layed compared to control preparations. These higher concentrations also had no significant action on electrical parameters compared to appropriate controls.

The d,l and d-aldosterone used in these studies were kindly supplied by CIBA Pharmaceuticals, Inc., Summit, N. J.

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## Relationship of Thymolytic and Glycogenic Activities of Adrenocorticoids. (27868)

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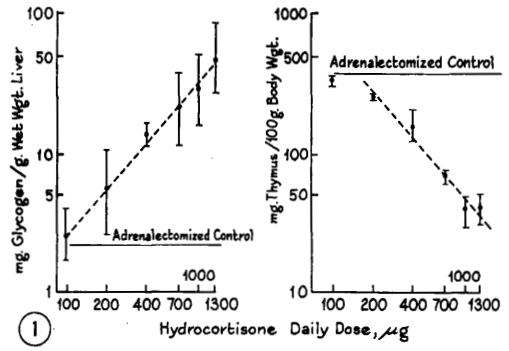
The disparity between estimates of adrenocorticosteroid efficacy, assessed by different criteria of response (*e.g.*, liver glycogen deposition, thymus involution, anti-inflammatory action) is prevalent in the bioassay literature. Stephenson(1) has stated, "ideally the various assays for glucocorticoid-like activity should be carried out simultaneously in test animals from the same group, and using the same route of administration and injection medium for all of the tests." Ringler *et al.*(2) indicated that liver glycogen deposition and thymus involution relative potencies compared favorably, suggesting that potency estimates of glucocorticoid activity would be similar if assessed simultaneously.

To study more critically the relationship between glycogenic and thymolytic efficacy ratios, 64 adrenocorticoids of varied structure were assayed utilizing a 5-day adrenalectomized rat bioassay.

*Materials and methods.* In a 5-day bioassay, liver glycogen deposition and thymus involution were simultaneously determined in adrenalectomized, immature (40-60 g Sherman strain) male rats. Animals were maintained with Purina Lab Chow and 1% NaCl drinking fluid *ad lib*. Six concentrations of test steroids* were assayed in parallel with 6 concentrations of hydrocortisone, using 4 rats per group. Twenty-four hours after adrenalectomy the rats were injected subcutaneously with steroid suspended in 0.2 ml of carboxymethylcellulose vehicle(3), excluding benzyl alcohol. Control animals received vehicle only. Steroid was administered daily for an additional 4 days. The rats were fasted 15 hours before and 7 hours after receiving the last injection to insure maximum depletion of liver glycogen. Animals were anesthetized with sodium pentobarbital and the livers quickly excised, weighed and hydrolyzed in 30% potassium hydroxide(4). Thymi were removed and weighed on a Roller-Smith torsion balance. Thymus response was expressed as mg thymus weight/100 g final body weight. Liver glycogen was measured

* Steroids kindly supplied by the Organic and Biochemical Research Sections, Lederle Laboratories.



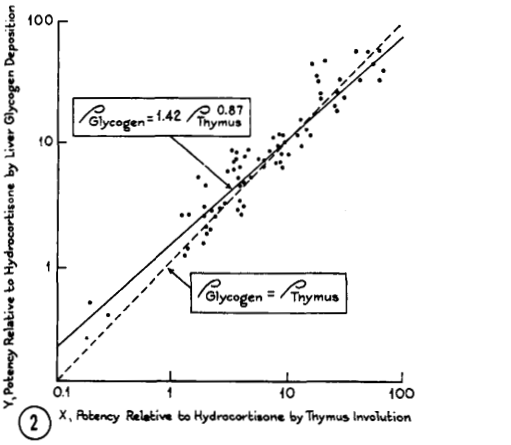




FIG. 1. Liver glycogen and thymolytic responses to hydrocortisone. Vertical bars represent standard deviation.

FIG. 2. Relationship of adrenocorticosteroid liver glycogen deposition and thymus involution relative potencies.

by the anthrone method of Seifter, *et al.*(5) and expressed as mg liver glycogen/g wet weight of liver.

The original data were statistically analyzed by fitting straight lines by the method of least squares. The use of 6 concentrations permitted elimination of responses not on the linear portion of the curve and left a sufficient number of points to provide a least squares estimate of the straight line equation. Potency estimates from several assays for a single compound were combined by the method of Wilcoxon and Haynes (unpublished). The solid line in Fig. 2, relating the 2 types of potencies, was calculated by the maximum-likelihood method of Acton(6), which allows for error in both X and Y, *i.e.*, uncertainty in thymolytic and glycogenic potencies.

