Removal of the ruptured follicle shortly after ovulation did not alter the response at the 19th hour in the uterus stage. The beginning of the plateau of the response curve approximately coincided with the time at which the oviducal egg became fully plumped, *i.e.*, reached its almost maximum size. The response curve was interpreted as the expression of a change in the mechanical relations between the uterus and the contained egg, rather than a change in intrinsic sensitivity due to the action of the ruptured follicle.

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Physiological Disposition of Penicillin G and K in Dogs.

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The unexpected low activity of penicillin K in experimental syphilis first observed by Chesney[1] has been confirmed.[2] In addition it has been demonstrated that penicillin K is less effective than G in the treatment of mice infected with pneumococcus Type I,[3] *Streptococcus pyogenes*[3] and *Borrelia novyi*.[4,5]

This low activity of penicillin K has been correlated with an apparently low plasma concentration and it has been suggested that this is a result of more rapid destruction in the animal body.[3,6] A more detailed study of the disposition of penicillin G and K in dogs has led us to a somewhat different conclusion, and has cast considerable doubt on the supposition that inactivation plays a major part in the immediate fate of injected penicillin K.

Recovery of Added Penicillin G and K from Body Fluids and Tissues. The recovery of added penicillin from various biological materials was studied by the customary cup test procedure of assay for penicillin concentration. Known amounts of each penicillin* were added to body fluids and to tissue mashes, and the actual assay figures in each case were related to the assay figures of the same concentration of penicillin added to saline.

Plasma was obtained by centrifuging oxalated or heparinized dog blood. Tissue mashes were prepared with a homogenizer, using 2 parts of saline for one of muscle, and equal parts of saline and tissue in all other cases. The grinder was immersed in ice water during the preparation of each tissue. One-tenth of a volume of an appropriate saline solution of penicillin was added to a series of samples of tissue mashes, plasma, and saline. Final concentrations of penicillin ranged from 0.3 μg per ml to 24 μg per

1 Unpublished observations quoted by Eagle and Musselman, *Science*, 1946, **103**, 618.

2 Rake, G., Dunham, W., and Donovick, R., to be published.

3 Eagle, H., and Musselman, A., *Science*, 1946, **103**, 618.

4 Burk, M., Farr, A. C., and Schnitzer, R. J., *Science*, 1946, **104**, 370.

5 Richardson, A. P., Loeb, P., and Walker, H. A., unpublished observations.

6 Coghill, R. D., Osterberg, A. E., and Hazel, G. R., *Science*, 1946, **103**, 709.

* We are indebted to Drs. Wintersteiner and Adler of the Division of Organic Chemistry, and Mr. Lott and Mr. Dolliver of the Division of Medicinal Chemistry for these preparations. Elementary analysis and bioassay values for both penicillins agreed well with theoretical values. Analysis by Craig distribution methods showed the G preparation to be at least 92% homogeneous, the remainder consisting of inactive components. The K preparations were at least 88% of "K type" penicillins, not more than 10% of an active fraction which was probably an "F type" penicillin, and the remainder was an inactive component.

