THE VARYING BLOOD LEVELS AFFORDED BY PENICILLINS F, G, K, AND X IN RABBITS AND MAN

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It has been shown in the preceding paper (1) that penicillins F, G, K, and X are all inactivated by serum, plasma, and whole blood, but at widely varying rates. Penicillin X was inactivated slowly (5 to 7 per cent per hour in 96 per cent human or rabbit serum at 37°C.); F and G somewhat more rapidly (8 to 10 per cent per hour); while the rate of inactivation of K varied with its concentration. In human serum, it averaged 10 per cent per hour at a concentration of 50 micrograms per cc. (115 units per cc.), increasing to 11, 14, and 50 per cent per hour at concentrations of 10, 2, and 0.4 micrograms per cc., respectively. The corresponding values in rabbit serum were 10, 16, 21, and 50 per cent per hour. The value of 50 per cent per hour at penicillin concentrations of 1:2,500,000 (0.4 microgram per cc.) had no quantitative significance because of the large error in the bioassay of penicillin at such low concentrations in the presence of serum; but the qualitative trend was definite.

As will be shown in the present paper, these results *in vitro* are precisely paralleled by the behavior of the same penicillins *in vivo*. When F, G, K, and X were injected into rabbits or man in gravimetrically equal amounts per kilo (and thus, approximately equimolar amounts), penicillin X regularly gave higher and more sustained blood levels than either F or G.[1] Penicillin K, on the other hand, regularly gave low and evanescent blood curves, associated with a low recovery in the urine, and suggesting a rapid inactivation *in vivo*.

The correlation between the varying rates of destruction of these penicillins in serum *in vitro*, and the correspondingly varying blood levels observed after their injection *in vivo*, strongly suggests a causal relationship.

The possibility that the substances in serum which inactivate penicillin may be present in even higher concentration in some of the body tissues is discussed in the text.

Methods and Materials

The penicillins used in these studies, and the methods of their assay, have been described in the previous paper. In addition to the penicillins there listed, there were used in the present

[1] There are several prior reports (3–5) that penicillin X gives higher and longer blood levels than commercial penicillin or G similarly injected. The penicillins in those experiments were, however, administered on a "unit" basis, which means that the dose of X was gravimetrically 1.8 times greater than that of G.

study (*a*) an impure lot of K in which approximately one-third of the solid was penicillin, but in which K was said to constitute more than 90 per cent of the active penicillin, and (*b*) a purified lot of K provided by Charles Pfizer and Company.

The assistance of Dr. R. C. V. Robinson, Dr. Thomas W. Farmer, Dr. H. A. Tucker, and Dr. E. V. Newman (from the Medical Clinics of The Johns Hopkins Hospital) in the procurement of serum and urine specimens from the human subjects is gratefully acknowledged.

EXPERIMENTAL RESULTS

A. The Blood Levels and Urinary Excretions of Penicillins F, G, K, and X Following Their Injection into Rabbits at 0.6 Mg. per Kg.

When rabbits were injected intramuscularly with gravimetrically equal doses of penicillins F, G, K, and X (0.6 mg. per kg.), one obtained the blood curves shown in Table I and Fig. 1. Each curve in the figure is the average of five to eight animals similarly injected at the same dosage.

It is apparent in the figure and table that penicillin K disappears from the blood far more rapidly than penicillins F, G, or X. After 1 hour, the blood levels of K averaged an apparent value of 0.02 microgram per cc., as compared with 0.11, 0.18, and 0.33 for F, G, and X, respectively. The therapeutically significant level of 0.1 microgram per cc. was maintained for approximately 0.5 hour with penicillin K, as contrasted with 1, 1.4, and 2.1 hours for F, G, and X, respectively. The hourly rate of fall in the blood penicillin level averaged 83, 73, and 53 per cent[1] for F, G, and X, respectively, and an indeterminate value greater than 98 per cent for K (*cf.* last two columns of Table I).

