scribed. The heat generated by the saw blade softens the plastic coverslip just enough to secure the section after the bone and surrounding plastic are completely cut through. The coverslip also prevents the section from warping. The damper serves to reduce a slight vertical flutter which appears in the saw blades when traveling at high speeds and also to minimize other extraneous vibrations. The keeper facilitates a fine adjustment of the pressure exerted upon the coverslip. The operating speed of the motor, and hence the saw blade speed, depends upon the type of bone being cut (degrees of hardness, inherent structure, etc.); the cross sectional area of the bone; the speed at which the bone is fed to the saw blade; and the thinness of the desired sections. Due to the extreme variability of these factors, it is difficult to set down definite rules for governing the saw blade speed; hence an insight into the problem must be acquired largely through experience.

The cut should be made in one firm smooth motion as rapidly as possible so that the section will be in contact with the saw blade a minimum period of time. It has been found that the bone should be moved against the saw blade at a speed approximately 0.5 cm/sec. for best results. After cutting, the sections can be removed from the coverslip by means of a thin razor blade.

If sections are being cut from a bone having a relatively small cross sectional area, a section will sometimes be lost because not enough of the coverslip is softened to secure the section. This loss can be prevented by stopping the cut before the trailing edge of the plastic is completely severed. The cut can then be finished with a thin razor blade without loss of the section. Figure 3 is a photomicrograph of a section of human bone cut by this method. The cut section is approximately 30 microns thick and was cut from a piece of human bone from an individual 30 years of age at the time of death, which occurred eighteen years ago. The bones were kept in a sealed glass jar in the dry condition for the eighteen-year period. They were prepared for sectioning by embedding in Bioplastic as described above without any other treatment of any kind. Several thousand sections have been cut by this method.

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Effect of Pilocarpine on Epinephrine Reversal Produced by 2-(N-p'toly-N-(m'-hydroxyphenyl)-aminomethyl)-Imidazoline. HCl (C-7337)* (17519)

JAMES L. MORRISON, RICHARD J. LANGSTON, AND ARTHUR P. RICHARDSON

From the Department of Pharmacology, Emory University School of Medicine, Ga.

The recent introduction of a number of new drugs, which antagonize the pressor action of epinephrine, has created renewed interest in the well known phenomenon of epinephrine reversal. This problem is of considerable practical importance since many of these newer agents are being used in the treatment of human disease as a possible substitute for surgical sympathectomy. There have been a number of hypotheses suggested which attempt to explain the phenomenon of epinephrine reversal, none of which seems adequate. Substances which produce an epinephrine reversal are considered to block some part of the neuro-muscular apparatus which is concerned with the excitor actions of epinephrine, thus uncovering the inhibitory functions of epinephrine. The antagonism of epinephrine reversal by a wide variety of agents has been reported in recent months.(1-3)