*Results.* Fig. 1 is representative of the glycogenic and thymolytic responses of immature adrenalectomized male rats subjected to hydrocortisone treatment. Steroid was administered at 6 dose levels (100-1300 μg) daily for 5 days. A linear relationship is demonstrated between dose of hydrocortisone and both thymus weight and liver glycogen concentration. Though the glycogen response was linear to doses ranging from 100-1300 μg, the response evoked by 100 μg hydrocortisone was not significantly different from control levels. Rats receiving 200 μg hydrocortisone exhibited significantly elevated liver glycogen levels. Thymolytic responses were evident in rats receiving 200-1300 μg steroid. Thus, using 4 rats per dose level, 200 μg hydrocortisone appears to be the minimum effective dose for both end-points of glucocorticoid action.

Values of $\lambda = s/b$, the widely used index of precision of a bioassay, for several steroids in 7 typical assays using hydrocortisone as the reference standard with one to 4 unknown compounds per assay are shown in Table I. The mean index of precision ($\lambda$) for the thymus assay was 0.150 with a standard error of 0.009. The mean index of precision for the liver glycogen assay was 0.188 $\pm$ 0.009. Though slopes of regression lines for liver glycogen deposition were greater than for thymus involution, so also were the residual standard deviations from the regression lines.

Since the ultimate test of assay precision is the observed reproducibility of potency estimates on the same unknown from assay to assay, hydrocortisone and triamcinolone were assayed 9 times over a period of 11 months (Table II). Estimates of thymolytic efficacy appear to vary less from assay to assay than those derived from the liver glycogen assay. Moreover, the mean liver glycogen deposition potency (6.1) of triamcinolone was 36% greater than the thymolytic potency (3.9).

From these data, along with those for other steroids assayed more than once, a measure of assay to assay variation was obtained. In logarithmic terms, the liver glycogen potency standard deviation was 0.137 and the thymus

TABLE I. Statistics from 7 Bioassays.

| Assay No. | No. of steroids | Glycogen regression s* | b† | s/b = λ‡ | Thymus regression s | b | s/b = λ |
|---|---|---|---|---|---|---|---|
| 49 | 2 | .188-.215§ | .990-1.441 | .149-.189 | .084-.110 | .978-1.135 | .074-.112 |
| 111 | 5 | .211-.261 | .874-1.271 | .166-.253 | .090-.135 | .642- .938 | .096-.191 |
| 140 | 4 | .152-.190 | 1.013-1.213 | .148-.185 | .084-.105 | .478- .984 | .085-.219 |
| 166 | 4 | .139-.273 | .884-1.243 | .157-.219 | .090-.110 | .669- .901 | .095-.164 |
| 178 | 4 | .119-.181 | .610-1.070 | .136-.264 | .055-.149 | .563- .959 | .097-.193 |
| 200 | 3 | .149-.179 | .805-1.224 | .121-.222 | .090-.176 | .692-1.002 | .089-.254 |
| 230 | 4 | .142-.259 | .933-1.350 | .105-.277 | .095-.152 | .605- .858 | .111-.251 |
| | Mean | | | .188 | | | .150 |
| | Stand. error | | | .009 | | | .009 |

* s = Residual standard deviation from regression line. † b = Slope. ‡ λ = Index of precision. § Range.

potency standard deviation was 0.113, each with 50 degrees of freedom. The corresponding coefficients of variation were 37% and 30%. The number of animals needed in the glycogen assay to achieve precision equal to the thymus assay was 47% greater.

Proceeding to the relationship of potencies among compounds, 64 steroids were assayed for thymolytic and glycogenic activities.

Regression of relative steroid liver glycogen deposition potencies on the thymolytic potencies is graphically presented in Fig. 2. Liver glycogen potency was 1.42 times the thymolytic potency raised to the 0.87 power. The line determined by this equation is shown in Fig. 2; the line for potency (liver glycogen deposition) = potency (thymus involution) also is shown. These two lines differ significantly. Values in the regions of observation are shown in Table III.

For steroids exhibiting thymolytic potencies equivalent to hydrocortisone, liver glycogen potencies average 40% higher and for compounds of greater potency, the divergence was smaller, reaching zero when the relative thymolytic potency was about 16. Beyond this potency a reversal was apparent; when the thymolytic potency was 100, liver glycogen activity was 25% lower.

