Chronic Potassium Depletion and Sensitivity to Tubocurarine

B. E. Waud, M.D.,* A. Mookerjee, M.D.,† D. R. Waud, M.D., D. Phil.‡

The effect of low potassium diets with and without deoxycorticosterone (DOCA), furosemide, chlorothiazide, or ethacrynic acid on sensitivity of isolated guinea pig lumbrical nerve-muscle preparations to d-tubocurarine was examined. The ED_{50} of d-tubocurarine was found to fall as the potassium level was lowered by dietary restriction. Addition of DOCA or furosemide was without effect while chlorothiazide reduced and ethacrynic acid enhanced the effect of diet alone. Acute restoration of potassium levels to 5.9 mM reversed considerably, but not completely, the effect of chronic depletion. Thus, a patient suffering from chronic potassium depletion would be expected to require a decreased dose of d-tubocurarine, and although acute replacement of potassium preoperatively would tend to return the dose requirement toward normal, such reversal might not be complete. (Key words: Ions: potassium, depletion. Neuromuscular junction. Neuromuscular relaxants: d-tubocurarine.)

ACUTE HYPOKALEMIA decreased the concentration of d-tubocurarine or pancuronium needed to produce neuromuscular block.[1] However, studies of the effect of chronic hypokalemia on the action of neuromuscular blocking agents have produced conflicting results. While chronic furosemide treatment in dogs had no effect on pancuronium or d-tubocurarine neuromuscular block,[2] chronic chlorothiazide treatment in cats decreased the dose of pancuronium required for neuromuscular block.[3] It is difficult to tell from such experiments whether the variability reflects a difference between diuretics or the difficulties inherent in controlling *in vivo* assay systems, (*e.g.*, cardiovascular parameters such as distribution of blood flow are both hard to measure and to control). Therefore, the effect of chronic hypokalemia of dietary and of drug-induced origin was examined in an *in vitro* system. The effect of acute potassium replacement was determined in a parallel series of experiments.

Methods

The experiments were performed in an isolated guinea pig nerve-lumbrical preparation suspended in Krebs' solution at 37°C as described previously.[1] Five groups of guinea pigs were studied. Each group contained a subgroup receiving normal diets and a second receiving low potassium diets. One group served as controls, and the remaining four groups received daily intraperitoneal injections of one of the following drugs; furosemide (1 mg/kg), ethacrynic acid (3 mg/kg or 10 mg/kg), chlorothiazide (1 mg/kg), and deoxycorticosterone acetate (1 mg) from 4 to 30 days. Following the pretreatment, the animals were anesthetized (see below), an approximately 2-ml blood sample was obtained by intracardiac puncture, and plasma potassium and sodium were assayed by flame photometry. Isolated nerve-lumbrical muscle preparations were prepared in two 50-ml baths of Krebs' solution, the compositions of which were identical, except that one had a potassium concentration of 5.9 mM (the usual value for Krebs' solution), and the other had a concentration equal to the plasma potassium determined earlier. The baths were bubbled with 95 per cent oxygen and 5 per cent carbon dioxide, and maintained at 37°C. The isometric twitch response to indirect stimulation was measured at 10-s intervals with a supramaximal stimulus of 0.3-ms duration. Once the twitch response had stabilized, d-tubocurarine was added in graded doses to generate twitch height *vs.* d-tubocurarine concentration-response curves. At the end of the experiment, the d-tubocurarine was washed out to confirm that the twitch responses had returned to control levels. A sigmoid curve was fitted to the results by an iterative nonlinear least squares technique, and values of the concentration reducing the twitch responses by 50 per cent (ED_{50}) were determined.

