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Abstract: The question, Is adrenal gland weight a useful index of adrenocortical secretion rate?, is answered by correlation analysis of gland weights and secretion rates in California ground squirrels. The weights and rates had a highly significant correlation coefficient of 0.75 in a combined sample of 12 females and 17 males. For the sexes separately the coefficients were significant at 0.55 and 0.56 respectively. Neither the correlation nor the regression coefficients showed any significant sex differences. A graph is presented showing sample sizs of adrenal weights required to distinguish mean differences in secretion rates at given confidence levels. It is concluded that gland weight is a useful index of secretion rate under steady state or slowly changing conditions. Apparent contradictions to this conclusion in the literature are resolved in discussion.

The adrenal cortex is often implicated in both parasitic and metabolic diseases. Hypercortical function lowers resistance to invading disease organisms and is associated with metabolic diseases such as glomerulonephritis and cardiac infarction.[5] Whether these hormonal relationships occur under natural conditions in wild animals is a subject of continuing research and debate. One difficulty is the lack of a proven simple index of adrenocortical secretion rate that can be used in field and laboratory studies.

Because the adrenocortical secretion rate is difficult to measure directly, many researchers have used the weight of adrenal glands as an index of secretion rates. However, some doubts exist regarding the validity of the weight-index,[9] and some investigators[4] have expressed cautions regarding the interpretation of weight changes. Since we planned to use the gland-weight index in forthcoming studies of populations of the California ground squirrel, *Spermophilus beecheyi*, it became pertinent to calibrate secretion rates in terms of gland weights.

This is a report of our calibration findings and is the second part of a three-part study. The first part[1] reported the verification of the tissue-incubation technique as a measure of adrenocortical secretion rate. The third study (Unpublished data) is concerned with behavioral correlates of adrenal gland size that can be used to monitor gland weight in longitudinal studies of free squirrels in their natural habitat.

METHODS

From January 25 to March 9, 1967, 12 female and 17 male squirrels were shot in the field and their adrenal glands were weighed to the nearest milligram on a torsion balance, incubated for 3 hours, extracted and dried, as described by Adams.[1] One USP unit of ACTH per 100 mg of tissue was added to the incubation medium. To the dried incubation extracts, trace amounts of radioactive cortisol and corticosterone were added to correct for losses during the thin-layer chromato-

graphy. The mean recoveries were 74 ± 1.7 (SEM) % for cortisol and 73 ± 1.9% for corticosterone. The samples were spotted on silica gel thin-layer plates (0.2 mm thickness) and chromatographed in the system $CHCl_3$:CH_3OH:H_2O (90:10:1) to separate cortisol and corticosterone. The steroids were eluted separately with absolute ethanol and after drying, each was quantified fluorometrically. Cortisol was measured by the method of Mattingly[7] and corticosterone by the method of Zenker and Bernstein.[12]

We incubated all gland pairs except a small section of one gland that was preserved separately for histological reference. The measure of secretion rate used in the correlation analysis was the total production of both cortisol and corticosterone, found by the fluorometric method, adjusted to allow for the production that the tissue section would have added if it had been included with the incubated tissue.

The relationship of gland weight to secretion rate was analyzed by standard methods of regression and correlation analysis. Correlation and regression coefficients were calculated for males and females separately and then for both sexes together, since the coefficients for individual sexes were not significantly different from each other.

RESULTS

Figure 1 shows the correlation and regression coefficients of secretion on gland weight for each sex separately and for the sexes combined. The correlation coefficients for each sex were significant

