Chlordiazepoxide and Diphenylhydantoin as Antagonists to ACTH Effect on Serum Calcium and Citrate Levels.* (31228)

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In earlier studies it was demonstrated that acid extracts of the anterior and posterior pituitary would lower serum calcium and elevate serum citrate levels in the rabbit(1,2). It was also shown that ACTH *per se* had this same property(3). Stress in rabbits would also cause an elevation of serum citrate levels and a slight but significant drop in serum calcium levels. Adrenaline causes a rise in citrate levels but has little effect on the calcium levels(2).

In view of the above, it was decided to study the effect of certain drugs which are used to relieve tension, to see whether they would antagonize the effect of ACTH in the rabbit. Chlordiazepoxide (Librium) was chosen for study because of its remarkable taming effect in animals(4,5), and its effect on reducing seizure frequency in convulsives (6). Diphenylhydantoin (Dilantin) was also chosen for study because of the long experience with this drug in controlling convulsive seizures(7).

Method. Male New Zealand rabbits weighing from 3-4 kg were fasted for 48 hours, water not being withheld. Blood specimens were obtained from the ear vein as described (8). Saline, chlordiazepoxide and ACTH were injected intramuscularly. Diphenylhydantoin was injected intravenously through the ear vein. A fasting blood specimen was taken and subsequent blood specimens were taken from the same ear vein at hourly intervals. Calcium and citrate levels were then determined on the serum obtained from the blood.

Normal saline (1 ml) was used in the controls (Fig. 1). To evaluate the potency of the ACTH preparation, the solid was dissolved in normal saline so that 1 ml comprised a dosage of 300 μg per kilo, of a preparation which assayed for 100 ACTH units per mg. The ACTH was obtained from Mann Research Laboratories, New York. A fasting blood specimen was taken and the ACTH was administered soon thereafter (Fig. 1).

Chlordiazepoxide (2 mg/kilo) was administered intramuscularly after taking the fasting specimen (Fig. 2). To study its effect in antagonizing the action of ACTH, the same experiment was performed except that the ACTH was administered directly after the first hourly blood specimen had been taken. Thus in Fig. 2 the one-hour values, in the second part of this experiment, are due to the effects of chlordiazepoxide alone. Subsequent values demonstrate the combined effect of chlordiazepoxide and ACTH on the serum calcium and citrate levels.

The experiments with diphenylhydantoin (Fig. 3) were carried out as before, 20 mg/kilo being administered intravenously after the fasting specimen had been taken. In studying the combined effect of diphenylhydantoin and ACTH, 30 minutes were allowed to elapse before ACTH was administered. Thus, in Fig. 3, in this experiment, the calcium and citrate values, after the fasting value, are influenced by the combination of these two substances.

The data are presented in the Figures as the mean per cent change from the fasting levels. Thus each rabbit's fasting level served as a control for subsequent values, since fasting calcium and citrate levels did not change significantly in the controls as seen in Fig. 1. The absolute changes can be obtained from Table I where the mean fasting values are given. This Table also lists the number of rabbits used in each experiment and the numbers which convulsed or expired in convulsions.

The chlordiazepoxide was used as the hydrochloride (Librium, Roche Laboratories, Nutley, N. J.). The diphenylhydantoin was used as the sodium salt (Dilantin, Parke, Davis & Co., Detroit, Mich.).

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Calcium levels were determined with the X-ray spectrometer(9). Reproducibility with this technique, as determined in the 61 rabbits used, comparing replicates on a total of 300 specimens was ± 3% (2σ). Citrate was determined by a method previously described (10). Reproducibility for this method was found in this study to be ± 6% (2σ).

Results. In Fig. 1 it is demonstrated that the preparation of ACTH used was effective in causing an elevation of citrate and depressing serum calcium levels in the rabbit. When the t test was applied, comparing citrate elevation under the influence of ACTH with the fasting levels, the mean differences were significant at the 0.001 probability level. This was also true for the minimum calcium level observed. The mean fasting calcium level for all experiments (60 rabbits) was 13.68 ± 1.4 (2σ). The mean fasting citrate level for the 60 rabbits was 4.69 ± 2.4 (2σ).

