The effect of genistin and its aglycone on weight gain in the mouse*

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The observations that reproductive disturbances occurring in sheep grazing subterranean clover pastures were associated with the oestrogenic activity of the plant (Bennetts, 1944; Bennetts, Underwood & Shier, 1946; Curnow, Robinson & Underwood, 1948) stimulated interest in compounds, other than those normally classified as nutrients occurring in plants, that may have an effect on the performance of animals. These observations, coupled with the isolation and identification of genistein (5, 7, 4′-trihydroxyisoflavone) as the compound in subterranean clover mainly responsible for its oestrogenic activity, gave rise to investigations into the soya-bean plant, since soya-bean meal contains genistin, the 7-glucoside of genistein (Walter, 1941), and reproductive disturbances had been reported in sheep and rabbits receiving soya-bean forage (Kendall, Salisbury & Vandemark, 1950; Schaub & Baver, 1944; Hunt, 1935). Soya-bean meal, genistin and the aglycone genistein, isolated and prepared from soya-bean meal, were subsequently found to be oestrogenically active (Carter, Smart & Matrone, 1953; Cheng, Story, Yoder, Hale & Burroughs, 1953; Carter, Matrone & Smart, 1955). Genistin has also been shown to affect reproduction of the mouse adversely (Carter *et al.* 1955) and at somewhat higher levels to depress growth (Matrone, Smart, Carter, Smart & Garren, 1956).

The objective of our study was to determine if the effects of genistin and genistein on growth are a result of their oestrogenic properties. To that end the growth-depressing effect, as reflected by weight changes in immature mice, of genistin and genistein prepared from soya-bean meal has been compared with that of stilboestrol and of oestradiol.

EXPERIMENTAL

Two levels of genistein were compared with several levels of oestradiol and stilboestrol, but only one level of genistin was used, since the response curve for genistin at various levels had been investigated previously by us (Matrone *et al.* 1956).

The levels used in the experiment were chosen so that the changes in body-weight were in the range of the linear log-dose relationship, in order to take advantage of well-known principles of bioassay.

The basal diet was the same in composition as that of Matrone *et al.* (1956). The

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experiment was conducted in two 4-week periods. In the first period the treatments were a control and four levels of stilboestrol; in the second, the treatments were the control, three levels of oestradiol, two levels of genistein and one level of genistin (cf. Table 1). The genistin and genistein were isolated or prepared from commercial soya-bean meal as described by Carter *et al.* (1953). The treatments of the first period were replicated ten times and those of the second eight times. Male and female 'Swiss albino' mice, about 3 weeks old, were divided into groups of uniform weight and of the same sex, and the treatments were assigned to groups at random.

The mice were housed individually in wire cages with screen floors. The test materials were given in the manner reported previously (Matrone *et al.* 1956).

Body-weights were recorded weekly. At the end of the experiment the mice were killed and fresh weights were determined of gonads, spleen, adrenals and kidneys.

RESULTS

The results are given in Table 1. Ten mice died during the experiment, one on the fourth or highest level of stilboestrol, two on the highest level of genistein and seven on genistin.

A regression analysis in which the final body-weight was considered as the dependent and the logarithm of the dose level as the independent variable indicated a significant ($P < 0.01$) linear decrease in final body-weight associated with increasing levels of genistein, oestradiol or stilboestrol. The level of genistin used gave results

Table 1. *Mean values for food intake, final body-weight, weight gain and organ weights of mice given various amounts of oestrogenic test materials in two 4-week periods*

Treatment	No. of mice/ group	No. that died	Food intake (g)	Body-weight		Organ weight					
				Final (g)	Gain (g)	Testes* (mg)	Ovaries* (mg)	Adrenals* (mg)	Kidneys* ♂ (mg)	Kidneys* ♀ (mg)	Spleen (mg)
Period 1											
Control	10	0	122	22·3	10·3	141·0	—	6·4	396·1	281·6	98·7
Stilboestrol (μg):											
0·32	10	0	118	20·9	8·7	150·4	—	6·8	364·6	268·2	96·1
3·20	10	0	110	16·9	5·0	71·5	—	7·1	265·8	221·1	77·7
32·00	10	0	103	13·6	1·3	38·3	—	5·6	245·7	193·2	53·9
320·00	10	1	65	8·0	−3·5	13·3	—	4·5	195·5	177·1	15·1
Period 2											
Control	8	0	110	23·0	11·4	174·2	6·1	5·2	344·8	283·0	105·4
Oestradiol (μg):											
5·84	8	0	101	19·0	7·6	120·9	2·6	5·6	279·6	258·0	66·4
58·40	8	0	82	14·6	2·9	48·7	3·4	5·9	250·7	226·2	40·4
584·00	8	0	72	11·9	0·6	22·0	4·7	5·6	170·3	202·8	38·1
Genistein (mg):											
5·6	8	0	101	17·8	6·2	127·5	4·6	5·9	308·1	204·8	65·6
45·0	8	2	69	10·9	−0·5	18·7	3·2	5·0	243·3	214·3	23·4
Genistin (mg):											
72·0	8	7	46	11·1	−1·5	24·6	—	4·4	153·6	—	25·8

* Both together.

similar to those reported by Matrone *et al.* (1956), i.e. loss in weight and the occurrence of deaths.

