amines including *p*-aminobenzoic acid, *i.e.*, by the same compounds that give color in the method of Bratton and Marshall.[4] Urea in pathological concentrations yields significant amounts of color with Ehrlich's reagent. Pyrrols and indoles, however, do not react with the reagent at the acidity attained in the test. Moderate hemolysis in the serum seems not to interfere seriously with color matching. The chief bars to accuracy appear at the present time to be (a) the possibility that the test papers or the standards may prove to be unstable under certain conditions of storage or exposure and (b) the fact that since the intensity of the color is influenced by protein and the test is empirically standardized for normal protein content of serum, alterations of the proteins in pathological sera might lead to inaccuracies. The first difficulty can be removed in part by keeping test papers in a cool place away from direct light and moisture. The other limitations as yet have not been adequately explored. Results of attempts to replace the phosphoric acid by oxalic or citric acids in the preparation of test papers indicate that modification in this direction is possible.

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The Chemotherapeutic Effect of Esters of Penicillin.*

KARL MEYER, GLADYS L. HOBBY, AND MARTIN H. DAWSON.
From the Departments of Ophthalmology and Medicine, College of Physicians and Surgeons, Columbia University, The Institute of Ophthalmology and the Edward Daniels Faulkner Arthritis Clinic, Presbyterian Hospital, New York.

In a recent communication[1] the preparation of esters of penicillin was reported. Although aliphatic esters show no bacteriostatic action *in vitro*, it was shown that they are highly effective chemotherapeutic agents in experimental infections due to hemolytic streptococci. Evidence was presented to show that they are effective *in vivo* both by the subcutaneous and by the oral route of administration. This activity is undoubtedly due to the hydrolysis of the esters with a slow liberation of active penicillin.

In this paper experiments will be described which confirm and extend the previous observations.

Materials and Methods. The penicillin employed throughout the study was prepared by the chloroform method.[2] The free acid was esterified with diazoethane or diazo-n-butane prepared from the corresponding derivatives of nitroso-urea. Remaining acidic fractions were separated from the ester by alkaline phosphate extraction.† Unless otherwise specified, the esters were dissolved in absolute alcohol before use and the solutions diluted with four volumes of propylene glycol.

In all experiments 15-hour blood broth cultures of a highly virulent strain of Group A hemolytic streptococci (strain C203Mv) were used. Mice were infected by the intraperitoneal route with 1 cc of varying dilutions of culture. Treatment was carried out by either the subcutaneous or the oral route of administration. All experiments were controlled with a series of untreated animals.

Subcutaneous Administration. In the first experiments mice infected with 1 cc of 10^{-3}, 10^{-4}, and 10^{-5} dilutions of hemolytic streptococci were treated with ethyl esters of peni-

* This work has been supported in part by a grant from the John and Mary R. Markle Foundation.

1 Meyer, K., Hobby, G. L., and Chaffee, E., *Science*, 1943, **97**, 205.

2 Meyer, K., *et al.*, *Science*, 1942, **96**, 20.

† Oily fractions removed from both the ethyl and butyl esters contained ethers and addition products of the diazo compounds, probably pyrazoline derivatives. These oily fractions were only weakly active *in vivo* and were locally irritating.

TABLE I.
Subcutaneous Administration of Ethyl Esters of Penicillin in Divided Dosage.

Total amt of ester (in mg)	Dilution of culture (Strain C203Mv)	No. of mice	No. died (<48 hr)	No. survived (>7 days)
1.4-4.5	10^{-3}	14		14
	10^{-4}	15	1	14
	10^{-5}	15	1	14
Total		44	2 (4.5%)	42 (95.5%)
0.6	10^{-3}	3	2	1
	10^{-4}	2	1	1
	10^{-5}	3	1	2
Total		8	4 (50%)	4 (50%)
Controls	10^{-7}	12	12 (100%)	

TABLE II.
Subcutaneous Administration of Ethyl Esters of Penicillin in Single Dosage.

Total amt of ester (in mg)	Dilution of culture (Strain C203Mv)	No. of mice	No. died (<48 hr)	No. survived (>7 days)
2.0	10^{-4}	3		3
	10^{-5}	3		3
	10^{-6}	3		3
	10^{-7}	3		3
Total		12		12 (100%)
Controls	10^{-7}	10	10 (100%)	

cillin given by the subcutaneous route. Treatment was started one-half hour after infection. Two to 4 injections of 0.1-1.0 mg were given on the first day and one to 2 injections of the same amount on the second day.

As shown in Table I, amounts equal to 1.5 mg of ethyl ester or more were adequate to protect 42 of 44 mice against 10^{-3}, 10^{-4}, and 10^{-5} dilutions of hemolytic streptococci (approximately 100-10,000 lethal doses). With 0.6 mg there was little protection.