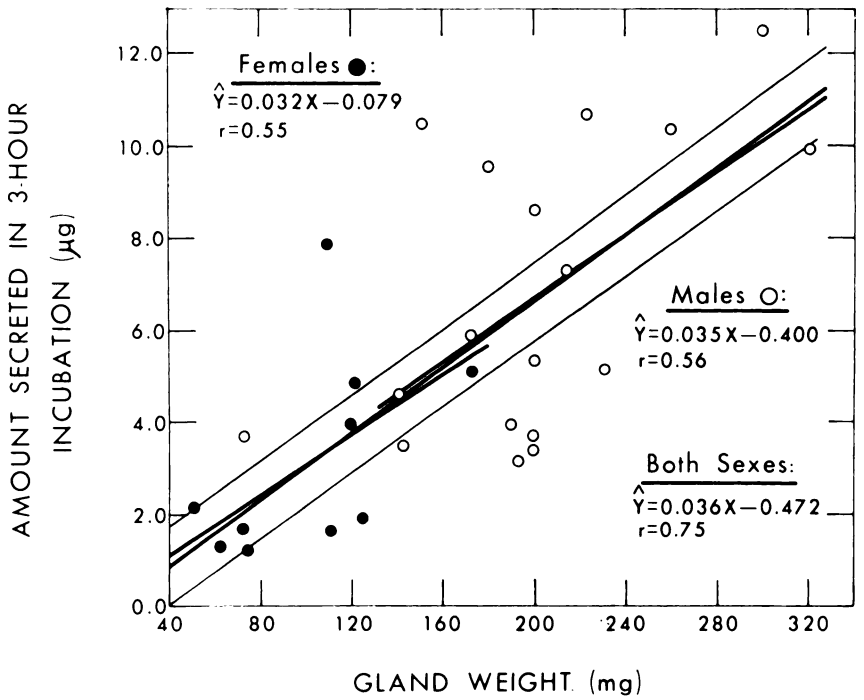

FIGURE 1. Regressions of adrenocortical secretion on weights of gland pairs. The three regression lines are for females only (short line at left), males only (short line at right), and both sexes (long line through both sexes).

at the 5% level (t-test); the coefficient for the sexes combined was significant at the 0.5% level of probability. With the degree of correlation we have from this calibration study, the question arises: How many gland-pairs would have to be weighed to distinguish the mean secretion rates of two populations with given differences? The curves presented in Fig. 2 give one kind of answer according to a

