

When hexosediphosphoric acid was precipitated only twice, for the first time as the acid barium salt at pH 3.8 and a second time as the neutral salt at pH 8.2, and when removal of inorganic phosphate was omitted, the results were as indicated in Table 2. These values may also more truthfully describe the quantitative proportions of hexosediphosphoric acid in both yeasts.

TABLE 2

	Hexosediphosphoric acid Total P content γ/1 gram dry weight	
	Fresh yeast	Fermented 15 min. at + 20° C.
Baker's yeast.....	62	214
Brewer's yeast.....	57	568

Yeasts employed contained few dead cells, the baker's yeast less than 0.5, per cent and the brewer's yeast 1.8-2.6 per cent.

Hexosediphosphoric acid must accordingly be considered as a normal intermediate in the fermentation of sugar both by baker's and brewer's yeast, even in an undestroyed fermentation system.

References

1. DEUTICKE, H. J. and HOLLMANN, S. *Z. physiol. Chem.*, 1939, 258, 160.
2. KERR, S. E. *J. biol. Chem.*, 1941, 139, 121.
3. MACFARLANE, M. G. *Biochem. J.*, 1939, 33, 565.
4. MEYERHOF, O. *J. biol. Chem.*, 1945, 157, 105.
5. RAPOPORT, S. *Enzymologia*, 1937, 3, 52.

Inactivation of Penicillins G and K by Liver and Kidney

JAY TEPPERMAN, N. RAKIETEN,
G. VALLEY, and EDNA W. LYON

Department of Pharmacology, Syracuse College of Medicine,
and Bristol Laboratories, Inc., Syracuse, New York

It has been shown (1, 2) that penicillin K disappears more rapidly from the blood of rabbits and men than do penicillins G, F, and X in comparable dosage. It has also been stated (1) that the recovery of penicillin K in the urine of rabbits and men was 30-35 per cent as compared with an average recovery for G, F, and X of 74 per cent in rabbits and 91 per cent in men. In agreement with these data, the curative dose for experimental rabbit syphilis has been found to be very much greater for penicillin K than for the other penicillins (1). Eagle and Musselman have ascribed the rapid disappearance of penicillin K from rabbit blood and its comparatively low recovery in the urine to its inactivation by a "relatively thermolabile, nondialyzable constituent of plasma." The following experiments were undertaken in an effort to discover whether or not the liver plays a role in the inactivation of penicillin K.

Crystalline penicillins K and G were used in these experiments.¹ These compounds were characterized by carbon, hydrogen, and nitrogen analyses and by determination of the subtilis-staphylococcus ratios. The assays for penicillin were

¹ The pure penicillin fractions were kindly supplied to us by Dr. W. C. Risser, Bristol Laboratories, Inc.

made by a modification of the Rammelkamp method (5). In some of the *in vivo* studies and in the *in vitro* experiments intermediate dilutions were made to detect small differences in penicillin concentration.

Rabbits anesthetized with sodium pentobarbital were used in the first phase of this study. Initially the rate of disappearance from the blood of penicillin K as compared with penicillin G was determined in renal-ligated preparations. In harmony with previously published reports (1, 2), penicillin K was found to disappear from the circulating blood far more rapidly than penicillin G (Table 1).

TABLE 1

PENICILLIN LEVELS IN BLOOD OF RABBITS TWO HOURS AFTER THE
INTRAVENOUS ADMINISTRATION OF 1,000 O.U./KG. OF
PENICILLIN G OR K

Penicillin G O.U./ml. of blood		Penicillin K O.U./ml. of blood	
Eviscerate, renal-ligated	Renal-ligated	Eviscerate, renal-ligated	Renal-ligated
7.5*	5.0	2.50	0.312
7.5*	2.5	2.50	0.156
5.0	5.0	2.50	0.156
5.0	2.5	1.25	0.156
2.5		1.87*	0.156
2.5			
2.5			
Mean 4.64	3.75	2.12	0.187
S.E. ±0.84	S.E. ±0.72	S.E. ±0.25	S.E. ±0.031

* Obtained by modifying the Rammelkamp method to include intermediate values in the usual geometric progression of serial dilution.

The disappearance rates of penicillins G and K were then compared in renal-ligated, eviscerated preparations. The dose of penicillin was 1,000 O.U./kg. of intact body weight as in the first experiment. The results, as shown in Table 1, suggest that the evisceration procedure slowed the inactivation of penicillin K considerably, although the rate of disappearance of penicillin K in the renal-ligated, eviscerated preparations may have been more rapid than the rate of disappearance of penicillin G under the same circumstances. In this connection it should be noted that in the eviscerate preparation used in this study the liver was allowed to remain in the animal with patent hepatic veins, and therefore the animals may have retained some residual liver function (4). This interpretation of the data is strengthened by studies of surviving liver slices under anaerobic conditions, to be reported below.

Experiments were undertaken to test the ability of surviving rabbit liver slices to inactivate penicillins K and G *in vitro*. In each experiment approximately 100 mg. wet weight of liver slice were suspended in 3 ml. of Krebs and Henseleit (3) phosphate buffer (pH 7.4) containing 0.5 O.U./ml. of either penicillin K or G and 200 mg. per cent of fructose. The QO_2 of the tissue was determined by the direct method. The flasks were filled with oxygen, incubated at 37.8° C., and shaken for 90 minutes at a rate of 120 per minute in a Barcroft-Warburg apparatus. A comparison of the inactivation of penicillin K with that of penicillin G under the same circumstances (Table 2) reveals that the total change in 90 minutes in penicillin K/100 mg. of liver tissue was 0.713 O.U., whereas the corresponding mean figure for penicillin G was 0.18 O.U.

