observed, nor was there any evidence of renal irritation.

Thirty cc of a 10% solution of the lithium salt were injected intramuscularly (15 cc into each buttock) in 3 patients. The injection was tolerated well in each case and no subsequent discomfort was noted.

One patient, not included in the above group, afforded the opportunity for massive oral and intravenous therapy. Subacute bacterial endocarditis with *Bacillus proteus* had been present for several months. In view of the activity of Nu-445 against this organism in the genito-urinary tract[1] and *in vitro,* it was thought worthwhile to attempt massive therapy in this case. After a 4-g initial dose, 1 g every 4 hours was administered for 4 days, 2 g every 4 hours for 4 more days, and then 3 g every 4 hours for 10 days. No reactions were noted. While the patient was receiving this oral dose, 18 g of the lithium salt of Nu-445 were given intravenously over a period of 4 hours. Two days later, with the patient still on the same oral dose, 12 g were given intravenously in 10 minutes. Pallor and sweating were noted for a few hours after this last dose and crystalluria and microscopic hematuria were noted for the ensuing 3 days. This was accompanied by a transient rise in the blood urea nitrogen. It is doubtful if the usual clinical requirements would ever indicate the administration of even a fraction of the dose administered to this patient.

Two other patients not included in the above group who had been hospitalized because of recent coronary occlusions, had fatal recurrences while receiving drug therapy. There was post-mortem confirmation in one of these. A third patient, recovering from a recent, severe, postoperative pulmonary embolization was being treated with Nu-445 in an attempt to clear his urinary tract of a *Bacillus proteus* infection, and while under treatment sustained a fatal recurrence.

Summary. A new sulfonamide preparation has been presented which, on the basis of its high solubility in buffer solution, would be expected to greatly diminish the danger of sulfonamide crystallization in the kidney, with or without the concomitant administration of alkali. Neither crystallization nor the signs of renal irritation in the absence of crystallization were observed (except in the patient to whom enormous doses were administered). The antibacterial activity *in vivo* and *in vitro* and the low toxicity in the experimental animal combined to recommend an investigation of the clinical pharmacology of this compound. The blood and urine levels attained were found to be satisfactory and it was found possible to achieve and maintain these levels with an 8-hour dosage schedule. The entry of this drug into the spinal fluid of the normal individual is low, but in the presence of meningeal inflammation it was markedly increased in the one patient studied. The toxicity of the compound is low.

16381

Action of Quaternary Ammonium Salts and the Theory of Competitive Inhibition of Acetylcholine.

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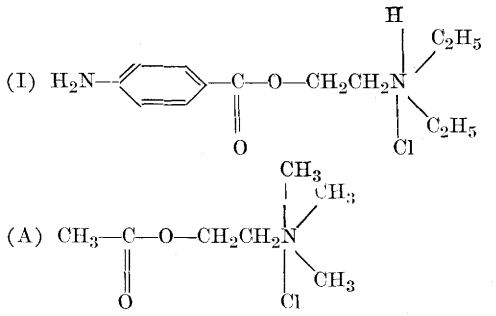
In several publications[1,2,3] attempts have been made to explain local anesthesia as a competitive inhibition of acetylcholine action upon the nerve. The theory, of course, assumes acceptance of the acetylcholine theory for the transmission of impulses[4] along the nerve fibre and the nerve ends. Some assume that similarity in chemical structure enables

a local anesthetic like procaine hydrochloride (I) to substitute for acetylcholine chloride (A) in the nerve tissue:



(A) $CH_3—C(=O)—O—CH_2CH_2N^+(CH_3)_2CH_3\ Cl^-$

However, it is hazardous to ignore the extreme differences in physiological and chemical properties between quaternary ammonium salts and salts of tertiary amines. A study of quaternary ammonium derivatives closely related to acetylcholine chloride and to procaine hydrochloride should shed some light on the problem.

If the theory cited is to be accepted, the nerve "receptor" cells must be assumed to be unable to distinguish between a tertiary amine salt and a quaternary ammonium salt; quaternary ammonium salts of the local anesthetic esters should either antagonize or potentiate the acetylcholine effect.