(These results, indicative of a marked qualitative difference between K and the other three penicillins, must, however, be qualified by the fact that the blood levels of K were actually much higher than the assays would indicate, due to the inhibitory effect of serum on penicillin assays, an inhibition which is particularly marked in the case of penicillin K. The lower the apparent concentration of penicillin in the serum, and thus, the more serum which must be used in the assay to have an inhibitory effect on the test organism, the greater is the error so introduced into the assay. However, the differences between penicillin K and the other three penicillins noted in the foregoing paragraphs are quantitatively far greater than could be explained on this basis.)

The rapid disappearance of K from the blood was not due to a more rapid excretion from the urine. On the contrary, the urinary excretion of penicillin K stopped more or less abruptly after the first hour, after it had largely disappeared from the blood; and the total recovery varied between 18 and 43 per cent, averaging 33 per cent, as compared with averages of 61, 87, and 74 per cent for penicillins F, G, and X (*cf.* Table II). Moreover, since penicillins F, G, and X all have maximal renal clearances (2), the more rapid disappearance of K from the blood must reflect its destruction *in vivo*.

One can only conclude that penicillin K is inactivated *in vivo* to a greater extent than F, G, or X; and the data of the preceding paper (1) indicate that at

TABLE I

Penicillin Blood Levels in Rabbits Injected Intramuscularly with Crystalline Penicillins F, G, K, and X at 0.6 Mg. per Kg.

After the first 15 to 30 minutes the penicillin level of the blood falls off at a rate generally conforming to the equation *blood level* $= ky^t$, where y is the multiplying factor given in this table, and t is the time in hours. The values given for y were obtained graphically from the straight line curves usually obtained in plotting the logarithm of the observed blood levels against the time in hours. A rate of fall of *e.g.* 0.15 corresponds to an 85 per cent drop in the blood level each succeeding hour.

Penicillin species	Rabbit No.	Time, *hrs.*				Rate of fall of penicillin blood level	
		¼	½	1	2	Hourly rate of fall (blood level = ky^t)	Average drop each succeeding hr.
		Blood levels					
		γ per cc.	*γ per cc.*	*γ per cc.*	*γ per cc.*		*per cent*
F	52-45	0.67	0.37	0.15	0.026	0.16	
	52-96	0.6	0.4	0.15	0.05	0.17	
	54-19	0.46	0.27	0.15	<0.033	0.24	83
	54-46	0.64	0.24	0.1	0.025	0.23±	
	54-95	0.35	0.13	<0.02	<0.015	—	
	Mean.....	0.54	0.28	0.11	0.02±	0.17	
G	52-38	1.33	0.23	—	0.028	—	
	52-41	1.8	0.45	0.25	0.021	0.08	
	52-54	—	0.3	0.2	<0.016	—	
	53-18	0.54	0.27	0.068	0.018	0.07	
	54-28	0.62	0.42	0.35	0.078	0.30	73
	54-45	0.33	0.23	0.14	0.088	0.31	
	54-67	—	0.3	0.13	0.011	0.11	
	57-51	0.4	0.26	0.13	0.033	0.22	
	Mean.....	0.83	0.31	0.18	0.044	0.27	
K	51-86*	0.82	0.41	<0.06	<0.06	—	
	52-10*	0.29	0.12	<0.07	<0.07	—	
	52-37	0.19	0.11	0.02	<0.02	0.053±	
	52-39*	0.4	<0.025	<0.025	<0.025	—	98
	52-42*	1.33	0.04	0.031	<0.025	0.104±	
	53-05	0.033	<0.036	<0.02	<0.02	—	
	53-19	0.4	0.2	0.029	<0.02	0.034±	
	54-47*	0.22	0.12	0.0125	<0.0125	—	
	Mean.....	0.46	0.13	0.02±	<0.02	0.02±	
X	52-58	0.64	0.43	0.19	0.042	0.21	
	53-27	0.8	0.69	0.3	0.1	0.3	
	54-49	0.46	0.25	0.125	0.066	0.4	53
	54-65	0.2	0.19	0.13	0.067	0.45	
	57-52	1.1	0.89	0.89	0.27	0.28	
	Mean.....	0.64	0.49	0.33	0.11	0.37	

* Injected with an impure specimen in which penicillin K was said to constitute 90 per cent of the active penicillin. Other rabbits in this series received a crystalline product (Abbott RP309P1).

least part of that inactivation takes place in the circulating blood, due to a thermolabile constituent of plasma (or serum).