In a subsequent experiment a small series of mice similarly infected with hemolytic streptococci were treated with a single subcutaneous injection of 2 mg of ethyl ester given one-half hour after infection.

As shown in Table II, this single injection was adequate to give complete protection against 10^{-4} dilutions of hemolytic streptococci (1000 lethal doses).

The chemotherapeutic effect of n-butyl esters was studied in a larger series of animals. Both unfractionated and more highly purified fractionated preparations of the ester were tested. Varying amounts of the unfractionated and fractionated butyl esters, ranging from 0.5 to 2.5 mg, were given in a single subcutaneous injection one-half hour after infection.

As shown in Table III, 1.5 mg of unfractionated butyl ester was adequate to give complete protection against 10^{-4} to 10^{-6} dilutions of hemolytic streptococci. With 0.5 mg only slight protection was obtained.

When more highly purified fractionated n-butyl esters were used, as shown in Table IV, 0.4 mg was adequate for prolonged life or complete protection of 100% of infected mice. With 0.2 mg there was only partial protection.

Oral Administration. Mice infected with hemolytic streptococci were also treated by the oral route of administration.

Table V shows a small series of which 13 of 16 mice were protected against 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} dilutions of hemolytic streptococci by a single dose of 5.0 mg of ethyl ester given by mouth. The remaining 3 mice showed prolonged life. With smaller amounts of ethyl ester, there was no protection.

Similar experiments were carried out in

TABLE III.
Subcutaneous Administration of Unfractionated n-Butyl Esters of Penicillin in Single Dose.

Total amt of ester (in mg)	Dilution of culture (Strain C203Mv)	No. of mice	No. died (<48 hr)	No. prolonged life (2-7 days)	No. survived (>7 days)
2.5	10^{-4}	3			3
	10^{-5}	3			3
	10^{-6}	3			3
1.5	10^{-4}	6			6
	10^{-5}	6			6
	10^{-6}	6			6
Total		27			27 (100%)
0.5	10^{-4}	7	7		
	10^{-5}	7		1	6
	10^{-6}	7	2	2	3
Total		21	9 (42.9%)	3 (14.2%)	9 (42.9%)
Controls	10^{-7}	9	9 (100%)		

TABLE IV.
Subcutaneous Administration of Fractionated n-Butyl Esters of Penicillin in Single Dosage.

Total amt of ester (in mg)	Dilution of culture (Strain C203Mv)	No. of mice	No. died (<48 hr)	No. prolonged life (2-7 days)	No. survived (>7 days)
0.5-1.5	10^{-4}	15		1	14
	10^{-5}	15			15
	10^{-6}	14		4	10
0.4	10^{-4}	4		1	3
	10^{-5}	4			4
	10^{-6}	3			3
Total		55		6 (10.9%)	49 (89.1%)
0.2	10^{-4}	4	2		2
	10^{-5}	4	1	1	2
	10^{-6}	4	1		3
Total		12	4 (33.3%)	1 (8.3%)	7 (58.4%)
Controls	10^{-6}	12	12		
	10^{-7}	12	12		
Total		24	24 (100%)		

TABLE V.
Oral Administration of Ethyl Esters of Penicillin in Single Dosage.

Total amt of ester (in mg)	Dilution of culture (Strain C203Mv)	No. of mice	No. died (<48 hr)	No. prolonged life (2-7 days)	No. survived (>7 days)
5.0	10^{-4}	2			2
	10^{-5}	4			4
	10^{-6}	7		3	4
	10^{-7}	3			3
Total		16		3 (19%)	13 (81%)
Controls	10^{-7}	10	10 (100%)		

TABLE VI.
Oral Administration of Fractionated n-Butyl Esters of Penicillin in Single Dosage.

Total amt of ester (in mg)	Dilution of culture (Strain C203Mv)	No. of mice	No. died (<48 hr)	No. prolonged life (2-7 days)	No. survived (>7 days)
4.0	10^{-4}	9	3	1	5
	10^{-5}	5			5
	10^{-6}	5			5
Total		19	3 (15.9%)	1 (5.2%)	15 (78.9%)
2.0-3.0	10^{-4}	10	2	3	5
	10^{-5}	10	3		7
	10^{-6}	10	3	3	4
Total		30	8 (26.7%)	6 (20%)	16 (53.3%)
1.0	10^{-4}	5	5		
	10^{-5}	5	2	2	1
	10^{-6}	5	2		3
Total		15	9 (60%)	2 (13.3%)	4 (26.7%)
Controls	10^{-6}	10	10		
	10^{-7}	10	10		
Total		20	20 (100%)		

which fractionated n-butyl ester was used. A single dose of varying amounts of ester was given by mouth one-half hour after infection.