The compounds used in this study were procaine hydrochloride (I) and the following closely related products, in which only the C_2H_5, H or Cl groups attached to the terminal N of I were varied:

I. C_2H_5, C_2H_5, H, Cl Procaine hydrochloride
II. C_2H_5, C_2H_5, C_2H_5, I Procaine ethiodide
III. C_2H_5, C_2H_5, CH_3, I Procaine methiodide
IV. CH_3, CH_3, H, Cl
V. CH_3, CH_3, CH_3, I *p*-Aminobenzoylcholine iodide
VI. CH_3, CH_3, C_2H_5, I

Compounds I and IV are hydrochlorides of tertiary amines, the rest quaternary ammonium derivatives. I, II, and IV, were first prepared by Einhorn.[5] Compound III was furnished by Mr. F. N. Minard. Compounds V and VI which appear to be new, were characterized as follows:

V. β-dimethylaminoethyl *p*-aminobenzoate methiodide. m.p. 254-5°. Anal. calcd. for $C_{12}H_{19}N_2O_2I$: N, 8.00. Found: N, 8.08.
VI. β-dimethylaminoethyl *p*-aminobenzoate ethiodide. m.p. 113-6°. Anal. calcd. for $C_{13}H_{21}N_2O_2I$: N, 7.69. Found: N, 7.58.

Rapp and Wessinger[2] had described a marked diminution of the depressor effect of acetylcholine by a preceding (presumably very large) dose of procaine. Their paper refers to only one experiment in a dog and does not state the amount of procaine given. Hence it seemed desirable to repeat their experiment. In our tests 5 dogs weighing 7-10 kg and 2 cats of 3.2 kg and 2.5 kg were used. Four dogs and 2 cats were under Pentobarbital (Nembutal) anesthesia; one dog had been decerebrated under ether. Acetylcholine, 1-5 γ, was injected repeatedly i.v. in order to cause reproducible blood pressure drops of 30-50 mm Hg. After this effect had been established, procaine hydrochloride was given i.v. and followed immediately by repeated acetylcholine injections. No change in response to acetylcholine was seen in the cats following the administration of 10-20 mg procaine hydrochloride. The same result was seen in the 4 anesthetized dogs when 40-50 mg of procaine hydrochloride were used; however, 100 mg in one of the dogs caused a reduction of response to acetylcholine by about 30%. In the decerebrated animal 40-60 mg of procaine hydrochloride inhibited acetylcholine action by 30-50% respectively. (See Fig. 1, part I) In only one of the dogs (6 kg) under nembutal anesthesia did we succeed in obtaining a significant decrease of the typical depressor effect of acetylcholine by smaller doses of procaine (4-10 mg) during one part of the experiment. We were unable to detect the circumstances which caused or prevented this responsiveness; anesthesia and height of blood pressure were not involved. Procaine hydrochloride itself in doses below 100 mg did not cause a visible effect upon the blood

[1] Thimann, K. V., *Arch. Biochem.*, 1943, **2**, 87.

[2] Rapp, G. W., and Wessinger, G. D., *The Burr.*, 1944, **44**, 58.

[3] Schueler, F. W., *J. Chem. Education*, 1945, **22**, 585.

[4] Nachmansohn, D., in Physical-Chem. Mechanism of Nerve Activity, *Ann. Acad. Science*, 1947, **47**, 395.

[5] Einhorn, A., *Ann.*, 1909, **371**, 125.