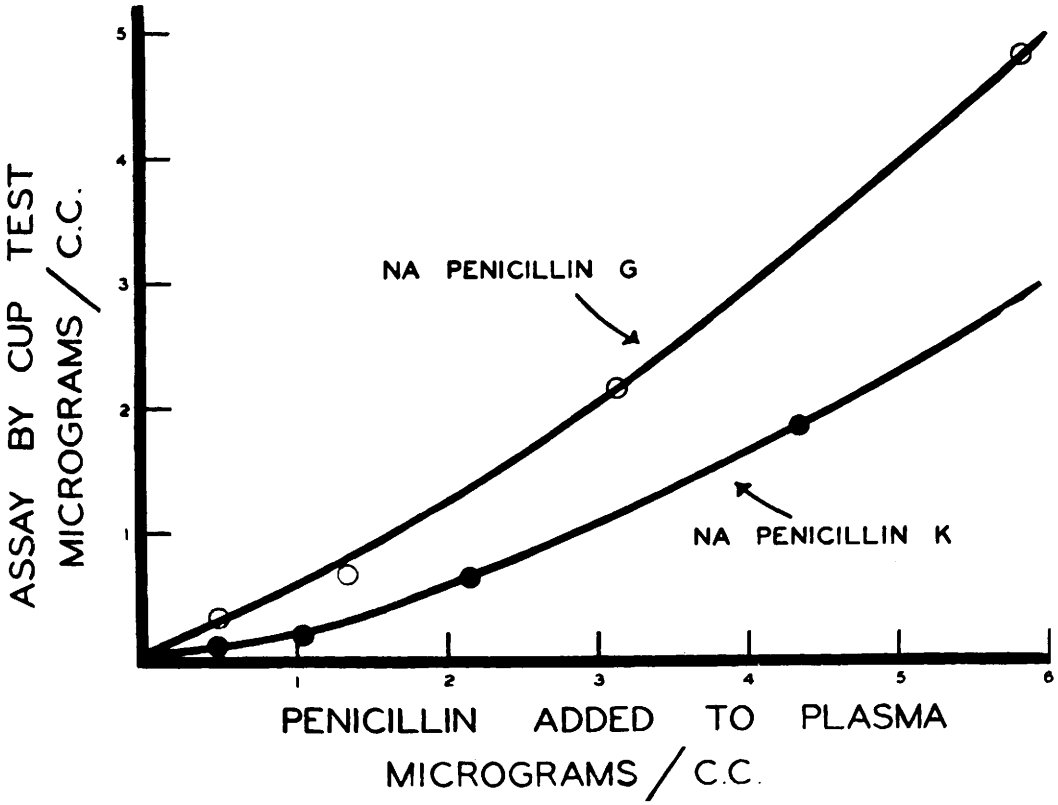

FIG. 1.
Recovery of Penicillin G and K from dog plasma, when assayed by the cup test procedure. Each curve represents an average of six experiments carried out on different days.

ml. The assays were then carried out by the usual cup test procedure.†

The recovery of penicillin added to saline was within 10% of the predicted concentration in all cases. With all tissues and plasma, the recoveries were incomplete and the extent of the loss was inversely proportional to the concentration of penicillin. Fig. 1 summarizes a portion of 6 typical experiments obtained with plasma, and is presented because it shows a marked difference in behavior of the 2 penicillins when added to this body fluid. It is quite obvious that cup test assays of penicillin K in plasma are subject to considerable error, unless a correction is made for incomplete recovery. Proof that the incomplete recoveries were due to the assay procedure used and not due to destruction of penicillin K was obtained by 2 experiments.

1. Simultaneous assay of representative samples of penicillin-plasma mixtures by the tube dilutions technics gave complete recoveries. For example, in one experiment in which 1.25 μg per ml of K was added to plasma, cup test assays gave 20% recovery, whereas tube dilutions assays gave 100%

TABLE I.
Average Per Cent Recovery of Penicillin G and K from Dog Plasma and Tissue Mash by Cup Test at Different Concentrations.

	5 γ/ml		1 γ/ml	
	G	K	G	K
Plasma	83.0	42.5	65.0	17.1
Liver	63.5	64.5	45.0	28.2
Kidney	62.5	55.5	35.0	25.0
Spleen	75.0	50.0	45.0	42.0
Brain	87.0	78.0	60.0	37.5
Muscle	73.0	80.0	60.0	54.0
Lung	54.0	66.0	45.0	44.0

† Bioassays reported in this study were carried out under the general supervision of the Division of Microbiology.

