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Effect of Certain Flavonoids on the Pituitary-Adrenal Axis.* (24472)

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Oral administration of a number of flavonoids to animals and humans has been shown to result in the urinary excretion of a variety of hydroxy aromatic acids(1,2,3). This finding suggested that, through the medium of their metabolites, flavonoids might influence the pituitary-adrenal axis in a manner comparable to that reported for salicylic acid and related compounds(4,5). The present report concerns an investigation of this possibility with the flavonoids quercetin, dihydroquercetin, and eriodictyol.

Methods. Albino rats from a colony maintained in this laboratory since 1931 were used. Young rats were weaned at about 21 days of age, weighed individually, and sexes segregated. Overweight and underweight rats were discarded. When selected rats were not more than 4 weeks old they were fed for 1 to 4 weeks a basal diet with or without addition of a flavonoid. The basal diet had the following percentage composition: corn meal 73, casein 10, linseed oil cake meal 10, alfalfa meal 2, cod liver oil 3, bone ash 1.5, and sodium chloride 0.5. The quercetin was prepared by acid hydrolysis of the rhamnoside quercitrin(6), and eriodictyol was prepared from dihydroquercetin‡ by the method of Pew(7). At end of feeding period all control and experimental rats were sacrificed, body weights and thymus weights determined, and all organs examined for evidence of gross changes. Comparable experiments were performed with groups of rats which had been adrenalectomized or hypophysectomized. Adrenalectomized rats were placed on the diets immediately after operation and supplied with 0.9% sodium chloride in the drinking water. Hypophysectomized rats were placed on the diets 4 days after operation.

Results. The data on fresh thymus weights, expressed as per cent of body weight for rats receiving quercetin, dihydroquercetin, or eriodictyol, and their respective controls are summarized in Table I. The smaller thymus weights of flavonoid-fed rats as compared with their respective controls are clearly significant. In the case of quercetin, where the largest number of rats was used, an analysis of the individual data, with construction of

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‡ Dihydroquercetin was supplied by Oregon Forest Products Lab., Corvallis.

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

		Avg thymus wt expressed as % of body wt						
Body wt, g	No. of animals	Thymus	SD	No. of animals	Thymus	SD	Difference ± SE	P
		Control ♀			Quercetin ♀			
60– 79	22	.309	.028	14	.276	.037	.0331 ± .0116	.01
80– 99	16	.301	.038	9	.271	.037	.0303 ± .0156	.07
100–119	11	.290	.023	5	.248	.021	.0413 ± .0118	<.01
		Control ♂			Quercetin ♂			
78–108	7	.359	.046	5	.269	.026	.090 ± .0208	<.01
		Control ♀			Dihydroquercetin ♀			
67– 92	29	.310	.031	15	.264	.026	.046 ± .0089	<.001
		Control ♂			Dihydroquercetin ♂			
72–100	7	.359	.046	8	.282	.028	.077 ± .0199	<.01
		Control ♀			Eriodictyol ♀			
69– 84	20	.316	.033	4	.243	.027	.073 ± .0152	<.001

SD = stand. dev.; SE = stand. error of difference; P = probability that such a difference would occur by chance.

regression lines, showed that relative thymus weight gradually decreases with increase in body weight, with gradual convergence of control and experimental groups. The apparent exception to this statement, as shown in the Table for rats in weights ranging 100-119 g is probably due to the small size of the samples. We believe that the difference in thymus weights between control and flavonoid-fed rats can be shown most satisfactorily with animals weighing 50 to 80 g.

In an experiment involving 74 female and 52 male rats as controls, and 43 female and 29 male rats fed 2% quercetin, thymus gland weights were determined after fixation in 10% formalin. Involution of thymus glands was again clearly evident as in the experiments with fresh thymus weights.

The data on thymus weights summarized in Table II clearly demonstrate that feeding quercetin does not affect the weight of thymus gland in adrenalectomized or hypophysectomized young rats. No abnormalities were noted in the various organs of any animals at time of sacrifice. Removal of adrenals or pituitaries was confirmed at time of autopsy by careful examination.

The nontoxicity of quercetin(8) and dihydroquercetin(9) suggests that stress due to ingestion of these flavonoids was not a causative factor in thymus involution.

Discussion. Involution of the thymus gland of young rats produced by feeding quercetin, dihydroquercetin, or eriodictyol, and failure to produce involution in adrenalectomized or hypophysectomized rats suggests that physiological effects of these flavonoids are mediated, in part at least, through an action on the pituitary-adrenal axis. The antioxidant action toward epinephrine(10,11) and ascorbic acid(12), and the inhibitory action on hyaluronidase(13) possessed by flavonoids in varying degrees have been used to explain the beneficial effects of flavonoids on capillaries in a variety of experimental and clinical conditions. This mechanism of action based upon properties of the intact flavonoid molecule, and the presently suggested mechanism involving stimulation of the pituitary-adrenal axis are complementary.

TABLE II. Thymus Gland Weights of Adrenalectomized or Hypophysectomized Female Rats Fed Control or Quercetin-Containing Diet.

	Control diet			Quercetin-containing diet		
	No. of rats	Avg body wt, g	Thymus, % of body wt	No. of rats	Avg body wt, g	Thymus, % of body wt
Adrenalectomized	10	64	.33	11	68.5	.32
Hypophysectomized	9	56	.28	9	53.5	.28

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Lipide Changes in Chylomicra and Subnatant Fractions of Rat Lymph During Cholesterol Absorption.* (24473)

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The effects of sodium taurocholate, oleic acid, and albumin, singly and in combinations, on absorption of exogenous and endogenous cholesterol in the rat were reported by Vahouny *et al.*(1,2,3). It was shown that lipide composition of lymph during absorption was related to composition of the test emulsion administered. Laurell(4) has reported the percentage distribution of lipides in chylomicra and lipoproteins of the lymph of rats which were fed one type of emulsion. Byers and Friedman(5) reported that dietary cholesterol travelled almost completely in the chylomicron fraction in the lymph of rats which were fed cholesterol in olive oil. Jobst and Schetter(6) isolated chylomicrons from chylic ascites and chylic pleural discharge of a patient suffering from Whipple's disease and found no differences in these types of chylomicra. In the experiment described below the lipide changes in the chylomicron and subnatant fractions of the lymph of rats which were administered various test emulsions were determined in order to evaluate more clearly the roles played by chylomicra and lipoproteins in lymph lipide transport.

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Methods. Adult male rats of the Carworth Farms strain, weighing between 175-250 g were employed. Collection of lymph, preparation and intragastric administration of the test emulsions were as described by Vahouny *et al.*(1). The basic emulsion contained 3 ml of isotonic saline solution and 50 mg of albumin. The test emulsions contained, in addition to the basic emulsion, amounts of cholesterol, oleic acid, triolein and sodium taurocholate shown in Table I. Ten groups of 3 or 4 rats each were used and they are numbered in the tables corresponding to the number of the emulsion shown in Table I which they received. Lymph was collected

TABLE I. Composition of Test Emulsions.

	Emulsion No.									
	1	2	3	4	5	6	7	8	9*	10
Albumin, 50 mg	+	+	+	+	+	+	+	+	+	+
Sodium taurocholate, 287.4 mg					+	+	+	+	+	+
Oleic acid, 290.9 mg			+	+			+	+	+	
Triolein, 303.9 mg										+
Cholesterol, 50 mg		+		+		+		+	+	+
.9% saline to 3 ml	+	+	+	+	+	+	+	+	+	+

* Oleic acid was increased to 878.7 mg.