In preliminary experiments, it was noted that high potassium values were obtained when the guinea pig was stunned prior to drawing the sample of blood by cardiac puncture. Therefore, we tried anesthesia prior to intracardiac puncture. The animals were given intraperitoneal thiopental (30 mg/kg) and when their righting reflex was lost, open drop halothane was added. Prior to induction, the animals were handled gently and allowed to become accustomed to being held and to sitting in the lap of the person holding the animal during the onset of anesthesia. When duplicate samples were taken, it was found that the second potassium measurement was very high (6–7 mM) if apnea or cardiac fibrillation followed the first cardiac puncture. Therefore, animals in which apnea or fibrillation occurred before the first blood sample could be taken, were not used. Anesthetic agents did not appear to affect results, since d-tubocurarine showed the same ED_{50} in animals receiving thiopental and halothane as those in a previous study in which the animals were stunned prior to removal of the two muscles.
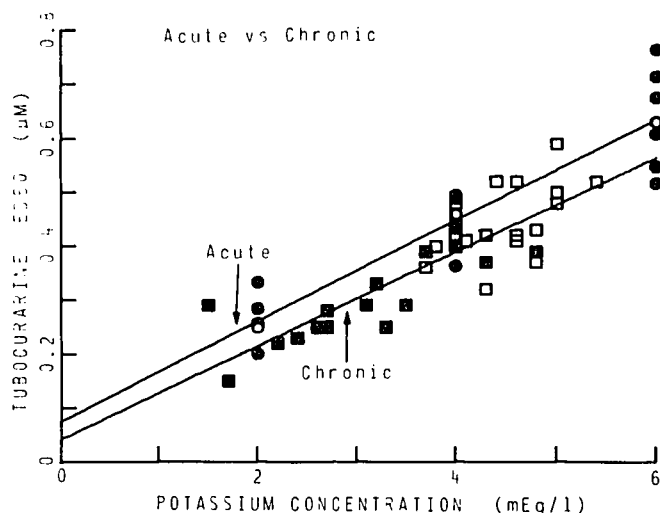
Fig. 1. Relationship of ED_{50} of tubocurarine to potassium levels. Ordinates: Values for ED_{50} of tubocurarine (μM). Abscissae: Concentrations of potassium (mEq/l) bathing the muscle. Open squares: values from animals on a normal diet: Muscle bathed in Krebs' solution of potassium level equal to the measured plasma level in that animal (n = 17). Closed squares: corresponding values from animals on a low potassium diet (n = 15). Closed circles: values from previous study in which muscles were removed from normal animals and bathed in Krebs' solution with the indicated potassium level (n = 18). Open circles: corresponding values from present study (n = 3). Regression lines fitted by least squares. Chronic depletion produced a reduction in tubocurarine dose requirement similar to that seen with acute depression but of a slightly greater magnitude.

Statistical Analysis

Most of the results are conveniently summarized in the form of a regression of ED_{50} of tubocurarine against concentration of potassium. However, when two or more such regressions are to be compared, a modified analysis of covariance is needed. The underlying logic is somewhat involved and is presented in the Appendix. Effects demonstrable at the 95 per cent probability level were considered significant.

Results

When the animals were treated gently during induction of anesthesia (see Methods), a potassium level of 4.5 ± 0.5 mM (SD) was obtained. This was taken as a "normal" level. When animals were kept on the low potassium diet, their plasma potassium levels were reduced. Muscles taken from such animals and mounted in a Krebs' solution of potassium concentration equal to the measured plasma level, showed an increased sensitivity to d-tubocurarine, $i.e.$, measured ED_{50} values were reduced. The extent of reduction depended on the potassium level as indicated by the closed squares in figure 1. These values appeared to be part of a continuum which included the values obtained in animals on a normal diet

(open squares in figure 1). Since these two populations should logically be expected to merge into each other, these two sets of results were pooled to form a group showing the relationship of d-tubocurarine ED_{50} to potassium level. In figure 1, this regression is compared to corresponding values (closed circles) obtained in an earlier study in which muscles from animals on a normal diet were exposed acutely in the organ bath to one of three potassium levels. Chronic depletion produced a significantly greater decrease in ED_{50}, but the change occurred equally at all levels. Because of the danger inherent in the use of historical controls, three muscles were studied acutely toward the end of the present study. The results appear in figure 1 as the open circles. Clearly, there is no sign of temporal drift.

When administered alone, neither DOCA nor any of the three diuretics examined significantly lowered potassium levels. Therefore, all four agents were studied in animals also receiving a low potassium diet. The results are shown in figure 2 which compares values obtained with each of the four drugs with those obtained by the diet alone. The values with DOCA showed considerable scatter but, to the level of precision possible given this background, they were not different from those found with diet alone. Similarly, furosemide showed no effect beyond what would be expected from the dietary level of potassium depletion. Chlorothiazide and ethacrynic acid, in contrast, showed significant drug-specific effects. With chlorothiazide, the ED_{50} was higher than expected, with ethacrynic acid, it was lower. In both cases, the drug regression was parallel to the control curve, indicating that the drug effect was unrelated to potassium depletion [c.f. case (2) in Appendix].

