Pharmacology Biochemistry & Behavior, Vol. 27, pp. 199–201. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

BRIEF COMMUNICATION

Premature Excess Release From the Alzet Osmotic Pump[1]

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Received 3 December 1986

KERENYI, S. Z. AND S. L. HARTGRAVES. *Premature excess release from the Alzet osmotic pump.* PHARMACOL BIOCHEM BEHAV **27**(1) 199–201, 1987.—This paper alerts investigators to the possibility of an excessive early release from the Alzet osmotic pump. In this research, rats were implanted with 7-day minipumps (model 2001) containing 24 mg/kg/day pyridostigmine, and blood samples were taken at intervals from 5 to 60 min after implantation. All samples showed that blood serum cholinesterase activity was markedly depressed as compared with those of controls. This finding demonstrated that animals were receiving the drug well before the pump's 4- to 6-hr start-up transient period. In a separate study, dye filled pumps were monitored for release when dropped into distilled water at 24°C or 37°C. The pump in the 37°C water released approximately 5% of its contents within 30 sec of submersion, while the pump in the 24°C water released virtually no dye. The early release appears to have been caused by expansion of the pump's contents when the pump, at room temperature, was implanted in the warmer animal.

Osmotic pump Premature release Pyridostigmine Blood serum cholinesterase

THIS paper identifies a potential problem for investigators using the Alzet osmotic minipump: The pump may release a significant amount of its contents immediately after implantation into an animal. Our initial study involved male Sprague-Dawley rats (350±50 g) that were implanted with osmotic minipumps filled with the anticholinesterase pyridostigmine. The continuous drug release from such pumps produced a constant inhibition of blood cholinesterase without the stress of multiple injections. However, we found that at the pyridostigmine dose level of 24 mg/kg/day (42 mg in the 0.2 ml pump), several rats died of cholinesterase poisoning within 5 min of pump implantation and 10 out of 12 died within 6 hours post implantation. According to the Alza Corporations's technical applications file, the Alzet minipump should not begin to release the drug until several hours after implantation. This delay is reportedly due to a start-up transient period, lasting about 4 hr, after which the dose level slowly increases to a stable plateau.

The Alza Corporation cites Theeuwes and Yum (1976) for basic technical information on the osmotic pump [4]. According to that information, the start-up transient phenomenon results from a combination of: "time lag for water permeation through the membrane, equilibration of the system's temperature and osmotic pressure, and thermomechanical relaxation of the membrane." No mention is made of a possibility of early release of the contents of the pump. The only start-up problem of which the manufacturer warns is the possible aspiration of body fluids through the flow moderator into the pump. We found the opposite problem: an efflux of the pump's contents within minutes of implantation.

We investigated the output of the minipump by both *in vivo* and *in vitro* techniques. In the *in vivo* experiment, pumps were filled with pyridostigmine (42 mg per 0.2 ml, measured by filling pump with a graduated 1.0 ml syringe) or vehicle (acetic acid-sodium acetate buffer titrated to pH=5), the exterior of the pump wiped dry, and implanted subcutaneously behind the animal's neck. Animals were then sacrificed by decapitation from 5 to 60 minutes after implantation, and trunk blood collected to determine cholinesterase activity (Ellman *et al.* 1960) [2]. The cholinesterase activity would decrease only if the animal received an acute dose of pyridostigmine from the minipump. Blood samples taken after implantation revealed a cholinesterase inhibition of approximately 80% compared to that of controls (Fig. 1). Furthermore, 7 out of 16 animals showed behavioral signs of severe cholinesterase poisoning prior to sacrifice. This observation, coupled with our original data, leads us to believe that the initial early spurt of drug out of the osmotic pump is at least an LD_{50} dose. The acute, 24 hr LD_{50} of pyridostig-

[1]The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by Institute of Laboratory Animal Resources-National Research Council.