Examination of Fig. 2 indicates that chlordiazepoxide depresses serum citrate levels in the rabbits. When the t test was applied,

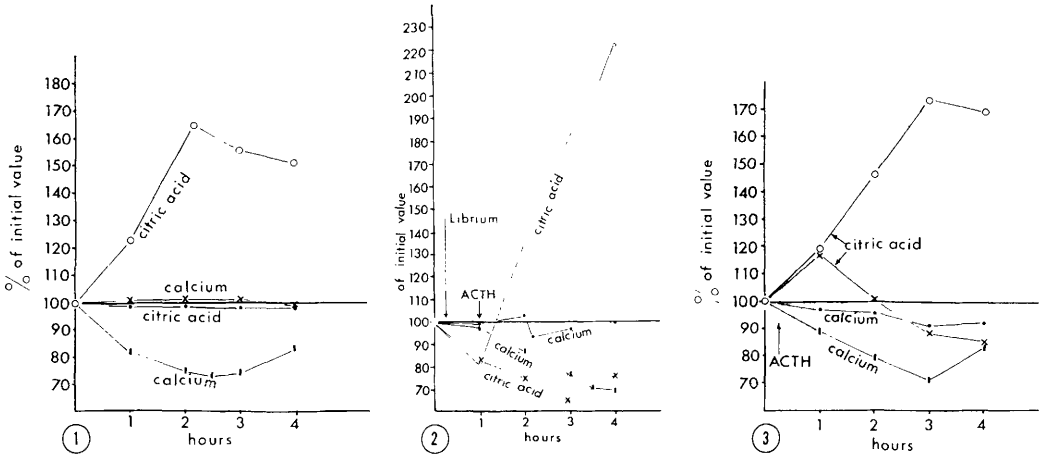

FIG. 1. Effect of ACTH administered intramuscularly on serum calcium (—I—I—) and citrate (—○—○—) levels in the fasting rabbit. Controls (—X—X— calcium, and —●—●— citrate) were injected with normal saline.

FIG. 2. Effect of chlordiazepoxide (Librium) on serum calcium (—●—●—) and citrate (—X—X—) levels in the fasting rabbit. ACTH injected after one hour resulted in curves indicated by —○—○— for citrate and —I—I— for calcium.

FIG. 3. Effect of diphenylhydantoin (Dilantin) on calcium (—●—●—) and citrate (—X—X—) levels in the fasting rabbit. Curves indicated by —■—■— for calcium and —○—○— for citrate resulted from injection of ACTH 15 min. after diphenylhydantoin administration.

TABLE I. Mean Fasting Levels for Serum Calcium and Citrate. Number convulsing and expiring in convulsions in the various experiments.

Substance	No. of rabbits	Fasting (mean), mg/100 ml	S.D. ±	Convulsed but recovered	Expired in convulsions
Saline (controls)	10	Ca 12.81 Cit 4.22	.44 .94	0	0
ACTH	12	Ca 13.03 Cit 4.39	.85 1.69	3	1
Chlordiazepoxide	10	Ca 13.00 Cit 4.16	.50 1.26	0	0
Chlordiazepoxide & ACTH	12	Ca 14.32 Cit 5.79	.90 1.00	1	7
Diphenylhydantoin	7	Ca 13.35 Cit 4.72	.56 1.38	0	0
Diphenylhydantoin & ACTH	10	Ca 14.39 Cit 4.86	.94 1.23	1	1

comparing the mean value obtained at the lowest point with the fasting level, the differences were significant at the 0.001 probability level. The changes in calcium level, however, were not significant at the 0.05 probability level. After ACTH injection, the rise in citrate was greater than that observed with ACTH alone. Depression of calcium with the ACTH was of the same order as that observed without chlordiazepoxide when Fig. 1 and 2 are compared.

The effect of the various combinations on the frequency of convulsions in the rabbits is listed in Table I. The mean fasting levels for citrate and calcium in these experiments are also listed. It is to be noted that one-third of the rabbits convulsed with ACTH alone, one expiring. When the ACTH was administered one hour after the chlordiazepoxide had been administered, two-thirds of the rabbits convulsed and seven died. Thus only 5 out of 12 rabbits survived the experiment, while 11 out of 12 survived with ACTH alone.

