TABLE I.
Bactericidal Combinations of Penicillin and Streptomycin for Various Pure Cultures.

Organism	% M.L.C.* Streptomycin	% M.L.C.* Penicillin	Sum
Hemolytic streptococcus C-203 MV	5.6	50.7	56.3
	10.0	25.3	35.3
	14.3	23.0	37.3
	28.7	15.3	44.0
	43.4	5.0	48.4
	56.5	2.5	59.0
Staphylococcus aureus No. 209	11.0	76.0	87.0
	13.0	61.0	74.0
	14.0	50.0	64.0
	28.0	38.0	66.0
	43.0	25.0	68.0
	56.0	12.0	68.0
Staphylococcus aureus Oxford H	15.3	50.0	65.3
	20.0	33.0	53.0
	25.3	16.0	41.3
	50.7	10.0	60.7
Staphylococcus aureus	10.0	76.1	86.1
	15.3	50.7	66.0
	25.3	20.0	45.3
	76.1	10.0	86.1
Staphylococcus aureus SM	10.0	76.1	86.1
	15.3	25.3	40.6
	25.3	20.0	45.0
	50.7	15.3	66.0
Staphylococcus aureus SM	10.0	50.7	60.7
	15.3	25.3	40.6
	25.3	20.0	45.3
	50.7	15.3	66.0
	76.1	10.0	86.1

* M.L.C. Minimum Lethal Concentration of each antibiotic alone for the particular organism determined to within 25% (*i.e.*, the organisms are known to grow at 75% M.L.C. or higher.)

Summary. Under the experimental conditions used, definite synergism was noted in the bactericidal action of penicillin and streptomycin upon staphylococci and streptococci *in vitro*.

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A Complement Fixation Test for Dengue.*

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Studies carried out by one of us (A. B. S.) since 1944 have established the fact that several immunologically distinct but related types of virus are responsible for the clinically typical and atypical forms of dengue.[1] Since the adaptation of dengue virus to mice,[2] Sabin and Schlesinger found that the neutralizing antibodies which developed following infection were so highly type-specific, that by means of the neutralization test[3] in mice one

could not diagnose infections caused by heterologous immunological types of virus which have not as yet been propagated successfully in mice. Continuous serial, intracerebral passage in mice has increased the titer of our Hawaii strain of virus from 10^{-1} to about 10^{-5} or 10^{-6}, and following intracerebral passage in 3- to 4-day-old mice (a procedure suggested by Dr. Gordon Meiklejohn) titers of 10^{-7} to 10^{-8} could be achieved. Satisfactory complement-fixing (C-F) antigens could be prepared from the brains of mice which were either 3 to 7 days old or approximately 14 days old at the time of inoculation with virus of highest titer (0.03×10^{-7}-0.03×10^{-8}) which could be obtained only from the younger suckling mice. Comparative tests on aliquot portions of 20% aqueous extracts of the infected mouse brains revealed that antigen prepared by benzene extraction of the lyophilized material[4,5] was no more potent as regards the number of antigenic units or the titer obtained with dengue antisera than that prepared only by centrifugation at 13,000 r.p.m. in the cold (Type SS-1 Swedish angle centrifuge—Sorvall), and both were equally effective in increasing the titer of complement. The 20% mouse brain suspension prepared by inoculation of 3- to 7-day-old mice contained 16 to 32 units of antigen per 0.25 ml as compared with 4 units (with two exceptions when it was only 1 unit and one when it was 8 units) for the similar suspension prepared in a similar manner from approximately 14-day-old mice. Almost all the work to be reported was carried out with benzene extracted antigens (containing 4 units), which, however, also had to be centrifuged at 13,000 r.p.m. for 1 hour to remove all the anticomplementary properties. The tests were set up in the usual manner, using 2 exact units of complement, and incubation for 16 to 20 hours in a refrigerator at about 5°C. The titers represent the highest original dilution of a serum, *i.e.*, before addition to the mixture, which yielded at least 2-plus fixation. Similarly prepared extracts from normal mouse brains were used as controls in all the tests.

Results. (1) Human volunteers, rhesus monkeys, and chimpanzees[6] inoculated with the homologous Hawaii strain of the *human* virus, or with immunologically identical *human* strains, developed C-F antibodies which reached peak titers of 1:64 to 1:256 within 2 to 6 weeks. These antibodies have been found to persist in high titer for many months, and in human volunteers, residing in dengue-free regions of the U. S. A., titers of 1:4 to 1:128 have been found 38 months after infection, and titers of 1:4 to 1:16 as long as 52 months after infection, the longest period tested.