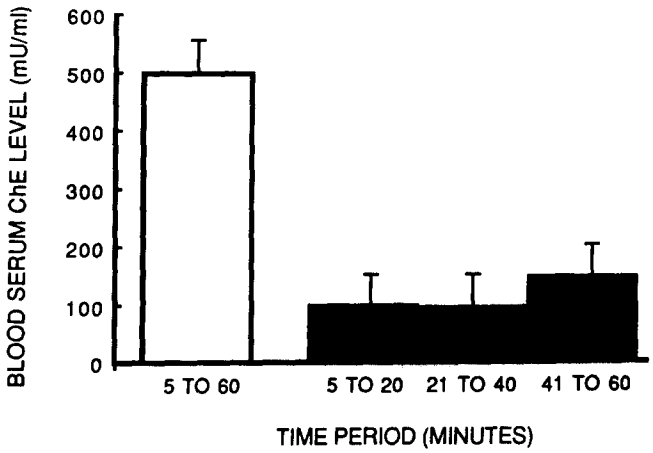

FIG. 1. Osmotic pump *in vivo* drug administration time test. Blood serum cholinesterase levels (mU/ml) were monitored from 5 to 60 min in rats implanted with minipumps filled with 24 mg/kg/day pyridostigmine (shaded columns, n=16) or vehicle (white column, n=8). Shaded columns are significantly different from white column, $p<0.005$, pooled t-test.

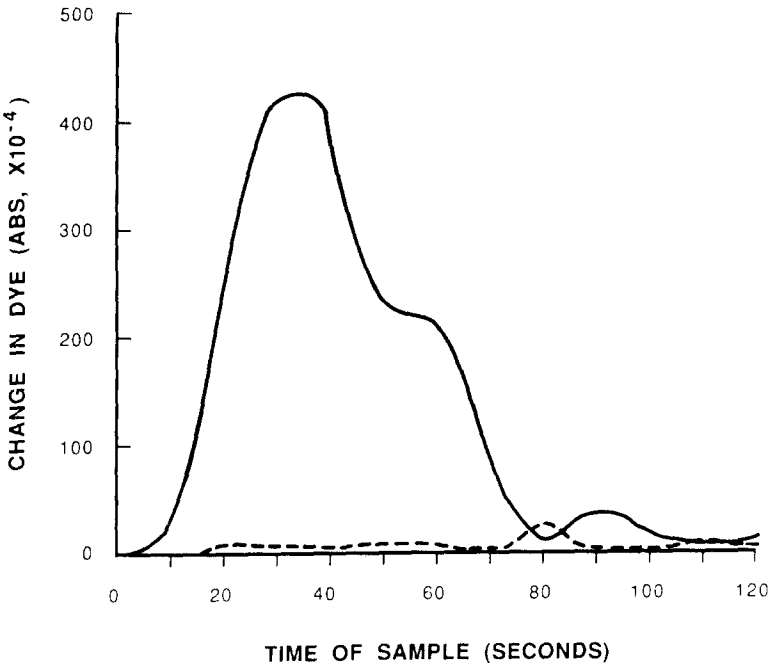

FIG. 2. Osmotic pump dye release at 24°C and 37°C. Dye-filled pumps were put into a beaker of water at 37°C (solid line) and at 24°C (broken line). Dye absorbance at 510 nm was measured at 5-sec intervals using a spectrophotometer.

mine in rats has been determined to be 2.69 mg/kg (IP) [5] and 2.73 mg/kg (IM) [3]. An early release of from 3 to 5% of the pump's contents (1.26 to 2.10 mg released in a 350 g rat, which equates to 3.6 mg/kg and 6.0 mg/kg, respectively) would be more than the IM or IP LD_{50} dose.

To further examine early release and a possible cause, we tested the effects of temperature change on the minipump *in vitro*. Two minipumps were filled with red dye (FD&C No. 40), and a spectrophotometer was calibrated to test for the peak absorption wavelength of the dye (510 nm). One pump was placed in a beaker of body-temperature (37°C) water while the other was placed in a beaker of room-temperature (24°C) water. In both beakers, the water was gently stirred, and samples were taken every 5 sec to test for the presence and amount of dye in the water.

The results from this experiment confirm an early release and suggest that temperature change is the cause of drug release from the pump immediately after implantation (Fig.

2). According to the spectrophotometric readings, release begins as a fast spurt immediately after the pump is put into a warm environment and, after 80 sec, slows considerably. This 80-sec spurt released approximately 5% of the pump's contents. The pump in the 24°C water released virtually no dye.

Other investigators have also noted a release problem with the Alzet osmotic minipump [1]. These investigators reported that rats were receiving "the equivalent of an initial bolus injection" immediately after minipump implantation. Our experiment results, coupled with the problem mentioned by these authors, suggest caution for investigators using the Alzet osmotic minipump. Early *in vivo* release of the contents from the minipumps can be easily alleviated by presoaking the filled pumps in 37°C water for 10 min prior to implantation (Anderson 1986, personal communication). This procedure may slightly decrease the contents of the filled pump and the length of time the animal receives drug, but will allow continued, confident use of an otherwise excellent means of continuous drug delivery.

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