ations on 3 normal plasmas and several pathological plasmas, using 15 determinations of each, gave a standard error of ± 1.9 sec. When clotting times of undiluted plasmas were longer than 60″, severe fibrinogenopenia was present. Such results are indicated as "no clot." Results have been checked against those with the standard "tyrosine method." Good correlation has been found in patients with normal, elevated or decreased plasma fibrinogen level and in severe hypofibrinogenemia (Table I).

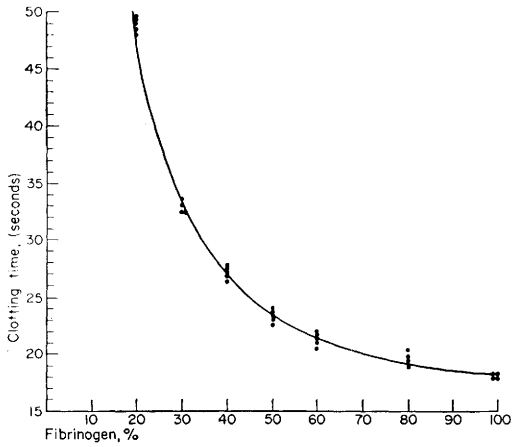

FIG. 1. Correlation of clotting times to plasma fibrinogen percentage values.*

* Clotting times are reported in sec. on ordinate; percentage levels of fibrinogen are reported on abscissa. Thus, clotting time values are readily translated into plasma fibrinogen percentages. The 5 points represent 5 actual experiments using the same system, with plasma pool where fibrinogen content was of 300 mg % by the tyrosine method. Continuous line is avg of the 5 values. As the normal plasma was originally diluted 1:5, the method can be used to study fibrinogen levels from 12 to 1200 mg %. No clot was obtained when plasma was diluted 1:25 or more.

TABLE I. Correlation of Present Method to Tyrosine Method.

No. of cases	Diagnosis	Tyrosine method, mg % (Avg values)	Present method, mg %* (Avg values)
1	Carcinoma of lung	430	388
2	Pulmonary infarction	380	360
2	Arterioselerosis	400	335
5	Myocardial infarction	292	305
3	Congestive heart failure	272	240
10	Mitral stenosis	368	332
2	Subacute bacterial endocarditis	285	302
2	Rheumatic heart disease	257	267
2	Intestinal obstruction	317	320
1	Fistula in ano	340	464
1	Perianal abscess	365	388
1	Infectious hepatitis	202	235
1	Cirrhosis of liver	100	104
3	Diabetes mellitus	224	220
1	Carcinoma of pancreas	214	168
2	Acute thrombophlebitis	330	360
1	Posthemorrhagic anemia	225	180
2	Fractures	425	447

* Values in mg % are calculated from curve of Fig. 1 where 100% value represented 300 mg % by the tyrosine method. Values were multiplied by 2 or 4 when plasma was diluted 1/10 or 1/20; or divided by 2 or 5 when plasma was diluted 1/2.5 or used undiluted.

Summary. A method is presented for rapid and, under certain conditions, accurate determination of plasma fibrinogen level. The method correlates well with standard tyrosine technic. This method should find its greatest use in rapid determination of plasma fibrinogen level in hemorrhagic catastrophes and especially in hemorrhagic accidents of pregnancy.

Received September 5, 1959. P.S.E.B.M., 1960, v103.

Experimental Penicillin Prophylaxis of Staphylococcal Infection in Rabbits. (25428)

JAMES K. WEAVER* AND GARDNER MIDDLEBROOK
Dept. of Research and Laboratories, National Jewish Hospital, and University of Colorado School of Medicine, Denver

The problem of *Staphylococcus aureus* infections in clean surgical wounds is receiving widespread attention. One method to control this is administration of penicillin. It is currently believed that, in addition to its undesirable side-effects, penicillin is of little or no

* Resident in Orthopedic Surgery, Univ. of Colorado Med. Center.

value for preventing penicillin-resistant staphylococcal infections in clean surgical wounds. To clarify this problem, evaluation of prophylactic penicillin administration in rabbits infected with "penicillin-resistant" staphylococci was undertaken.

