to provide certain advantages over the tanned sheep cell hemagglutination method in the study of the rheumatoid factor. Euglobulin preparations from 3 rheumatoid arthritis sera have been shown by the BDB hemagglutination method to react with human, rabbit, bovine, equine, porcine and guinea pig gamma globulins; significant reactivity with chicken gamma globulin was not demonstrated. It has been shown that the reactivity with rabbit, bovine and equine gamma globulins is associated with rheumatoid factor activity both by euglobulin precipitation and ultracentrifugal fractionation. These reactivities with the animal gamma globulins were absorbed from rheumatoid sera by a preparation of insoluble HGG. The reactivities with the animal gamma globulins therefore are felt to represent a part of the rheumatoid factors of these sera. These findings are consistent with the hypothesis that the rheumatoid factor is antibody to human gamma globulin with cross-reactivity with the gamma globulins of other mammalian species.

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Quercetin Inhibition of Specific Histidine Decarboxylase. (29314)

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Control of histamine formation *in vivo* should be of value in medicine. One of the authors (GJM) investigated the bioflavonoids as histidine decarboxylase inhibitors in 1949(1). At that time, the studies were carried out using an enzyme prepared from kidney; today, it is known that the enzyme involved in histamine production *in vivo* differs from this. The enzyme in kidney extracts is primarily an aromatic L-amino acid decarboxylase with strong activity against 5-hydroxytryptophan and only a weak action on histidine; the enzyme obtained from mast cells (via mast cell tumors or a transplantable rat hepatoma) is a specific histidine decarboxylase, highly active against histidine and with no activity against 5-hydroxytryptophan or dihydroxyphenylalanine(2-5).

It seemed indicated to recheck the bioflavonoid molecules against the specific histidine decarboxylase, both *in vivo* and *in vitro*.

Methods. Histidine decarboxylase activity. Tissues (1 g each) homogenized in 5 ml cold phosphate buffer (0.1 M, pH 7.5 or 8.3)

and centrifuged. The supernate was used as the enzyme source. To 4 cc enzyme, 0.1 cc pyridoxal-5'-phosphate (0.2%), 0.1 ml aminoguanidine HCl (1%), 0.025 ml benzene, 1.0 ml L-histidine-2 ring C14 (Nuclear Research Chemicals—1 uc/mg—0.1%) were added with buffer to desired volume. In some instances, quercetin was added for analysis as an inhibitor. The mixture was shaken at 37°C for 4 hours, centrifuged with an equal volume of 20% TCA and 1 cc of histamine (0.002%) and the supernate analyzed for histamine-2 ring-C14. Histamine was isolated by the method of Lubschey(10) utilizing diazotization and organic solvent fractionation with methyl isobutyl ketone. In all instances, the amount of diazotization and extraction reagents was sufficient for complete reaction with the amount of histidine-histamine present. The ketone layer containing histamine diazo salt was placed in a planchet and isotope levels determined.

The effect of quercetin on induced mouse skin histidine decarboxylase was investigated by producing local irritation with xylene, sacrificing the animal within 15 minutes and utilizing skin homogenate as enzyme source (pH 8.3).

The effect of quercetin on gastric histidine decarboxylase was investigated in rats (250 g—Wistar strain) anesthetized with pentobarbital (30 mg/kg i.p.). The stomach was ligated and 2.5 cc of solution (containing 40 mg histidine C14, 80 mg histidine carrier—100 cc H_2O) was injected into the stomach with and without quercetin. In one hour the stomachs were removed, homogenized in an equal volume of H_2O, and an equal volume 20% TCA added. The mixture was centrifuged and an aliquot of the supernate analyzed for histamine-C14 content.

The effect of quercetin on overall histamine synthesis via decarboxylase activity was determined by administration of 0.25 cc histidine C14 solution (40 mg histidine C14, 50 mg histidine carrier—5 cc of H_2O) intravenously. Urine was collected in glass metabolism cages over a 48 hour period. Animals were given free access to food and water. Quercetin was administered intraperitoneally in some groups (5 rats/group). The urine flasks were washed with 5 cc histamine solution (0.002%), and an equal volume 20% TCA added. After centrifugation, an aliquot of the supernate was analyzed for histamine C14.

