efficiency during the fattening period (*1–3*). Recently, naturally occurring estrogenic substances have been detected in varying amounts in various plant herbages, both pasture and hays, fed to cattle and sheep (*4–6*). Genistein (5,7,4′-trihydroxyisoflavone) has been suggested by Curnow and Bennetts as the chemical constituent responsible for estrogenic activity in one of these herbages, namely, subterranean clover (*7*). The glucoside of genistein, genistin (5,4′-dihydroxy-7-glucosidoisoflavone) has been shown by Walter (*8*) to be present in substantial amounts (0.1%) in soybean oil meal. Since soybean oil meal is used rather widely in livestock feeding, it appeared advisable to investigate the estrogenic activity of both genistin and genistein in ascertaining their probable significance in livestock feeding.

Commercial soybean oil meal (solvent process) was extracted with methanol according to the method of Walter (*8*). Genistin was isolated as pale yellow, thin rectangular plates having a melting point of 256° C. Upon hydrolysis of genistin with hydrochloric acid in methanol, genistein was obtained. It was crystallized from hot 60% ethanol as white rectangular rods having a melting point of 298° C.

The estrogenic activity of these compounds was determined by the mouse uterine weight method described in detail in an earlier paper (*6*). The chemicals under study were either fed directly to immature female mice or were injected subcutaneously. Since neither genistin nor genistein is soluble in water, they were injected as their sodium salts. Six mice were used in each group, and the treatments were given once daily for 4 days. The mice were sacrificed 24 hr after the last treatment, their uteri dissected, fixed in Bouin's fluid, and weighed. The results obtained are presented in Table 1.

Feeding 2.5 and 5.0 mg of either genistin or genistein per day per mouse resulted in increased uterine weights. Injecting genistein at 1- and 2-mg levels respectively also increased uterine weights consistently over the corresponding weights of control animals. Whereas the injection of 1 mg of genistin did not have a measurable effect, the injection of 2 mg proved quite effective. It should be noted that these responses are similar to those due to the injection of 0.02–0.04 μg respectively of diethylstilbestrol, as shown in Table 1. The estrogenic activity of genistein can accordingly be estimated as approximately equivalent to 1/50,000 the activity of diethylstilbestrol. Genistin activity on a weight basis was slightly lower than that of genistein. However, the two compounds appeared to have approximately equal activity on a molecular basis.

Experiments are in progress with fattening lambs to determine whether the estrogenic activity of genistin as found in soybean oil meal is as beneficial as the estrogenic activity of stilbestrol, which has been shown experimentally to be valuable in lamb feeding. Although the estrogenic activity of genistin per unit of weight is small compared with that of diethylstilbestrol, the relatively large amount of genistin present in soybean oil meal, coupled with its presence in small amounts in certain hays, suggests the likelihood that the amounts present in certain cattle and sheep rations may be sufficiently large to exert major beneficial influences.

TABLE 1

ESTROGENIC ACTIVITY OF GENISTIN AND GENISTEIN

Group	No. of mice	Treatment*	Av uterine weight, mg
1	6	Normal control	9.7 ± 2.8
2	6	Feeding genistin, 2.5 mg	12.9 ± 4.4
3	6	Feeding genistin, 5.0 mg	39.8 ± 8.9
4	6	Injecting genistin, 1 mg	9.2 ± 1.7
5	6	Injecting genistin, 2 mg	14.6 ± 3.6
6	6	Feeding genistein, 2.5 mg	21.6 ± 13.4
7	6	Feeding genistein, 5.0 mg	22.6 ± 4.6
8	6	Injecting genistein, 1 mg	13.2 ± 2.4
9	6	Injecting genistein, 2 mg	17.0 ± 7.3
10	6	Injecting stilbestrol, 0.02 μg	13.2 ± 2.6
11	5	Injecting stilbestrol, 0.04 μg	18.3 ± 6.9

* Treatment was given daily for 4 days.

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The Reduction by Reactivating Light of the Frequency of Phenocopies Induced by Ultraviolet Light in *Drosophila melanogaster*

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Phenocopies (*1*), or abnormalities of adults simulating mutations, are readily induced in *Drosophila melanogaster* by irradiating eggs, larvae, or pupae with ultraviolet light (*ca.* 2600 A) (*2, 3*). Visible and near-ultraviolet light (3600–4900 A) prevents ultraviolet-induced killing or mutation in bacteria and other organisms (*4, 5*). Such reversal of ultraviolet effects has been found in various organisms (see [*5*] for references) including, recently, *Drosophila,* where reactivating light lowered the incidence of ultraviolet-induced lethal mutations (*6*).