* This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

1. Chen, G., and Russell, D., *Fed. Proc.*, 1949, v8, 280.

2. King, T. O., and Koppanyi, T., *J. Am. Pharm. Assn.*, 1949, v37, 346.

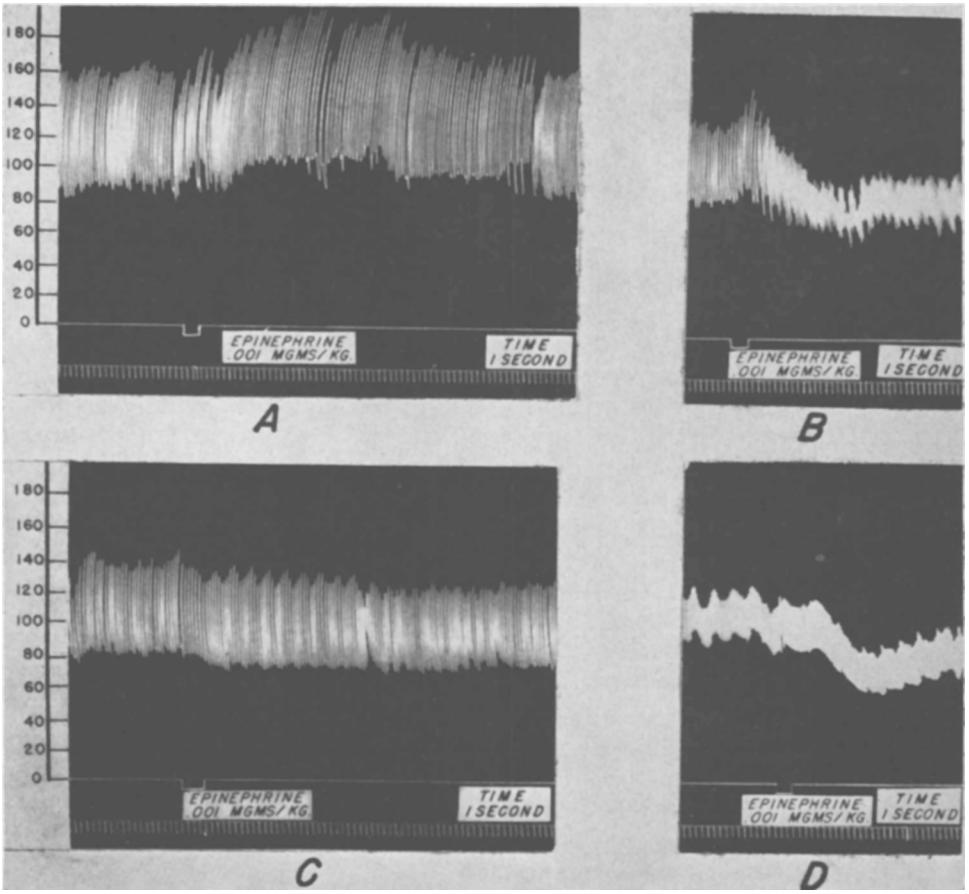

FIG. 1.
Unanesthetized dog. Events reading in sequence from left to right. Blood pressure recorded from femoral artery. A—Control injection of epinephrine. B—Epinephrine after 4.0 mg per kilo of C-7337. C—Epinephrine after C-7337 and 0.25 mg per kilo of pilocarpine. D—Epinephrine after C-7337, pilocarpine and 10 minutes after 1.0 mg per kilo of atropine.

Although there has been little similarity in the chemical or physiologic action of most of these agents, there has been some agreement that pilocarpine and other parasympathomimetic agents were most active. Since this phenomenon seems to us to have an important bearing on our concepts of autonomic physiology and pharmacology we have quantitated this effect using a new adrenolytic agent C-7337.(4)

Methods. Unanesthetized dogs were tied to an operating table and under procaine local anesthesia, a femoral artery and vein were exposed and cannulated. The artery was connected to an Anderson glass capsule manometer(5) for recording of arterial pressure and pulse. The vein was connected to a system which permitted easy injection of drugs. All drugs were given intravenously and washed in with 10 ml of normal saline. Each animal was heparinized by the injection of 1 mg/kg of heparin and the manometer system was filled with heparinized saline. Recordings were made on smoked kymograph paper. Epi-

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4. Meier, R., Yonkman, F., Craver, B. N., and Grass, F., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 70.

5. Anderson, F., *J. Lab. Clin. Med.,* 1941, v26, 1520.

nephrine was injected in a dose which produced approximately 40 mm rise in pressure, following which 4 mg per kg of C-7337† was injected. This dose of adrenolytic agent is approximately 4 times that required to block the action of epinephrine(4,6) and produces effects which last for at least several hours. Following the injection of the adrenolytic agent, epinephrine was injected again in the same dosage as used in the control period. This invariably produced a fall rather than a rise in blood pressure. Gradually increasing doses of a variety of substances were then injected to test their effect on the epinephrine reversal. Dosages were continued until the epinephrine reversal was abolished or toxic symptoms were produced. Because of the fact that the injection of epinephrine after an adrenolytic agent produces an effect on the circulation comparable to that of isopropyl norepinephrine, it seemed of interest to determine the effect of pilocarpine on the depressor action of the latter compound.

Results. We have found that pilocarpine, physostigmine, and choline all possess the ability of abolishing epinephrine reversal. Kymograph records from a typical experiment are illustrated in Fig. 1. Since pilocarpine produced the most striking results, this agent was studied in sufficient detail to describe the relationship between dosage and effect. A total of 5 dogs were used and the results of these studies are summarized in Fig. 2.

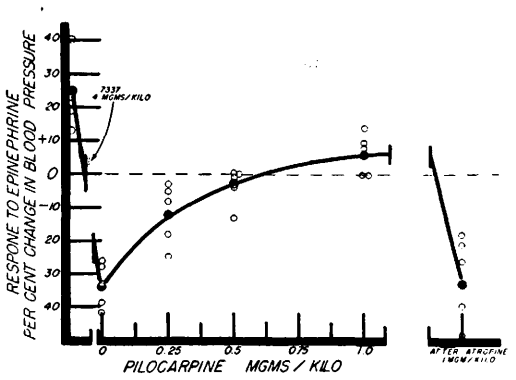

FIG. 2.

Summary of 5 experiments in unanesthetized dogs showing the relationship between dosage of pilocarpine and antagonism of epinephrine reversal by C-7337.

