and eosinophil count varying with dose used. A dose equivalent to 10 g of brain tissue caused no change (Case Wl). A dose equivalent to 20 g caused small rises in blood glutathione and eosinophil levels (Cases Wl and Kr); doubling the dose caused further rises in these measurements (Cases Wl and Kr).

In all experiments, measurements returned to control level some weeks after cessation of injections. Placebo injections had no effect (Case J). Placebos in this and other cases were extracts made either from beef ovary, liver, or skeletal or cardiac muscle.

Discussion. Our data showed that brain tissue contains a substance that causes biochemical changes in schizophrenic patients identical with those produced by certain pineal extracts. As far as can be judged from these crude experiments, concentration of this material in brain is about a fifth to a tenth of that found in the pineal gland.

No such activity was found in extracts of beef liver, ovary or skeletal or cardiac muscle.

The question whether brain manufactures this material, or whether it is distributed through the brain from the pineal gland cannot be answered. However Holmgren has shown that secretory granules leave the pineal gland and migrate to the third ventricle. If secretory material entered the spinal fluid in this fashion it could readily be distributed through the entire central nervous system. In this connection it is interesting that melatonin, another product of the pineal gland, is also widely distributed in nervous tissue(6).

Summary and conclusions. 1) Extracts from whole steer-brain (excluding pineal gland) cause the same changes in schizophrenic patients as do pineal extracts made the same way. Concentration of active material in brain tissue is very small. Substances which originate in the pineal gland appear to be widely distributed in the central nervous system. 2) Beef ovary, liver and skeletal and cardiac muscle contained no active material.

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Action of Flavonoid Metabolites on Pituitary-Adrenal Axis.* (25851)

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We showed that certain flavonoids accelerate thymus involution through action on pituitary-adrenal axis, while others lack this effect(1,2). This comparison permitted some conclusions relating this action to structural configuration. Metabolic studies with several flavonoids showed they are readily absorbed and degraded to a variety of phenolic acids (3-9). The effect of phenolic acid metabolites of certain flavonoids and compounds related to these metabolites on thymus involution is reported here.

Methods. Female albino rats, 22-26 days old were fed for 12-14 days a semi-synthetic diet previously described(2) with or without addition of a phenolic acid (1%). They were

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TABLE I. Effect of Phenolic Acids on Thymus Involution in Normal Female Rats.

Acid fed	No. of rats	Final age, days	Mean final body wt, g	Mean thymus wt, % of body wt		
				Mean ± S.E.	Diff. ± S.E.	P*
None, basal alone	61	38	84.5	.354 ± .005		
3,4-Dihydroxybenzoic (protocatechuic)	11	41	82.5	.299 ± .007	.055 ± .009	<.001
2,4-Dihydroxybenzoic	10	35	77.1	.341 ± .013	.013 ± .014	
m-Hydroxybenzoic	9	38	81.7	.384 ± .028	−.030 ± .028	
p-Hydroxybenzoic	10	41	74.5	.335 ± .010	.019 ± .011	
3-Methoxy-4-hydroxybenzoic (vanillic)	9	38	76.6	.326 ± .050	.028 ± .050	
3,4-Dihydroxyphenylacetic	10	39	80.2	.318 ± .014	.036 ± .015	<.02
m-Hydroxyphenylacetic	10	35	76.0	.336 ± .013	.018 ± .014	
3,4-Dihydroxycinnamic (caffeic)	10	37	80.4	.320 ± .011	.034 ± .012	<.01
3-Methoxy-4-hydroxycinnamic (ferulic)	9	38	83.8	.351 ± .010	.003 ± .011	
m-Hydroxyphenylpropionic	10	35	69.2	.367 ± .018	−.013 ± .019	
p-Hydroxyphenylpropionic	10	35	68.6	.339 ± .012	.015 ± .013	

