shown by Proskauer *et al.*[6] one must accept the possibility of a systemic error due to elevation of the rats' body temperature.

In order that we might compare the 2 methods we have replaced the tail holder with a simple plethysmograph and have simultaneously recorded cuff pressures by both carbon button and plethysmographic endpoints. Over a large number of trials we have found general agreement within 5 to 15 mm, the carbon button endpoint nearly always being the higher. Often the readings were identical.

Discussion. The method described may be used on either anesthetized or unanesthetized animals. If anesthesia is not objectionable a very satisfactory endpoint may be more simply obtained by inserting 26-gauge syringe needles about 3 cm apart into the tail distal to the pressure cuff. These needles serve as electrodes and are connected to a sensitive direct current wheatstone bridge. Thus the actual resistance of the rat's tail is measured and is found to decrease when blood flows into it.

Summary. A new endpoint for the indirect measurement of systolic blood pressure in the caudal artery of the rat has been described. The change in volume of the rat's tail which occurs when the pressure in an inflated cuff at the base of the tail is allowed to drop to arterial pressure is mechanically transferred to develop pressure on a carbon telephone transmitter button. The electrical resistance of the button is continuously observed, a decrease in resistance indicating the endpoint for the blood pressure measurement.

[6] Proskauer, G. G., Neumann, C., and Graef, I., *Am. J. Physiol.*, 1945, **143**, 290.

15579

Chemotherapeutic Action of Various Forms of Penicillin on Hemolytic Streptococcal Infections in Mice.*

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The existence of at least 4 distinct forms of penicillin was first revealed in 1945.[1,2] The various penicillins differ widely in biological activity as evidenced by the fact that their potency varies from approximately 900 to 2300 Oxford units per mg when assayed *in vitro* against a standard strain of *Staphylococcus aureus*. Differences in the biological activity of these penicillins *in vivo* have also been reported recently by Eagle and Musselman[3] and by Coghill, Osterberg, and Hazel.[4] The penicillin in clinical use during the past 4 years has contained varying proportions of these penicillins as well as varying amounts of unidentified impurities.[5] It has been suggested by Dunham and Rake[6] and by Lewis[7] that the impurities in commercial penicillin may play a role in the total efficacy of the drug *in vivo*.

In 1943 it was observed by one of us

* This work was presented at a Penicillin Conference held under the auspices of the U. S. Public Health Service, Food and Drug Administration, and National Research Council, Washington, D.C., on March 26-27, 1946.

[1] Dale, Sir Henry, *Science*, 1945, **101**, 23.

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[5] Coghill, R. D., *Chemical and Engineering News*, 1945, **23**, 2310.

[6] Dunham, W. B., and Rake, G., *Am. J. Syph.*, 1945, **29**, 214.

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(G.L.H.)[8] that a total of 60 Oxford units of preparations of crude or commercial penicillin gave partial protection against hemolytic streptococcal infections in white mice, whereas a total of 100 Oxford units afforded complete protection to 90 to 95% of the animals. In similar experiments in 1944 it was observed that the administration of 3 times as much crystalline penicillin G (*i.e.*, 300 units) was necessary to obtain the same degree of protection in comparably infected mice. Subsequent comparisons of certain of these preparations which were originally tested in 1943 with other preparations of crystalline penicillin G showed similar significant differences in therapeutic efficacy. Accordingly, studies were undertaken to determine the relative value of the various forms of purified penicillin and of "commercial" penicillin.

Crystalline penicillin G and preparations containing predominantly or only penicillin X, F, and K were studied as well as random samples of material manufactured for general clinical use.

Preliminary observations on the bacterial spectrum of these preparations gave scant information. Whereas certain differences could be detected by the *Bacillus subtilis-Staphylococcus aureus* differential ratio† as determined by the Oxford cup plate method,[9] this technic was not practicable with most pathogenic organisms. Only with certain strains, such as *Eberthella typhosus* which appeared to be more sensitive to penicillin X than to the other penicillins, was it possible to demonstrate a difference in any of the forms of penicillin. Using a modified dilution technic, differences in the sensitivity of various species of microorganisms to penicillin may be easily demonstrated.[10-12] However differences in the sensitivity of an individual organism to the different forms of penicillin were not sufficiently great to be demonstrable by this procedure. As it appeared that the relative value of the various forms of penicillin could not be satisfactorily determined *in vitro*, the following studies *in vivo* were conducted.

