tensity of the reaction will be indicated only by plus and minus signs, with the arbitrary degrees 1, 2 and 3. (Table I). The pattern of response was markedly uniform in all animals, with the exception of the lung which behaved erratically towards quinine (no inhibition, one case; slight to moderate inhibition, 3 cases; complete suppression of the reaction, 4 cases).

The histochemical results are in good agreement with previous findings on the *in vitro* behavior of hepatic and pancreatic lipase towards quinine and arsanilate and on the similarity between the pancreatic and gastric enzymes, both being activated by bile salts.[9] On the other hand, the effect of several substances, markedly active in test tube experiments, such as urethane, butyraldehyde, acetophenone, hexylresorcinol and NaCl, cannot be observed under the conditions of the histochemical experiment. An interesting new finding is the difference in sensitivity to arsanilate between the enzyme of the interstitial cells (testis) and that of the spermatic elements. Another curious observation was a marked diffusion of the reaction around the sites of activity in the pancreas and the stomach, but nowhere else, when cholate was added to the substrate. In some cases such large numbers of lead sulfide granules were embedded in the protecting collodion membrane and on its surface as to make the exact localization of the enzyme virtually impossible. This finding may indicate an increased diffusibility of pancreatic and gastric lipase in the presence of bile salts.

Summary. Differences in the behavior of esterases (lipases) from various sources towards activator and inhibitor substances, similar to those found previously in *in vitro* experiments, can be observed also in tissue sections stained for lipase.

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Biological Activity of Crystalline Procaine Penicillin *In vitro* and *In vivo*.*

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In a recent communication Salivar, Hedger, and Brown[1] described the preparation and chemical properties of crystalline procaine penicillin. The present report deals with the biological activity and with the absorption and excretion of this form of penicillin and of other relatively insoluble salts of penicillin.

Materials and Methods. Unless otherwise specified, crystalline procaine penicillin G and dihydro-F, prepared from crystalline sodium penicillins G and dihydro-F, respectively, were used throughout this study. The potencies of these preparations were determined by the Oxford cup plate method;[2] the per cent G, non-G penicillin by a modification of the N-ethyl piperidine method.[3] Sensitivity determinations were carried out by a quantitative broth dilution technic. The method of Tompsett, Schultz, and McDermott,[4] using *Strepto-*

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1 Salivar, C. J., Hedger, F. H., and Brown, E., *J. A. C. S.*, 1948, in press.

2 Schmidt, W. H., Ward, G. E., and Coghill, R. D., *J. Bact.*, 1945, **49**, 411.

3 *Federal Register*, April 4, 1947, v. **12(67)**, p. 2222, 141.5 (f).

4 Tompsett, R., Schultz, S., and McDermott, W., *J. Bact.*, 1947, **53**, 581.

coccus hemolyticus as the test organism and crystalline sodium penicillin G as standard, was used for determination of the concentrations of penicillin in blood and urine. Mouse protection tests were carried out using *Streptococcus hemolyticus* and *Diplococcus pneumoniae* as the test organisms and the method described by Hobby *et al.*[5] in a recent communication.

Activity of Crystalline Procaine Penicillin In Vitro. Two preparations of procaine penicillin G, having potencies of 1066 and 1025 units per mg, and 2 preparations of procaine penicillin dihydro-F, having potencies of 1010 and 987 units per mg, were used.† Likewise 3 preparations containing mixtures of crystalline procaine penicillins G and dihydro-F as well as certain naturally occurring penicillin pigments were used.‡ These showed potencies of 930 u/mg, 975 u/mg, and 950 u/mg, respectively. The sensitivity of a variety of organisms to these penicillins was determined as follows:

Six-hour plain broth cultures of *Streptococcus hemolyticus* (strain C230Mv), *D. pneumoniae* (strain I/230), *Staphylococcus aureus* (strain H), *Bc. subtilis, Streptococcus viridans, E. coli,* and *A. aerogenes* were used throughout. Cultures were diluted with broth to a constant density immediately prior to use. A density equivalent to a MacFarland $BaSO_4$ No. 1 standard and allowing 78% transmission on a Photovolt Lumetron No. 400 was arbitrarily chosen as standard. For each gram negative organism tested a series of 9 tubes were set up containing 0.1, 0.15, 0.2, 0.25 . . . . 0.5 ml of broth containing 400 units of procaine penicillin per ml. In a few instances, a concentration of 800 units per ml was essential. For the more sensitive gram positive organisms, concentrations of 0.1 unit per ml were used. The total volume of each tube was adjusted to 0.5 ml with sterile broth and 0.5 ml of a 10^{-3} dilution of the standardized culture was then added to each. The final concentration of organisms was, therefore, about 150,000 per ml. Incubation was carried out at 37°C for a period of 24 hours. The sensitivity of an organism was accepted as the least amount of penicillin causing complete inhibition of growth, as evidenced by absence of gross turbidity, after 24 hours incubation.

Crystalline sodium penicillins X, G, dihydro-F, and K§ were tested simultaneously for their activity against the same group of organisms.

As shown in Table I, crystalline procaine penicillins G and dihydro-F are highly effective antibacterial agents *in vitro*. The activity of crystalline procaine penicillin G at times may differ quantitatively, however, from that of the crystalline sodium salt of penicillin G. Under the experimental conditions used in this study, all organisms tested were slightly more sensitive to crystalline sodium penicillin G than to crystalline procaine penicillin G. No differences were observed in the sensitivities of these organisms to the sodium salt of penicillin dihydro-F as

[5] Hobby, G. L., Burkhart, B., and Hyman, B., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 296.

† The theoretical potencies of crystalline procaine penicillins G and dihydro-F are calculated to be 1041 and 986 units per mg respectively, based on crystalline sodium penicillins G and dihydro-F at 1667 and 1600 units per mg, respectively. Each mg of crystalline procaine penicillins G and dihydro-F contains 0.42 and 0.43 mg procaine base, respectively.

‡ On crystallization of procaine penicillins G and dihydro-F from impure penicillin containing approximately 70 to 80% G and 20 to 30% dihydro-F, certain of the naturally occurring impurities are precipitated with the penicillins. Nevertheless the penicillins appear to be in the crystalline form.

§ The crystalline sodium salts of penicillin used were identical with the preparations used by one of the present investigators (G.L.H.) in a previous study and have been described in detail elsewhere.[5] The preparation of crystalline penicillin G had a potency of 1634 units per mg by the bioassay method and a *Bc. subtilis, Staphylococcus aureus* differential ratio of 1.0. The polariscopic assay of this preparation was 1635 units per mg. Ultraviolet absorption indicates 100% G. The crystalline penicillin K used showed a potency of 2182 units per mg and a differential ratio of 0.36; the crystalline penicillin X, a potency of 1069 units per mg, and a differential ratio of 1.39; the purified penicillin dihydro-F, a potency of 1675 units per mg and a differential ratio of 0.57.

TABLE I.
Comparative Activity of Crystalline Procaine and Sodium Penicillins *in Vitro*.

Organism	Sensitivity in units per ml						
	Procaine penicillins			Sodium penicillins			
	G	Dihydro-F	Mixed*	G	Dihydro-F	X	K
D. pneumoniae (I/230)	0.085	0.042	0.033	0.030	0.040	0.005	0.022
Strep. hemolyticus (C203Mv)	0.022	0.020	0.015	0.015	0.025	0.004	0.012
Strep. viridans	0.220	0.200	0.150	0.100	0.200	0.075	0.100
Staph. aureus (H)	0.100	0.050	0.047	0.050	0.050	0.060	0.040
Bc. subtilis	0.025	0.035	0.018	<0.005	0.035	0.005	0.025
A. aerogenes	110.000	120.000	53.000	40.000	160.000	80.000	>200.000
E. coli	160.000	160.000	87.000	60.000	200.000	40.000	>200.000

* Crystalline procaine penicillins G and dihydro-F mixed, with accompanying naturally-occurring impurities.

compared to its procaine salt. Furthermore, their sensitivities to crystalline sodium penicillin G and to mixtures of crystalline procaine penicillins G and dihydro-F, with accompanying impurities, were identical.