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

sacrificed thereafter and body and thymus weights obtained. Body organs were examined for gross changes. Adrenalectomy (bilateral) was performed on 27-day-old females; these were immediately maintained on test diets and saline postoperatively for 12-14 days before sacrifice. Hypophysectomized females were 26 days old when pituitaries were removed; they were then put on test diets and sacrificed 2 weeks later. 3, 4-Dihydroxyphenylacetic and m-hydroxyphenylacetic acids were obtained by demethylation of corresponding methyl ethers. The following derivatives of cinnamic acid were prepared from corresponding benzaldehydes by condensation with malonic acid(10): m-hydroxycinnamic (m-coumaric), p-hydroxycinnamic (p-coumaric), 3,4-dihydroxycinnamic (caffeic), and 3-methoxy-4-hydroxycinnamic (ferulic) acids. Derivatives of phenylpropionic acid were prepared from corresponding cinnamic acids by hydrogenation using palladium over charcoal. Oxidation of vanillin with fresh silver oxide gave 3-methoxy-4-hydroxybenzoic (vanillic) acid(11). The following were purchased: m- and p-hydroxybenzoic and 3,4-dihydroxybenzoic (protocatechuic) acids.

Results. Effect on thymus involution of several phenolic acid metabolites of flavonoids or compounds related to these metabolites is shown in Table I. Average body weights and ages of different groups at time of sacrifice are also indicated. Accelerated involution is evident in groups receiving catecholic acids: 3,4-dihydroxyphenylacetic, caffeic and protocatechuic. Gross organ examination and growth rate of groups receiving these acids showed no changes from control group. Other acids in Table did not accelerate involution. Table II shows that either adrenalectomy or hypophysectomy abolished thymolytic response to 3,4-dihydroxyphenylacetic acid. Protocatechuic and caffeic acids were not tested in hypophysectomized or adrenalectomized rats.

Discussion. Our findings shed light on whether the action of flavonoids on the pituitary-adrenal axis is due to intact flavonoids or their metabolites or both. The flavonoids quercetin, dihydroquercetin, eriodictyol and luteolin all possess orthodihydroxyphenyl (catecholic) groupings and had thymolytic action(1,2); of these, only quercetin and dihydroquercetin yield a catecholic metabolite in the rat, namely, 3,4-dihydroxyphenylacetic acid(3,4,7,8). This acid accelerated thymus involution (Table I), through the pituitary-adrenal axis (Table II). Major metabolites of eriodictyol(5) and luteolin‡ in the rat, on

‡ Unpublished observations from this Laboratory.

TABLE II. Thymus Gland Weights of Adrenalectomized or Hypophysectomized Female Rats Fed Control Diet or Diet Containing 3,4-Dihydroxyphenylacetic Acid.

	Control diet			Diet containing 3,4-dihydroxyphenylacetic		
	No. of rats	Mean final body wt, g	Thymus, % of body wt	No. of rats	Mean final body wt, g	Thymus, % of body wt
Adrenalectomized	10	64.9	.460 ± .030	12	59.8	.474 ± .026
Hypophysectomized	9	49.6	.354 ± .014	8	51.1	.348 ± .009

the other hand, are m-hydroxyphenylpropionic and m-coumaric acids but no catecholic acids. m - Hydroxyphenylpropionic acid (which readily forms m-coumaric acid in the body) did not accelerate involution (Table I). Thus, it may be inferred that this action of flavonoids is due in part at least to the intact molecule and, in certain instances, may also be due to the metabolites they yield.

The flavonoids hesperetin, naringenin, and morin, which have no catecholic groups do not cause thymus involution(2) and produce phenolic metabolites(5,6,9), which also fail to cause thymus involution (Table I).

Certain non-catecholic phenolic acids such as salicylic, gentisic and γ-resorcylic markedly affect level of circulating corticosteroids(12, 13). Whether flavonoids possessing radicals of these acids (constituting the prime ring of flavonoid) also stimulate adrenal function merits consideration.

Summary. (1) Several phenolic acid metabolites of flavonoids and compounds related to these metabolites were tested for thymolytic action by feeding in the diet to young normal rats. (2) Catecholic acids 3,4-dihydroxyphenylacetic, protocatechuic and caffeic, accelerated thymus involution. Several other phenolic acids did not. (3) 3,4-Dihydroxyphenylacetic acid did not accelerate thymus involution in either hypophysectomized or adrenalectomized rats. (4) Whether the action of flavonoids on thymus involution is due to intact flavonoids or their metabolites is discussed.

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Serum Lactic Dehydrogenase Activity in Experimental Endotoxic Shock.* (25852)

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Elevations of plasma lactic dehydrogenase (LDH) activity in experimental hemorrhagic shock have been described(1). This paper demonstrates elevations of serum LDH activity after endotoxin administration.

Materials and methods. One MLD_{100} of

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