Figure 3 shows the reversibility of the effect of diet. When muscles were studied in Krebs' solution containing the usual 5.9 mM potassium, the ED_{50} of d-tubocurarine was closer to normal than in comparison preparations tested at a potassium level matching that assayed in their plasma just prior to removal of the muscle.

Figure 4 shows that acute replacement of potassium behaves similarly in the muscles from animals pretreated with DOCA, furosemide, or chlorothiazide; in all three cases, bathing the muscle in 5.9 mM K^+ produced an upward shift of the regression, $i.e.$, the muscle behaved more like normal muscles, but the effect of chronic depletion was not completely reversed. Ethacrynic acid also showed partial reversal, but even in animals not on a low potassium diet (at the right-hand end of the curve in fig. 4D), the ED_{50} was lower than normally seen (the mean is normally 0.575 mM ± 0.0195 SE).

There was no relationship seen between the measured plasma sodium level and ED_{50} of d-tubocurarine, nor was there any influence of plasma potassium on control developed twitch tension.
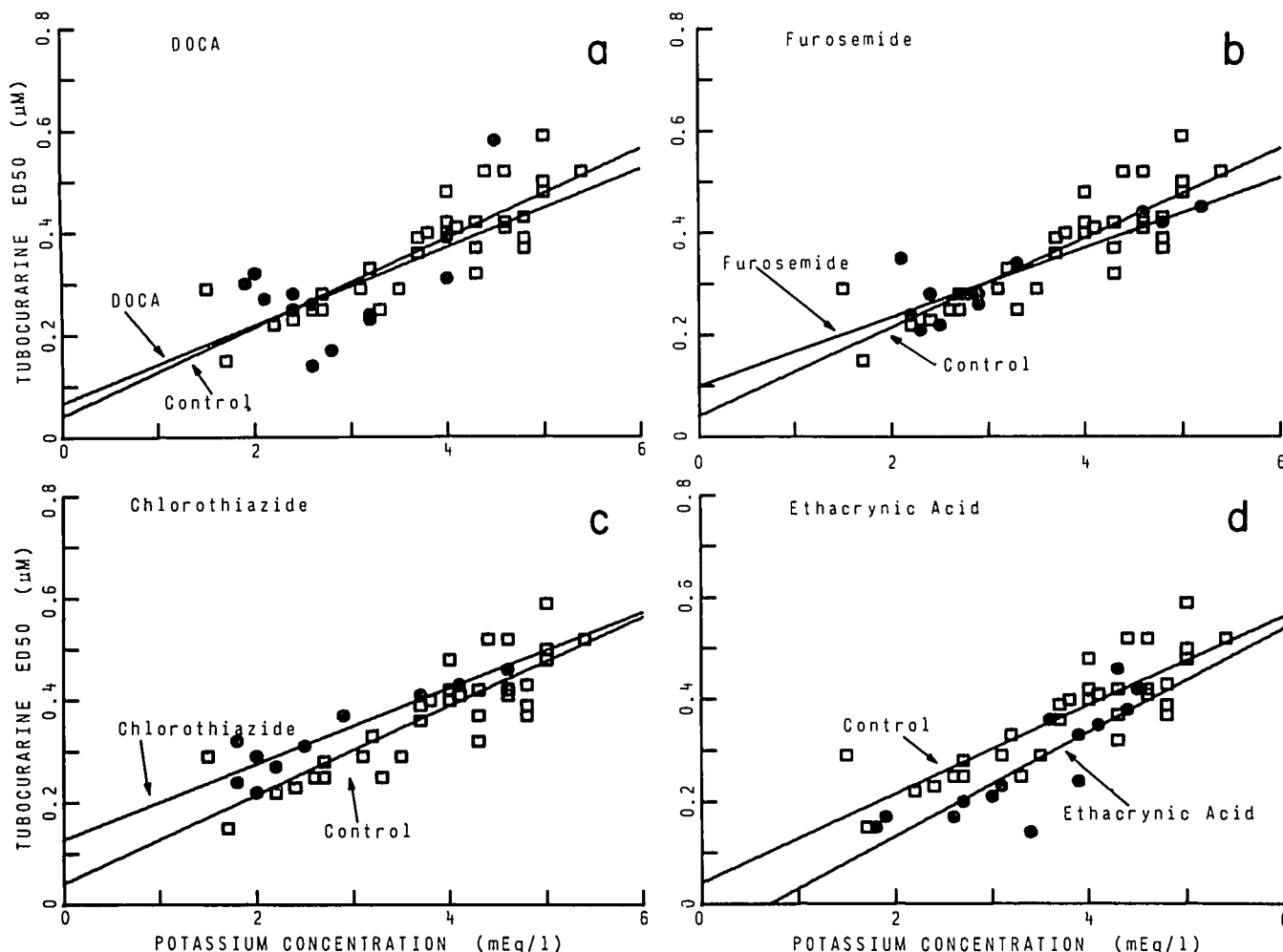
FIG. 2. Effects of DOCA (n = 13), furosemide (n = 12), chlorothiazide (n = 10), and ethacrynic acid (n = 14) on ED_{50} of tubocurarine. Ordinates and abscissae as in figure 1. Circles: values obtained when drug indicated was added to the dietary pretreatment. DOCA and furosemide produced no effect beyond that seen with diet alone while chlorothiazide produced slightly less and ethacrynic acid slightly more reduction in tubocurarine ED_{50} as the potassium concentration fell.