Materials and methods. A. *Test organism.* 1. *Characteristics.* A strain of *Staphylococcus aureus* was obtained from the nasopharynx of a ward nurse. It was hemolytic, mannitol fermenting, coagulase positive, and of phage type 80/81. This strain was highly resistant to penicillin by routine disc-drug-susceptibility tests. *In vitro* penicillin resistance also was established by serial tube dilution technics using nutrient broth and reading after 24 hours incubation. With large inocula (5×10^5 to 5×10^7 viable bacterial units/ml) this particular strain was resistant to 250-500 penicillin units/ml. With small inocula (5×10^2 to 5×10^3 viable bacterial units/ml), the strain proved susceptible to 1 penicillin unit/ml, but not susceptible to concentration of 0.5 penicillin unit/ml. This phenomenon of increase in *in vitro* penicillin-susceptibility as size of inoculum decreases is characteristic of penicillinase-producing, resistant staphylococci(1). 2. Staphylococci were prepared for inoculation into wounds in 2 ways: a. *No penicillin* (*np*). Five ml were taken from a heavy 4 to 6 hour culture and grown 2 hours in nutrient broth to which was added 2% glucose. The preparation was aerated during growth in roller tubes turning at 16 rpm. b. The preparation was the same except that 100 units of crystalline penicillin G (Squibb)/ml were added at beginning of 2 hour growth period and additional 100 penicillin (p) units/ml were added after first hour of growth. Both suspensions were then diluted to standard nephelometric density of 20 units (Coleman Nephelo-colorimeter), from this standard, 10^{-4} dilutions in saline were made for inoculation into wounds. 3. *Estimation of inoculum size.* Dose of organisms in each wound was estimated as follows: A sample of suture material identical to that used to close the wound was soaked in the suspension of staphylococci for 30 minutes, then transferred to sterile nutrient broth and allowed to soak for 2 hours. All remnants of the suture used to close the wound were soaked in similar fashion. Aliquots of each broth specimen were plated on nutrient agar and colony counts obtained. Some multiplication occurred during 2 hours of soaking. Difference in number of colonies from the silk prior to closure and the number obtained from suture remnants, after closure, was accepted as a rough approximation of size of wound inoculum. Values for the *np* inoculum were 600, 200 and 50 organisms, and for *p* inoculum 200, 150 and 0 organisms. It seems fair to assume that the dose was *less* than 1000 viable bacterial units in all instances. B. *Technic.* Clean surgical wounds, each approximately 3 cm in length and extending beneath the superficial muscular layer, were done in the backs of albino New Zealand rabbits of similar age and weight (1.5 - 2.0 kg). Infection was consistently produced by a technic similar to that used by Elek(2) in human subjects; namely by soaking silk suture in 10^{-4} dilution of standard suspension (1 to 5×10^4 viable units/ml) and using this suture to close the wound. Fifty cm of 00 non-capillary, braided, Ethicon silk was prepared for each wound closure. The suture was kept completely submerged in 10^{-4} diluted broth for 30 minutes at room temperature, and allowed to drip until free of adherent droplets, and placed immediately in the wound. A 2 layer closure was used with continuous silk in the fascia, and with a running subcuticular stitch to approximate the skin margins. Approximately 12 cm of total 50 cm were used in each wound. Animals were handled in groups of 4 and sites of placement of the differently prepared populations alternated. The type of population placed in each wound was recorded and the records kept in such a way that during postoperative period the observer was unaware of the pattern of wound inoculation. Two animals in each group were treated with procaine penicillin and 2 were not. The treated (*P*) animals received 10,000 units/kg intramuscularly twice daily for 4 days, including day prior to surgery, day of surgery and 2 days after surgery. Wounds were inspected daily. Onset of infection was determined by presence of abscess formation as detected by palpation for induration and fluctuance and by inspec-

tion for erythema. At end of 14 days animals were sacrificed and wounds and viscera inspected. Cultures of all infected wounds were taken at time of autopsy. The infected population was identified in each wound by culture and tests for mannitol fermentation and coagulase production. In selected cases (2 *np* and 2 *p* populations) the strain was further identified by its level of penicillin-susceptibility as determined by tube-dilution technic. To confirm that results obtained were not due to genetic change, the procedure was repeated using a population obtained from a *Pp* abscess in one animal in the initial group.

Results. There were 11 animals in the group which did not receive penicillin (*NP*) (Table I). Each of these animals had 4 wounds placed in its back. One wound was closed with a suture which had been soaked in a suspension of *np* staphylococci, and one wound was closed with suture which had been soaked in suspension of the *p* population. For each of these wounds a contralateral wound was created as a control and closed with sterile silk. There were no instances of infection in control wounds. All 11 wounds infected with *np* staphylococci became infected as did all 11 wounds infected with *p* organisms. These infections became apparent within 6 to 11 days. There was no significant difference in time of onset or clinical appearance of the wounds.

There were 11 animals in the *P* group (which received parenteral penicillin); one animal was eliminated because of wound dehiscence on first postoperative day. There were no infections in control wounds in these animals. In the 10 wounds inoculated with *np* populations there were only 2 infections. These 2 infections were produced in one experiment, and were very early in onset suggesting that the inoculum size may have been larger than in other experiments.

TABLE I. Penicillin Prophylaxis of Staphylococcal Infections.

Animals	Population* (np)	Population (p)
No penicillin prophylaxis (NP)	11/11†	11/11
Penicillin prophylaxis (P)	2/10	10/11

* Staphylococci (np) derived from population not previously exposed to penicillin, and (p) derived from population cultivated with penicillin.
† Wounds infected/wounds inoculated.

TABLE II. Penicillin Prophylaxis of Staphylococcal Infections. Population obtained from a *Pp* abscess, then prepared for inoculation in the same manner as the population in Table I.