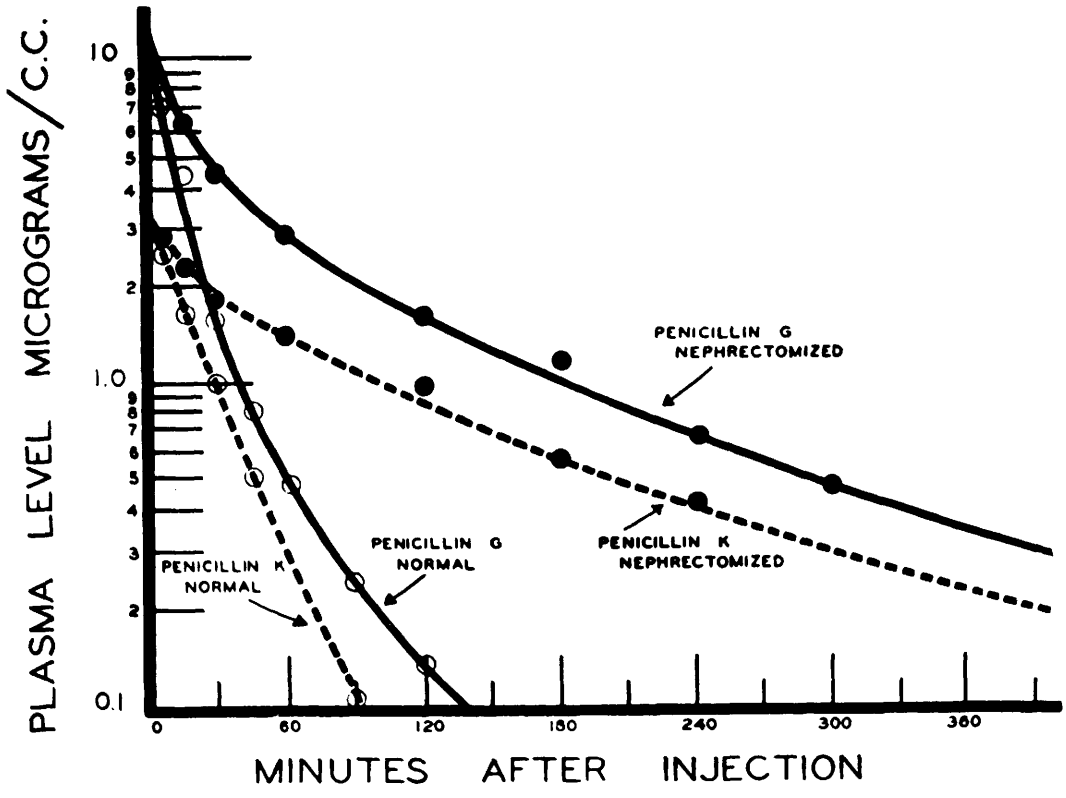

FIG. 2.
The rate of disappearance of penicillin G and K from the plasma of dogs following intravenous injection of 2 mg per kilo. Each curve is the mean of experiments in 6 dogs. All figures have been corrected for error in the cup test assay.

recovery.

2. Incubation of plasma-penicillin mixtures for 3 hours at 22°C or 24 hours at 4°C gave the same recovery as when the penicillin was added immediately preceding assay. Thus if destruction had been responsible for the low cup test assay it must have occurred immediately and then stopped within a few minutes.

The results of all recovery experiments with plasma and tissues are summarized in Table I. The per cent recovery for each material was obtained by plotting added penicillin against assay results in a form similar to Fig. 1. Per cent recovery of the 2 concentrations was determined from the curves thus obtained. All results reported later in this paper have been corrected by the use of such curves to give "true" penicillin concentration.

Rate of Disappearance of Penicillin G and K from the Plasma of Normal and Nephrectomized Dogs. The rate of disappearance of a drug from the plasma of an intact animal is the net result of distribution, excretion and destruction. In the case of penicillin it is well recognized that rate of disappearance is conditioned largely by renal excretion. Therefore, the rate of fall of plasma-penicillin in a nephrectomized dog should reflect distribution, destruction and extrarenal excretion.

Experiments were set up as follows: each dog was injected intravenously with 2 mg per kg of the appropriate penicillin. Blood samples were taken at short intervals of time until the plasma concentration approached zero. The following day a nephrectomy was performed, and after 24 hours recovery, the same dose of the same penicillin was injected intravenously and rate of disappearance from plasma again determined. Six

TABLE II.
Distribution of Penicillin G and K Between Plasma and Tissues of Dogs Following Continuous Intravenous Injection.

Dog No.	Plasma level, γ/ml		Plasma-tissue ratios after 2 hrs infusion						
	After 1 hr Infusion	After 2 hr Infusion	Cerebrospinal Fluid	Brain	Muscle	Spleen	Lung	Liver	Kidney
			Penicillin G—0.1 mg/kilo/minute.						
25	13.8	23.5	151.0	47.0	5.2	5.9	2.1	3.3	0.25
26	15.0	24.7	185.0	62.0	5.1	9.5	2.0	2.7	1.1
27	11.2	16.0	191.0	32.0	4.4	3.8	1.4	3.6	0.42
28	17.3	14.6	91.0	18.4	6.1	6.1	2.1	1.4	0.43
29	24.3	28.1	220.0	56.0	5.9	4.5	2.7	2.7	0.64
30	15.1	16.7	230.0	69.0	10.6	5.2	2.4	1.9	0.46
Mean	16.1	20.6	176.0	47.4	6.2	5.8	2.1	2.4	.55
			Penicillin K—0.2 mg/kilo/minute.						
31	12.5	18.3	62.0	18.3	4.7	4.6	2.7	1.8	0.55
32	17.5	24.8	220.0	20.6	8.3	9.6	4.8	1.9	0.91
33	13.6	13.5	151.0	34.0	6.1	9.1	3.2	1.1	0.52
34	12.6	13.5	135.0	22.5	6.4	8.5	3.8	1.2	0.81
35	19.5	27.0	270.0	45.0	*	*	*	1.2	0.82
Mean	15.1	19.4	167	28.1	6.4	7.9	3.8	1.4	0.72