Discussion

The resting plasma level of 4.5 mM is lower than many reported in the literature. However, measurements of plasma potassium in the guinea pig are easily overestimated. The animals are readily excited and presumably there is release of adrenal medullary catecholamines which leads to a shift of potassium from the intracellular pool into the plasma. Anoxia following stunning an animal might have a similar effect. Therefore, we suspect higher values elsewhere probably reflect a contribution from superimposed excitement and/or anoxia associated with the sampling procedure. We still cannot be sure our levels do not reflect a slight residual overestimate, but feel its magnitude is probably small since our measures designed to keep our animal calm prior to sampling were clearly reaching the point of diminishing returns.

The effect of chronic potassium depletion was very similar to that seen previously with acute changes in potassium concentration. However, in figure 1, the curve of chronic depletion lies below the regression for the acute experiments at all potassium levels. This means the difference in curves is nonspecific, i.e., unrelated to potassium level alone [c.f. case (2) in Appendix]. A likely explanation relates to the unattractive taste of the low-potassium diet. Guinea pigs on this diet seemed to eat less enthusiastically and gained weight less than when on a normal diet. Hence, it is possible that a nutritional contribution may underlie the lower position of the chronic curve of figure 1 relative to the acute results, although there was no relationship between weight of animal and either plasma potassium or d-tubocurarine ED_{50}.

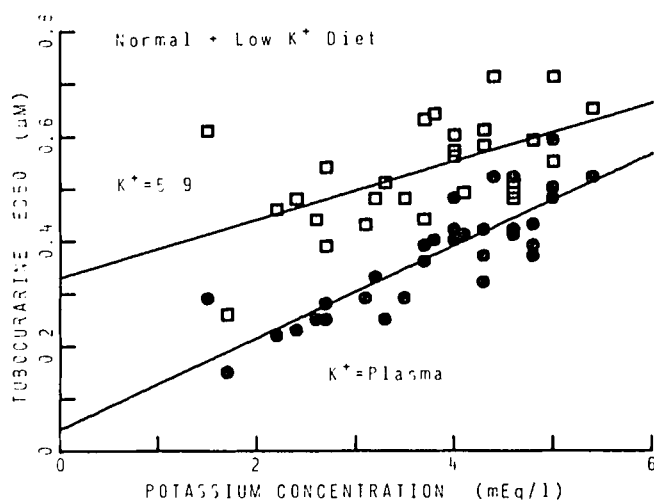The inability of DOCA and the three diuretics to

Fig. 3. Reversibility of effect of diet. Ordinates and abscissae as in figure 1. Circles: responses obtained with chronic dietary pretreatment and muscle bath at potassium level equal to that measured *in vivo* prior to the *in vitro* assay (n = 32). Squares: responses obtained in a paired muscle assayed in Krebs' solution with a potassium concentration of 5.9 mEq/l (n = 30). Acute restoration of the potassium level of the bath fluid to normal levels produced a substantial but not complete reversal of the effect of chronic dietary potassium depletion.

lower potassium levels probably reflects simply the fact that the normal guinea pig food used contains abundant (1.46 per cent by weight) potassium. This conclusion is in line with experience in people where adequate dietary potassium protects against depletion. Thus, the combination of drug plus low potassium diet used in this study provides a good model for the patient who comes to surgery on diuretics and with a low potassium level.