Hemolytic streptococcus, strain C_{203}Mv, was used as the infecting organism throughout the study. One cc of 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions of a 14-hour rabbit's blood broth culture was injected intraperitoneally into each of a series of 20 g white mice. A minimum of 6 to 10 mice was used for each dilution of each series. Treatment was started 1 to 2 hours after the infecting dose and the penicillin was administered subcutaneously in peanut oil[13] in divided dosage. Forty per cent of the total dose was administered 1 to 2 hours, 40% 7 to 8 hours, and 20% 24 hours after infection. Total dosages of 15, 30, 60, 100, 150, 210, 250, 300, 500 and 600 units were used. A control series of untreated animals was always included in each day's experiment. The untreated infection was always uniformly fatal within 18 to 48 hours. The results may be seen in Tables I, II, and III.

Purified Penicillins.‡ Crystalline penicillin G afforded little or no protection in total dosages of 30, 60, or 100 units. With 150 or 210 units, there was partial protection

[8] Hobby, G. L., and Dawson, M. H., unpublished data.

† Hereinafter designated differential ratio. The differential ratios of crystalline penicillin X, G, F, and K are accepted as 1.2-2.0, 1.0, 0.66, and 0.38 respectively. The differential ratio does not indicate the penicillins present in mixtures. In the absence of penicillin X, a ratio of 1.0 probably indicates penicillin G only whereas a ratio below 1.0 indicates the presence of more than one form.

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‡ With the exception of penicillin X, all purified or crystalline preparations of penicillin were obtained through the kindness of Dr. R. Pasternack, Dr. E. V. Brown, and Dr. P. Regna of the Research Laboratory of Chas. Pfizer and Co., Brooklyn, N.Y.

We are indebted to Dr. Henry Welch of the Food and Drug Administration for the sample of crude penicillin X and to Lederle Laboratories, Inc., for the sample of crystalline penicillin X.

TABLE I.
Chemotherapeutic Action of Crystalline and Purified Penicillins on Hemolytic Streptococcal Infections (Group A) in Mice.

Form of penicillin		X	X	G	F	K	Controls
Oxford units per mg *Bc. subtilis*		1069	170	1667	960	2017	
Staph. aureus		1.39	1.16	1.0	0.66	0.38	
Dilution of culture*	Total dosage in units	Therapeutic effect: % survival					
10^{-4}	30	40	40	25			3
10^{-5}		30	70	0			1
10^{-6}		60	80	0			6
10^{-7}		70	80	50			10
10^{-4}	60	87.5†	80†	32			
10^{-5}		100	90	22			
10^{-6}		100	90	66			
10^{-7}		100	100	66			
10^{-4}	100	87.5	70	60	57		
10^{-5}		100	100	54	80		
10^{-6}		100	100	69	57		
10^{-7}		100	100	90	100		
10^{-4}	150			30	20		
10^{-5}				80	60		
10^{-6}				70	10		
10^{-7}				86	90		
10^{-4}	210	100		80			
10^{-5}		100		70			
10^{-6}		100		90			
10^{-7}		100		90			
10^{-4}	300	100		90†	80†	40	
10^{-5}		100		100	80	50	
10^{-6}		100		100	90	80	
10^{-7}		100		100	100	80	
10^{-4}	500					100†	
10^{-5}						80	
10^{-6}						90	
10^{-7}						100	

* A minimum of 6 to 10 mice were used for each dilution in each set. One cc of a 10^{-7} dilution contained 1-10 lethal doses of hemolytic streptococci; 10^{-6}, 10-100; 10^{-5}, 100-1000; and 10^{-4}, 1000-10,000.

† Curative $dose_{90}$ = Level at which 90% of infected animals survived.

and with 300 units almost complete protection. The preparation of penicillin G used had a potency of 1667 o.u./mg and a differential ratio of 1.0.

Crystalline penicillin X (potency 1069 o.u./mg, differential ratio 1.27 to 1.39) gave partial protection with only 30 units and almost complete protection with 60 units. Thus a comparable degree of protection was supplied by 60 units of penicillin X and 300 units of crystalline penicillin G.

Essentially the same results were obtained with a low potency preparation thought to contain only penicillin X. This preparation had a potency of 170 o.u./mg and a differential ratio of 1.16. The action was apparently not influenced by the presence of a high concentration of impurities.

A high potency preparation of penicillin K (potency of 2017 o.u./mg, differential ratio 0.38), which contained relatively little impurity, gave appreciably less protection than either penicillin G or X. A total dosage of 300 units of K was required to afford protection comparable to that supplied by 100 to 150 units of penicillin G or 30 units of penicillin X. A larger dosage, equivalent to 500 units of penicillin K, gave almost complete protection. The protective action of 500 units of penicillin K was equivalent to that of 300 units of crystalline penicillin G or 60 units of crystalline penicillin X.

