subjects who were given a 1 cc injection, subcutaneously, of the calcium phosphate-virus suspension containing the virus adsorbed from 1 cc of formalinized allantoic fluid containing Type A virus and 1 cc of fluid containing Type B virus. The sites of inoculation were examined over a period of 18 days. The reactions observed were similar to those seen in 5 other subjects who had received a corresponding dose of allantoic fluid suspension of both viruses. The sharp stinging pain that followed the injection of the formalinized allantoic fluid did not occur after the injection of the adsorbed material which was free of formaldehyde.

The stability of the hemagglutinating capacity of the formalin-inactivated virus adsorbed on calcium phosphate and of the original formalinized allantoic fluid suspension of virus, stored at 4° C., room-temperature and 37° C., has been tested over a period of 4 months. The results are shown in Table 2. Whether

TABLE 2

STABILITY OF HEMAGGLUTINATING CAPACITY OF CALCIUM PHOSPHATE—ADSORBED AND UNADSORBED FORMALINIZED INFLUENZA VIRUS, TYPE A (PR8), AFTER STORAGE FOR 4 MONTHS AT DIFFERENT TEMPERATURES

Temperature	Preparations	Final dilutions								
		40	80	160	320	640	1280	2560	10,240 5120	
4° C.	*Adsorbed	+	+	+	+	+	+	+	±	0
	*Unadsorbed	±	+	+	+	+	+	+	±	0
Room temperature	Adsorbed	+	+	+	+	+	+	+	±	0
	Unadsorbed	+	+	±	0	0	0	0	0	0
37° C.	Adsorbed	+	+	+	+	+	+	0	0	0
	Unadsorbed	0	0	0	0	0	0	0	0	0

* Titers after 4 months at 4° C. were the same as at the start of the experiment.
Symbols: + = complete agglutination;
± = partial or slight agglutination;
0 = no agglutination.

the greater stability of the adsorbed-virus is due to removal from prolonged contact with formaldehyde or to some protective effect of adsorption will be determined.

Further studies are in progress to determine the mechanism of the adjuvant effect and the quantitative relationship between dosage and antigenic activity of free and adsorbed virus as measured by antibody response and immunity to infection. A more detailed report of these studies in other hosts as well as mice will be made.

JONAS E. SALK

PROLONGATION OF PENICILLIN ACTIVITY BY MEANS OF ADRENALIN[1]

THE rapid absorption and excretion of penicillin following its injection by various routes is well known, and interest has been shown in methods to prolong effective blood concentration levels of this antibiotic. Thus the use of penicillin in beeswax-peanut oil mixtures[2] and the application of ice bag chilling at the site of injection[3] have been reported as procedures which prolong penicillin absorption. It occurred to

AVERAGE BLOOD CONCENTRATION LEVELS OBTAINED IN RABBITS FOLLOWING THE INJECTION OF AQUEOUS AND ADRENALIN SOLUTIONS OF CALCIUM PENICILLIN

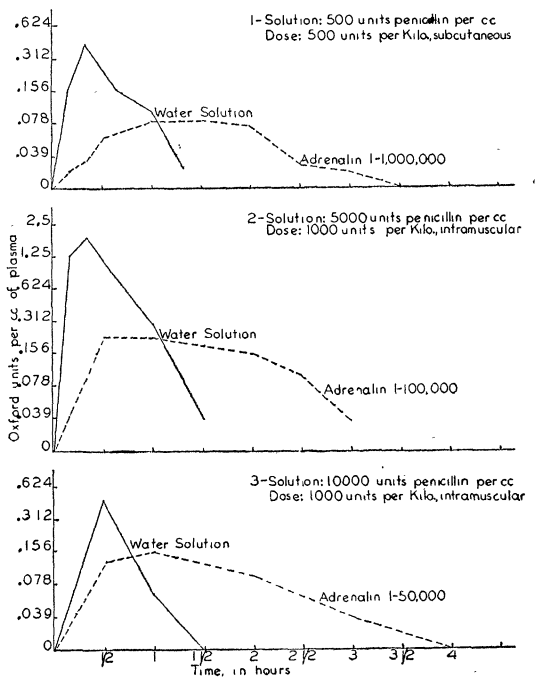

FIG. 1. (1) Each curve represents an average of values obtained from 10 determinations made on 5 rabbits. (2) Each curve represents an average of values obtained from 3 determinations made on 3 rabbits. (3) Each curve represents an average of values obtained from 5 determinations made on 5 rabbits.

the authors that adrenalin, because of its vasoconstricting properties, might afford a practical method for accomplishing this effect. The behavior of adrenalin is well established and the injection of this substance is not attended by the objectionable local tissue reactions[4] sometimes resulting from the use of vehicles such as vegetable oils which are being employed to hinder the absorption of certain therapeutic agents.

