cast serious doubt upon a role of acetylcholine and its esterase in the mediation of conduction along the nerve fiber. Nevertheless, the importance of this substance in the mediation of the stimulus at nerve endings cannot be completely excluded. In our experiments we were not uniformly able to repeat the inhibition of the depressor action of acetylcholine by procaine as reported by Rapp and Wessinger, but it appeared that under the influence of relatively large doses of procaine a moderate decrease of the acetylcholine effect could be obtained. Our results on the isolated rabbit intestine were completely negative in this respect. We doubt that the above data can properly be interpreted as supporting the theory of a competitive inhibition of acetylcholine by procaine on the nerve ends.

As pointed out, procaine is the hydrochloride of a tertiary amine and cannot justly be considered a quaternary ammonium compound; II, III, V, and VI are true quaternary ammonium derivatives, and are therefore chemically more like acetylcholine than procaine; this is particularly true of compound V which contains 3-methyl substituents as does acetylcholine. None of the quaternary derivatives possessed the effect typical of acetylcholine, nor did they interfere with its action upon blood pressure or isolated rabbit intestine. The compounds exerted, in many respects, rather a marked nicotine-like effect.

If partial inhibition of acetylcholine action by procaine is to be ascribed to similarity in chemical structure, such inhibitory effects should logically be expected to increase in the case of the closely related quaternary ammonium derivatives. This was not observed.

Summary. The methyl and ethyl quaternary ammonium salts of procaine and of the analogous compound in which the ethyl groups are replaced by methyl groups were prepared. They were studied pharmacologically, especially with regard to their ability to modify some of the effects of acetylcholine, and their properties were compared with those of the hydrochlorides of the tertiary amines. No support was found for the theory that inhibition of acetylcholine action in this series is dependent upon similarity in chemical structure.

16382

Fate and Distribution of Penicillin in the Body. I. Circulation of Penicillin in the Lymph.*

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The presence of penicillin in the urine[1,2] and in the tissues[3,4] long after its disappearance from the blood stream, posed a number of questions concerning the fate and distribution of the antibiotic in the body. This led us to study the penicillin content of the lymph and its relation to the blood levels.

We expected to find an explanation for the missing correlation between blood, urine, and tissue levels in the lymphatic circulation.

Material and Methods. Dogs of various breeds, of 9-12 kg body weight, were anesthetized with either nembutal (35 mg/kg,

* Part of this material was presented at the Meeting of the American Societies of Experimental Biology, Atlantic City, May, 1948.

1 Romansky, M. J. and Rittman, G. E. *New England J. Med.*, 1945, **233**, 577.

2 Cohn, A., Kornblith, B. A., Gruenstein, I., Thomson, K. J. and Freund, J. PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 145.

3 Ercoli, N., Hueper, W. C., Landis, L., Lewis, M. N. and Schwartz, B. S. *Am. J. Med. Sci.*, 1948, **215**.

4 Schwartz, B. S., Lewis, M. N., Whitehead, M. and Ercoli, N., in press.

TABLE I.
Penicillin Content of the Lymph and Serum in Dogs Treated with 20,000 u. Penicillin in Saline.

Dog No.	Route	Hr	Units/cc	
			Serum	Lymph
1	Intramuscular	2¼	.5	2.0
		4-5	0	.5
		7	0	2.0
11		6	.03	.125
		7	0	.03
17		7	.03	.06
		8	.03	0
23		3½	.06	.125
		4	0	.06
		5	0	.06
10	Intravenous	3	.125	.25
		4	0	.125
		5	0	.06
		6	0	.03
14		1	2.0	4.0
		2	1.0	2.0

i.v.) or sodium phenobarbital (120 to 160 mg/kg, i.p.). A glass cannula was inserted and tied into the thoracic duct at its entrance into the vein and the lymph was permitted to flow continuously. Samples of blood (femoral vein) and lymph (cannula) were taken periodically and assayed for penicillin. To rule out the possible inherent bacteriostatic properties of these fluids, samples collected before treatment were used as control. For

