of Mayer who found that "pyribenzamine, a substance exhibiting strong and specific antihistamine properties, exerts a definite activity in experimental dermatitis".[3]

The localization and concentration of trypan blue following an intravenous injection, in areas of skin injected intradermally with antihistamine emphasizes the fact that substances other than histamine may cause a localization of this dye.

Summary The hyperemia that follows the local application of xylene apparently is not modified by the intravenous and intraperitoneal injections of the antihistamine preparations, Pyrrolazote and Thenylene. Likewise, the localization and concentration of trypan blue in the xylene treated areas of skin are not affected by these preparations of antihistamine. Trypan blue also localizes and concentrates in areas of skin injected intradermally with these preparations of antihistamine.

Received July 5, 1949. P.S.E.B.M., 1949, **71.**

17282. Studies on Elimination of Penicillin G in Dogs.*

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Rammelkamp and Keefer[1] found that only about 60% 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%.[2]

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

Methods. Healthy mongrel dogs weighing from about 6 to 12 kg were used. Crystalline penicillin G§ was injected intravenously in all experiments; the blood samples were obtained from another vein and the serum penicillin assayed against Staphylococcus 209 by a serial dilution technic.

Diffusion of Penicillin from Blood to Tissues. Results. A dog which had been bilaterally nephrectomized and the cystic and common bile ducts ligated was given an intravenous injection of 25,000 u/kg of crystalline penicillin G. The penicillin concentration decreased rapidly during the first hour but very slowly thereafter (Fig. 1). It is assumed that this initial decrease was due to

The diffusion of penicillin from blood to tissues

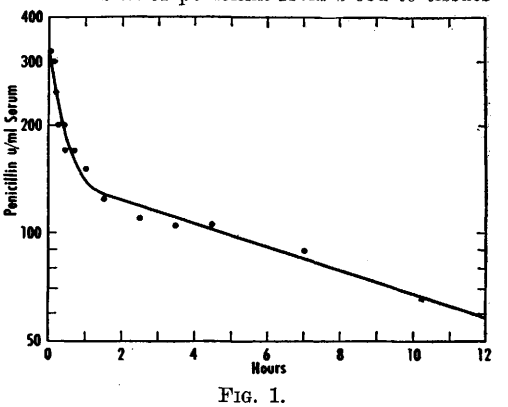

FIG. 1.

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.

* 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.

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1 Rammelkamp, C. H., and Keefer, C. S., *J. Clin. Invest.*, 1943, **22**, 425.

2 Herrell, W. E., Penicillin and Other Antibiotic Agents, W. B. Saunders Company, 1945.

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

TABLE I.

Dose units/kg	c ∞ units/ml	c ∞ units/ml dose units/g 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	

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.

diffusion of penicillin from the blood stream into the tissues.

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 u/ml of serum.

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. This means that 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 4 times as great when diffusion equilibrium was established. This relationship can be expressed as:

Serum penicillin concentration = 4 x amount of penicillin per gram body weight. The formula has been established for the blood concentrations between 20 and 400 u/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. When allowance was 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 u/ml to 1 u/ml. A curve demonstrating this relationship is shown in Fig. 2.

Bilateral nephrectomy was performed in 3 dogs which were then given a single intravenous injection of penicillin. These dogs were still able to eliminate penicillin from the blood stream, although at a considerably lower rate (Fig. 2). The average rate of elimination was found to be ¼ as rapid in the nephrectomized as in the normal dogs. This indicates that approximately three-fourths of the penicillin was eliminated through the kidney and one-fourth elsewhere. From the variable velocities obtained in normal and nephrectomized dogs, a considerable variation was to be expected in the fraction of penicillin inactivated extra-renally.

The rate of elimination of penicillin after various surgical procedures

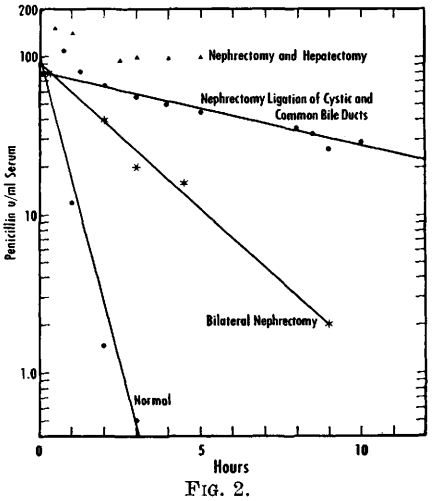

FIG. 2.

All dogs were given 25,000 units of penicillin per kilogram in a single intravenous injection.

Site of the Extra-Renal Elimination. The rate of elimination was not significantly altered by the removal of the intestine from the 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]

Following complete evisceration the serum penicillin concentration remained constant, within the limits of experimental error; 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 by these dogs. The velocity constants indicating the relative decrease in penicillin concentration, or total amount of penicillin per hour, are shown in Table II. The velocity constants were found from the curves by the formula:

$$K = \frac{\ln (c_{t1}/c_{t2})}{t_1 - t_2}$$

Mechanism of Hepatic Elimination of Penicillin. In 2 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 Fig. 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 was only 4% of the normal rate. This finding seemed to indicate that the penicillin was excreted in the bile rather than destroyed by the liver. If 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 still appeared possible that destruction might 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% of the injected penicillin. It could be

TABLE II.
The Velocity Constant Indicating the Relative Decrease in Penicillin Concentration in the Serum After Various Surgical Procedures.

