

# A Comparative Study of Some Effects of Aminoglutethimide Phosphate on Serum Potassium and Sodium

GEORGE P. POLLOCK, Indiana State University

## Abstract

A comparative study of the effects of 2-(p-aminophenyl)-2-ethyl glutethimide phosphate on serum potassium and sodium concentrations was conducted. *Rattus norvegicus*, *Coturnix japonicum*, *Rana pipiens* and *Chrysemys picta* were used. A significant increase in serum potassium and a significant decrease in serum sodium were observed in the homeothermic species. No significant changes in the two electrolytes were observed in the poikilothermic species.

## Introduction

The compound 2-(p-aminophenyl)-2-ethyl glutethimide phosphate (AGP) has been used clinically as an anticonvulsant in the treatment of generalized seizure and petit mal and temporal lobe epilepsy (1). Administration of the drug has resulted in some undesirable side effects. Changes in endocrine function similar to those produced with amphenone B were reported by Hertz et al (4). Pittman and Brown (5) observed hypertrophy of thyroid, ovarian, and adrenal tissues. Dexter (2) indicated a steroidogenic block with AGP prior to  $\Delta$ -5-pregnenolone. The specificity for the site of this block was indicated since addition of the latter compound allowed normal synthesis to occur. The experiments of Eversole and Zimmerman (3) suggested that abnormal mineralocorticoid synthesis occurred in rats following AGP treatment, since they reported deviations in urinary and blood electrolyte values in these animals. The present investigation was conducted to determine if similar effects of AGP are found in the serum electrolytes of other classes of vertebrates. Representative mammals, birds, amphibians, and reptiles were used in this study.

## Materials and Methods

Female *Rattus norvegicus*, white rats of the Charles-River strain, *Coturnix coturnix japonicum*, Japanese quail, *Rana pipiens*, grass frogs, and *Chrysemys picta*, painted pond turtles, were used in this experiment. All animals used were fasted 24 hours prior to treatment but were allowed water *ad libitum*. The AGP solution administered was prepared in a concentration of 100 mg of AGP/ml of distilled water. Each animal was given two injections of AGP in a dosage of 100 mg/kg body weight at 12-hour intervals. The injections for the rats, quail, and turtles were given subcutaneously in the neck region. The frogs were injected in the dorsal lymph sacs. Each control animal received an injection of distilled water comparable to the volume of AGP that it would have received as an experimental animal. Twenty-four hours after the first injection, the animals were sacrificed by guillotining. The blood was collected, and the serum was separated by centrifugation. Serum electro-

lytes were measured by flame photometry using the "Coleman Flame Photometry Manual" method. This procedure required serum dilutions of 1:50 for potassium and 1:200 for sodium samples. Since small quantities of serum were obtained from the frogs and quail, all sodium samples were prepared from the previously tested potassium samples to maintain consistency in all tests.

The standard "t" test was used for the statistical evaluation of the data. A "p" value of less than .01 was considered significant.

### Results

The AGP injections caused extreme inactivity in the rats and quail with many specimens unresponsive to external stimuli. This condition was more severe and prolonged in the quail than in the rats. Only initial irritation, shown by considerable kicking, was evident in the frogs and turtles. AGP caused significant differences in serum potassium and sodium concentrations in both the rats and quail. These differences were expressed as an increase in potassium and a decrease in sodium. No variations in these electrolytes were observed in the frogs and turtles. These results are cited in Table 1.

TABLE 1. *Serum Sodium and Potassium Concentrations.*

<i>Species</i>	$Na^+$		$K^+$	
	<i>Control</i>	<i>AGP</i>	<i>Control</i>	<i>AGP</i>
<i>R. norvegicus</i> (12)	147.0 ± 2.2	104.6 ± 3.1*	6.2 ± .3	8.1 ± .3*
<i>C. japonicum</i> (10)	149.2 ± 3.4	124.2 ± 3.6*	5.5 ± .3	8.1 ± .3*
<i>R. pipiens</i> (24)	114.8 ± 2.0	114.2 ± 1.5	2.6 ± .04	2.7 ± .06
<i>C. picta</i> (24)	118.5 ± 1.0	119.3 ± 1.2	2.4 ± .08	2.5 ± .04

P < .01.