The effect of quercetin on endotoxin-induced histidine decarboxylase was determined by administration of 10 μg *E. coli* lipopolysaccharide (0111:B4, Difco) intravenously. In some groups (10 mice per group) quercetin was administered intraperitoneally 1 hour prior to endotoxin. In 5 hours the mice were sacrificed, various organs removed, homogenized in buffer (pH 8.3). Following centrifugation the supernate was analyzed for histidine decarboxylase activity with and without added quercetin.

The effect of quercetin *in vivo* on the histidine decarboxylase activity of the Furth Mastocytoma tumor (solid line)(11) was investigated in mice (LAF, strain) by injecting 0.1 cc histidine C14 solution (0.15%) into the subcutaneous tumor (2 weeks old) with and without quercetin. In one hour, the tumors were removed and homogenized (10%), then centrifuged with an equal volume of 20% TCA added, and the supernate analyzed for histamine C14 content.

Results. As is evident in Tables I and II,

TABLE I. Effect of Quercetin on Histidine Decarboxylase of Various Rat Tissues.

	Histidine decarboxylase source		
Quercetin, mg/ml	Liver pH 8.3	Kidney pH 8.3	Pylorus-duodenum pH 7.5
.0158	56	0	—
.033	76	0	—
.142	83	71	—
.183	100	79	—
.0138	—	—	0
.030	—	—	16
.073	—	—	43

Results in terms of % inhibition of enzyme *vs* control.

TABLE II. Effect of Quercetin on Inducible Histidine Decarboxylase of Mouse Skin.

Quercetin concentration per system (mg/ml)	% inhibition
.0158	0
.033	0
.142	60
.183	77

TABLE III. Effect of Quercetin on Rat Gastric Histidine Decarboxylase *in vivo*.

Quercetin (mg/g stomach tissue)	Histamine activity (cpm)	
	Per g tissue	Per stomach
0	294	1488
.43	144	836
1.47	67	450

TABLE IV. Effect of Quercetin *in vivo* on Rat Histidine Decarboxylase Activity.

Quercetin (mg/kg)	Histamine activity (cp/10 min per total vol urine)	Aqueous phase* activity
2	3720	66,500
5	3780	63,100
10	0	47,500
0	11,960	127,500

* TCA sol, diazo salt insol. in methyl isobutyl ketone.

quercetin is an effective *in vitro* inhibitor of the nonspecific histidine decarboxylase of rat liver, kidney, pylorus, duodenum, and locally induced enzyme in mouse skin.

Quercetin administered orally inhibited histidine decarboxylase activity of the rat gastric area *in vivo* (Table III) and when administered intraperitoneally modified overall histamine synthesis via decarboxylase activity (Table IV) as reflected in urinary histamine levels.

The flavonoid was an effective inhibitor of endotoxin-induced histidine decarboxylase in mouse spleen, lung and liver *in vitro* (Table V) in animals with and without quercetin pretreatment. The data in Table VI demonstrate that quercetin does not significantly interfere with histidine decarboxylase synthesis (endotoxin-induced) but will inhibit the enzyme reaction.

TABLE V. Effect of Quercetin on Endotoxin-Induced Histidine Decarboxylase Activity of Mouse Spleen, Lung and Liver.

Organ	Quercetin (mg/ml of test system)	% inhibition		
		Group 1	Group 2	Group 3
Liver	.0158	7.4	0	15.4
	.033	56.2	16.8	41.8
	.142	60.8	56.1	58.1
	.183	71.2	69.3	76.2
Spleen	.0158	0	16.4	0
	.033	26.6	26.9	21.5
	.142	35.5	45.3	—
	.183	73.3	59.1	100
Lung	.0158	0	0	0
	.033	53.2	7.0	19.8
	.142	65.7	19.8	30.2
	.183	75.3	41.8	53.5

Group 1—0; Group 2—2 mg; Group 3—10 mg per kg quercetin I.P. prior to endotoxin.