Of the other three penicillins, X gave consistently higher blood levels, and of longer duration, than either F or G, which behaved similarly. The levels of X ½, 1, and 2 hours after the injections (0.49, 0.33, and 0.11 microgram per cc., respectively) were 1.5 to 5 times greater than those provided by F or G; and the therapeutically significant level of 0.1 microgram per cc. was maintained

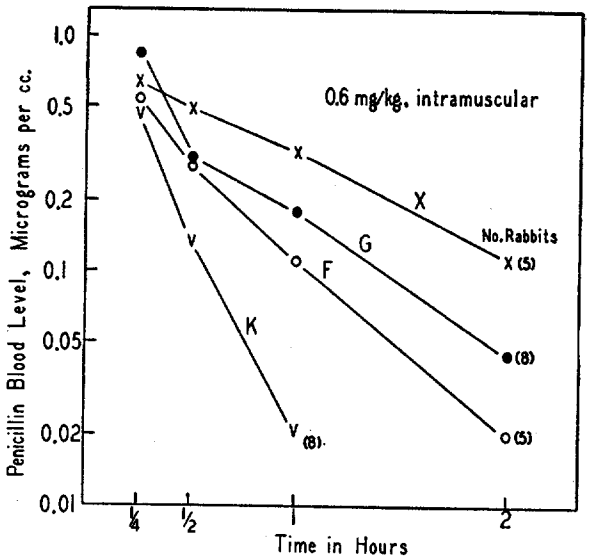

FIG. 1. The average penicillin blood levels obtained in rabbits after the intramuscular injection of equal amounts of penicillins F, G, K, and X (0.6 mg. per kg.). Each curve in the figure is the composite curve obtained by averaging the results in five to eight animals as indicated in Table I.

for an average of 2 hours, compared with an average of 1.0 and 1.4 hours for F and G, respectively. These findings conform to the observation that X is inactivated by serum more slowly than the other penicillins. By virtue of the higher and more sustained blood levels, one might anticipate that its therapeutic activity *in vivo* relative to F or G would be somewhat greater than is suggested by its bactericidal activity *in vitro*.

It should be pointed out that these qualitative differences between penicillins F, G, K, or X were actually lessened by injecting the penicillins on a gravimetric basis, in milligrams per kilo, instead of units per kilo. The selected dose of 0.6 mg. per kg. represents 1000 units per kg. of G. The latter amount of X would be approximately 1.1 mg. per kg., and the resulting blood levels would have been almost twice the levels shown in the figures; while 1000 units of K

TABLE II

The Urinary Excretion of Crystalline Penicillins F, G, K, and X in Rabbits

Unless otherwise stated, all animals received 0.6 mg. per kg. intramuscularly.

Penicillin species	Rabbit No.	Time, *hrs.*					
		½	1	2	3	4	6
		Cumulative percentage of penicillin excreted					
F	52-45		57	67	70	73	73
	52-96		40	40	40	40	40
	54-19	32	36	48	52		54
	54-46	18	29	54	62		72
	54-95	23	50	61	66		68
	Mean.....	24	42	54	58		61
G	51-59*		47	71	78	82	83
	51-87*		49	65	74	75	75
	52-54		78	89	89	90	90
	53-18		100+	100	100	100	100+
	54-45	20	37	56	69		80
	54-28	39	59	69			
	54-67	14	36	46	48		
	Mean.....	24	58	71	82	87	87
K	51-86‡		18	26	27	27	27
	52-10‡		33	35	35	35	35
	52-39‡		42	43	43	43	43
	52.42‡		32	41	42	42	42
	53-05		16	18	18	18	18
	53-19		29	31	31	31	32
	Mean.....		28	32	33	33	33
X	52-52		23	35	37	38	39
	52-58		43	60	63	64	65
	53-27		70	90	90	98	100
	54-65		11	20	26		54
	54-49	54	85	104	109		112
	Mean.....		46	62	65		74

* Injected with 1.4 mg. per kg. instead of 0.6.

