

Estimation of Estrogenic Activity of Genistein Obtained from Soybean Meal. (20693)

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(Introduced by W. L. Boyd.)

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The association of infertility of sheep in Australia with the estrogenic properties of subterranean clover(1,2,6,8) has stimulated interest in compounds of plant origin showing estrogenic activity. Bradbury and White(4) identified the isoflavone genistein, 4', 5, 7 tri-hydroxyisoflavone, as the major constituent of subterranean clover responsible for its estrogenic activity. Their data also indicated that the chemical properties of the genistein isolated from the chloroplast fraction of subterranean clover were similar to those of the genistein isolated by Walz(10) and Walter(9) from soybeans. Walter(9) reported that dry hexane extracted soybean flakes contained 0.10% of the glucoside of genistein (genistin), an amount 50 times greater than was reported (4) to be present in fresh subterranean clover.

These quantitative considerations gave rise to the idea that soybean meal could be used as a source of genistein for biochemical investigations providing the genistein isolated was biologically active. Preliminary tests in the present investigation indicated that genistein isolated from soybean meal possessed estrogenic activity. The work reported herein deals primarily with a quantitative bioassay of the estrogenic activity of genistein isolated from soybean meal.

Experimental. Genistin was extracted from soybean meal,[†] and hydrolysed to its aglucone, genistein, according to the method of Walter(9). The test doses of the crystallized aglucone were dissolved in a minimum volume

of ethanol and transferred to peanut oil[‡] in a 25-ml Erlenmeyer flask. The ethanol was removed under vacuum leaving the genistein dissolved in the peanut oil. (The genistein isolated had a melting point of 291-294°C and a specific absorption coefficient of 133 at 2625 Å). The bioassay of the estrogenic activity of genistein was conducted with immature female mice§ (3 weeks of age) employing uterine weight response as the criterion. Diethylstilbestrol|| was used as the reference standard preparation. The test material as well as the diethylstilbestrol standard were injected subcutaneously twice daily for a period of 3 successive days. Twenty-four hours after the last injection the animals were sacrificed, the uteri removed, pressed lightly on a filter paper to eliminate uterine fluid and weighed. In preliminary tests the uterine-weight response curve of either the standard or the genistein preparation exhibited linearity over a wider range when plotted against the log of the dose rather than the arithmetical progression. Dose levels used in the subsequent assay were selected from the linear portion of the preliminary response curves. The assay was arranged as a 2-dose factorial(3) with 6 replications. The doses of genistein injected were 3 mg and 6 mg, and of diethylstilbestrol 0.015 µg and 0.030 µg. Each dose was dissolved in 0.6 ml of peanut oil and injected in 6 0.1-ml portions (representing 0.2 ml of oil per mouse per day). Controls were injected with oil alone.

Results. The results of the assay are presented in Table I. The mean uterine weight of the negative controls was 8.2 mg as compared with 23.8 mg for the genistein injected

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[†] Solvent Extracted Soybean Meal.

[‡] Charlotte's Peanut Oil, Charlotte Refining Co., Charlotte, N. C.

[§] Mice furnished by M. P. Bailey of the North Carolina State Laboratory of Hygiene.

^{||} Diethylstilbestrol obtained from Jensen-Salsbery Laboratories, Inc., Kansas City, Mo. (Oil solution, each cc containing 2 mg)

TABLE I. Estrogenic Activity of Substances Tested. 6 mice in each series.

Treatments	No. mice	Mean uterine wt, mg
Injected		
Neg. control	6	8.2
Diethylstilbestrol		
.015 μ g	6	20.3
.030 μ g	6	32.4
Genistein		
3 mg	6	15.5
6 mg	6	32.2
Feeding		
Soybean meal with genistin extracted	5	8.1
Commercial soybean meal (solvent process)	6	23.0

TABLE II. Analysis of Variance of Two-Dose Factorial Assay.

	d.f.	S.S.	M.S.
Total	23	1876.53	
Replications	5	273.06	54.61*
Treatments	3	1311.45	437.15†
Difference between standard and genistein	1	36.75	36.75*
Combined slope of dose effect curve	1	1242.72	1242.72†
Divergence in slope	1	31.97	31.97*
Error	15	565.08	37.67

* Not significant ($P > .05$).† Highly significant ($P \leq .01$).

mice. The slope of the response curve of diethylstilbestrol was not different from that of genistein over the range tested indicating that the biological action of the 2 preparations was similar over the range tested (Table II). The estrogenic potency of genistein computed from the data in Table I was 4.44×10^{-6} times the potency of diethylstilbestrol. Confidence limits (95%) placed on this estimate of potency gave a lower limit of 3.48×10^{-6} and an upper limit of 5.56×10^{-6} .

Discussion. The mean estimated potency of genistein obtained in this experiment seems to be different from that of genistein isolated from subterranean clover(4) (10^{-5} times as potent as estrone). This apparent difference probably is due to the fact that the estrogenic activity of estrone and diethylstilbestrol is different. Diethylstilbestrol has been reported(7) to be 16 times more effective than

estrone when injected subcutaneously.

Cheng *et al.*(5) estimated the potency of genistein obtained from soybeans as 2×10^{-5} times that of diethylstilbestrol. This estimate of potency is approximately 4.5 times the potency obtained in this experiment. The difference apparently is not due to a difference in uterine response to genistein but to the fact that Cheng(5) obtained a lower uterine response per unit of diethylstilbestrol than in the assay reported here.

In preliminary studies using the feeding method of bioassay, soybean meal from which genistein had been extracted was estrogenically inactive, whereas unextracted soybean meal was active (Table I). These observations indicate that the method of extraction was effective and point up the possibility of studying the biological effects of genistein with the unextracted meal.

On the basis of these current findings, nutritional studies are now in progress to determine the effects of estrogenic substances of plant origin on growth and reproduction.

Summary. Genistein of soybean origin was found to be 4.44×10^{-6} times as potent in estrogenic activity as diethylstilbestrol when measured by uterine weight responses of injected immature female mice. Commercial solvent extracted soybean meal also was found to be estrogenically active when fed.

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