Procaine penicillin may be prepared by the interaction of procaine hydrochloride and sodium penicillin. Procaine hydrochloride in low concentration has in itself no bacteriostatic action against this group of test organisms. It is chemically derived from para-aminobenzoic acid, and has been shown by Woods and Fildes[6] and by others,[7-11] to be capable of inhibiting the *in vitro* and *in vivo* bacteriostatic action of sulfadiazine against *Streptococcus hemolyticus* and certain other organisms. The possibility that procaine at times may serve as an essential metabolite and thus alter the concentration of penicillin necessary for inhibition of growth was, therefore, suggested. Procaine hydrochloride, however, in concentrations of 0.02, 1.0, and 100 mcg per ml failed to alter the sensitivity of *Streptococcus hemolyticus* to crystalline sodium penicillins G or dihydro-F.

Activity of Crystalline Procaine Penicillin In Vivo. Preparations of crystalline procaine penicillins G and dihydro-F in oil, diluted to contain 1,000 units of penicillin per ml, were used throughout this study. In a few instances preparations containing mixtures of crystalline procaine penicillins G and dihydro-F, with certain of the accompanying impurities of partially purified penicillin, were used. Crystalline potassium penicillin G in oil and beeswax was also tested for comparison.

Fifteen-hour blood broth cultures of a highly virulent strain of Group A hemolytic streptococcus (strain C203Mv) and of pneumococcus type I (Strain I/230) were used throughout. Mice were infected by the intraperitoneal route with one cc of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} dilutions of culture. Treatment was carried out by the subcutaneous route, a single injection of penicillin being administered 2 hours after infection. All experiments were controlled with a series of untreated animals. In all instances the penicillins used were administered in sesame or peanut oil, a concentration of 1,000 units of penicillin per ml being used.

As shown in Table II, procaine penicillin is an effective chemotherapeutic agent against hemolytic streptococcal and pneumococcal infections. Three hundred units of procaine penicillin G, administered in a single injection 2 hours after infection, is adequate to protect approximately 70% of animals against 10 to 10,000 lethal doses of hemolytic streptococci or pneumococci. Similar protection against hemolytic streptococcal infections was ob-

6 Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

7 Woods, D. D., and Fildes, P., *Chem. and Industry*, 1940, **59**, 133.

8 Boroff, D. A., Cooper, A., and Bullowa, J. G. M., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 182.

9 Casten, D., Fried, J. J., and Hallman, F. A., *Surg. Gynecol. Obstet.*, 1943, **76**, 726.

10 Keltch, A. K., Baker, L. A., Krahl, M. E., and Clowes, G. H. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 533.

11 Legge, J. W., and Durie, E. B., *Med. J. Australia*, 1943, **29**, 561.

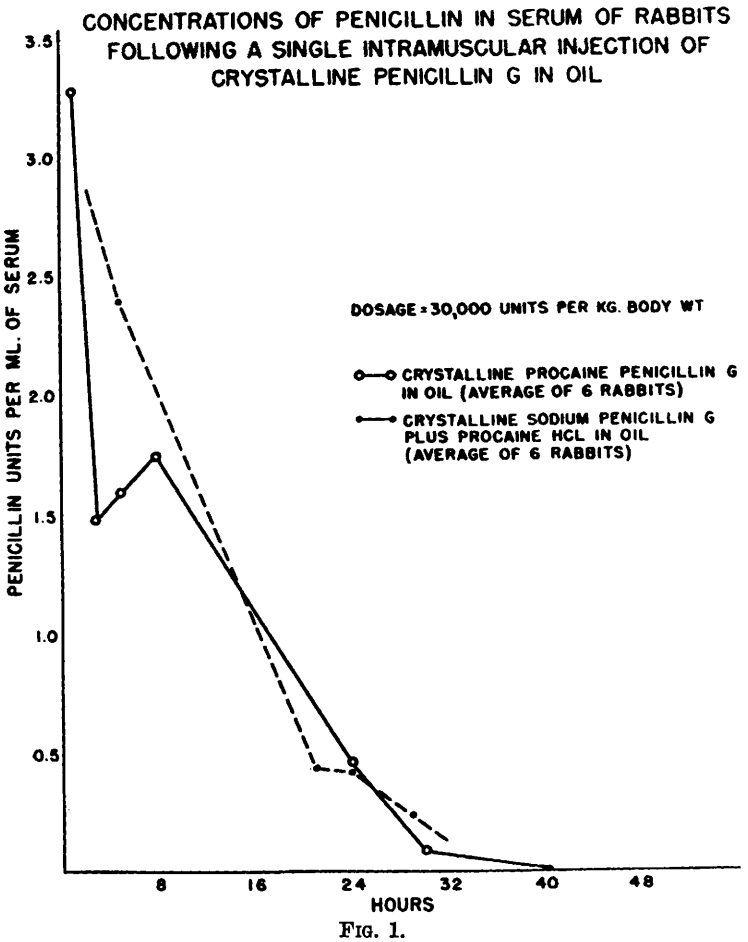

FIG. 1.

served with crystalline procaine penicillin dihydro-F.