‡ Injected with an impure specimen in which penicillin K was said to constitute 90 per cent of the active penicillin. Other rabbits in this series received a crystalline product (Abbott RP309P1).

would be approximately 0.44 mg. per kg., and the resulting blood levels would have been only three-fourths of the low levels observed.

B. The Blood Levels and Urinary Excretions of Penicillins F, G, K, and X in Man

In Tables III and IV and Figs. 2 and 3 are summarized a series of experiments in human volunteers injected with penicillins G, K, and X at 0.6 mg. per kg. Half the subjects were injected intravenously, and half intramuscularly. As in rabbits, penicillin X again provided consistently higher levels than did G or K, whether after intravenous or intramuscular injection. In order to control the significant individual variations in the rate of urinary excretion, three subjects receiving X were also injected with G. In all three, X gave higher levels, of longer duration, than did G in the same subject. The blood levels of X ½, 1, and 2 hours after intramuscular administration averaged 0.61, 0.37, and 0.14 microgram per cc., compared with 0.73, 0.23, and 0.061 for G. (The values at one-quarter hour are of dubious significance because of the rapidly changing levels at that time.) A level of at least 0.1 microgram per cc. was maintained for an average period of 23 hours, compared with 16 hours for G. The hourly decrease in the blood penicillin level was 82 per cent for G and 60 per cent for X.

That these differences were not due to varying rates of absorption from the intramuscular depot is shown by the fact that qualitatively similar results were obtained after intravenous injection. The average blood levels of X ½, 1, and 2 hours after the injection were 0.41, 0.15, and 0.04 microgram per cc., compared with 0.3, 0.11, and an indeterminate value for G. A level of 0.1 microgram per cc. was maintained for 13 hours, compared with 1 hour for G. Finally, the hourly drop of the penicillin blood level averaged 80 per cent for X, and 94 per cent for G.

Penicillin K behaved in man as it did in rabbits, in conformity with its rapid inactivation by serum *in vitro*. Two lots of K were used for human injection: one was an impure preparation in which K constituted approximately 90 per cent of the active material. The second was a relatively pure crystalline preparation, more than 90 per cent of which was actually K. Both lots behaved similarly. The blood levels of K 1 hour after intramuscular injection averaged 0.16 microgram per cc., compared with 0.23 and 0.37 for G and X, respectively. The corresponding values after intravenous injection were < 0.02 microgram per cc. for K, as compared with 0.11 and 0.15 for G and X. A therapeutically effective level of 0.1 microgram was maintained for 0.5 hour, after intravenous injection, as compared with 1 and 1.3 for G and X.

(The foregoing results must be qualified, as in rabbits, by the indeterminate error introduced into the assays of penicillin K by the necessary presence of serum. Although that error is large, it is not believed to affect these results qualitatively.)

It is of interest that although penicillin K consistently gave lower blood levels than G or X in the human subjects, the divergence was not as great as it was in rabbits.

TABLE III

Penicillin Blood Levels in Men Injected with Crystalline Penicillins G, K, and X at 0.6 Mg. per Kg.