The slopes of the weight curves as determined by the regression analysis indicated that tenfold increases of oestradiol and stilboestrol were required to obtain the same decrease in weight as twofold increases of genistein. On testing the divergence of slope by the method described by Bliss (1952), it was found that the divergence in slope between the genistein and oestradiol curves was significant ($P < 0.05$). There was no apparent divergence of slope between the oestradiol and stilboestrol curves.

Stilboestrol, oestradiol and genistein had a depressing effect on weights of spleen, gonads and kidneys. With stilboestrol the tests only were considered, owing to difficulty experienced in dissecting the ovaries. The effect of genistein on the ovaries and kidneys, however, differed somewhat from that of oestradiol. The smallest ovaries occurred on the lowest level of oestradiol, and the ovaries increased in weight as oestradiol was increased, but with genistein the smallest ovaries occurred at the highest level. An analysis of the kidney weights indicated a significant sex–treatment interaction ($P < 0.05$). Kidney weights of males decreased with increasing levels of either oestradiol or genistein. Although oestradiol was observed to have a similar effect on kidney weights of females, the depressing effect of genistein was apparently maximum at the lowest level.

DISCUSSION

The results substantiate the earlier findings of Matrone *et al.* (1956) that genistin has a detrimental effect on survival and growth of the mouse. It was also found that genistein, the aglycone of genistin, has a detrimental effect on survival and weight gain in the mouse. Although stilboestrol and oestradiol retarded the weight gain of the mouse, they did not appear to have the same adverse effect on survival as did genistin or genistein.

Even though the retardation in weight gain caused by these substances was coincident with a decrease in food intake, it seems unlikely that taste of the test substances *per se* was a significant factor, inasmuch as the test materials were given in small amounts of the basal ration before *ad lib.* feeding with it. Thus the decrease in food intake may have been a result of a decreased weight gain rather than its cause. The fact that treatment differences remained significant ($P < 0.05$) after adjustment for food intake also indicates that the food consumed was not utilized as efficiently.

The weight-response curve for genistein did not parallel those for oestradiol and stilboestrol, whereas the weight curves for the two oestrogens were similar. Statistical tests for common slope (Bliss, 1952) indicated that the genistin and genistein curves were similar and that the stilboestrol curve was different from that for genistin. The values for the genistin curve were obtained from the previous study (Matrone *et al.* 1956). The fact that the slopes of the weight curves for genistin and genistein differed from those for stilboestrol and oestradiol indicates that their action on growth is different.

There were other responses indicating that the action of the plant oestrogens is not the same as that of oestradiol and stilboestrol. Oestradiol elicited its maximum effect of decreasing ovary weight at the lowest level given, a level that had the least effect on

body-weight; whether or not partial restoration of ovarian weight might have been due to an increase in luteal tissue is not known, since histological examination of the ovaries was not done. With genistein, however, the smallest ovaries occurred at the highest level. Apparently the oestradiol had depressed the gonadotrophin output before depression of body-weight occurred, which would be expected of this oestrogenic compound. On the other hand, the fact that genistin did not cause depression of gonadotrophin output before growth depression indicates that the effect did not conform to that expected of an oestrogen. This idea is in harmony with the findings of Matrone *et al.* (1956) that stilboestrol decreased testis weight before it had an appreciable effect on body-weight, whereas in contrast the effect of genistin on body-weight preceded the effect on testis weight.

The fact that the mortality rate was higher after administration of genistin or genistein than after oestradiol or stilboestrol may indicate that the metabolism of the animal is affected more drastically by the plant oestrogens, perhaps by inhibition or blocking of key metabolic reactions, and that if growth hormone is affected the effect follows or is even a result of some such primary action.

SUMMARY

1. Comparisons were made of the effects of genistin and genistein with those of stilboestrol and oestradiol on weight gain, in an effort to determine whether or not genistin and genistein depressed growth in a manner similar to the oestrogens.

2. Various levels of these compounds were fed to immature male and female mice for 4 weeks. The levels were chosen so that the effects on body-weight would be in the linear log-dose range.

3. The weight curves obtained with mice demonstrated that the effects of oestradiol and of stilboestrol were different from those of genistein and genistin.

4. Oestradiol had an apparently different effect on the ovaries from that of genistin and genistein.

5. Fewer of the animals receiving genistin or genistein survived.

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