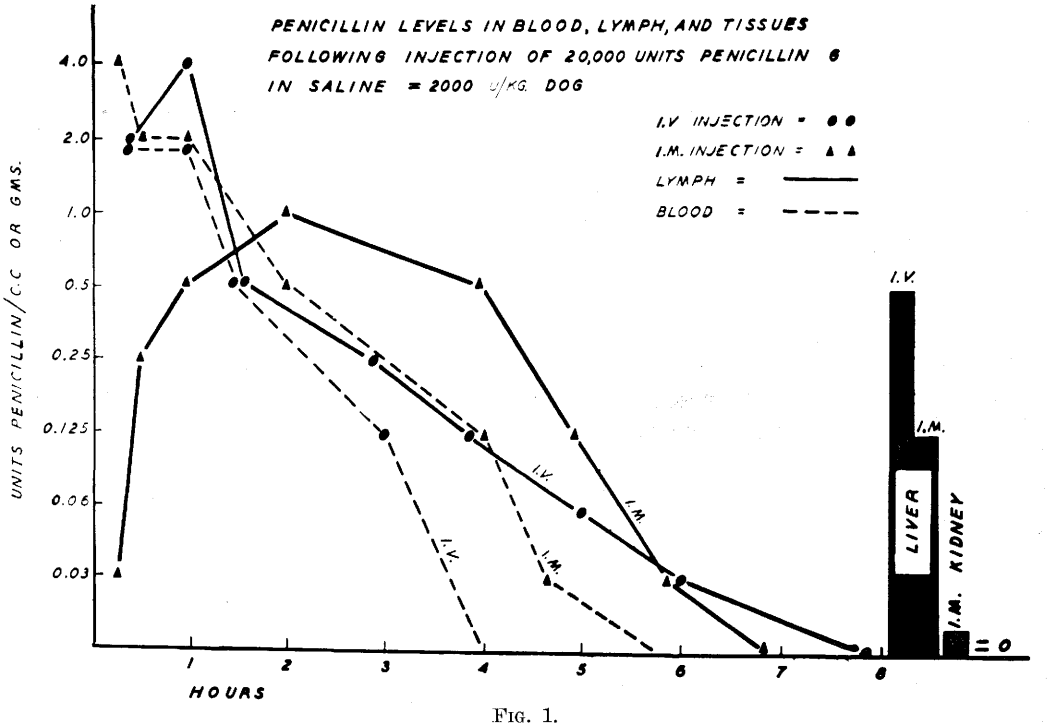

FIG. 1.

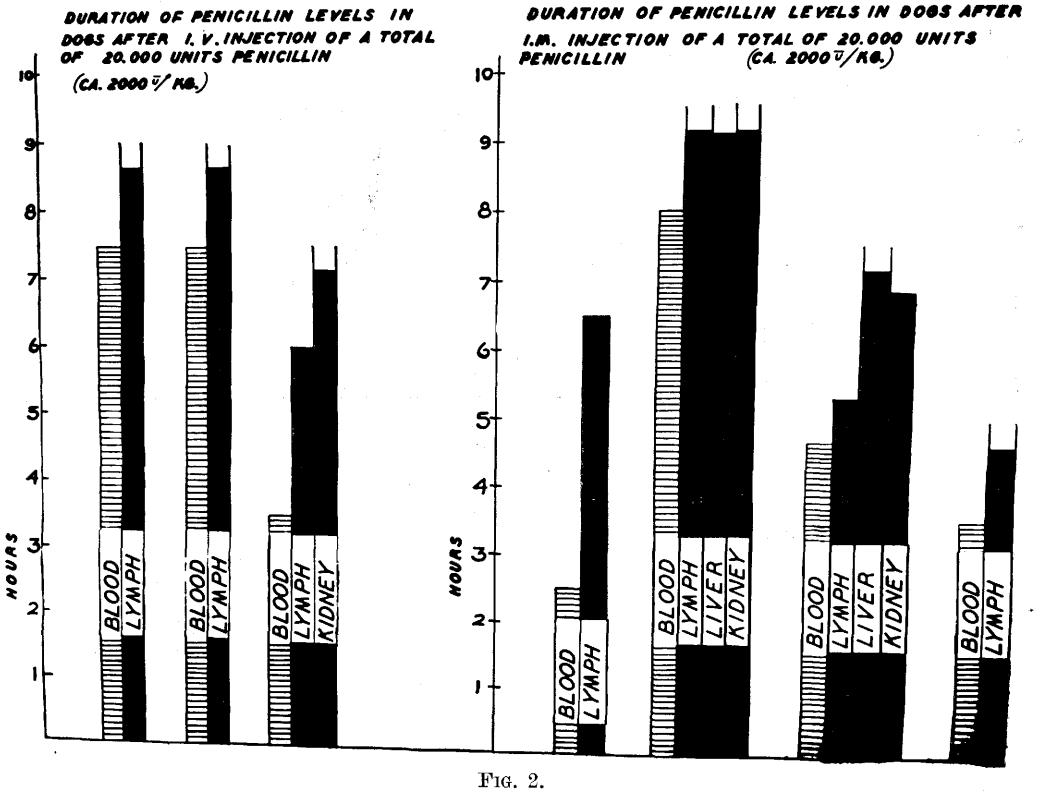

FIG. 2.