Route of administration	Penicillin species	Subject	Time, *hrs.* ½	¾	1	2	Rate of fall of penicillin blood level: Hourly rate of fall (blood level = ky^2)	Average drop each succeeding hour
			Blood levels					
			γ per cc.	*γ per cc.*	*γ per cc.*	*γ per cc.*		*per cent*
Intravenous	G	H	0.97	0.27	0.135	<0.07	0.070	
		RW	0.59	0.17	0.04	<0.012	0.027	94
		DC	1.07	0.49	0.14	0.026	0.07	
		Mean.....	0.88	0.3	0.11		0.06	
	K	H*	1.15	0.12	<0.036	<0.036	<0.01	
		MB	0.8	0.1	<0.01	<0.01	<0.015	>99
		LB	0.4	0.1	<0.01	<0.01	<0.01	
		Mean.....	0.78	0.11	<	<	<0.01	
	X	T	0.48	0.28	0.17	0.034	0.24	
		G	0.65	0.3	0.14	0.05	0.12	80
		RW	0.53	0.19	0.1	<0.012	?	
		DC	1.1	0.46	0.18	0.05	0.23	
		Mean.....	0.69	0.31	0.15	0.04	0.2	
Intramuscular	G	W	1.8	1.07	0.33	0.06	0.14	
		D	1.14	0.8	0.2	0.083	0.22	
		BH	0.73?	0.33	0.15	0.04	0.24	82
		Mean.....	1.22	0.73	0.23	0.061	0.18	
	K	W*	0.64	0.48	0.06	<0.036	0.042±	
		D*	0.96	0.48	0.144	<0.036	0.080	89
		JW	0.93	0.76	0.29	<	0.2	
		Mean.....	0.84	0.57	0.16	<	0.11	
	X	S	0.6	0.66	0.3	0.1	0.28±	
		JW	0.6	0.45	0.3	0.111	0.30	62
		BH	0.84	0.73	0.5	0.22	0.46	
		Mean.....	0.68	0.61	0.37	0.14	0.38	

* Injected with an impure specimen in which penicillin K was said to constitute 90 per cent of the active penicillin. Other subjects in this series received a crystalline product (Charles Pfizer and Company).

As in the rabbits, the rapid disappearance of K from the blood in man was not due to a more rapid urinary excretion (*cf.* Fig. 4). The total urinary ex-

TABLE IV

The Urinary Excretion of Penicillins G, K, and X in Man

(Single dosage of 0.6 mg. per kg. throughout)

Penicillin species	Route of administration	Subject	Time, *hrs.*						
			½	¾	1	2	3	4	6
			Cumulative percentage of penicillin excreted						
G	Intravenous	H			85.3	92.6	94.4		95.4
		WB	48.2	71.8	87.5	96.5	101.9		
		RW		68.4	78.4	82.1	83	83.3	
	Intramuscular	W			61.5	69.8	72	72.3	73.2
		D				83.7		96.7	98.9
		BH		10.6	59.0	62.7	64.7	65.3	
		Mean.....			74.3	81.2	83.3	85.6	86.3
K	Intravenous	H*		30.5	35.9	38.3	39.0	39.2	39.3
		WB	13.6	20.4	26.4	28.9	29.7	30.1	30.6
		LB		12.2	13.7	14.6	14.8		14.8
	Intramuscular	W*			19.6	28.4	30.1	30.8	31.1
		D*			11.4		20.4	21.4	21.7
		JW		19.4	25.1	28.2	29.0		30.9
		Mean.....		20.6	22.0	27.7	27.2	27.6	28.1
X	Intravenous	T	54.6	80.1	93.1	100.1	104.8	105.9	106.4
		G	45.6	69.4	82.1	86.4	92.7	93.8	95.1
		RW		76.8	84.4	90	91.7	92.5	
	Intramuscular	S			61.5	85.1	91.2	95.6	
		JW			36.2	66.6	79.5	80	80.2
		BH		11.3		67.9	82.3	86.1	
		Mean.....			71.5	82.7	90.3	92.3	92.6

* Injected with an impure specimen in which penicillin K was said to constitute 90 per cent of the active penicillin. Other subjects in this series received a crystalline product (Pfizer).

cretion of K in six subjects varied only from 15 to 39 per cent, averaging 28 per cent, as compared with an average recovery of 86 and 93 for G and X, respectively, each tested in six subjects. As in rabbits also, the urinary excretion usually fell off sharply after the first hour, coincident with its almost complete disappearance from the blood.