Procedure	K 1/hr	Avg
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 one 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	

[3] Reid, R. D., Felton, L. C., and Pittroff, M. A., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 438.

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 common duct 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 (Fig. 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.

The slow elimination after bilateral nephrectomy and ligation of cystic and common bile ducts

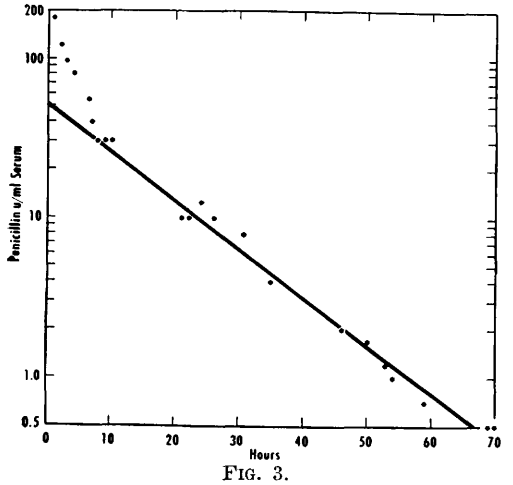

FIG. 3.

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.

The inactivation by bile *in vitro*

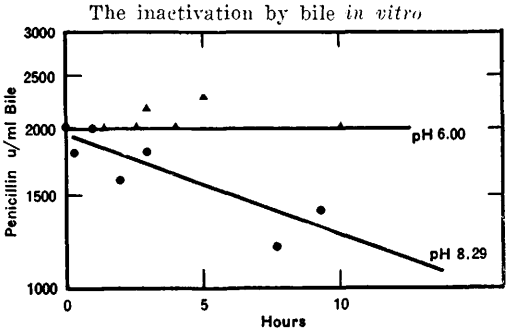

FIG. 4.

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.

Bile and serum penicillin concentration after an intravenous injection of penicillin in a nephrectomized dog

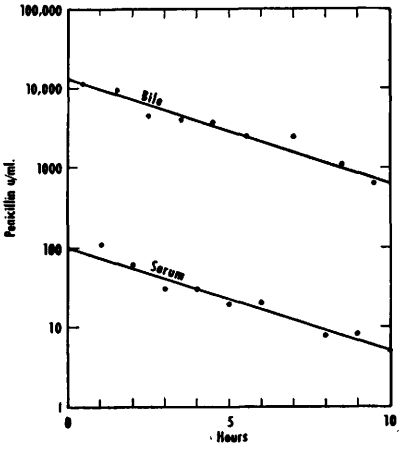

FIG. 5.

The bile is seen to contain about 130 times as much penicillin per milliliter as the serum, independent of the serum concentration.

Relationship of Penicillin Concentrations in Serum and Bile. In Fig. 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 7 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.

[4] Brodersen, R., *Trans. Farad Soc.*, 1947, **43**, 351.

TABLE III.
Correlation Between Bile pH and the Bile Penicillin-serum Penicillin Ratio.

	Penicillin concentration of bile
pH 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. A cannula was placed in the gall bladder of a normal dog, 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 re-injected. The volume of bile in the gall bladder was determined every hour and a small sample taken out for penicillin assay. The total amount of penicillin found in the gall bladder is shown in Fig. 6.

Resorption and inactivation of penicillin in the gall bladder

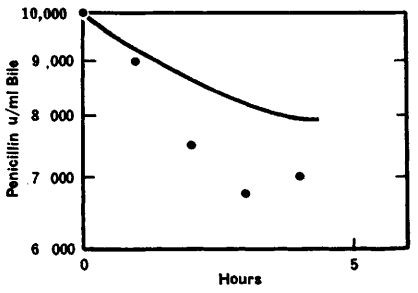

FIG. 6.

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.

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 demonstrate the resorption from the gall bladder. Within the 4 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.

Summary. Bilaterally nephrectomized dogs eliminated penicillin G at a considerable rate from their blood stream after an intravenous injection. This elimination took place at essentially the same rate when either the intestines were removed, or when the common bile duct was ligated and the stomach removed. But no inactivation could be demonstrated after complete evisceration or hepatectomy in nephrectomized animals. A major portion of the injected penicillin was excreted in the bile. Appreciable inactivation was found in the bile, especially in the case of a strongly alkaline bile.

The remaining portion of penicillin may have gone either to the gall bladder, where only a slow but significant resorption took place, or to the intestine where it was partially resorbed and partially inactivated.[5]

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 secretion of digestive juices.

[5] Seeberg, V. P., Illg, P. I., Brown, D. J., *J. Am. Pharm. Assn.*, 1946, **35**, 280.

Received June 17, 1949. P.S.E.B.M., 1949, **71**.