The fact that certain impurities are capable of enhancing the action of crystalline sodium penicillin G has been discussed in previous communications.[5,12] Preparations containing mixtures of crystalline procaine penicillins G and dihydro-F with certain of the naturally occurring penicillin mold pigments or other impurities have shown a similar enhanced action. Whereas 300 units of crystalline procaine penicillin G were capable of protecting 70% of animals against infection due to *Streptococcus hemolyticus*, this amount of the mixed procaine penicillins G and dihydro-F, with accompanying impurities, was capable of protecting 90% of animals. One hundred and fifty units of these preparations of mixed crystalline penicillins were almost as effective as 300 units of crystalline procaine penicillin G or dihydro-F. The enhanced action of these mixed penicillins was not apparent in the small series of animals tested with pneumococcus.

Absorption and Excretion of Crystalline Procaine Penicillin. Crystalline procaine penicillins G and dihydro-F were prepared in a concentration of 300,000 units per ml peanut or sesame oil. Similar preparations of mixtures of crystalline procaine penicillins G and dihydro-F and accompanying impurities were also used. All experiments were carried out in normal male rabbits, weighing approximately 3 kg. In all instances 30,000 units per kg body weight were administered by the intra-

[12] Hobby, G. L., Lenert, T. F., and Hyman, B., *J. Bact.*, 1947, **54**, 305.

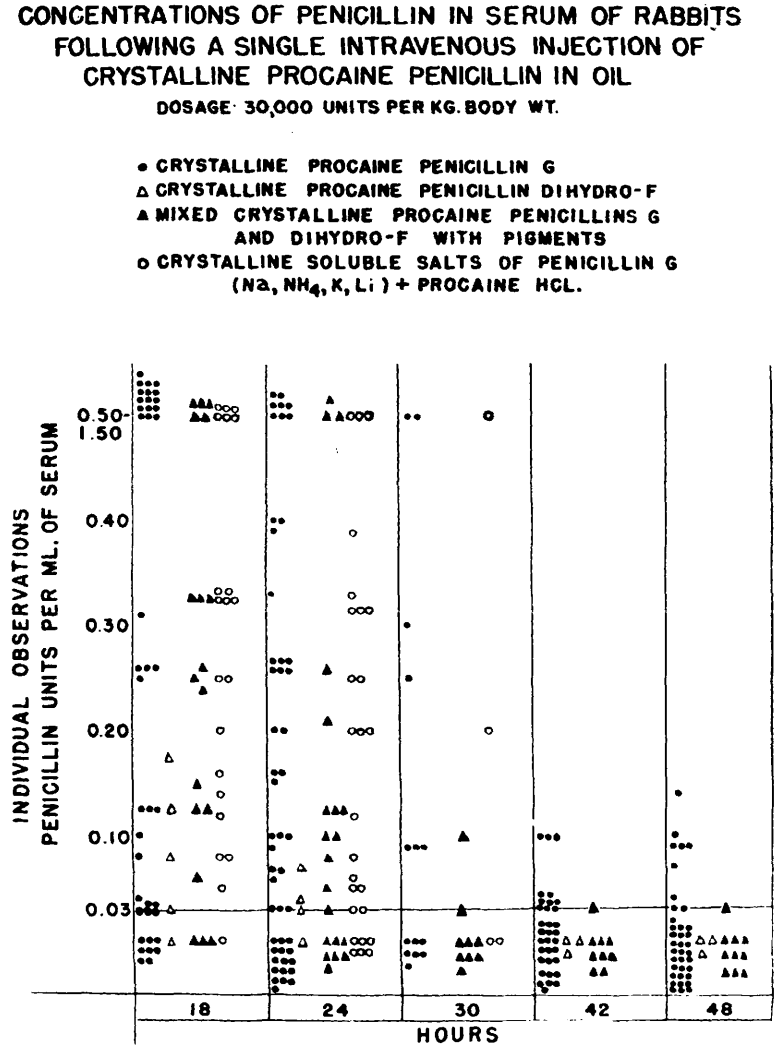

FIG. 2.