DOCA proved to be an unsatisfactory tool for producing potassium depletion—too much scatter came into the results. This may reflect the difficulty of administering a preparation of insoluble crystals or of getting consistent absorption of them. In any case, the ED_{50} of *d*-tubocurarine did not differ from that obtained from animals on diet alone. The other three drugs, all powerful diuretics, gave more stable results. While the values of ED_{50} from those animals treated with furosemide did not differ from the controls, chlorothiazide produced less change and ethacrynic acid produced more change than would be predicted from diet alone. These differences, while significant statistically ($P < 0.05$ and $P < 0.01$, respectively), are probably insufficient to be detected clinically. Thus, it appears that the anesthesiologist need not be particularly concerned about what caused a potassium depletion but only about its magnitude.

The experiments in which, following pretreatment, the muscle was returned to a medium in which the potassium level was 5.9 mM, indicate that the effects of chronic potassium depletion can be reversed significantly

with replacement of the ion. The reversal was not complete, however. In the case of ethacrynic acid, the ED_{50} was reduced even in animals on a normal diet and having potassium levels in the normal range. Presumably, whatever nonspecific factor depressed the ethacrynic regression in figure 2D, is also operative in the results in figure 4D. Thus, although the experimental design was not such as to shed light on specific mechanisms, the results in the two series of experiments are consistent.

Taken together, our results suggest chronic potassium depletion, if severe, might be expected to reduce dosage requirement for *d*-tubocurarine to about one-third of the normal. Acute replacement of potassium could reverse this effect significantly but probably less successfully, if the patient were receiving ethacrynic acid. The anesthesiologists faced with a patient receiving a diuretic and with a low potassium diet, would be advised, even when potassium supplement is administered preoperatively, to choose the initial dose of competitive neuromuscular blocking agent conservatively so as to be less likely to overshoot when titrating the drug against the patient. We agree with the referee who said "careful monitoring in such situations would make a lot of sense!"

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APPENDIX

Slopes of the regressions can be compared by first fitting two lines with separate intercepts and slopes and then fitting with two intercepts and a single slope. If the first fit is not significantly closer than the second, then the curves are not demonstrably non-parallel. In this case, the curves can next be compared to see if their heights (*i.e.*, intercepts) differ. This can be done in the usual fashion[4] by comparing two lines fitted with two intercepts and a single slope with a single line fitted with just a single intercept and slope.