Quercetin administered in situ inhibited *in vivo* the specific histidine decarboxylase activity of the mastocytoma tumor as estimated by histamine synthesis (Table VII).

Discussion. Schayer(6) offers evidence that histamine mobilization resulting from the activity of an induced form of histidine decarboxylase is the intrinsic regulator of the autonomous changes of the microcirculation, satisfying requirements of tissues for blood. The slow phase of the inflammatory reaction and shock itself result from uncontrolled histamine production. This same mechanism of induced histidine decarboxylase production with subsequent action doubtless plays a major role in all histamine related clinical states. An agent controlling the induction phase for the enzyme and/or its subsequent action should provide a most interesting agent for study; quercetin would seem to be one such agent.

Primarily, it represents a molecule effective both *in vitro* and *in vivo* against the nonspecific and the specific histidine decarboxyl-

TABLE VI. Effect of Quercetin Administered Intraperitoneally on *in vitro* Activity of Isolated Liver and Lung. Endotoxin-induced histidine decarboxylase.

Quercetin (mg/kg I.P.)	Relative activity	
	Liver	Lung
0	1.0	1.0
2	1.3	.7
10	1.3	.7

TABLE VII. Effect of Quercetin on Histidine Decarboxylase Activity of Mouse Mastocytoma Tumor *in vivo*.

Quercetin (mg/tumor)	Histamine conc (cpm)		% inhibition
	cpm/tumor	cpm/g tissue	
1	1420	322	36.9
2	1141	300	41.3
2.5	540	200	60.7
0	3007	510	—

ase. While remaining significant, all studies with the non-specific enzyme are in need of repetition with the specific form which is the *in vivo* controlling homeostatic factor. There are few reports in the literature of chemicals active *in vivo* in inhibition of specific or induced histidine decarboxylase. Kahlson *et al* (7) found α-methylhistidine an *in vivo* inhibitor of histamine formation. The method employed measured urinary excretion of C^{14}-histamine following injection of known amounts of C^{14}-histidine. A second active molecule has been reported effective in animals(8) and clinically(9). Quercetin is the third. The clinical results of Bezian and Pautrizel(9) offer much encouragement for utility of histidine decarboxylase inhibitors. The agent they used, 1-(3-amino-4,5,6-triethyoxyphthalidyl)-2-methyl-6,7-methylenedioxy-8-methoxy-1,2,3,4-tetrahydroisoquinoline, was effective in pollinosis, aperiodic rhinitis, Quincke's edema and urticaria but proved disappointing in asthma and eczema. While the series was small (22 cases) and uncontrolled, the study offers hope that agents of this type will prove non-toxic and effective in therapeutics.

Summary. Quercetin by an isotopic method has been shown to be an effective inhibitor of non-specific and specific (induced) histidine decarboxylase both *in vivo* and *in vitro*.

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Hypocholesterolemic Effect of an Androsterone Analogue. (29315)

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Interest in androsterone and androsterone-like steroids for treatment of hypercholesterolemia was initiated by the report of Hellman *et al*(1) who observed that androsterone administered parenterally to hypercholesterolemic patients reduced the serum cholesterol level about 20%. They further noted that the most pronounced reductions in circulating cholesterol levels were observed in myxedematous patients and proposed that androsterone possessed some ill-defined "thyromimetic" activity. These observations have been confirmed by Cohen *et al*(2), and Furman and Howard(3), and others.

The implication that androsterone is thyromimetic(1) must be true only in an indirect sense. Certainly, all groups(1-3) have reported the most profound effects of androsterone to occur in hypothyroid individuals; but Cohen *et al*(2) found the steroid had no effect on serum protein-bound iodine. It is well established that the thyroid hormone promotes the conversion of testosterone to androsterone(1). Thus, either a deficiency of the circulating thyroid hormone or of androsterone precursors might lead to a reduction in amount of androsterone available for normal sterol metabolism. Therefore, it seems logical to propose that the hypocholesterolemic response to androsterone therapy may be the result of supplementation of an un-