muscular route. Animals were bled immediately prior to and at varying intervals after injection. The concentration of penicillin in the blood was determined by the method of Tompsett, Schultz, and McDermott,[4] using undiluted serum as well as serum diluted 1:4 and 1:20. *Streptococcus hemolyticus* was used throughout as the test organism and crystalline sodium penicillin G as standard.‖ The minimum level detectable by this method varied from 0.02 to 0.04 units per ml. No attempt was made to determine the urinary excretion of penicillin from the rabbits injected.

As indicated in Fig. 1 and 2, a single intramuscular injection of 30,000 units of crystalline procaine penicillin G in oil per kg of body weight produces in the majority of experimental rabbits detectable blood levels for periods of 24 to 30 hours. Blood levels ranging from 0.03 to 1.5 units per ml were ob-

‖ In a few instances crystalline procaine penicillin G as well as sodium penicillin G was used as standard. The levels on this basis were at times slightly higher due to the fact that the sensitivity of the test organism (*Streptococcus hemolyticus*) to crystalline procaine penicillin G may be less than its sensitivity to crystalline sodium penicillin G.

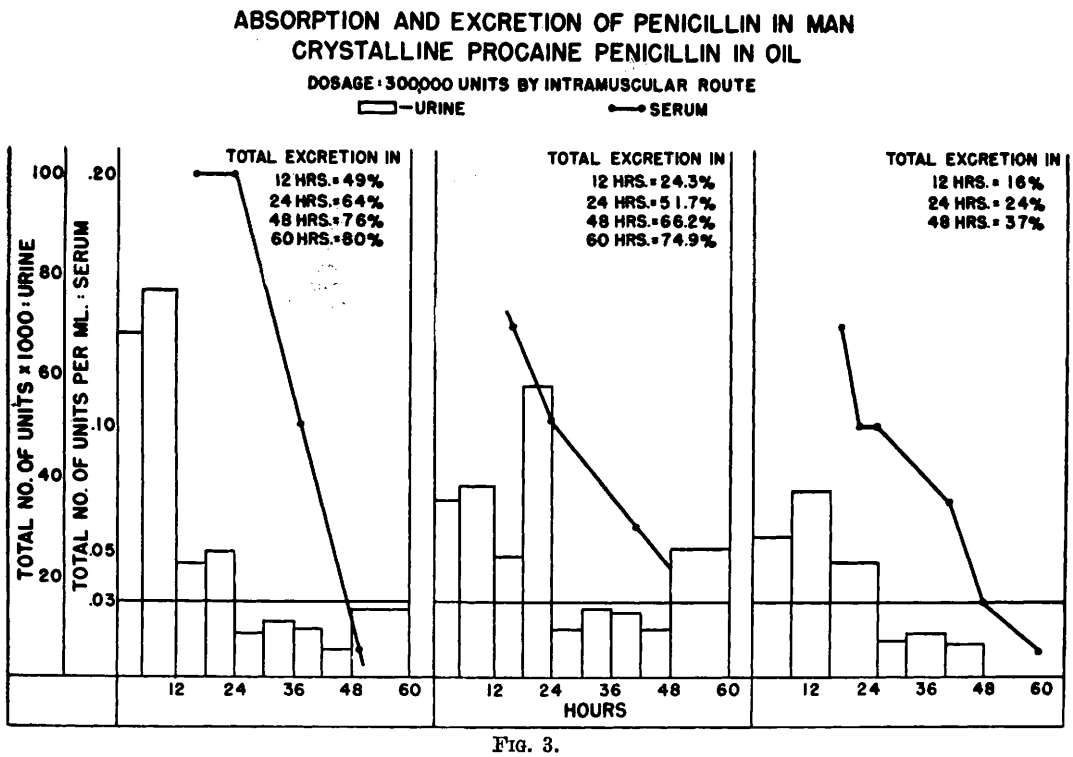

FIG. 3.