If the curves are not parallel, a general "cookbook" approach is no longer appropriate—one must work from a more specific model which takes into account the known pharmacology of the system. Thus, in the present experiments, it is reasonable to expect that the simplest shift of one curve from the other would be a rotation about the point corresponding to a normal level of potassium. In figure 1, for example, the ED_{50} obtained at a normal potassium level should be the same whether that value
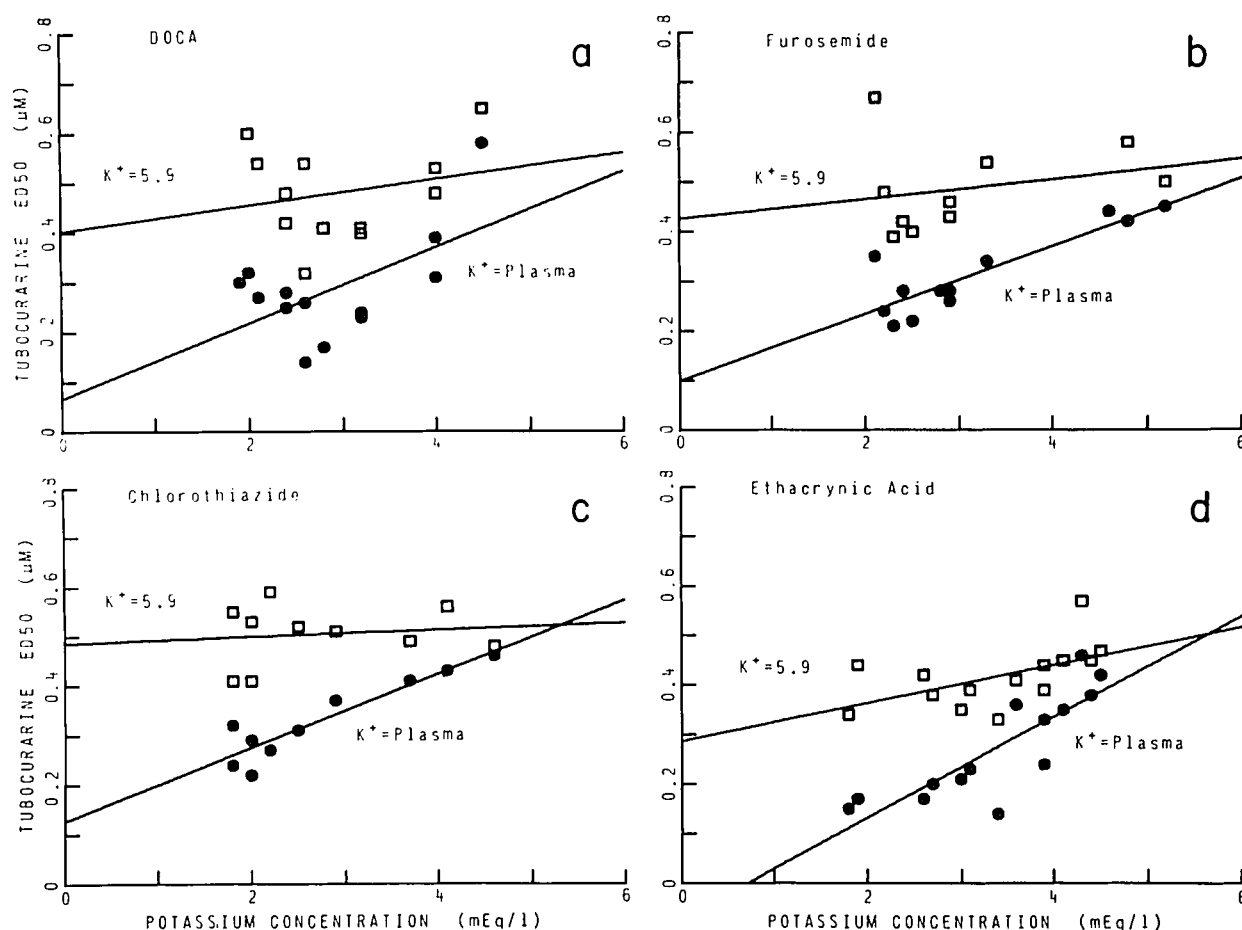
FIG. 4. Effect of acute replacement of potassium on muscles from animals pretreated with both diet and a diuretic. Ordinates and abscissae as in figure 1. Circles: responses obtained with combined drug and dietary pretreatment and muscle bathed in Krebs' solution having a potassium level equal to that measured in vivo prior to isolation of the preparation. Squares: responses of paired muscles from the same animals assayed at K^+ = 5.9 mEq/l. In all cases the effect of chronic treatment could be reversed considerably by potassium replacement, but the reversal was less when ethacrynic acid was used.

was reached acutely or chronically, since no perturbation would have occurred in either case. The first step in the analysis then is to move the axis of ordinates over to the right so that it passes through the scale of abscissae at K^+ = 4.5 (the mean normal level observed in our experiments), i.e., effectively to measure potassium concentrations as deviations from the mean normal level. Now, if the only effect is a rotation of one line relative to the other, the intercept with the axis of ordinates will not change significantly. To test for a change, one can now simply fit two lines with separate intercepts and slopes, then two lines with a common intercept and slope. If the former fit is not significantly better, the curves differ only in slope. This analysis can be generalized to analyze several curves when appropriate.

In summary, the logical flow chart for analysis of a pair of regressions is (a = intercept, b = slope, y = a + bx).

Are the slopes different? (i.e., do 2 as and 2 bs give a better fit than 2 as and 1 b?)

(a) No

Are the heights different? (i.e., do 2 as and 1 b give a better fit than 1 a and 1 b?)

(1) No—There is no difference at all.

(2) Yes—There is a nonspecific effect, i.e., one not depending on potassium level. For example, the experimental intervention may reduce the ED_{50} by a mechanism having nothing to do with effects on potassium levels.

(b) Yes

Are the intercepts different? (i.e., do 2 as and 2 bs give a better fit than 1 a and 2 bs?)

(3) No—There is a difference which is purely potassium-related.

(4) Yes—There is a combination of cases (2) and (3).