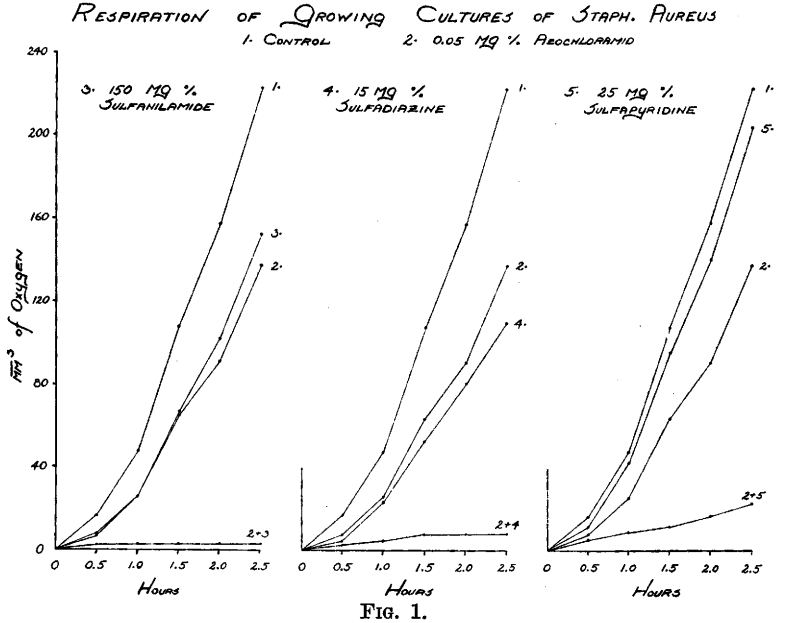

FIG. 1.

and sulfonamides could be due to a general lowering of the threshold for sulfonamide of the bacteria due to injury by Azochloramid.

TABLE III.

Effect of Azochloramid and Para-amino Benzoic Acid on the Inhibition of *E. coli* by Sulfanilamide.

Concentrations mg %			Turbidity (16 hr)
Sulfanilamide	Para-amino benzoic acid	Azochloramid	
0	0	0	34.0
0	0	.1	31.0
50	0	0	1.8
50	.1	0	33.5
50	.05	0	26.0
50	.01	0	2.0
50	.1	.1	1.5

TABLE IV.

Effect of Azochloramid and Peptone on the Inhibition of *E. coli* by Sulfanilamide.

Concentrations			Turbidity (16 hr)
Sulfanilamide mg %	2% Peptone ml	Azochloramid mg %	
0	0	0	43.
50	0	0	2.5
50	.5	0	30.0
50	.1	0	5.0
0	.5	.1	42.0
50	.5	.1	2.6

The specificity of the synergistic effect as well as the extremely small concentrations of Azochloramid required, directed our attention to the possibility that the action of Azochloramid might be due to the inactivation of the sulfonamide inhibitor. That this might actually be the case is shown in Tables III and IV.

Other chlorine compounds give similar results. Here, however, depending upon the higher reactivity of the halogen compound employed, higher concentrations may be required.

Of particular interest is the action of sulfanilamide and Azochloramid upon *Staph. aureus*, an organism notoriously resistant to sulfanilamide. In Table II it is shown that in the presence of a minute amount of Azochloramid, which is practically ineffective of itself, sulfanilamide has a striking bactericidal effect upon a large inoculum of this organism. Likewise in Fig. 1-A a minute amount of Azochloramid permits an otherwise ineffective sulfanilamide concentration to inhibit respiration of a large inoculum of growing staphylococci. McLeod[2] has published several exploratory experiments, one of which shows that his strain of Staphylococcus was characterized by its ability to release into the medium relatively large amounts of inhibitor. This might account for the resistance of this organism to sulfanilamide. The induced ability of sulfanilamide to kill large inocula of this organism in the presence of Azochloramid may be due to the latter compound's ability to inactivate the native inhibitor of the Staphylococcus, enabling sulfanilamide to kill this organism.

McLeod also showed that in some "sulfonamide-fast" strains the resistance of the organisms is accompanied by a great increase in the production of inhibitor. An examination was therefore made of the effect that small concentrations of Azochloramid might have upon the sulfonamide resistance of a "sulfonamide-fast" strain. Such a strain was quickly obtained in the following manner: Inocula of *E. coli* decreasing gradually from 1 ml to 1 loopful were transferred to tubes of a synthetic medium containing 50 mg % of sulfanilamide. Only the largest inoculum yielded growth in the first transfer. With this culture the process was repeated, slowly increasing the sulfanilamide concentration in the medium up to 100 mg %. However, upon continued culture from the smallest inoculum which gave growth in the sulfanilamide medium a culture was obtained in 6 transfers that grew well from a small number of organisms in the presence of 100 mg % of sulfanilamide.

By the addition of .04 mg % Azochloramid the resistance of this

[2] McLeod, C. M., *J. Exp. Med.*, 1940, **72**, 307.

TABLE V.
Effect of Azochloramid and Sulfanilamide upon Sulfanilamide-fast *E. coli*. Inoculum 2,000,000 *E. coli*/ml.

Sulfanilamide mg %	Azochloramid mg %	Turbidity 15 hr
0	0	49
100	0	45
25	0	47.5
0	.04	44.0
5	.04	0.5

culture was destroyed and it was again rendered sensitive to 5 mg % sulfanilamide as is shown in Table V.

Comment. In order to increase the accuracy of these experiments low concentrations of these agents were studied. The time of exposure is thereby lengthened and inaccuracies due to the time factor are eliminated. In order to produce these effects with short contact periods as would be desirable for clinical application appropriately increased concentrations of the agents are required.

Summary. These data indicate that Azochloramid potentiates the bactericidal effect of sulfonamides. This effect may be due to the inactivation of sulfonamide inhibitors by the chlorine compound.

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Measurement of Mean Blood Flow in Arteries and Veins by Means of the Rotameter.*

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In experiments designed to determine the accuracy of the thermostromuhr,[1, 2] the need arose for a simple and accurate method by means of which blood flow in arteries and veins of anesthetized dogs could be immediately quantitated from moment to moment for a considerable time period. For this purpose the rotameter[3] seemed

* The expenses of this investigation were defrayed by a grant from the Commonwealth Fund.

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1 Gregg, D. E., Pritchard, W. H., Eckstein, R. W., Shipley, R. E., Steege, T. W., and Wearn, J. T., *Am. J. Physiol.*, in press.

2 Shipley, R. E., Gregg, D. E., and Wearn, J. T., *Am. J. Physiol.*, in press.

3 Schoenborn, E. M., and Colburn, A. P., *Transactions of the American Institute of Chemical Engineers*, 1939, **35**, 359.