JCI The Journal of Clinical Investigation

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J Clin Invest. 1949;28(5):821-825. https://doi.org/10.1172/JCI102159.

Research Article

THE ELIMINATION OF PENICILLIN G IN BILATERALLY NEPHRECTOMIZED DOGS [1, 2]

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Rammelkamp and Keefer (1) found that only about 60 per cent of intravenously administered penicillin could be recovered from the urine of man. This finding has been confirmed by later reports, although the recovered amounts vary from 40 to 99 per cent (2).

This paper deals with the mechanism of the elimination of penicillin in bilaterally nephrectomized dogs.

Diffusion of Penicillin from Blood to Tissues

A dog, in which the elimination of penicillin [5] had been blocked by bilateral nephrectomy and ligation of the cystic and common bile ducts, was given an intravenous injection of 25,000 units/kg. of crystalline penicillin G. Blood samples were taken from another vein at intervals from two minutes to 10 hours after the injection. The serum concentrations of penicillin were determined using a serial dilution technique.

In Figure 1, the serum penicillin concentrations have been plotted on a logarithmic scale as a function of time after the injection. The penicillin concentration decreases rapidly during the first hour but thereafter very slowly. For the time being we shall consider only the first portion of the curve. We may assume that this initial decrease was due to diffusion of penicillin from the blood stream into the tissues.

One can imagine this process as taking place through a thin membrane from one phase to another. At the time $t = 0$, all penicillin is in the one phase having the concentration c_0. If the concentration in this phase is c_t at the time t and c_∞ when equilibrium is established we have:

$$\ln (c_t - c_\infty) = \ln (c_0 - c_\infty) - Kt\frac{c_0}{c_0 - c_\infty}, \qquad (1)$$

where ln denotes the natural logarithm and K is the velocity constant.

From the data in Figure 1, K is found to be 5 1/hours. The curve in the figure is drawn according to equation (1), this value being used for K and allowance being made for the inactivation in the later part of the experiment. Agreement is found between the calculated curve and the experimental points. The value, 5 1/hours, for K was confirmed in another experiment of similar nature as the one depicted in Figure 1.

If there were no further elimination of penicillin from the blood, the concentration would decrease to a constant value which would be reached when diffusion equilibrium was established. This concentration may be found by drawing the straight line representing the inactivation back to intersection with the axis of the ordinate which, in this case, was 143 units/ml. of serum.

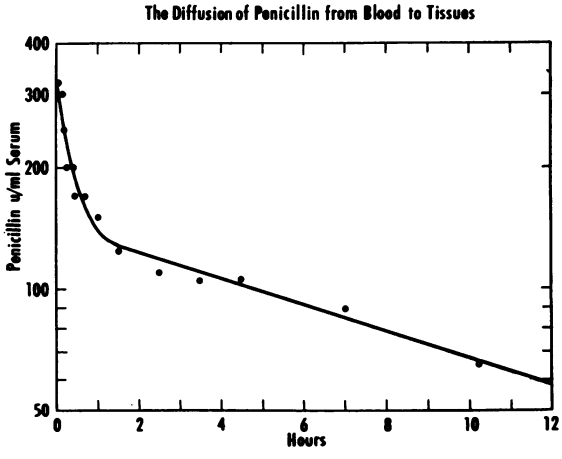

FIG. 1. THE SERUM PENICILLIN CONCENTRATION AS A FUNCTION OF THE TIME AFTER A SINGLE INTRAVENOUS INJECTION

The inactivation has been partially blocked by bilateral nephrectomy and ligation of the cystic and common bile ducts.

[1] Presented at the Second National Symposium on Recent Advances in Antibiotics Research held in Washington, D. C., April 11–12, 1949, under the auspices of the Antibiotics Study Section, National Institutes of Health, Public Health Service, Federal Security Agency.

[2] This investigation was supported (in part) by a research grant from the Antibiotics Study Section of the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service and (in part) by the A. B. Kuppenheimer Foundation and the Douglas Smith Foundation of the University of Chicago.