(2) Human volunteers inoculated with immunologically distinct strains of *human* dengue virus, who developed little or no neutralizing antibodies for the Hawaii virus,[1] nevertheless, developed C-F antibodies in titers no higher than 1:16; however, 6 months after inoculation these antibodies were not demonstrable in serum dilutions of 1:4 in some of the individuals tested.

(3) Rhesus monkeys inoculated with immunologically distinct strains of *human* dengue virus, which developed little or no neutralizing antibodies for the Hawaii virus, nevertheless developed C-F antibodies in titers as high as 1:512; however, the fixation was not complete (*i.e.*, only 2- to 3-plus) even in the lowest dilutions of such sera, suggesting that only about 1 unit of the serologically related component was present in the antigens used. Furthermore, in some of the monkeys tested,

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1 Sabin, A. B., in Viral and Rickettsial Infections of Man, edited by T. M. Rivers, J. B. Lippincott Co., Philadelphia, 1948.

2 Sabin, A. B., and Schlesinger, R. W., *Science*, 1945, **101**, 640.

3 Sabin, A. B., Diagnostic Procedures for Virus and Rickettsial Diseases, Am. Pub. Health Assn., 1948, 289-293.

4 DeBoer, C. J., and Cox, H. R., *J. Immunol.*, 1947, **55**, 193.

5 Espana, C., and Hammon, W. McD., *J. Immunol.*, 1948, **59**, 31.

6 Paul, J. R., Melnick, J. L., and Sabin, A. B., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 193.

thus far, these antibodies disappeared as early as 6 to 10 weeks after inoculation.

(4) Rhesus monkeys inoculated intracerebrally with the homologous *mouse-adapted* Hawaii virus, developed C-F antibodies in titers as low as 1:16 which in some monkeys disappeared as early as 6 weeks after inoculation, although neutralizing antibodies in high titer persisted for many months.

(5) Human volunteers inoculated with dengue vaccine, consisting of the living mouse-adapted Hawaii virus, who developed neutralizing antibodies and resisted infection with the unmodified, human virus[1,7] *failed* to develop C-F antibodies.

(6) Sera obtained from individuals 2 years after a natural attack of dengue in Hawaii or Japan, which neutralized the Hawaii virus in high titer, also fixed complement in titers of 1:16 to 1:64. Positive results also have been obtained with sera of individuals who had dengue in Singapore, Java, and New Guinea.

(7) Sera collected from American marines, 11 months after a single, primary natural attack of dengue on Guam, gave positive C-F tests in titers of 1:2 to 1:8 with the Hawaii antigen, although they had no significant neutralizing antibodies[7] for this virus.‡

(8) Human volunteers infected with phlebotomus (pappataci, sandfly) fever virus developed no C-F antibodies for the dengue antigen.

(9) Rhesus monkeys which had no C-F antibodies for dengue 7 weeks after inoculation with the French neurotropic yellow fever virus, developed such antibodies in titers of 1:4 to 1:32, 8 to 10 days after a large booster dose of either the Asibi viscerotropic or French neurotropic strains of yellow fever virus.§ These results indicate an antigenic, if not an immunogenic,[1] relationship between the viruses of yellow fever and dengue.

[7] Sabin, A. B., and Schlesinger, R. W., unpublished studies.

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§ We are indebted to Dr. Max Theiler, of the International Health Division of the Rockefeller Foundation, for the sera from these monkeys.

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Influence of Choline, Cystine and Methionine on Toxic Effects of Pyridine and Certain Related Compounds.

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In a study of the pharmacological effects produced by the ingestion of various pyridine derivatives by the rat[1,2] pyridine and coramine (nikethamide) were found to be the most active from the standpoint of the production of marked changes in liver size and composition. Coramine produced a great increase in liver weight without causing very much damage to the liver or modifying its composition. Pyridine has proved to be somewhat more toxic than many of its derivatives[1,3] and it must be included in the list of substances known to produce severe liver and kidney damage.[4,5] Methionine and cystine have been shown to have some liver protective

[1] Coulson, R. A., and Brazda, F. G., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 1.

[2] Brazda, F. G., and Coulson, R. A., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 37.

[3] Brazda, F. G., and Coulson, R. A., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 19.

[4] Baxter, J. H., *Proc. Am. Fed. Clin. Research*, 1945, **2**, 77.

[5] Baxter, J. H., *Am. J. Path.*, 1948, **24**, 503.