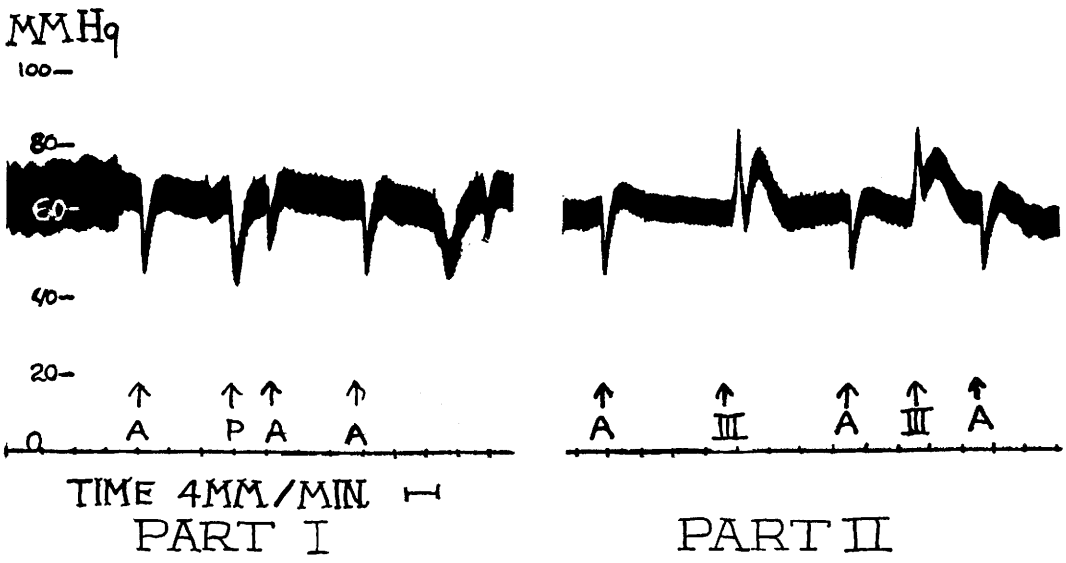


FIG. 1.
Dog, 6 kilo, decerebrated blood pressure taken from the carotid artery. A = 5 gamma acetylcholine. P = 40 mg procaine HCl. III = 4 mg cpd. No. III.

pressure in dogs. At 100 mg it produced a slight and transitory depression. If any effect upon the response to acetylcholine occurred, it lasted but 3 minutes.

Similar experiments were carried out with compound IV. When this dimethyl analogue of procaine was injected in doses of 50 mg in a cat weighing 2.5 kg, no reduction or an only insignificant one, in the typical response of blood pressure to acetylcholine occurred.

Acetylcholine is known to cause a marked contraction of the isolated rabbit intestine *in vitro* by peripheral parasympathicotropic stimulation. When 1.0 mg procaine hydrochloride was added to a bath containing 20 cc of Tyrode solution, a moderate reduction in tone and amplitude of contractions occurred, but the response to 20 γ acetylcholine remained unimpaired.

Richards, Roth and Kueter[6] have reported that compound II possesses definite nicotine-like properties and produces a marked pressor effect frequently followed by a short depressor phase in dogs; in cats the depressor action prevailed. Further work, which will be described elsewhere in detail, indicates that compounds III, V, and VI have a similar pressor effect in both species. These substances are completely devoid of local anesthetic activity, but have curare-like properties.

The action of acetylcholine upon blood pressure was studied in 2 anesthetized cats and 3 anesthetized and one decerebrated dog before and following single or repeated doses of 1-4 mg of Compounds II, III, and V, immediately after the blood pressure had returned to the base line and then again about 1-2 minutes later. In no case did we notice a change of the typical acetylcholine effect. Fig. 1, part II shows a typical experiment using Compound III. The same results were obtained with Compounds II, V, and VI.

In experiments on isolated rabbit intestine, analogous to those described above, Compound II did not exert an effect in doses of 1.0 mg, Compound V produced a transitory stimulation, and neither affected the typical response of the gut to acetylcholine.

Discussion. Recent publications such as the ones by Gerard[7] and Heymans,[8] have

[6] Richards, R. K., Roth, L. W., and Kueter, K. E., *Fed. Proc.*, 1947, **6**, 364.

[7] Gerard, R. W., in Physical-Chemical Mechanism of Nerve Activity, *Ann. New York Acad. Science*, 1947, **47**, 575.

[8] Heymans, G., and Jacob, F., *Arch. Intern. Pharm.*, 1947, **74**, 233.

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.