Following the abolition of epinephrine reversal, atropine in a dosage of 1 mg/kg was injected intravenously. Epinephrine injected within 5 minutes after atropine produced only slight effect upon blood pressure. If, however 10 minutes were allowed to elapse before epinephrine was reinjected, it invariably produced the same depressor effect which is observed immediately following administration of the adrenolytic agent.

In 6 experiments, using isopropyl norepinephrine, it was found that pilocarpine in a dosage of 0.5 mg/kg blocked the action of .001 to .004 mg/kg of isopropyl norepinephrine.‡ Fig. 3 illustrates one of these experiments. Of interest also was confirmation of the observations of Raymond-Hamet(7) that pilocarpine antagonizes the action of acetylcholine as shown in Fig. 4.

Further evidence of the similarity of action of isopropyl norepinephrine and the inhibitory effects of epinephrine following an adrenergic agent was shown by the fact that the pilocarpine blockading action could be reversed by atropine. Similar results have been reported by Fromherz.(8)

That antagonism of C-7337-epinephrine reversal by pilocarpine was not limited to this one adrenolytic agent was obtained from experiments showing that dibenamine-epinephrine reversal was also blocked by pilocarpine.

Discussion. The results presented above are difficult to explain in terms of present concepts of function of peripheral autonomic mechanisms. The similarity of action of isopropyl norepinephrine and the cardiovascular inhibitory actions of epinephrine after an adrenolytic agent suggests a common mode of action. The effect of parasympathetic stimulants and depressants on these phenomena suggest some relationship between the receptive mechanisms of the inhibitory sympathetic end organs and the receptive substance of the

† Generously supplied by Ciba Pharmaceutical Products, Inc.,

6. Walker, H. A., Morrison, J. L., and Richardson, A. P., unpublished observations.

‡ Generously supplied by Sterling-Winthrop Research Institute.

7. Raymond-Hamet, C. R.., *C. R. soc. biol.*, 1934, v115, 1076.

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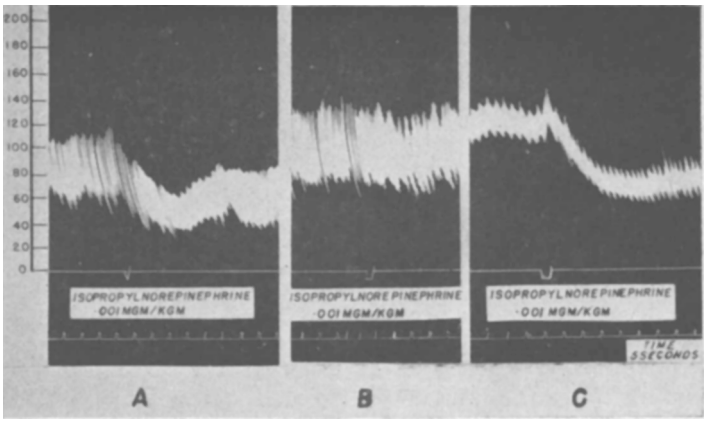

FIG. 3.

Unanesthetized dog. Events leading in sequence from left to right. Blood pressure recorded from femoral artery. A—Control injection of isopropyl norepinephrine. B—Isopropyl norepinephrine after 0.5 mg per kilo of pilocarpine. C—Isopropyl norepinephrine after pilocarpine and after 1.0 mg per kilo of atropine.

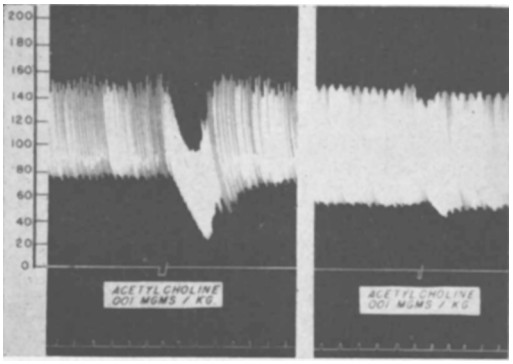

FIG. 4.

Unanesthetized dog. Blood pressure recorded from femoral artery. Left—Control injection of acetylcholine. Right—Same dose of acetylcholine 10 minutes after 1 mg per kilo pilocarpine.

parasympathetic system. This relationship, however, is not immediately clear, but points to the need for a more complete study of the interaction of autonomic drugs.

Conclusion 1) Epinephrine reversal produced by 2-(N-p'tolyl-N-(m'-hydroxy-phenyl)-aminoethyl)-Imidazoline HCl (C7337) may be abolished by the injection of pilocarpine and other parasympathomimetic agents.

2) The reversing action of such an adrenolytic agent may be reinstated by injection of atropine.

3) Pilocarpine is also effective in decreasing the depressor action of isopropyl norepinephrine and of acetylcholine.

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