Tests were run by *in vitro* methods to determine

[1] From the Collis P. and Howard Huntington Memorial Hospital, Pasadena, and the Departments of Bacteriology and Pathology, School of Medicine, University of Southern California, Los Angeles, California. Valuable technical assistance was provided by Patrice Morrow.

[2] M. J. Romansky and G. E. Rittman, SCIENCE, 100: 196–198, 1944.

[3] M. Trumper and A. M. Hutter, SCIENCE, 100: 432–434, 1944.

[4] R. C. Page and E. J. DeBeer, *Am. Jour. Med. Sciences*, 205: 812–814, 1943.

the stability of penicillin in adrenalin solutions at different temperatures and animal experiments were carried out to learn the effect of adrenalin on the rate of penicillin absorption as measured by blood level concentrations obtained following subcutaneous or intramuscular injections.

Calcium penicillin which assayed at 500 Oxford units per mg and which was prepared from surface fermentation cultures of *Penicillium notatum* was used in all animal experiments. Solutions containing 500 units of calcium penicillin per cc and adrenalin in concentrations not exceeding 1–16,000 showed no loss of activity, compared with aqueous control solutions, when kept for 24 hours at 37° C, 5° C or at room temperature. The assays for penicillin stability were run by the cylinder plate method using *Bacillus subtilis* as the test organism.

Animal experiments were run with rabbits which were injected alternately with experimental preparations and aqueous control solutions. Injections were made subcutaneously or intramuscularly and blood samples collected from the ear veins. Penicillin assays were run on plasma by the broth dilution method of titration using a culture of *Streptococcus pyogenes* as the test organism.

The injection of aqueous solutions of penicillin resulted in blood level concentrations which showed maximum bacteriostasis after about 20 minutes with little or no activity remaining in plasma collected after 1 or 1½ hours. The addition of adrenalin to penicillin solutions produced a significant change in the blood level concentrations obtained in all experiments. As may be seen in Fig. 1, the effect of adrenalin was to flatten the blood activity curves so that compared with control tests, lesser concentrations of penicillin were found in early bleedings and a prolongation of activity was observed in samples collected from 1½ to 3 hours after injection. This occurred when the material was administered by either the subcutaneous or the intramuscular method. Small amounts of adrenalin added to aqueous solutions of penicillin usually doubled the time during which a bacteriostatic concentration could be detected in blood samples.

The outcome of these experiments suggested the clinical use of adrenalin in penicillin therapy and preliminary trials indicate that humans may be expected to respond in a manner similar to the experimental animals employed. The effect of adrenalin was tested in 7 subjects[5] injected by the intramuscular route with freshly prepared solutions of 50,000 units of sodium penicillin dissolved in 4 cc of 1–50,000 adrenalin and in 2 patients who received 20,000 units contained in 2 cc of 1–25,000 adrenalin. Six control subjects were injected by the same method with saline solutions of penicillin and blood level titrations were run by a method similar to that of Rammelkamp.[6] The prolongation of bacteriostatic serum levels produced by adrenalin, which may be seen from the results summarized in Table 1, was sufficiently great to indicate further investigation with this vasoconstrictor. Until the most efficient adrenalin and penicillin mixtures can be worked out, it would appear from observations made in the present study that the use of small amounts of adrenalin in conjunction with penicillin may find immediate application in the usual method of administering this antibiotic by repeated intramuscular injections.

TABLE 1

BLOOD LEVEL CONCENTRATIONS OF PENICILLIN FOLLOWING THE INTRAMUSCULAR INJECTION OF ADRENALIN SOLUTIONS IN HUMAN SUBJECTS

Subject	Treatment	Hours after injection							
		½	1	1½	2	2½	3	3½	4
1	50,000 units sodium penicillin in 4 cc of saline	.624		.039		0		0	
2		.312		.039		0		0	
3		.624	.624		.078		0		0
4		1.25	.624		.039	0	0		0
5		1.25	.624		.039	0	0		0
6	50,000 units sodium penicillin in 4 cc of saline plus .08 cc of 1–1000 adrenalin	.156		.156		.039		0	
7		.624		.312		.039		0	
8			.624		.156		0		0
9		.312	.624		.624		.312		
10		1.25		.312		.078		0	
11		.624	.624		.156	.039	0		0
12		.312	.624		.156	.039	.039		0
13	20.000 units, 2 cc saline	.624	.156		0		0		0
14	20,000 units as above plus .08 cc of 1–1000 adrenalin	.039	.078		.039		0		0
15		.078	.078	.039	.039	.015	0		

ROY T. FISK
ALVIN G. FOORD
GORDON ALLES

[5] The penicillin blood concentration studies on human subjects were made possible through the generous cooperation of Doctors Herbert Cowper and Harold Mazur of the Los Angeles City Health Department and Mr. Charles Arthur of the Pasadena City Health Department.

[6] C. H. Rammelkamp, *Proc. Soc. Exp. Biol. and Med.*, 51: 95–97, 1942.