Only partially purified preparations of penicillin F were available. Three hundred units of a preparation containing 960 o.u./mg (differential ratio 0.66) gave approximately

TABLE II.
Chemotherapeutic Action of Commercial Preparations of Penicillin on Hemolytic Streptococcal Infections (Group A) in Mice.

Preparation of penicillin‡		I	II	III	IV	V	VI	VII	Controls
Oxford units per mg *Bc. subtilis* / *Staph. aureus*		281 / 1.0	685 / 0.58	955 / 0.80	1004	1071	1057	1175 / 0.65	
Dilution of culture*	Total dosage in units	Therapeutic effect: % survival							
10^{-4}	30		20	50					3
10^{-5}			10	44					1
10^{-6}			40	27					6
10^{-7}			70	72					10
10^{-4}	60	50	60		100†		75	90†	
10^{-5}		80	90	67	100	17	75	80	
10^{-6}		90	80	84	100	100	100	90	
10^{-7}		100	100	67	100	100	100	100	
10^{-4}	100	100†	90†		100		100†	100	
10^{-5}		100	100	84†	100	67†	100	100	
10^{-6}		80	100	84	100	100	100	100	
10^{-7}		100	100	100	100	100	100	100	
10^{-4}	150	100	100				100	100	
10^{-5}		100	100				100	100	
10^{-6}		80	100				100	100	
10^{-7}		100	100				100	100	

* A minimum of 6 to 10 mice were used for each dilution in each set. One cc of a 10^{-7} dilution contained 1-10 lethal doses of hemolytic streptococci; 10^{-6}, 10-100; 10^{-5}, 100-1000; and 10^{-4}, 1000-10,000.

†Curative $dose_{90}$ = Level at which 90% of infected animals survived.

‡ Preparations I and V were prepared from *Penicillium notatum*, strain 832; preparations IV and VI from *Penicillium chrysogenum*, strain 1951B25; preparations II and III from *Penicillium chrysogenum*, strain X-1612; and preparation VII from *Penicillium chrysogenum*, strain Q176.

TABLE III.
A Comparison of the Chemotherapeutic Action of Various Forms of Penicillin in Hemolytic Streptococcal Infections in Mice.

Penicillin	Curative $dose_{90}$		Relative activities	
	Units	μg*	Biological	Gravimetric
Penicillin X	60	67	500	270
" G	300	180	100	100
" F	slightly >300	>195	<100	<92
" K	500	220	60	82
Prep. I (crude)†	100	60	300	300
Preps. II-VII (crude)	60-100		300-500	

* Based on potencies of 900, 1667, 1550, and 2300 units per mg for X, G, F, and K respectively.

† $\frac{\textit{Bc. subtilis}}{\textit{Staph. aureus}}$ differential ratio on Prep. I = 1.0; gravimetric values calculated on basis of pure penicillin G at 1667 Oxford units per mg.

the same degree of protection as 300 units of crystalline penicillin G.

On a unitage basis, the relative chemotherapeutic efficacy of penicillins X, G, F and K in hemolytic streptococcal infections in mice was on the order of 500, 100, slightly less than 100, and 60 respectively.¶ Con-

¶ Preliminary experiments carried out after this manuscript was submitted for publication indicate that, on a unitage basis, the relative efficacy of penicillin dihydro F to crystalline penicillin G is approximately 143 to 100.

verting to a gravimetric basis, the relative order of their efficacy was 270, 100, slightly less than 92, and 82 respectively§ (Table I and III).

Commercial Penicillin.‖ Preparations of "commercial" penicillin were chosen at random from material manufactured between 1943 and 1946. Samples of penicillin produced by all of the 4 major strains of Penicillium used in production during that time were included. The potency of these preparations varied from 281 o.u./mg to 1175 o.u./mg. Likewise the differential ratio of these preparations varied from 0.58 to 1.0. In all cases the penicillin was recovered from the fermentation liquor by adsorption onto activated carbon, elution with organic solvents, and subsequent extraction into chloroform or chlorinated hydrocarbons. This procedure would presumably eliminate penicillin X.

Preparation I was among those originally tested in 1943. On retesting one and again 2 years later, partial protection was obtained with only 60 units of this penicillin, whereas 100 units gave almost complete protection. One hundred units of preparation I was as effective as 300 units of crystalline penicillin G, tested simultaneously with the same dilutions of the same culture on the same days. The results were constant. It was after this observation had been confirmed that the differential ratio of preparation I was determined and found to be 1.0—the same as the value for crystalline penicillin G. In the absence of penicillin X this information suggested that probably only penicillin G was present in preparation I.