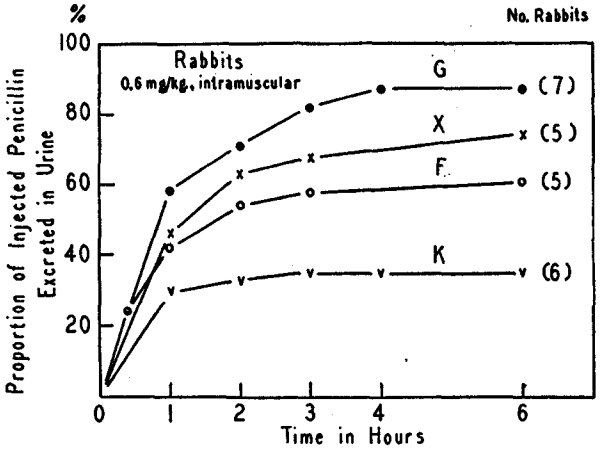

FIG. 2. The urinary excretion of penicillins F, G, K, and X in rabbits (from data of Table II).

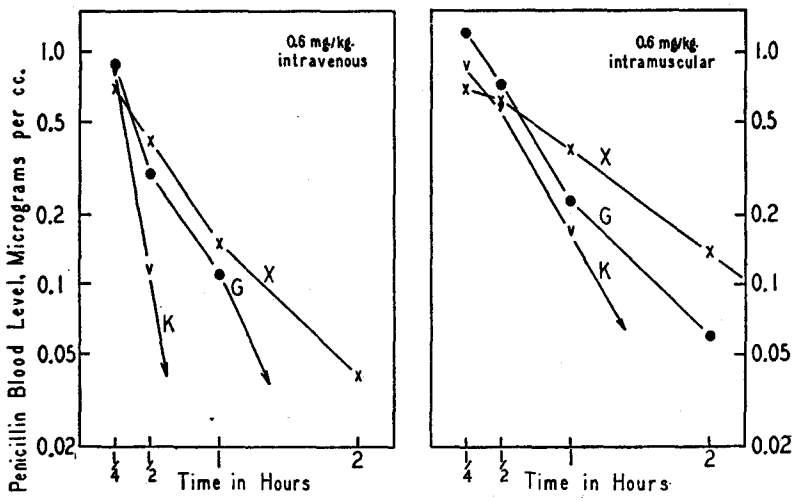

FIG. 3. The average penicillin blood levels observed in man after the intramuscular and intravenous injection of equal amounts of penicillin G, K, and X (0.6 mg. per kg.). The arrows in the figure signify that there was no demonstrable penicillin in the next time period tested. Each curve is average of three subjects similarly injected.

DISCUSSION

The rate at which penicillins F, G, K, and X disappear from the blood after their intravenous injection clearly depends on the rate at which they are excreted or destroyed. Preliminary experiments in this laboratory indicate that in both rabbits and man, the renal clearances of F, G, and X are of the

same order of magnitude. It follows that any significant differences between the magnitude of the blood levels afforded by these penicillins after their intravenous injection in equimolar amounts per kilo must reflect a varying degree of destruction *in vivo*. This is probably equally true of intramuscular injections of the aqueous solution, since there is no evidence that these penicillins differ with respect to the rate of absorption from such depots.

In both rabbits and man the highest and most sustained blood levels (minimum destruction *in vivo*) were afforded by penicillin X^1, the lowest and most evanescent (maximum destruction *in vivo*) by penicillin K, with penicillins F and G intermediate. These are precisely the relative susceptibilities of these penicillins to inactivation by serum *in vitro*, and one may reasonably postulate

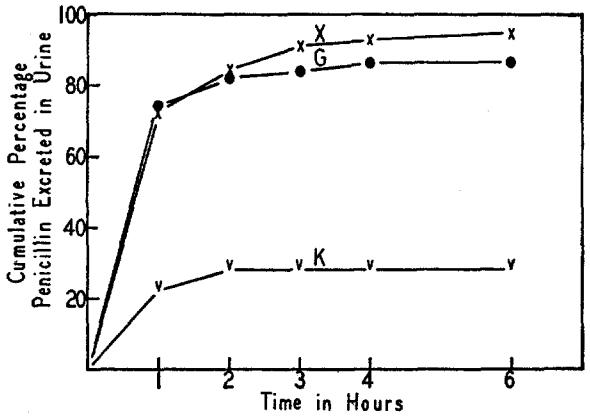

FIG. 4. Cumulative urinary excretions in man of penicillins G, K, and X (from data of Table IV). Each curve is the average of six subjects, three injected intramuscularly and three intravenously, all at 0.6 mg. per kg.