penicillin determination, the dilution method,[5] with defibrinated rabbit blood as indicator of hemolysis and β hemolytic streptococcus strain 4 (Group A, Type 3), received in 1946 from Dr. R. J. Schnitzer, was used as test organism. With his method penicillin concentrations of 0.015-0.03 u/cc, are detectable. The tissues collected at the end of the experiment were mechanically ground, taken up in 2 volumes of phosphate buffer (pH 6) and assayed for penicillin. Crystalline potassium penicillin G was administered in saline by various routes and by repository injection. For the latter purpose, we injected (intramuscularly) a suspension of crystalline penicillin in oil plus epinephrine, which gives a high prolongation of blood and organ levels.[3] (A commercial preparation—"Intracillin"—containing 300,000 u penicillin and 0.3 mg epinephrine per cc oil, was used.)

1. *Penicillin in Aqueous Medium.* (a) *Parenteral administration.* A total of 23 dogs were anesthetized and prepared for collection of blood and lymph samples. Table I shows the results of experiments in dogs treated with 20,000 units of penicillin, either intramuscularly or intravenously (approximately 2000 u/kg). By both methods of administration the retention of penicillin in the lymph was longer than in the blood. The lymph and blood level curves did not show the same general direction. One and one half hours after injection, the penicillin content of the blood was rapidly descending; this was not the case in the lymph, as appears in Fig. 1. The duration of detectable penicillin levels in the blood, lymph, and organs is represented in Fig. 2. (b) *Intraduodenal administration.* After laparotomy, the aqueous solution was injected into the duodenum with a hypodermic needle. A 9.5 kg dog, treated with 1,000,000 units, showed a high penicillin blood concentration (0.5 u/cc) within 5 minutes after administration; in ½ hour the concentration increased to 4.0 u/cc. After 8

[5] Rammelkamp, C. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 95.