* Not determined.

dogs were used for each penicillin. Scrutiny of the data (Fig. 2) shows a number of points of interest:

1. In as short a time as 5 minutes after injection the plasma level of penicillin K is less than one-half that seen when the same dose of G was injected. This finding suggests that K is either destroyed within 5 minutes or some other factor is responsible for the disposition of K as compared to G.

2. From a given plasma level the rate of disappearance of the 2 penicillins is not greatly different. If destruction had been a major factor in determining the fate of one penicillin as compared to another, the slope of these curves should have been different. Such was not the case.

3. Extending the time-plasma level curve back to zero permits a calculation of the apparent volume of distribution of each penicillin. For G this was estimated as 20.2% or corresponds roughly to total blood volume plus extra-cellular tissue fluid. In the case of penicillin K the apparent volume of distribution was 57.1% which at once suggested localization in tissues or some body fluid, other than blood.

Distribution of Penicillin G and K in Body

TABLE III.
Distribution of Penicillin G and K Between Dog Plasma and Saline After 20 Hr Dialysis at 4°C.

Concentration added to saline γ/ml	No. of exp.	Mean concentration after dialysis—γ/ml		Apparent % "bound"
		Plasma	Saline	
		Na Penicillin G.		
3.0	8	3.6	2.3	46.7
30.0	6	46.3	28.6	36.1
		Na Penicillin K.		
2.3	9	3.6	1.0	71.0
23.0	6	39.1	11.2	70.6

Fluids and Tissues After Continuous Infusion. Since calculation of the apparent volume of distribution of penicillin K suggested localization, it was obviously necessary to determine the distribution of the 2 penicillins in tissues. There are only a few data available in the literature on the distribution of penicillin in tissues of animals, and in all cases the studies[7,8] have been carried out with impure preparations. In our studies dogs were lightly anesthetized with sodium pentobarbital following which a cannula was inserted into a femoral vein and attached to a burette, so that an appropriate concentration of penicillin could be infused at a constant rate of 1 ml per minute. Plasma concentration was determined at the end of one hour and again at 2 hours, following which infusion was discontinued, the animal was killed and representative tissues removed and prepared for assay as described above. Each penicillin was administered in a dose which was calculated to maintain the plasma concentration between 15-20 μg per ml for the period of infusion so as to obtain equilibrium between plasma and tissues. To accomplish this, the rate of infusion of G was maintained at 0.1 mg per kg per minute. For K, 0.2 mg per kg per minute was required.

Results of these experiments are summarized by Table II. As was expected kidney had the highest concentration, whereas brain and spinal fluid had the lowest levels. Lung and liver had higher concentrations than were predicted on the basis of blood and tissue fluid content, thus suggesting some localization. The most striking difference in distribution of the 2 penicillins was noted in the liver assays. The concentration of K in this organ approached the concentration in plasma, and was nearly twice as high as that of G.

Adsorption of Penicillin G and K by Dog Plasma. The error in cup test assays of penicillin K in plasma described above indicated the likelihood of binding by some constituent of plasma. A cellophane bag containing 7.5 ml of plasma was suspended in a dialysis tube and equilibrated with 15 ml of saline containing a desired concentration of penicillin. Dialysis was carried out on a rocking machine at 4°C for 20 hours, following which penicillin concentration in each fluid was determined.