With diphenylhydantoin, at 10 times the dosage of chlordiazepoxide, a preliminary rise of citrate (15%) followed by a drop was observed to a level about 15% below the initial fasting level. Comparing the citrate maxima and minima with the fasting level, both were barely significant at the 0.05 probability level. The drop in serum calcium levels observed (9%) was not significant at the 0.05 probability level. When ACTH was injected 15 minutes after the diphenylhydantoin, the effect on calcium levels was not significantly different (29%) from that observed with ACTH alone (26%). Citrate elevation was slightly higher (74%), as compared with that observed with ACTH alone (65%). Comparing these two values by means of the t test no significant difference was obtained. Thus both citrate and calcium levels seemed to change under the influence of ACTH, independent of the diphenylhydantoin administered.

Discussion. Citrate levels will rise markedly both in humans(11) and in rabbits under the influence of adrenaline(8). On the other hand, chlordiazepoxide will depress citrate levels significantly (Fig. 2). This drop in citrate level suggests that adrenaline levels in the rabbit blood may be decreased under the influence of chlordiazepoxide. This needs to be confirmed by direct study of serum adrenaline levels.

When the effect on citrate levels was observed, it was expected that chlordiazepoxide would act as an antagonist to the action of ACTH in rabbits with regard to the elevation of their serum citrate levels. This expectation was fortified by the observation of others on rats stressed by means of electric shock. With electric shock, fatty acid levels in the rat serum were elevated. Pretreatment of the rats with chlordiazepoxide prevented this effect(12).

This expectation was not realized in our experiments. On the contrary, the effect of this drug was to exaggerate the effect of ACTH on the rabbit serum citrate levels (Fig. 2). The effect on the animals seemed to be such that their natural ability to overcome the effect of ACTH on calcium and citrate levels was diminished. This can be seen from Table I where 7 out of 12 rabbits, after ACTH injection, expired in convulsions in the presence of chlordiazepoxide while only one expired in its absence. No significant effect of chlordiazepoxide on either alleviating or exaggerating the depression of serum calcium levels, with ACTH treatment, was noted.

The physiological action of diphenylhydantoin is quite distinct from that of chlordiazepoxide(7). Difference in their action on serum citrate levels can be observed in comparing Fig. 2 and 3. The effect of diphenylhydantoin on citrate levels is slight, causing a moderate rise followed by a fall in citrate levels. Its effect on calcium levels is also small. Diphenylhydantoin does not seem to affect the action of ACTH on citrate or calcium levels in these experiments (Fig. 3). Also, in Table I it can be seen that diphenylhydantoin did not seem to affect significantly the ability of the animals to overcome the effect of the ACTH, as far as recovery is concerned. These observations should be compared to those made by others where it was demonstrated that diphenylhydantoin effected no significant change either in the basal plasma 17-hydroxycorticosteroid level or in the

magnitude of the rise occurring after ACTH administration(13).

The observations reported above indicate that there is an interaction between the effects of ACTH and chlordiazepoxide. This is apparently not significant in regard to ACTH and diphenylhydantoin. Since the mechanism by which ACTH affects the rabbit's serum calcium and citrate levels is unknown, it is apparent that the unexpected effects observed with chlordiazepoxide cannot be interpreted at this time. It will be noted in Fig. 1 that at 3 to 4 hours the rabbit is overcoming the effect of ACTH on calcium and citrate levels. Under the influence of chlordiazepoxide, as seen in Fig. 2, citrate levels continue to rise and calcium levels continue to fall. It can therefore be suggested that by whatever mechanism the rabbit overcomes the effect of ACTH on calcium and citrate levels, this is inhibited by chlordiazepoxide.

Summary. ACTH (100 U/mg) caused a 64% elevation of citrate and a 26% drop of serum calcium levels at 2 hours after administration of the hormone, intramuscularly, in fasting rabbits. One out of 12 rabbits died in convulsions. Chlordiazepoxide (2 mg/kilo) administered intramuscularly caused a 33% depression of serum citrate levels at 3 hours. This substance had no significant effect on serum calcium levels. ACTH administered one hour after chlordiazepoxide injection caused a greater elevation of citrate levels (110%) which persisted longer than with ACTH alone. In these experiments 7 out of 12 rabbits died in convulsions within 4 hours after ACTH administration. Calcium depression due to the ACTH in this experiment was the same as without the chlordiazepoxide but persisted for a longer period. Diphenylhydantoin (20 mg/kilo) did not cause marked changes in the serum citrate or calcium levels. ACTH administered intramuscularly after diphenylhydantoin injection intravenously depressed calcium levels and elevated citrate levels to the same degree as in the absence of the diphenylhydantoin. Diphenylhydantoin had no significant effect on the number of rabbits which survived the experiment.

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