TABLE II. Repeated Estimates of Triamcinolone* Potency.

| Assay | Liver glycogen deposition | Thymus involution |
|---|---|---|
| 49 | 8.9 (6.4 and 12.4)† | 4.0 (3.4 and 4.8) |
| 53 | 7.4 (5.9 " 9.3) | 3.8 (3.3 " 4.5) |
| 91 | 6.4 (4.7 " 8.9) | 3.2 (2.5 " 4.1) |
| 105 | 6.5 (5.3 " 7.9) | 4.2 (3.6 " 4.9) |
| 107 | 5.4 (4.0 " 7.4) | 4.1 (2.8 " 6.1) |
| 117 | 6.5 (3.6 " 11.7) | 4.3 (3.3 " 5.4) |
| 122 | 8.8 (5.3 " 14.5) | 4.8 (3.4 " 6.8) |
| 155 | 9.0 (3.7 " 22.0) | 5.0 (2.9 " 8.4) |
| 163 | 2.6 (1.3 " 5.2) | 4.0 (2.7 " 6.1) |
| Mean | 6.1 (5.5 " 6.7) | 3.9 (3.6 " 4.3) |

* $\Delta^1$-9α-fluoro-16α-hydroxyhydrocortisone
† (95% confidence limits)

*Discussion.* The ubiquity of corticosteroid action is manifest in numerous laboratory measurements of biological activity, *e.g.*, electrolyte excretion, liver glycogen deposition, thymus involution, eosinopenia, granuloma pouch inhibition (and other anti-inflammatory bioassays), life-maintenance, adrenal suppression and others. Data are available suggesting that permutations of the hydrocortisone molecule may affect physiological actions differently. 16α-Hydroxylation of $\Delta^1$, 9α-fluorohydrocortisone, though reducing glucocorticoid activity, completely negated its sodium retention property(7). This dissociation of action also was observed in man; however, with the exception of prednisone and prednisolone, animal anti-inflammatory bioassays have proved to be poor quantitative, and in some instances qualitative, indicators of effects in man. Though claims have been made for dissociation of carbohydrate

TABLE III. Relationship of Liver Glycogen Deposition Relative Potency to Thymus Involution Relative Potency.

| Potency ($\rho_G$) Liver glycogen deposition | Potency ($\rho_T$) Thymus involution | $\rho_G/\rho_T$ |
|---|---|---|
| .80 | .50 | 1.60 |
| 1.42 | 1.00 | 1.42 |
| 10.6 | 10.0 | 1.06 |
| 75.0 | 100.0 | .75 |

regulating and anti-inflammatory actions for dexamethasone(8), no substantiation either in the clinic(9) or other laboratory assays has been recorded. Divergence in potency estimates between animal and man should not be unexpected when one recognizes the wide disparity in glucocorticoid potency estimates from different assays.

Ringler and Dulin(10), utilizing 2 different liver glycogen bioassay methods, reported that the glycogenic efficacy of a steroid was a function of the assay procedure, and that the degree of divergence was a direct function of the magnitude of the steroid liver glycogen deposition potency; the greater the potency the greater the divergence in the activities estimated by the 2 assay methods.

The present study was conceived to ascertain if a dissociation in thymolytic and glycogenic actions of a steroid could be realized. On the contrary, glycogenic and thymolytic efficacy studies of 64 steroids of diverse chemical structure have illustrated a strong relationship in these estimates when assessed concurrently in the same animal. Though regression analysis showed the glycogenic and thymolytic potencies were not equal (except at $\rho = 16$), inspection of the difference in potency estimates at the extremes of observation for thymus involution ($\rho = 0.5$ and 100.0) indicate potency assessed by liver glycogen deposition and thymus involution vary only 60% and 25%, respectively.

*Summary.* In a 5-day bioassay, liver glycogen deposition and thymus involution were simultaneously determined in adrenalectomized, immature male rats. The mean index of precision ($\lambda$) for the thymus measurement was $0.150 \pm 0.009$, whereas the index for the glycogen measurement was $0.188 \pm 0.009$. Glycogenic and thymolytic efficacy ratios of 64 steroids of diverse chemical structure illustrated a strong relationship in these studies. In brief, steroids exhibiting a thymolytic potency equivalent to hydrocortisone, possessed liver glycogen activity averaging 40% higher and for compounds of greater potency the divergence was smaller, reaching zero when the relative thymolytic potency was about 16. Beyond this potency, a reversal was apparent, thus when the thymolytic potency was 100, liver glycogen activity was 75.

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## Potency of Vitamin A Acid in the Vaginal Smear Assay. (27869)

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Vitamin A acid fulfills some of the functions of Vitamin A but is neither stored in the body nor converted to Vit. A(1) and attempts to measure its potency have produced extremely variable results. Arens and Van Dorp (2), by means of the growth assay, found Vit.