It became of interest to determine whether phenocopies induced by ultraviolet light in *Drosophila* were also affected by reactivating light, especially since the induction of phenocopies was perhaps a more complex phenomenon than those studied before.

TABLE 1

EFFECT OF REACTIVATING LIGHT ON SURVIVAL AND ON THE INCIDENCE OF PHENOCOPIES IN ULTRAVIOLET-IRRADIATED PUPAE

No. of pupae	Ultraviolet light (min)	Reactivating light (min)	Percentage of pupae hatching	Percentage of phenocopies in adults
56	0	0	84	0
86	5	0	68	76.5
47	5	35	66	41.5
43	5	70	72	32.2
43	5	120	81.5	38.0

The L-S strain of wild-type *Drosophila melanogaster* was cultured on molasses-cornmeal-yeast-agar medium. Eggs laid in a 2-hr period were segregated and incubated at 25° C for 7 days. Immediately after pupation, pupae were distributed over the bottom of a dry Petri dish and irradiated with ultraviolet light from a 15-watt low-pressure mercury lamp (GE germicidal) emitting chiefly radiation at 2537 A. The pupae were irradiated for 5–7 min at 20-cm distance from the bulb, then immediately exposed for 30 min or longer to reactivating light from a 100-watt ribbon filament tungsten bulb. The reactivating light was cooled by passing it through 2 cm of water. Care was taken not to disturb the pupae between irradiations, to make certain that the same region of the pupae exposed to ultraviolet light was exposed to visible light. After irradiation, the pupae were exposed to diffuse room light for not more than 1½ min. The dish with the pupae was placed in a bowl containing a large food chip, the bowl was covered with gauze netting, and the pupae were incubated in the dark at 25° C until they hatched. "Dark" controls were treated similarly, except that they were not exposed to reactivating light after ultraviolet radiation. The adult flies were etherized and examined with a 20 × microscope. Any visible abnormalities in the flies were scored as phenocopies.

An ultraviolet dose was chosen which gave abundant phenocopies without decreasing survival markedly. A typical experiment is shown in Table 1. Reactivating light increased survival. The frequency of phenocopies in pupae treated with visible light was reduced to about half that of the "dark" control.

In the experiment shown in Table 1 any fly with an abnormality, whether severe or slight, whether in one area or in several, was scored as one phenocopy. But, if the average number of abnormalities per fly was taken as the measure of phenocopy induction, the effect of reactivating light was more pronounced than indicated in Table 1. In one experiment the average number of abnormalities per fly in the "dark" control was 1.78, many flies having three or four abnormalities, e.g., in the abdomen, wings, eyes, and bristles; in the reactivated group it was 0.65. In the latter group none of the flies had more than two areas of the body abnormal. Moreover, the severity of the abnormalities in the group treated with visible light was always less than in the "dark" control.

The types of phenocopies were, in general, similar to those reported in the literature, namely, distortion and irregular banding in the abdomen (a very common abnormality), abnormal wings, distorted eyes, missing, fused, or "singed" hairs and bristles. One-sided effects, similar to those found by Geigy (*2*) in which only one side of the animal was abnormal, were common.

Several experiments were made which indicated that the photoreversal of the induction of phenocopies was a phenomenon essentially similar to photoreactivation in bacteria.

Thus, if a glass filter (Corning No. 3385) cutting off wavelengths below 4700 A was interposed in the path of the light from the tungsten lamp, there was no reduction in phenocopies, whereas blue and violet light (obtained by the use of Corning filter 5850, transmitting only wavelengths below 4900 A) was effective. It should be noted that, contrary to our experience in photoreactivating bacteria, a GE AH-5 medium pressure mercury lamp was not a suitable source of reactivating light, since the radiation from this lamp was itself lethal to pupae. This lethality was perhaps due to the abundant radiation at 3650 A present in the AH-5 lamp.

Treating the pupae with visible light *before* ultraviolet light had no effect on phenocopies. If ultraviolet-irradiated pupae were allowed to incubate in the dark before exposure to visible light, the incidence of phenocopies was no longer reduced. After about 20 hr, visible light had little effect.

In summary, the ability of ultraviolet light of wavelength 2537 A to induce phenocopies in *Drosophila melanogaster* is markedly reduced by exposure to visible light. And, since the phenomenon appears similar to photoreactivation in bacteria, we may expect the same basic mechanism of action to be involved in both organisms.

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