Subsequent experiments showed that 1.05 O.U. of penicillin

K was inactivated in the presence of 100 mg. of kidney tissue aerobically. Smaller but appreciable amounts of penicillin G apparently disappeared from the buffered solution in the

TABLE 2
DISAPPEARANCE* OF PENICILLINS G AND K FROM MEDIUM IN THE PRESENCE OF SURVIVING LIVER AND KIDNEY SLICES

Tissue	Liver			Kidney		
	O ₂		N ₂	O ₂		N ₂
Gas						
Penicillin	K	G	K	K	G	K
	0.60	0.27	0.60	1.05	0.60	0.75
	0.75	0.27	0.60	1.05	0.60	0.75
	0.75	0.27	0.60	1.05	0.60	0.75
	0.60	0.27	0.93	1.05	0.27	0.51
	0.60	0.0	0.93	1.05	0.27	0.51
	0.60	0.0	0.93	1.05	0.27	0.75
	1.02		0.75			
	0.75		0.75			
	0.75		0.90			
Mean.....	0.713	0.18	0.77	1.05	0.433	0.67

* Disappearance is calculated as O.U./100 mg. of wet weight of tissue. Triplicate analyses on individual rabbits are grouped.

presence of surviving kidney slices. Further studies revealed that the inactivation of penicillin K by liver and kidney slices occurs in an atmosphere of nitrogen as well as aerobically. The oxygen uptake by the tissue slices suspended in penicillin K-containing buffer was exactly the same as that observed in the case of the slices in penicillin G buffer.

Additional studies are now in progress to determine whether penicillin K is inactivated by other tissues, and whether intact liver and kidney cellular structure is essential for the effect noted above.

Summary. The inactivation of penicillin K is more rapid in the renal-ligated rabbit than in the renal-ligated, eviscerate preparation. Inactivation of penicillin K occurs in the presence of surviving liver and kidney slices. Small amounts of penicillin G were inactivated by liver slices; larger amounts disappeared in the presence of kidney slices. The inactivation of penicillin K in the presence of rabbit liver and kidney slices is demonstrable anaerobically as well as aerobically.

References

1. COGHILL, R. D., OSTERBERG, A. E., and HAZEL, G. R. *Science*, 1946, 103, 709.
2. EAGLE, H., and MUSSELMAN, A. *Science*, 1946, 103, 618.
3. KREBS, H. A., and HENSELEIT, K. *Z. physiol. Chem.*, 1932, 210, 33.
4. PETERSON, J. M. *Physiol. Rev.*, 1934, 14, 586.
5. RAMMELKAMP, C. H. *Proc. Soc. exp. Biol. Med.*, 1942, 51, 95.

IN THE LABORATORY

Measurements of Underwater Noise Produced by Marine Life¹

M. B. DOBRIN

Naval Ordnance Laboratory, Washington, D. C.

That certain fish species make noise under water has been common knowledge among fishermen since ancient times. For at least a century, observations on this phenomenon have been published by naturalists and zoologists. Until recently, however, all observations upon noise produced in this way were incidental and qualitative. No physical measurements of its frequency distribution or intensity are reported anywhere in the biological literature. It was not until the recent war that a need was felt for exact quantitative data on biological water noise. The introduction during the war of underwater acoustic equipment, such as listening devices, submarine detecting

gear, acoustic mine mechanisms, and homing torpedoes, raised questions as to the interference that might be expected from natural background noises in the water. For this reason, information was required on the nature and magnitude of the water noise to be expected at various localities and under various conditions. Since no data of the type needed were available in the general literature, it was necessary for war research agencies working in underwater sound to make their own measurements. A large body of data was accumulated in this way which should considerably augment previously available knowledge of natural water noise and its production.

Although waves, wind, and tidal currents give rise to a measurable amount of water noise, this is seldom of a higher order of magnitude than 1 dyne/cm. 2 in an octave band and is usually much lower. Biological sources, on the other hand, can be responsible for sustained noises with an octave pressure of several hundred dynes per square centimeter.

MEASUREMENT OF NATURAL WATER NOISE

The Naval Ordnance Laboratory carried on background measurements at several field locations where biological noises were particularly intense, and it has recorded the highest natural water-noise levels that have been observed anywhere. At the same time, a systematic effort was made to identify the species giving rise to the different kinds of fish noises recorded. This involved elaborate tests on segregated fish species, both in aquaria and in experimental ponds.

¹ The field measurements and data reductions upon which this report is based were carried on by the following staff members of the Naval Ordnance Laboratory: L. G. Swart, G. E. Brown, L. C. Bell, D. L. Bobroff, R. F. Grunwald, G. R. Irish, W. E. Loomis, and the author. The consulting biologist for much of the work was Cdr. Charles J. Fish, USNR, of the Mine Warfare Operational Research Group. Walter H. Chute, director of the John G. Shedd Aquarium, gave the Laboratory substantial cooperation in its measurements there, and H. F. Prytherch, director of the U. S. Fishery Biological Laboratory, Beaufort, North Carolina, generously granted use of facilities for the field measurements and segregation tests reported from that area.