served in 80% of animals tested at 18 hours after injection. Sixty-seven per cent showed levels within this range at 24 hours, 50% at 30 hours, 33% at 42 hours, and 25% at 48 hours. Comparable results were obtained with crystalline sodium (potassium, ammonium, or lithium) penicillin G when mixed with procaine hydrochloride in oil in an amount equivalent to that present in crystalline procaine penicillin G. Likewise similar results were observed with other local anesthetics such as procaine buterate and procaine borate. Prolonged but lower levels resulted from the administration of crystalline procaine penicillin dihydro-F in oil, in the same dosage. The use of aqueous rather than oil suspensions of procaine penicillin gave less prolonged penicillin blood levels.

Comparative studies of crystalline potassium penicillin in oil and beeswax, using the same dosage and experimental conditions as those used for procaine penicillin, resulted in detectable blood levels lasting from 18 to 24 hours only.

Preliminary Observations on the Absorption and Excretion of Crystalline Procaine Penicillin in Man. Preliminary observations in man indicate that a single injection of 300,000 units of procaine penicillin G in oil will result in detectable concentrations of penicillin in the blood for periods of 24 to 48 hours, while high concentrations of penicillin may exist in the urine for at least 48 hours (Fig. 3).**

Toxicity of Crystalline Procaine Penicillin. Determination of the toxicity of crystalline procaine penicillins G and dihydro-F in animals is limited by the solubility of the compound. Solutions containing 6,000 units per ml have produced no toxic reaction when administered by the intravenous route to white mice in dosages of 1,400 units per 20 g mouse. Furthermore, rabbits receiving 4,000 units

** Since this paper was submitted for publication, a report has appeared by Herrell and his associates (Herrell, W. E., *et al., Proc. Staff Meet. Mayo Clinic,* 1947, **22**, 567), who have administered procaine penicillin in oil to man in dosages of 300,000 units, and have observed prolonged blood levels similar to those reported herein.

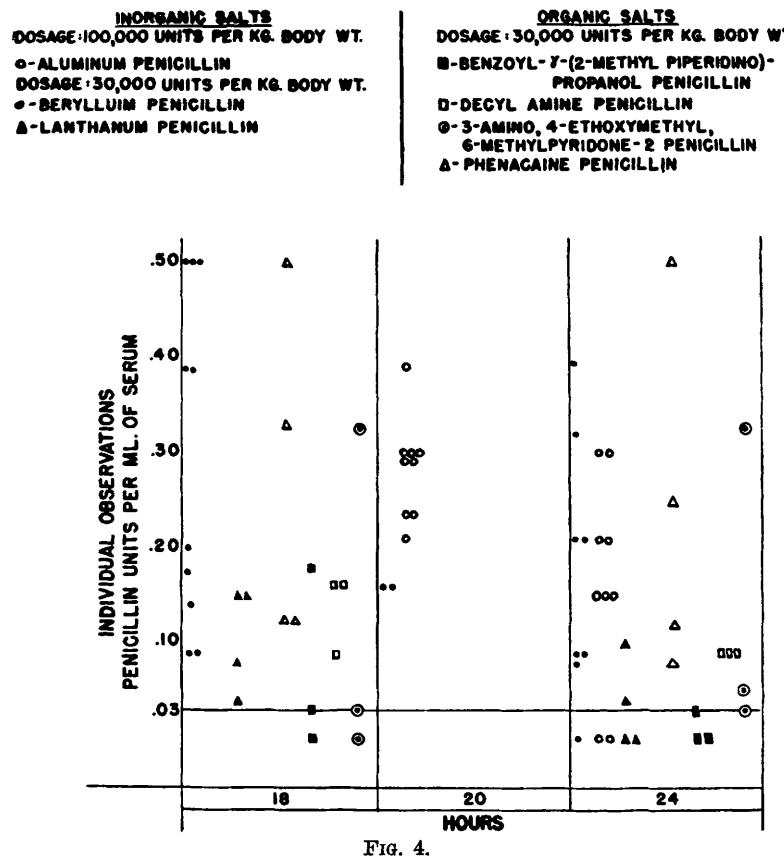

FIG. 4.

intravenously per kg body weight have shown no toxicity. Suspensions containing 40,000 units per ml have caused no reactions in dosages of 40,000 units per 20 g mouse when administered by the subcutaneous route.