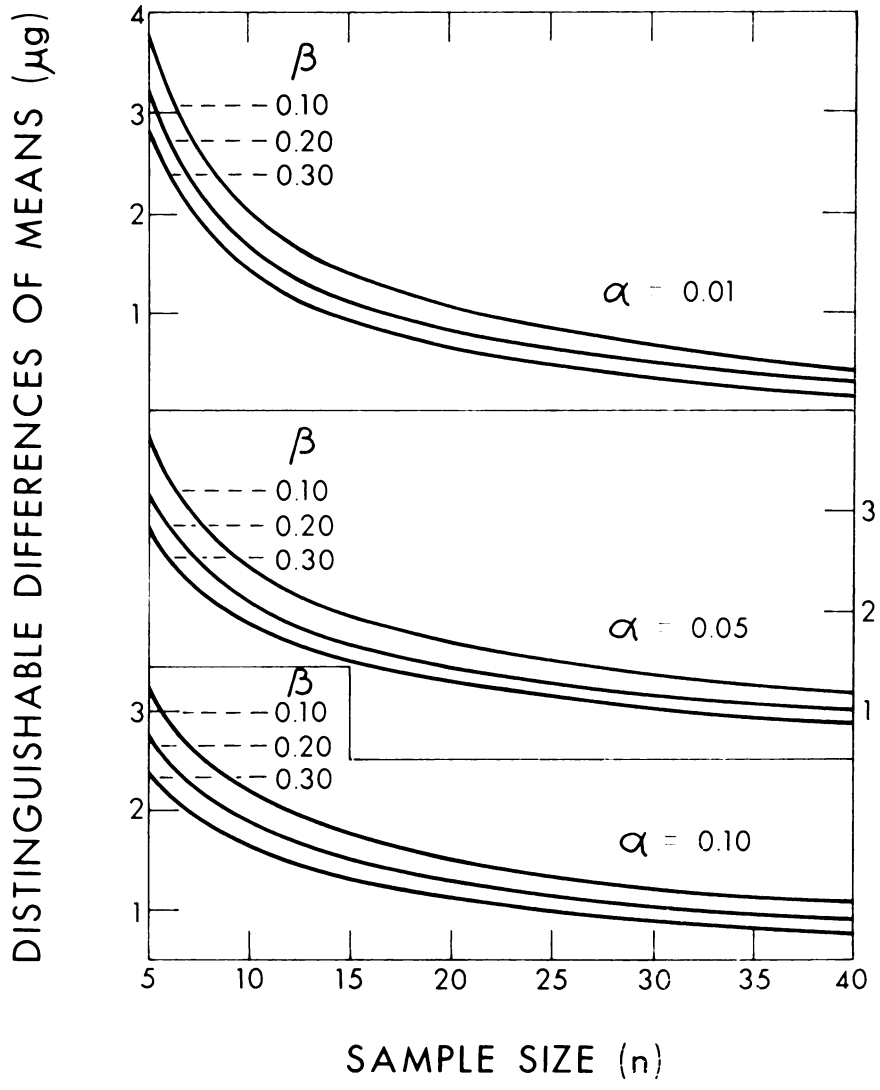

FIGURE 2. Sample sizes required to distinguish mean differences in mean secretion rates at various levels of confidence. Ordinates refer to the mean volume (μg) of cortisol secreted in 3 hours of incubation. α and β refer respectively to probabilities of errors of the first and second kind.

method proposed by Hodges and Lehmann.[7] These curves show for given levels of alpha and beta, how many gland weights (n) would have to be measured to distinguish between pairs of population means whose differences are of given amounts ($\triangle$). For example, a sample of 26 (=n) gland weights would be required from each of two squirrel populations to distinguish mean secretion rates of 1.5 μg ($=\triangle$) with $\alpha = 0.05$ and $\beta = 0.10$.

DISCUSSION

The results suggest that adrenocortical secretion rates of the California ground squirrel are proportional to gland size. The use of ACTH in the incubation media places a special restriction on this conclusion. The ACTH in the media stimulates secretion by the gland tissue. In effect, then, we measured the secretion potential of the glands at the given level of ACTH supplement. This is not necessarily the level of secretion that would prevail if the glands were still *in vivo*. It would be informative to repeat our experiment using a range of effective ACTH levels in the media commensurate with a range of ACTH that prevailed in the adrenocortical blood supply during steady-state conditions prior to death. Such an experiment requires more elaborate facilities than those available to us. For the present we can only point out that our conclusion, that gland size reflects secretion rate, is subject to the restriction that only one level of ACTH was used in incubation and other levels may give other correlations.

Since the calibration of secretion rate to adrenal weight involves the variance expected in most biological phenomena, studies of secretion rate using the gland-weight index must be statistically designed. However, the sample sizes required for good estimates of mean differences of secretion rates are not prohibitive for most purposes.

Much doubt about using adrenal weight as an index of secretion rate stems from a misunderstanding of the way the index should be used and the nature of the studies that seem to contraindicate the index. The weight index should not be used in cases of acute or emergency stimulation of the gland, but only in chronic conditions, because acutely stimulated glands may secrete hormones at rates disproportionately greater than the gland sizes would indicate. Perhaps a release of stored hormones from the gland or the rapid synthesis of corticosteroids takes place. In a previous study,[1] it took 2 or 3 days for the glands to increase in weight when ACTH was injected. Possibly they were secreting at elevated rates immediately after injection started. Sanzari *et al.*[10] reported lack of correlation between gland weight and secretion rate in the dog but they measured secretion only 5 minutes after injecting ACTH.

Gordon *et al.*[6] fed triparanol to experimental animals and got enlarged adrenals without increased secretion. Triparanol inhibits biosynthesis of the adrenocorticoid precursor cholesterol. Without the precursor the adrenocorticoid level dropped, stimulating increased ACTH production which caused gland enlargement. Without the precursor the gland was unable to secrete the hormones. The result was large adrenals with small secretion.

In contrast to these findings, van der Vies[11] found good correlation between secretion rate and gland size under chronic conditions in laboratory rats. Bronson and Eleftherieu[2,3] found strong correlations between gland weight and secretion rate in experiments with white-footed mice and house mice under steady-state or gradually changing conditions.

CONCLUSIONS

Adrenal gland weight is a useful index to secretion rate, within limits of sampling error. The sampling requirements to distinguish mean gland weights between populations with different secretion rates are small enongh to be practicable in most experimental designs. The results of our experiments and of experiments reported elsewhere suggest that the weight-rate relationship in adrenal glands may hold in other species as well.

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