[3] Research Fellow, U. S. Public Health Service.

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[5] The crystalline penicillin was supplied by Abbott Laboratories, Commercial Solvents Corporation, Lederle Laboratories, and Schenley Laboratories, Inc.

The equilibrium concentration was determined in a number of experiments and collected in Table I. In the last column of this table are the ratios between c (serum concentration of penicillin at equilibrium) and the dose of penicillin per gram of dog. In 16 experiments this ratio was found to be larger than unity; that is, the penicillin did not distribute equally throughout the tissues of the dog; but was present in a higher concentration in the plasma than in the remainder of the dog. The average ratio was about four times as great, when diffusion equilibrium was established. The reason for this is not clear.

TABLE I

The serum concentration (c_∞) of penicillin after diffusion equilibrium has been established with the tissues

Values are corrected for any elimination or inactivation of penicillin taking place.

Dose units/kg.	c_∞ units/ml.	c_∞ units/ml. dose units/gram of dog
85,000	400	4.7
80,000	250	3.1
30,000	130	4.3
25,000	90	3.6
25,000	70	2.8
25,000	80	3.2
25,000	100	4.0
25,000	85	3.4
25,000	100	4.0
25,000	110	4.4
25,000	80	3.2
25,000	100	4.0
25,000	140	5.6
14,000	60	4.3
14,000	60	4.3
7,000	20	2.9
Average		3.9
Maximum		5.6
Minimum		2.8

From Table I we obtain the formula:

Serum penicillin concentration = 4
× amount of penicillin per gram body weight. (*2*)

—provided that diffusion equilibrium is present. This equation shows the relationship between the amount of penicillin present in the body and the serum concentration. This could be used to estimate the former when the latter is known. The formula has been established for the blood concentrations between 20 and 400 units/ml. and may not be valid for smaller concentrations.

The Renal and Extra-Renal Elimination

Blood serum concentrations of penicillin were determined at intervals after a single intravenous injection in a number of normal dogs. If allowance is made for the diffusion equilibrium to be established, it was found that the logarithm of the concentration decreased as a linear function of time (first order reaction). This relationship was found to be valid from the initial concentration of about 100 units/ml. to 1 unit/ml. A curve demonstrating this relationship is shown in Figure 2.

Bilateral nephrectomy was performed in three dogs which were then given a single intravenous injection of penicillin. The relationship between serum concentration and time showed that these dogs were still able to eliminate penicillin from the blood stream, although, as was to be expected, at a considerably lower rate (Figure 2). Here also, the inactivation took place as a reaction of the first order. The rate of elimination was, as an average, found to be one-fourth as rapid in the nephrectomized as in the normal dogs. This means that approximately three-fourths of the penicillin was eliminated through the kidneys and one-fourth somewhere else. From the variable velocities obtained in normal and nephrectomized dogs, a considerable variation is to be expected in the fraction of penicillin inactivated extra-renally.

The Site of the Extra-Renal Elimination

The rate of elemination was not significantly changed by the removal of the intestine from the