It is recognized that the acute mouse toxicity of the soluble salts of penicillins is a direct measure of the concentrations of cation present. In like manner, the minimum lethal dose (LD_0) of crystalline procaine penicillin G (1400 units = 1.35 mg procaine penicillin = 0.56 mg procaine base) is in close agreement with that of procaine hydrochloride (LD_0 = 0.60 mg).

Preliminary observations in man indicate that procaine penicillin in oil is nonirritating on injection and produces no local pain or soreness. It seems probable that crystalline procaine penicillin in oil possesses no greater toxicity than its component parts.

Preliminary Observations on Other Water-Insoluble Salts of Penicillin. Crystalline procaine penicillins G and dihydro-F are water-insoluble salts of penicillin. Their maximum solubilities have been found to be 6,700 units (6.5 mg) and 15,000 units (15.2 mg) per cc, respectively. In view of the fact that procaine penicillin in oil prolongs penicillin blood levels, it seemed likely that other insoluble salts of penicillin might act similarly. That this is true is indicated by the work of Monash[13] who has recently reported detectable blood levels in rabbits at 18 and 24 hours following the intramuscular injection of silver

penicillate, mercury penicillate, and ferric penicillate in oil. Furthermore, Bohls and his associates[14] have indicated that detectable blood levels may be attained for prolonged periods after the intramuscular injection of aluminum-penicillin in oil.

In the present investigation a small series of other water-insoluble organic and inorganic salts of penicillin was tested in experimental rabbits, using the procedure previously described. A dosage of 30,000 units per kg body weight was used throughout. The results are indicated in Fig. 4. Significant levels were observed at 18 and 24 hours in all instances. Beryllium penicillin, the least soluble of the compounds tested, showed the highest and most prolonged levels.

Discussion. Early studies[15-17] on the bacteriostatic and bactericidal properties of the blood of animals treated with aqueous penicillin, as well as subsequent studies on the absorption and excretion of penicillin in man,[18-20] have indicated that penicillin remains in the blood stream for only 2 to 3 hours after injection. These observations have been amply confirmed by subsequent investigators during the past 4 years.

Although it was originally assumed that effective bacteriostatic concentrations of penicillin should be maintained constantly in the circulating blood, as was the case with the sulfonamides, it was soon recognized that 2 injections per day were sufficient for the control of certain experimental infections in animals.[17,21] Furthermore, it was demonstrated by Tillett[22] and more recently by Finland,[23] and by Tompsett and McDermott[24] that pneumococcus pneumonia may be treated successfully with penicillin regimens which afford detectable levels in the blood during only a fraction of each day.

The preponderance of clinical experience with penicillin, however, has been obtained with dosage schedules which maintain continuous or nearly continuous measurable concentrations of penicillin in the blood throughout the period of treatment. For this purpose penicillin in oil and beeswax has been used to advantage.[25-27] It has been amply demonstrated that a single injection of 300,000 units of penicillin in oil and beeswax will maintain detectable concentrations of penicillin in the blood for 12 to 24 hours; however, the reported irritation and hypersensitivity reactions following the administration of this form of penicillin have indicated the need for other methods by which prolongation of penicillin blood levels can be attained.

The fact that such prolongation may result from the use of water-insoluble salts of penicillin, suspended in oil, is of interest. Whereas the majority of these salts are only slowly absorbed, the possible toxicity of many of them limits their use. The toxicity of procaine has been reviewed by Graubard and his associates[28] in a recent communication dealing with the use of procaine intravenously in man. The toxicity of procaine injected intravenously varies with animal species. The minimal lethal dose in rabbits, guinea pigs, and dogs is reported to be in the vicinity of 40 mg per kg body weight; in man, the toxicity of procaine is thought to be dependent upon the