a causal relationship. What is surprising is that the differences *in vivo* were so clear cut. After intramuscular injection, penicillins F, G, and X disappeared from the blood of rabbits at the rate of approximately 83, 73, and 53 per cent per hour, far the greatest part of which reaction was due to their urinary excretion. Their inactivation in the circulating blood would be merely superimposed on this rapid excretion; and one might have anticipated difficulty in demonstrating such minor differences as that represented by inactivation rates of *e.g.* 5 and 10 per cent per hour for X and G, respectively. Similarly, although penicillin K is inactivated by serum far more rapidly than either F, G, and X, and although the rate of that inactivation accelerates as its concentration diminishes, one could not have anticipated that only 15 to 45 per cent of penicillin K would be recoverable in active form in the urine, implying the inactivation *in vivo* of 55 to 85 per cent of the amount injected, as compared with an average of less than 20 per cent for F, G, or X.

These considerations suggest that although penicillins F, G, K, and X are

inactivated by serum *in vitro*, and although they are probably similarly inactivated in the circulating blood *in vivo*, the same inactivating agent (or agents) may be present in even higher concentrations in one or more of the body organs.

The therapeutic implications of the present observations are clear. By virtue of the higher and more sustained blood levels provided by penicillin X, compared with F or G injected at the same (gravimetric) dosage, one would expect it to be significantly more active *in vivo* than is indicated by its bactericidal activity *in vitro* relative to those penicillins. Penicillin K, on the other hand, because of the evanescent blood levels observed after its injection in therapeutic dosage, should be far less active than its (high) bactericidal activity *in vitro* would imply. As is discussed in the following paper, the therapeutic activities of penicillins F, G, K, and X in streptococcal and pneumococcal infections conform to these speculations. It has already been shown by Chesney (6) and Mahoney and Arnold (7) that penicillin K is relatively inactive in the treatment of experimental syphilis.

SUMMARY

1. In both man and rabbit, penicillin X provided higher and more sustained blood levels than did penicillins F or G similarly administered in equal dosage (0.6 mg. per kg.); while penicillin K gave lower and evanescent levels.

(*a*) One hour after intramuscular injection, the blood levels in rabbits averaged 0.11, 0.18, 0.02, and 0.33 for F, G, K, and X, respectively; and levels of 0.1 mg. per kg. were sustained for 1, 1.4, 0.5, and 2.1 hours, respectively.

(*b*) In man, the blood levels of G, K, and X averaged 0.23, 0.16, and 0.37 mg. per kg. 1 hour after intramuscular injections at 0.6 mg. per kg., and 0.11, 0.02, and 0.15 mg. per kg. 1 hour after intravenous injection.

(*c*) In man, a level of 0.1 microgram per cc. was sustained for 1.6, 1.2, and 2.3 hours after the intramuscular injection of G, K, and X, respectively, and for 1, 0.5, and 1.3 hours after their intravenous injection.

2. The total urinary recovery of penicillins F, G, and X varied between 68 and 100 per cent, averaging 61, 87, and 74 per cent, respectively, in rabbits. In man, the urinary recovery of G and X averaged 86 and 93 per cent, respectively. In sharp contrast, the urinary recovery of penicillin K averaged 33 per cent in seven rabbits and 28 per cent in six human volunteers. The major portion of the penicillin appeared in the first 30 to 60 minutes. This suggests a rapid inactivation of penicillin K *in vivo*.

3. The therapeutic significance of these data is discussed in the text, and in greater detail in the following paper.

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