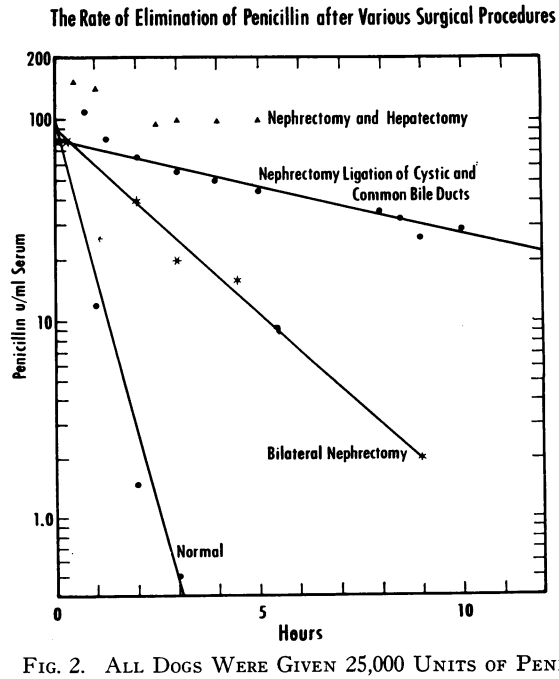

FIG. 2. ALL DOGS WERE GIVEN 25,000 UNITS OF PENICILLIN PER KILOGRAM IN A SINGLE INTRAVENOUS INJECTION

ligament of Treitz to within an inch of the anus, nor by the removal of the stomach and ligation of the common bile duct. This showed that, in these dogs, the intestinal tract was not the primary site of the extra-renal elimination, as suggested by the findings of Reid (3).

The serum penicillin concentration remained constant, within the limits of experimental error, following complete evisceration; thus the site of the elimination was somewhere in the viscera.

A two stage hepatectomy and bilateral nephrectomy were performed in a dog. Here too, no inactivation could be shown after the equilibration of penicillin between the blood stream and the tissues. In another dog, both kidneys and all the viscera except the liver were removed. The hepatic artery was left intact by dissecting away the hepatico-duodenal ligament. The portal vein and the common bile duct were ligated and the gall bladder left in connection with the biliary system. This dog eliminated penicillin at a rate very similar to the dogs in which only bilateral nephrectomy was done. It could be concluded that the liver was responsible for the extra-renal elimination of penicillin in these dogs.

The velocity constants were found from the obtained curves by the formula:

$$K = -\frac{\ln (c_{t_1}/c_{t_2})}{t_1 - t_2}. \qquad (3)$$

The results, in reciprocal hours, are found in Table II. The velocity constant indicates the relative decrease in penicillin concentration, or total amount of penicillin, per hour.

TABLE II

The velocity constant indicating the relative decrease in penicillin concentration in the serum after various surgical procedures

Procedure	K 1/hrs.	Average
Normal dogs	1.85 1.90 2.40 1.90 1.25 1.15 1.45	1.70
Nephrectomized	0.46 0.38 0.40	0.41
Nephrectomized and the intestine removed from the ligament of Treitz to within an inch of the anus	0.33 0.35	0.34
Nephrectomized, bile duct ligated and complete gastrectomy	0.40	
Eviscerated, except for the liver	0.53	
Complete evisceration	0.0	
Hepatectomy, nephrectomy	0.0	
Cystic and bile ducts ligated; nephrectomized	0.07	
Bile duct cannulated—excreted in bile	0.31 0.25	
Inactivation in bile at $-\log cH+ 8.5$, 40° C.	0.10	

The Mechanism of the Hepatic Elimination of Penicillin

In two dogs the kidneys were removed, the cystic and common bile ducts were ligated twice and cut. Similar curves were found in both dogs; one curve is shown in Figure 3. It was noted that a certain elimination of penicillin seemed to occur during the first 10 hours; however, the rate of elimination decreased after this to a very low value. Thus, penicillin was still present in appreciable amounts 70 hours after the intravenous injection. The final rate of elimination here was only 4 per cent of the normal rate. This finding seemed to indicate that the penicillin was excreted in the bile rather than destroyed by the liver; if

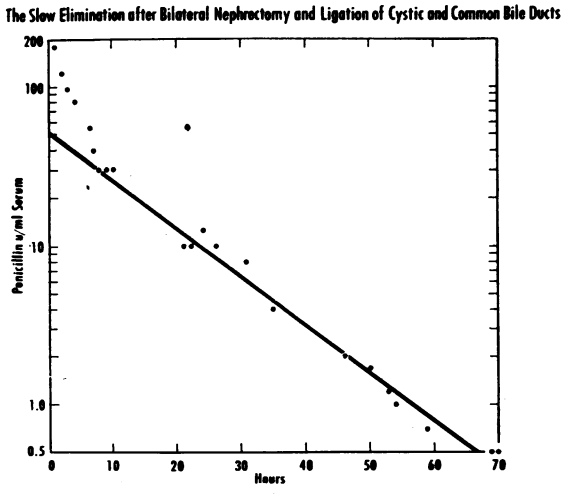

FIG. 3. THE ELIMINATION DURING THE FIRST 10 HOURS MAY BE DUE TO EXCRETION OF PENICILLIN CONTAINING BILE INTO THE BILIARY SYSTEM TO THE POINT OF DISTENTION