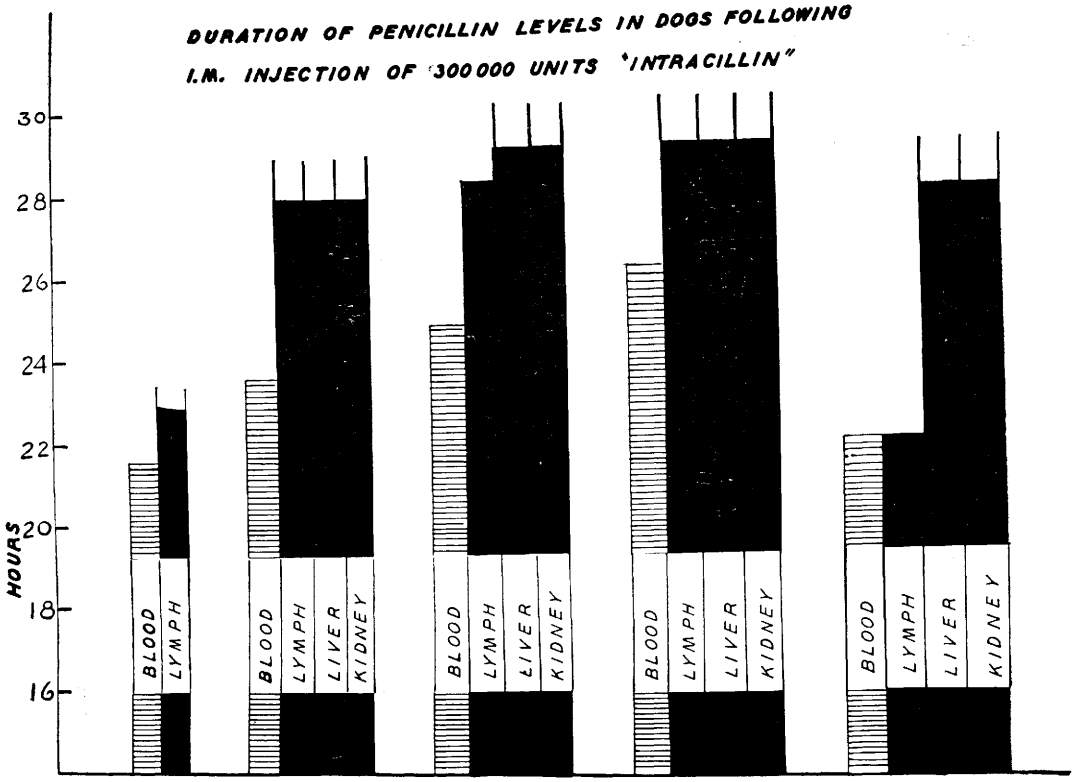

FIG. 3.

hours the blood still contained 0.25 u/cc penicillin. The penicillin did not appear in the lymph until 10 minutes after injection; after this period the lymph level followed the blood level for the entire duration of the observation (8 hours). In a second dog, weighing 9.0 kg, treated with 20,000 u penicillin, 0.5 u/cc was found in the lymph and in the serum during an observation period of 3 hours. A third dog, weighing 12.5 kg treated with the same dose, showed penicillin in the lymph for the complete observation period of 7 hours, whereas the penicillin remained in the blood for only 6 hours. In this experiment the penicillin appeared in the lymph more than one hour after administration, when the blood already contained high penicillin concentrations (0.5 u/cc).

2. *Repository Treatment.* Fifteen dogs were injected intramuscularly with 1 to 3 cc Intracillin, corresponding to 300,000 to 900,000 units crystalline penicillin G. In one group of experiments, the lymph and blood levels were followed immediately after injection; in another group, the dogs were anesthetized for cannulation of the thoracic duct 16 hours after penicillin treatment. During most of the period when penicillin was still detectable in the blood stream, the concentrations in the blood and lymph were of approximately the same value. However, toward the end period of the blood level curve, the lymph concentration was higher in the majority of cases. In relation to this, the duration of the penicillin in the lymph was significantly longer than in the blood. The duration of the detectable penicillin levels in the blood, lymph, liver, and kidney is presented in Fig. 3. The difference in duration between blood and lymph was probably greater than it would appear from these figures, since in most of these long-lasting experiments the lymph level was not followed to the end.

3. *The Time of Appearance of Penicillin in the Lymph.* In a certain number of experiments, the time of appearance of penicillin in

TABLE II.
Time of Appearance of Penicillin in the Lymph and in the Serum.

Minutes after intramusc. inject.		Saline, 20,000	Procaine penicill., 300,000 u	Intracillin, 300,000 u.
5	Lymph	0	0	0
	Serum	0.5	1.0	8.0
10	Lymph	0.25	0.125	0.125
	Serum	2.0	2.0	8.0
15-25	Lymph	—	2.0	2.0
	Serum	—	2.0	8.0

TABLE III.
Organ, Lymph, and Blood Levels of Dogs Treated with Penicillin.

Administration		Dosage, × 10,000	Assay at end of exper. hr	u/cc			u/g	
				Blood	Lymph	Urine	Liver	Kidney
Saline	i.m.	2	6½	.25	.03		.25	.5
,,	,,		9	.5	.25		.5	.12
,,	i.v.	1	4½	.25	.25	8<	.25	1.0
Intracillin,	i.m.	30	23	.25	.25		.5	4.0
			29	0	.5	10	.12*	.5
			30	0	.015		.5	0
		90	36	.06	.12		.125	.125

* Lung and spleen = 0.12 u/g.

the blood and lymph was noted. In every case studied—as in the previously mentioned intraduodenal experiments—the penicillin appeared first in the blood, then, 5 to 10 minutes later, in the lymph. (Table II). These results suggest that the absorption of penicillin takes place through the blood capillaries. It is noteworthy that the recently described insoluble procaine penicillin suspended in oil[6] gave the same result as the soluble salts.

4. *Lymph and Organ Levels.* The duration of penicillin in the liver and kidneys was generally longer than in the lymph. Occasionally it was observed that the duration of the penicillin was more prolonged in the liver than in the kidneys. (Figs. 2 and 3.)