Animals	Population* (np)	Population (p)
No penicillin prophylaxis (NP)	7/7†	6/7
Penicillin prophylaxis (P)	2/7	6/7

* Staphylococci (np) derived from population not exposed to penicillin after animal passage, and (p) cultivated in presence of penicillin after animal passage.
† Wounds infected/wounds inoculated.

In animals given penicillin (*P*) wounds which were inoculated with *p* populations became infected in 10 out of 11 instances (Table I). Three infections occurred in the *Pp* sites earlier than infection was apparent in other wounds, including wounds in animals which did not receive penicillin. These *Pp* infections also gave the *clinical impression* of being more erythematous and hemorrhagic. The evidence available did not permit statistical validation of this impression, but was consistent with the findings of Tacking(3).

No evidence of genetic change was detected in bacterial populations recovered from the animals. Those which were tested (in 7 experiments) retained the same level of *in vitro* resistance to penicillin they had manifested prior to animal passage. This was true irrespective of whether they had been passed through a penicillin-treated or a non-penicillin-treated animal. Additional evidence that no genetic change had occurred was obtained in 14 animals that were inoculated with a population obtained from a *Pp* abscess (Table II). The results obtained were similar to those obtained in the original investigation. All groups were combined for final statistical evaluation of the results.

Discussion. Our results indicate that a population of staphylococci which has been exposed to penicillin immediately prior to inoculation has a greater capacity for initiating infection in spite of prophylactic penicillin treatment (16/17) than one of equal "resistance" which has not (4/17), a probability

value$<$0.001. They also indicate that penicillin is effective in preventing wound infections due to "penicillin-resistant" staphylococci not previously exposed to penicillin (18/18 as compared to 4/17) provided the inoculum size is small and the penicillin dosage is adequate ($P<0.001$).

As originally observed by Kirby(1) the resistance of staphylococci to penicillin depends upon ability of penicillinase, synthesized by the bacteria, to inactivate penicillin before the bacterial cell itself is irreversibly injured. The individual cell in such a penicillin-resistant population is relatively susceptible to penicillin. The inoculum must be large enough to provide and maintain sufficient penicillinase in the environment to inactivate a given concentration of penicillin. This is the probable explanation for the difficulty in demonstrating penicillinase production when working with low density populations of staphylococci.

To demonstrate *in vitro,* a difference in penicillin-susceptibility of populations used, the large populations which were cultivated in broth for only 2 hours, with and without penicillin, were studied by tube-dilution technic. No *in vitro* evidence of modification of penicillin-resistance was observed. This seemed surprising in view of the partially substrate-adaptive nature of penicillinase synthesis by staphylococci(4,5). However, it has been observed(6) that synthesis of penicillinase by staphylococci is significantly influenced by the composition of the nutritional environment. Therefore, increased penicillinase production stimulated by previous exposure to the substrate, penicillin, could have occurred in the more complex nutritional environment *in vivo* in spite of the fact that it was not manifested *in vitro.*

In the continuing effort to control hospital infections due to "penicillin-resistant" staphylococci, it appears that control of penicillin in the environment may be as important as the distribution and genetic character of the staphylococci. This appears to be an instance in which the phenotype, as well as the genotype, of a pathogen is important.

Summary. Experimental wound infections with small inocula of staphylococci were regularly produced in rabbits. Prophylactic penicillin was effective in preventing such experimental wound infections due to "penicillin-resistant" staphylococci when size of inoculum was small and when the infecting organisms were derived from environment containing *no* penicillin. Under similar conditions penicillin was not effective in preventing wound infection due to small inocula of "penicillin-resistant" staphylococci which were derived from an environment *containing penicillin.*

The authors acknowledge technical assistance of Charmion Carnes.

1. Kirby, W. M. M., *J. Clin. Invest.,* 1945, v24, 165.
2. Elek, S. D., Cohen, P. E., *Brit. J. Exp. Path.,* 1957, v38, 573.
3. Tacking, R., *Nature,* 1954, v173, 726.
4. Geronimus, L. H., Cohen, S., *J. Bact.,* 1957, v73, 28.
5. ———, *ibid.,* 1957, v74, 507.
6. Bondi, A., de Saint Phalle, M., Kornblum, J., Moat, A. G., *Arch. Biochem.,* 1954, v53, 348.

Received September 8, 1959. P.S.E.B.M., 1960, v103.

Intimal Lesions in Arteries of Vit. E Deficient Rats.* (25429)

NORMAN M. SULKIN AND DOROTHY F. SULKIN (Introduced by Camillo Artom)
Dept. of Anatomy, Bowman Gray School of Medicine, Wake Forest College

During an investigation on effects of Vit. E deficiency on nerve cells in the rat, it was incidentally observed that in the few instances in which carotid arteries were removed and processed simultaneously with the superior cervical ganglia, the intimal lining of these vessels was characterized by proliferative changes. Since the rat is generally con-

* This work was supported in part by Grant B-342 from the National Institute of Health, U. S. Public Health Service and in part from a grant from the Hoffmann-La Roche, Inc.