The slower inactivation throughout the 70 hours of the experiment can be explained by the destruction of penicillin due to the alkalinity of the bile.

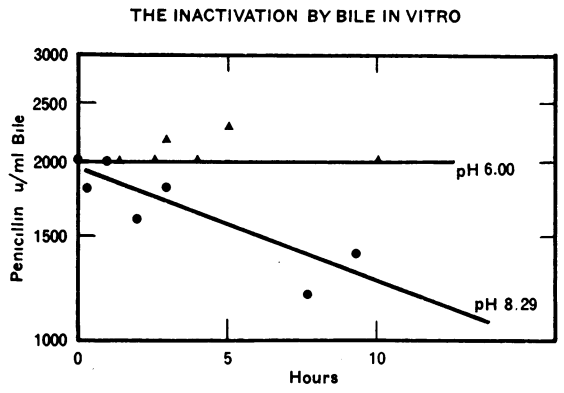

FIG. 4. THE LINES SHOW THE INACTIVATION, AT THE GIVEN pH VALUES AS CALCULATED FROM THE DATA OF BRODERSEN (4), IN GOOD AGREEMENT WITH THE EXPERIMENTAL POINTS

The two samples of bile were obtained from the gall bladder (pH 6.00) and from a common duct fistula (pH 8.29).

The pH values were determined electrometrically by comparison with 0.01 m. HCl + 0.5 m. NaCl (pH 2.00). The hydrogen ion concentrations were found to be independent of temperature in the range of 20 to 40° C.

so, the initial drop in serum concentration might be due to excretion of bile into the biliary system to the point of distention. However, it appeared still possible that destruction may have taken place in the liver, but that liver function was impaired by the biliary obstruction.

In another nephrectomized dog, the common bile duct was cannulated and the secreted bile collected hourly after an intravenous injection of penicillin. Very high concentrations of penicillin were found in the bile samples. The total amount recovered in the bile was 80 per cent of the injected penicillin. It could be concluded that, at least in this dog, the main part of the extra-renal elimination took place as excretion in the bile.

Inactivation by Bile In Vitro

A sample of dog's bile (pH 8.29), obtained from a fistula, was mixed with penicillin and incubated at 37° C. A rather slow inactivation was found to take place. Another sample drawn from the gall bladder (pH 6.00) did not inactivate penicillin at a demonstrable rate. This suggested that the inactivation was due to the alkalinity of the fistula bile. The rate of inactivation to be expected in this case was calculated (4) and found to be consistent with the experimental values (Figure 4). The rate of inactivation in the bile can thus be calculated at known values of temperature and hydrogen ion concentration on the basis of the known destruction rate by alkali.

At the highest pH values found in the bile (8.65), the rate of inactivation found in the dogs with ligated bile ducts (Figure 3) could readily be accounted for as taking place in the bile standing in the intra-hepatic ducts.

The Relationship of Penicillin Concentrations in Serum and Bile

In Figure 5 are plotted the concentrations of penicillin in cannulated bile and in blood serum taken simultaneously from a nephrectomized dog. There was a constant ratio between the two concentrations, the bile containing 130 times as much penicillin per ml. as the serum at serum concentrations ranging from 100 to 5 units/ml. This ratio was found to vary considerably. In seven experiments carried out in two dogs, values ranging from 10 to 330 were obtained. From the figures in Table III it is seen that the high ratios generally were found at high pH values in the bile and the low ratios at the low pH values.