Six other preparations of penicillin manufactured during 1944, 1945, and 1946 gave comparable results as indicated in Table II. In general 60 to 100 units of crude penicillin was as effective as 300 units of crystalline G. Crude penicillin with a potency of less than 300 o.u./mg was as effective against hemolytic streptococcal infections in mice as material with a potency of 1000 o.u./mg and vice versa. Likewise, crude preparations with a low differential ratio were as effective as those with a high ratio.

Preparation VII, having a low differential and known to contain a high percentage of penicillin K in addition to other penicillins, gave complete protection with fewer units than was necessary for a comparable degree of protection when either crystalline penicillin G or highly purified K were used. Crystalline penicillin G, highly purified penicillin K, and preparation VII were tested on the same day, using the same experimental conditions throughout. Although relatively pure penicillin K was less effective than crystalline penicillin G, the presence of penicillin K in the crude or commercial preparation did not alter its protective action against this particular experimental infection.

It appears that crude or commercial preparations contain a substance which enhances the action of the penicillins present. All of the crude preparations tested were more effective against hemolytic streptococcal infections in mice than crystalline penicillin G or purified preparations of penicillin F or K. Although the crude preparations were all more effective than penicillin K, they were no more effective than crystalline penicillin X. (Tables II and III).

Summary. The comparative efficacy of 4 forms of penicillin and random samples of "commercial" penicillin has been studied in experimentally-produced hemolytic streptococcal infections in mice. On a unitage basis the relative chemotherapeutic efficacy of penicillin X, G, F and K under the experimental conditions used, was on the order of 500, 100, slightly less than 100, and 60 respectively. On a gravimetric basis, the relative order of efficacy was 270 for X, 100 for G, slightly less than 92 for F and 82 for K. The values for penicillin F were

§ These values were based on CD_{90} levels. On a CD_{50} basis the relative order of efficacy of penicillin X, G, F, and K was gravimetrically 127, 100, 57, and 40 respectively. Eagle and Musselman[3] have recently reported on this basis values of 260, 100, 50, and 9. The relative order is the same in the two series. The difference in the actual values reported by Eagle and Musselman and by ourselves may be due at least in part to the vehicle used for administration of the penicillin.

‖ All commercial penicillins used in these studies were prepared by Chas. Pfizer & Co., Brooklyn, N.Y.

obtained with a preparation which contained impurities, the action of which is not known.

All of the samples of "commercial" penicillin were 3 to 5 times as effective as crystalline penicillin G in protecting the animals against the infection. Moreover, the protective action exerted by these impure penicillins was of the same order regardless of the unitage per mg or the value of the *Bacillus subtilis-Staphylococcus aureus* differential ratio. The protective power of pure X is as great as that observed following the administration of the impure "commercial" penicillins. As the latter were manufactured by a process which presumably excludes the presence of X, it would not seem that the superiority of the impure penicillins over crystalline G could be attributed to the presence of X in the impure material.

15580 P

Nicotinic Acid and Pantothenic Acid Content of Sunflower Seed Meal and Some Oil Seed Products.*

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Interest in the nutritional significance of sunflower seed meal has been aroused by recent reports, based on feeding experiments, that it is a good source of B complex vitamins[1] and protein.[2,3] Such procedures indicated that the content of pantothenic acid is relatively lower than most of the other B complex vitamins. Also, chemical analyses for nicotinic acid in this product gave uncertain results. Therefore, in this investigation particular attention was given to the determination of these 2 vitamins by microbiological methods. Several products in addition to sunflower seed meal were analyzed.

Well known analytical methods for pantothenic acid[4,5] and nicotinic acid[6] were used.

The materials assayed were commercial products. All, except one, had been defatted by solvent extraction processes.

The analytical results, as shown in Table I, reveal that sunflower seed meal contains approximately 150% more preformed nicotinic acid than does the best grade of wheat germ analyzed, and it is approximately 500% richer in this vitamin than is corn germ or solvent-extracted soybean meal. Of the materials analyzed, only sunflower seed meal showed the presence of the "alkali-labile precursor" of nicotinic acid.[7] Since the precursor is biologically active in both dogs and chicks,[7] the total nicotinic acid value for sunflower seed meal is approximately the same, if not higher, than that of peanut meal.[8] This is of considerable interest because the latter has been rated as an outstanding source of nicotinic acid, as compared with other edible oil seed and cereal products.[9]

The pantothenic acid content of the sunflower seed meal is similar to that of the solvent-extracted soybean meal but it is ap-

* Aided by a grant from the VioBin Corporation.

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