As shown in Table VI, 15 of 19 mice were protected against 10^{-4}, 10^{-5}, and 10^{-6} dilutions of hemolytic streptococci by 4.0 mg of fractionated n-butyl ester. With 2.0 to 3.0 mg protection was less regular, whereas with 1.0 mg there was only slight protection.

The administration of esters by divided dosage does not affect the total amount necessary for protection. When a strain which kills within 48 hours (as C203Mv) is used, the entire therapeutic dose must be given within the first five to six hours after infection.

Similar experiments have been carried out with a type II strain of pneumococci (D/39). It was found that these esters are also effective against this organism.

Toxicity. Experiments were carried out in white mice to determine the toxicity of ethyl and n-butyl esters. Varying amounts of preparations of ethyl ester and n-butyl ester were injected into normal white mice by the subcutaneous and by the oral route. Both esters, when given in sufficient amount, produced in mice a narcotic effect resulting either in gradual recovery or in death after 2 to 30 hours. Different preparations of ester showed slight variations in the degree of toxicity. It was found that by either route of administration the LD_{50} of the ethyl ester of penicillin is about 6-7 mg per 18 g mouse, whereas the LD_{50} of the n-butyl ester ranges from 8 to 10 mg. When divided dosage is used over a period of 18 to 20 hours, the LD_{50} remains unchanged. Apparently there is a cumulative effect.

Rabbits, weighing 6-7 lbs each, have been injected by the subcutaneous and intravenous routes with alcoholic solutions containing amounts up to 100 mg of n-butyl ester with no untoward results.

Discussion. The use of penicillin has been seriously hampered by its rapid excretion and by the consequent necessity for frequent administration in order to maintain an adequate blood level. In a previous communication,[3] experiments were described in which a slow release of penicillin could be obtained in the body by the subcutaneous administration of penicillin suspended in oil or in the form of dry pellets. These methods, however, have

[3] Hobby, G. L., Meyer, K., and Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 285.

not been suitable for use in human therapy.

The preparation of esters of penicillin and the demonstration of their chemotherapeutic activity introduces a group of substances which can be changed from an inactive to an active form by hydrolysis within the animal body. The hydrolysis of these esters with the liberation of active penicillin takes place slowly in the body. A single injection is therefore adequate to give complete protection against hemolytic streptococcal infections in mice.

The ethyl and n-butyl esters of penicillin appear to be stable. In contrast to penicillin, they are not destroyed at the pH of the stomach and are highly active when administered by mouth. Absorption from the stomach is less regular, however, and the therapeutic dose is approximately ten times greater by the oral route than by the subcutaneous.

The toxicity of the ethyl and n-butyl esters of penicillin is 2 to 3 times that of penicillin. However, when the subcutaneous route of administration is used, the toxic range is far above the therapeutic range. When the oral route is used, the therapeutic dose more closely approaches the toxic level. In the case of the n-butyl ester the range is sufficiently wide, however, for satisfactory use of the substance.

Conclusions. Although inactive *in vitro*, ethyl and n-butyl esters of penicillin have been shown to be highly effective chemotherapeutic agents in mice infected with large doses of virulent hemolytic streptococci. These esters are apparently hydrolyzed slowly with the liberation of active penicillin. They are effective when given by the subcutaneous or by the oral route of administration.

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Antigenically Different Strains of Virus from a Localized Influenza Outbreak.

THOMAS P. MAGILL AND JOHN Y. SUGG.

From the Department of Bacteriology and Immunology, Cornell University Medical College, New York City.

The purpose of this paper is to present additional evidence that antigenically different, although related, strains of virus may be prevalent during the same outbreak of influenza. It previously has been observed that cases of influenza that occur during the same large epidemic are not all caused by antigenically identical agents.[1] The present data confirm that previous observation, and are of especial interest because they deal with an outbreak of influenza that was a particularly well localized one.

The influenza outbreak with which the investigation was concerned and which consisted of 40 clinically diagnosed cases of epidemic influenza, was observed in the nurses' infirmary of The New York Hospital during January and February, 1941. Throat washings obtained from 11 of the persons were tested for virus, and in 5 instances strains of influenza virus were isolated and adapted to Swiss mice. The 5 strains were compared with each other and with the PR8[2] by means of mouse protection tests in which each strain of virus was tested against the same group of antiserums. The antiserums were obtained from 6 rabbits each of which had been inoculated with a different strain of virus: bleedings were obtained from each rabbit 9 or 10 days and 3 to 4 weeks after a single intraperitoneal injection of a suspension containing 2% infected mouse lung in 0.85% saline. The virus suspensions used in the different protection tests were all comparable in that they contained the same magnitude of lethal doses of virus (from 300 to 600) for mice. The results of the tests are presented in Chart 1.

[1] Magill, T. P., and Francis, T., Jr., *Brit. J. Exp. Path.*, 1938, **19**, 273.

[2] Francis, T., Jr., *Science*, 1934, **80**, 457.