13 Monash, S., *Science*, 1947, **106**, 370.

14 Bohls, S. W., *et al.*, *Texas State J. Med.*, 1945, **41**, 342; *J. Ven. Dis. Inform.*, 1946, **27**, 69.

15 Chain, E., *et al.*, *Lancet*, 1940, **2**, 226.

16 Abraham, E. P., Florey, H. W., *et al.*, *Lancet*, 1941, **2**, 177.

17 Hobby, G. L., Meyer, K., and Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 277.

18 Rammelkamp, C. H., *et al.*, *J. Clin. Invest.*, 1943, **22**, 425.

19 Kirby, W. M., *et al.*, *J. Clin. Invest.*, 1944, **23**, 789.

20 Dawson, M. H., and Hobby, G. L., *Ann. Int. Med.*, 1943, **19**, 707.

21 Dawson, M. H., and Hobby, G. L., *J. A. M. A.*, 1944, **124**, 611.

22 Tillett, W., *et al.*, *Bull. N. Y. Acad. Med.*, 1944, **20**, 142.

23 Finland, M., personal communication, 1947.

24 Tompsett, R., and McDermott, W., personal communication, 1947.

25 Romansky, M., and Rittman, G. E., *Science*, 1944, **100**, 196.

26 Romansky, M., and Rittman, G. E., *New Eng. J. Med.*, 1945, **233**, 577.

27 Romansky, M., Murphy, R. J., and Rittman, G. E., *J. A. M. A.*, 1945, **128**, 404.

28 Graubard, D. J., *et al.*, *N. Y. State J. Med.*, 1947, **47**, 2187.

TABLE II.
Chemotherapeutic Action of Crystalline Procaine Penicillin on Hemolytic Streptococcal (Group A) and Pneumococcal Infections in Mice.

Culture	Total dosage in units	Procaine penicillin in oil: G	Procaine penicillin in oil: Dihydro-F	Procaine penicillin in oil: Mixed	Potassium penicillin G in oil and beeswax	Untreated controls
		Therapeutic effect: % survival				
Streptococcus hemolyticus (C230Mv)	600		75.0			
	450		69.4			
	300	70.0	67.4	90.0	83.3	
	150	51.7	30.0	63.3	43.3	
	50	23.4	20.0	45.0	23.4	8.8
D. pneumoniae (I/230)	500	95.0				
	400	80.0		70.0		
	300	72.5		77.5		
	150	65.0		42.5		
	50	30.0		20.0		

* A minimum of 10 to 15 mice was used for each dilution in each set. One ml of a 10^{-7} dilution contained 1-10 lethal doses of hemolytic streptococci or pneumococci; 10^{-6}, 10-100; 10^{-5}, 100-1,000; 10^{-4}, 1,000-10,000. In all instances mice were injected intraperitoneally with 1 ml of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} dilutions of culture.

percentage concentration administered.

The administration of penicillin in 1% procaine hydrochloride has been used frequently to eliminate local irritation and soreness following injection.[29] Crystalline procaine penicillin contains a high concentration of procaine (120 mg per 300,000 units). In preliminary studies it has produced little or no toxicity however.

That crystalline procaine penicillin is a highly efficient antibacterial agent both *in vitro* and *in vivo* has been demonstrated. Furthermore, the fact that this form of penicillin when suspended in oil will prolong blood levels in animals for 24 to 30 hours, or more, following the intramuscular injection of 30,000 units per kg has been shown. Preliminary observations in man indicate that a similar prolongation of blood levels occurs.

The mechanism by which procaine penicillin acts is not known. It is probable that the action is dependent upon at least 3 factors: (1) the low solubility of procaine penicillin in aqueous fluids, (2) the protective action of the oil surrounding the particles of procaine penicillin, and (3) the pharmacological activity of procaine on the tissues at the site of injection.

Conclusions. Crystalline procaine penicillins G and dihydro-F are highly effective antibacterial agents *in vitro* and *in vivo*.

Crystalline procaine penicillins G and dihydro-F, in oil, when injected intramuscularly in rabbits in a single dose of 30,000 units (0.1 cc) per kg body weight, in most instances produces blood levels lasting 24 to 30 hours or longer.

Preliminary observations in man indicate that a single intramuscular injection of 300,000 units of crystalline procaine penicillin G in oil may produce detectable blood levels lasting from 24 to 48 hours while penicillin may be excreted in the urine for at least 48 to 60 hours.

Preliminary observations suggest that the toxicity of crystalline procaine penicillin is probably low.

Other water-insoluble salts of penicillin when suspended in oil also produce marked prolongation of blood levels.

[29] Buckles, D. L., *Bull. U. S. Med. Dept.*, 1947, **7**, 648.