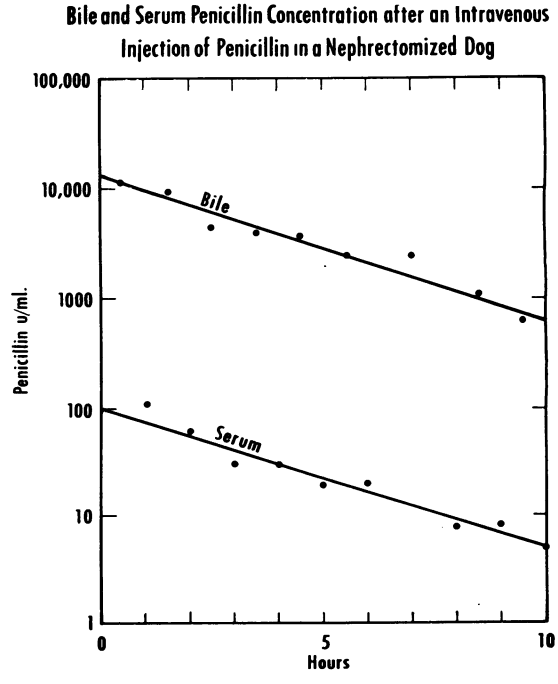

FIG. 5. THE BILE IS SEEN TO CONTAIN ABOUT 130 TIMES AS MUCH PENICILLIN PER MILLILITER AS THE SERUM, INDEPENDENT OF THE SERUM CONCENTRATION

TABLE III

The correlation between bile pH and the bile penicillin-serum penicillin ratio

pH of bile	Penicillin concentration of bile / Penicillin concentration of serum
8.65	330
8.50	130
8.48	270
8.29	10
8.20	16
8.04	11
7.93	11

Storage of Penicillin in the Gall Bladder

In a normal dog, a cannula was placed in the gall bladder and the common bile duct ligated in order to close the outlet from the gall bladder. The bile was drawn from the gall bladder, mixed with 100,000 units of penicillin and reinjected. Every hour the volume of bile in the gall bladder was determined and a small sample taken out for penicillin assay. The total amount of penicillin found in the gall bladder is shown in Figure 6. The pH values were determined at the same time and from these the spontaneous inactivation calculated as shown by the curve. The difference between this curve and the points demonstrates the resorption from the gall bladder. Within the four hours of the experiment the resorbed amount is seen to be rather small. That a certain resorption does take place was shown by the fact that penicillin was present in the urine of the dog.

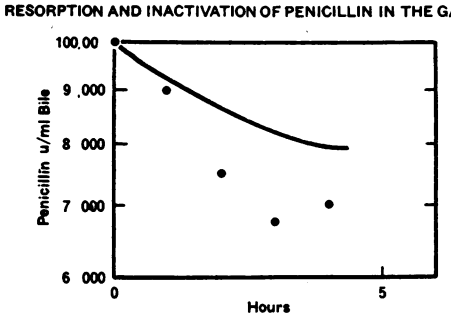

FIG. 6. THE CURVE INDICATES THE CALCULATED INACTIVATION

The experimental points are seen to deviate relatively little from this curve, indicating that only a slow resorption of penicillin takes place from the gall bladder.

SUMMARY AND CONCLUSIONS

Bilaterally nephrectomized dogs were found to eliminate penicillin at a considerable rate from their blood stream after an intravenous injection. This process took place even if the intestines were removed, whereas no inactivation could be demonstrated after complete evisceration or hepatectomy. A major part of the penicillin was found to be excreted in the bile. A certain inactivation took place in the bile, especially when it was strongly alkaline. The remaining portion went either to the gall bladder, where only a slow resorption took place, or to the intestine, where it was partially resorbed and partially inactivated.

Caution must be exercised in drawing generalized conclusions from these results. Since the nephrectomized dogs do not eat, it seems possible that normal dogs might inactivate certain amounts of penicillin in their intestine because of a greater excretion of digestive juices.

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