\* Indicates significant difference.

All serum concentrations are in mEq/1 of solution.

### Discussion

Some individuals question the use of guillotined animals for electrolyte determinations because of simultaneous collection of body fluids. The results of this experiment are all relative values since all samples were obtained in the same manner. It is to be noted that the control values correspond with those reported by Prosser (6) for the rats, frogs, and turtles.

The results obtained in the experiments by Eversole and Zimmerman (3) in rats were similar but less profound than those found here in both rats and quail. These differences might be expected in the experiments of those authors since they gave one injection per 24 hours as compared with two injections per 24-hour period for the present experiment. The increased response might then be attributed to 1. a pharmacological booster effect of the AGP titer in the bloodstream, or 2. an increased response due to more AGP being present per 24-hour period.

The evidence that AGP produces a steroidogenic block prior to  $\Delta$ -5-pregnenolone, prepares one to assume a marked reduction or absence of mineralocorticoid secretion after treatment with the drug. The changes in the electrolytes of the rats and quail found here would not be unexpected. Furthermore one might expect identical changes in frogs and turtles since identical hormones have been isolated from these vertebrates. The question then arises as to why there were no changes in the electrolyte balance of these animals following treatment with AGP.

Several possible answers to this question exist, but they are highly speculative. This is due in part to lack of a method for qualitative determination of AGP in the bloodstream of these animals. If one assumes that AGP is absorbed and acts in cold-blooded animals in the same way as in warm-blooded animals, then one cannot overlook the idea of the existence of an alternate pathway of synthesis for corticosteroids unaffected by AGP treatment. There are tissues of unknown function which are suspected of steroidogenic action in both amphibians and reptiles (7).

A second area that needs investigation is that of absorption of the compound by these animals. The degree of vascularity in the subcutaneous neck region is not as extensive in the turtle as in the rats and quail. The results obtained would indicate that absorption of the compound was negligible for both the frogs and the turtles. When the mass of the turtle shell is considered as part of the total mass, and the injections were given on a body weight basis, the dose given to the turtles was the highest received by any of the animals. The frog injections were given in the lymph sacs and should have reached the general circulation more rapidly than in any of the other animals. This method of injection almost insures the presence of AGP in the bloodstream, and since no response was elicited, it then seems logical to assume the drug does not block steroidogenesis in the frog. Metabolic alteration of AGP by the frog and turtle exists as a possible cause for their lack of response. If the compound is altered to some intermediate before it can cause its block, then no change could occur. To be able to support this hypothesis, some means of qualitative determination of AGP must be developed.

#### Literature Cited

1. BAUER, R., and J. S. OLEYER. 1960. Clinical evaluation of Elipten, J. Mich. Med. Soc., 59:1829-1832.
2. DEXTER, R. N., L. M. FISHER, A. C. BLACK, and R. L. NEY. 1966. Inhibition of adrenal corticoid synthesis by aminoglutethimide. Clinical Res. 14:16 (abstr.)

3. EVERSELE, W. J., and R. E. ZIMMERMAN. 1968. Effects of aminoglutethimide on water and electrolyte metabolism. *Fed. Proc.* **27**:691.
4. HERTZ, R., W. W. TULLNER, J. A. SCHRICKER, F. G. DHYSE, and L. F. HALLMAN. 1955. *Recent Prog. Hormone Res.* **11**:119.
5. PITTMAN, J. A., and R. W. BROWN. 1964. Antithyroid and antiadrenocortical activity of aminoglutethimide. *J. Clin. Endo. and Metal.* **26**:1014-1016.
6. PROSSER, C. L. 1966. *Comparative Animal Physiology*, ed. 3. W. B. Saunders Co., Philadelphia.
7. TURNER, C. D. 1966. *General Endocrinology*, ed. 4. W. B. Saunders Co.,