Conclusions. This study reveals that the penicillin lymph level is more prolonged than the blood level. The appearance of penicillin in the blood earlier than in the lymph suggests that the absorption of penicillin takes place through the blood capillaries. It seems to be a regular occurrence that penicillin, after its passage from the blood into the lymphatic system and into the tissues, is retained longer in the last two.

That there is a definite accumulation of penicillin in the organs, particularly following repository injection, is clearly demonstrated by our data on dogs and further substantiated by other rat experiments.[4] The elimination through the lymphatics of this penicillin accumulated in the organs might be responsible for the prolongation of the lymph levels.

The penicillin found in the thoracic duct reaches the blood stream where, following its greater dilution, it becomes bacteriologically undetectable. In all probability, this undetectable penicillin is concentrated in the kidneys and then reappears in high concentrations in the urine.

By analogy, we may assume, from the frequent presence of penicillin in the organs (liver, kidney) after the end of the detectable lymph level, that the penicillin circulation in the lymphatic system is probably longer than can be demonstrated by the methods used.

The therapeutic interest of the prolonged penicillin lymph level is obvious considering that the total volumes of the lymph and of

[6] Hobby, G. L., Brown, E., and Patelski, R. A., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 6.

the blood are of the same magnitude,[7] and that the "lymph comes closer to reflecting the actual environment of the body cells than any other fluid that can be collected" (Drinker and Joffey[8]).

The well known role played by the lymphatic system in certain infections adds further interest to this observation.

[7] Abel, J. J., Hampil, B., Jonas, A. F. and Chalian, W., *Bull. Johns Hopkins Hosp.*, 1938, **62**, 522.

[8] Drinker, C. K., and Joffey, J. M. Lymphatics, lymph and lymphoid tissue. *Harvard University Press*, Cambridge, Mass. 1941.

16383

Influence of Sodium Tetrathionate on Rate of Blood Flow to the Digits.

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Sodium tetrathionate (Tetrathione) has been used extensively by Theis and Freeland,[1-4] primarily for peripheral vascular disorders characterized by deficient oxygenation of the blood and tissues. Most prominent among these are Buerger's disease.[5] Theis and Freeland[1] show, during the active stages of thromboangiitis obliterans, either a decrease in the oxygen capacity or in the degree of oxygen saturation of the arterial blood, as well as increased sedimentation rates without significant departures from the normal in erythrocyte counts and hemoglobin percentages. In these cases venous blood taken from the involved extremities was abnormally dark, showed an increase in viscosity and sedimentation rate, rapid coagulation without an increase in fibrinogen, increased alkalinity and a low degree of oxygen saturation with a low carbon dioxide content. A subnormal content of the oxidation catalyst, the reduced form of glutathione, was noted in the venous blood. Sodium tetrathionate administered intravenously was of value in restoring to normal these abnormal blood findings. Since this work, some controversy over the value of sodium tetrathionate in the treatment of peripheral vascular disorders has arisen.

The present studies were carried out to determine whether or not sodium tetrathionate influenced the rate of blood flow, skin temperature, or pulse rate in normal individuals and in patients with peripheral vascular disease.

Methods and Materials. The rate of blood flow was determined, using a digital pneumoplethysmograph.[6] The occluding cuff was placed at the ankle or wrist. The venous occlusion was accomplished by inflating a cuff with approximately 60 mm of mercury pressure. The rate of volume change was measured by taking the angle between the base line before and after venous occlusion. The volume of the part was measured using the volumometer, as described by Burch.[6] The rate of flow was then determined after a period of rest, not less than 45 minutes. Tracings were taken after the injection of 10 cc of distilled water intravenously, after injection of 0.6 g of sodium tetrathionate in 10 cc of distilled water, and after placing

* All opinions held are those of the author and are not necessarily those of the Veterans Administration.

[1] Theis, F. V., and Freeland, M. R., *Arch. Surg.*, 1939, **28**, 191.

[2] Theis, F. V., and Freeland, M. R., *Arch. Surg.*, 1940, **40**, 190.

[3] Theis, F. V., and Freeland, M. R., *Ann. Surg.*, 1941, **113**, 411.

[4] Theis, F. V., and Freeland, M. R., *Surg.*, 1942, **11**, 101.

[5] Robinowitz, H. M., *Am. J. Surg.*, 1933, **21**, 260.

[6] Burch, G. E., *Am. Ht. J.*, 1947, **33**, 48.