The results summarized in Table III show that in all instances the concentration of penicillin in plasma was higher than in the corresponding saline solution. Per cent of "bound" penicillin was calculated by the usual method of subtracting concentration in saline from concentration in plasma and dividing by total plasma concentration. Interpretation of the data is difficult because of an apparent high recovery of both penicillins in these experiments, which could not be explained by irregularities in the assay procedure. In spite of this there is little question but that K was bound to a far greater extent than G; thus furnishing a reasonable explanation for the difficulty of determining true concentration of penicillin K in plasma by the cup test procedure.

Discussion. The foregoing experiments leave little doubt as to the fact that penicillin K is handled by the intact animal in a quantitatively different manner than G. The fundamental basis for these differences is not clear at the moment in terms of the commonly recognized chemical properties of the 2 penicillins. However, data on the physical characteristic of these penicillins are not now available, and it may be that information along these lines will clarify the problem.

Regardless of the explanation of the differences in properties of these 2 penicillins, K has an apparent disadvantage for the treatment of systemic infections. This arises from the greater difficulty in maintaining a given total plasma concentration, and because it is likely that adsorption decreases the effective plasma level. Cup test assays may give a misleading picture of total penicillin K content in plasma. However, they may well give a truer indication of the concentration of "free" penicillin available for therapeutic effect.

[7] Stuble, G. G., and Bellows, J. G., *J. A. M. A.*, 1944, **125**, 685.

[8] Cutting, W. C., Luduena, F. P., Fiese, M., Elliot, H. W., and Field, J., *J. Pharm. and Exp. Therap.*, 1945, **85**, 36.

Summary. 1. As determined by the customary cup test procedure it is not possible to obtain complete recoveries of penicillin G and K when added to plasma and tissues. A considerable error is involved in the assay of penicillin K in the presence of a high concentration of plasma. Such low recoveries have been shown not to be due to destruction of penicillin K. 2. For a given dose, the plasma level of penicillin K 5 minutes after intravenous injection is less than one-half that with penicillin G. The rate of disappearance from a given plasma concentration, however, is not greatly different for the 2 penicillins. 3. The apparent volume of distribution of G is 20.2% of the body weight, whereas with K it is 57.1%. Both of the penicillins are localized in lung and liver to some extent. Penicillin K is localized to a considerably greater extent in the liver than is G. 4. As determined by dialysis experiments penicillin K is adsorbed by some component of dog plasma to a much greater extent than is penicillin G.

15656

Subtilin-Antibiotic Produced by *Bacillus subtilis*.* V. Effect on *Streptococcus pyogenes* Infections in Mice.†

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In a previous communication[1] subtilin was found to exert a pronounced antibiotic action against a large number of species of Gram-positive organisms, including *Streptococcus pyogenes* (hemolytic) by the agar cup-plate procedure. The agent was shown to be bacteriostatic in high dilution and bactericidal in more concentrated solution. Subtilin exhibited an extremely low toxicity index to living embryonic chick heart tissue fragments cultivated *in vitro*[2] and exerted a powerful *in vivo* action on the course of experimental pneumococcus infections in mice[3] and anthrax in guinea pigs.[4]

TABLE I.
Treatment of *Streptococcus pyogenes* Infections in Mice.

		6 mice used in each group				
Date	Time	Control	Treated immediately	Treated after 3 hr	Treated after 6 hr	Treated after 9 hr
8-13-46	9 a.m.	Infected	Infected	Infected	Infected	Infected
"	9 "	—	0.5 cc subtilin	—	—	—
"	12 m.	—	" " "	0.5 cc subtilin	—	—
"	3 p.m.	—	" " "	" " "	0.5 cc subtilin	—
"	6 "	—	" " "	" " "	" " "	0.5 cc subtilin
"	11 "	All dead	1.0 " "	1.0 " "	1.0 " "	1.0 " "
14	9 a.m.	—	" " "	" " "	" " "	" " "
"	12 m.	—	" " "	" " "	" " "	" " "
"	4 p.m.	—	" " "	" " "	" " "	" " "

* This investigation was aided by a grant from Eli Lilly and Co., Indianapolis, Indiana.

† The subtilin preparation used in these experiments was kindly supplied by the Western Regional Research Laboratory, Albany, Calif.

1 Salle, A. J., and Jann, Gregory, J., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 60.

2 Salle, A. J., and Jann, Gregory J., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 23.

3 Salle, A. J., and Jann, Gregory J., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 40.

4 Salle, A. J., and Jann, Gregory J., PROC. SOC. EXP. BIGL. AND MED., 1946, **63**, 41.