Further Studies on Flavonoids and Thymus Involution. (25107)

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Our recent report showed that feeding of quercetin, dihydroquercetin, and eriodictyol to rats induced thymus involution, mediated through the pituitary-adrenal axis(1). Results of similar studies with other flavonoids are here presented.

Materials and methods. Treatment of animals was similar to that of previous study(1), except that a different basal diet was used with the following percentage composition: degerminated corn meal 65, sucrose 10, casein 15, corn oil 4, U.S.P. No. XIV salt mixture 4, adequate vitamin mixture triturated in dextrose 2. This semi-synthetic diet was used in testing all flavonoids reported here, except in the case of morin where the non-synthetic diet used previously(1) was employed. Young female albino rats, 25-28 days old from our colony were fed the basal diet with or without addition of flavonoids for 12-14 days. The rats were then sacrificed, their organs examined carefully for gross changes and the fresh thymus weights recorded, except in the case of hesperidin and appropriate controls, where thymus weights were determined after fixation in formaldehyde. Naringenin, hesperetin, luteolin, morin, eriodictyol, and quercetin were fed at dietary level of 2%. Hesperidin and "Calcium Flavonate Glycoside, Lemon"†, which contains eriodictyol glycoside(2) as the main flavonoid component, as well as minor amounts of luteolin, quercetin and other glycosides (Horowitz, R. M., personal communication), were fed at level of 4%. Hesperidin and its aglycone hesperetin, naringin and morin were obtained from commercial sources. Naringenin was prepared from its glycoside naringin by acid hydrolysis. Since naringin is bitter, only the non-bitter aglycone naringenin(3) was tested. Luteolin was prepared from eriodictyol by the method of Lorette *et al.*(4). Quercetin and eriodictyol were prepared as previously reported(1).

Results based on fresh thymus weights expressed as % of body weights are summarized in Table I. Luteolin ($P<0.01$) and "Calcium Flavonate Glycoside, Lemon" ($P = 0.02$) caused significant thymus involution. The data on quercetin and eriodictyol confirm that reported previously(1) and provide a comparison with other data of the present report. Hesperetin, hesperidin, naringenin, and morin failed to elicit this response. No gross changes in rats receiving flavonoids could be observed at time of sacrifice. Growth of rats receiving flavonoids was comparable to that of those fed basal diets alone.

Discussion. Although final body weights in control and experimental groups were variable, especially in rats receiving eriodictyol, food intakes and rates of growth over the experimental period were comparable. Consequently thymus involution in certain experimental groups could not be due to the stress of anorexia.

A demonstrable thymolytic action by the flavonoids tested to date was observed only with those which possess a hydroxyl group on both the 3′ and 4′ positions of the flavonoid molecule (*cf.* quercetin, dihydroquercetin, eriodictyol and luteolin *vs.* hesperetin, naringenin and morin). On the other hand, a double bond between carbons 2 and 3 is not essential for the thymolytic action (*cf.* quercetin and luteolin *vs.* dihydroquercetin and eriodictyol); nor is the presence of a hydroxyl group on carbon 3 (*cf.* quercetin and dihydroquercetin *vs.* eriodictyol and luteolin). The structures of these flavonoids are shown in Fig. 1.

It is interesting that hesperetin, which is the 4′-methyl ether of eriodictyol, lacks the thymolytic action of the latter. It might be predicted that diosmetin, the 4′-methyl ether of luteolin, would also lack thymolytic action.

The relationship of the metabolic fate of

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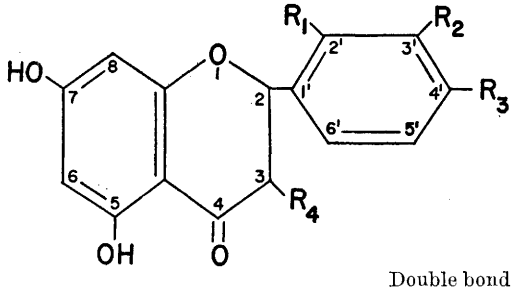
† A commercial preparation distributed by Sunkist Growers, Products Dept.

TABLE I. Thymus Gland Weights of Control and Flavonoid-Fed Rats.

Flavonoid fed	No. of rats	Range of final body wt, g	Mean body wt, g	Avg thymus wt, % of body wt		
				Mean ± S.E.	Diff. ± S.E.	P*
None (basal, semi-synthetic diet)	40	67–99	83.7	.350 ± .006		
Hesperetin	10	64–87	78.9	.340 ± .011	.010 ± .013	
Naringenin	10	77–90	84.7	.326 ± .017	.024 ± .018	
Luteolin	9	62–90	77.7	.313 ± .010	.037 ± .012	<.01
Ca-flavonate glycoside	10	74–101	85.2	.319 ± .014	.031 ± .012	.02
Eriodictyol	10	54–81	67.1	.295 ± .012	.055 ± .014	<.001
Quercetin	10	77–98	89.0	.303 ± .008	.047 ± .010	"
None (basal, non-synthetic diet)	5	65–90	79	.297 ± .07		
Morin (non-synthetic diet)	5	69–90	80	.306 ± .08	.009 ± .11	

* P = Probability that such a difference would occur by chance. Only significant values of P are indicated.



	R_1	R_2	R_3	R_4	Double bond 2:3
Hesperetin	H	OH	OCH_3	H_2	No
Naringenin	H	H	OH	H_2	"
Luteolin	H	OH	"	H	Yes
Eriodictyol	H	"	"	H_2	No
Quercetin	H	"	"	OH	Yes
Dihydro-quercetin	H	"	"	H, OH	No
Morin	OH	H	"	OH	Yes
Diosmetin	H	OH	OCH_3	H	"

FIG. 1. Generalized structure of flavonoids. Substituents for individual flavonoids are tabulated above.

various flavonoids to their thymolytic action will be discussed later.

Summary. (1) Hesperidin, hesperetin, naringenin, morin and luteolin were fed to young rats to determine their ability to induce thymus involution. Only luteolin was effective, and in this respect resembles quercetin, dihydroquercetin, and eriodictyol. (2) A commercially available preparation, "Calcium Flavonate Glycoside, Lemon," caused thymus involution. Some relationships between structure and thymolytic action of flavonoids are discussed.

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1. Masri, M. S., DeEds, F., PROC SOC. EXP. BIOL. AND MED., 1958, v99, 707.

2. Horowitz, R. M., *J. Am. Chem. Soc.*, 1957, v79, 6561.

3. Ting, S. V., *J. Agr. and Food Chem.*, 1958, v6, 546.

4. Lorette, N. B., Gage, T. B., Wender, S. H., *J. Org. Chem.*, 1951, v16, 930.

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Demonstration of Glucose-6-Phosphatase in Mammalian Pancreas.* (25108)

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Previous workers demonstrated non-specific alkaline phosphatase in pancreatic ductules (1,2,3) and non-specific acid phosphatase in islets as well as in exocrine pancreas(4,5). Glucose-6-phosphatase has been demonstrated in liver, kidney and intestinal tract(6,7) but has been stated to be absent from pancreas (7). This enzyme is heat labile, inactivated at

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