In 2 patients hepatic bile was analyzed and in both patients only traces of deoxycholic acid were found. One of these chromatograms is given in Fig. 3 case D. Case E in Fig. 3 is a patient with familial hypercholesterolemia. The 2 major bile acids were chenodeoxycholic acid and cholic acid. Only traces of deoxycholic acid were found in this patient together with small amounts of lithocholic acid.

Conclusions. The major bile acids in samples of bile as determined with the aid of gas-liquid chromatography were cholic acid, chenodeoxycholic and deoxycholic acid. Regarding the proportions of the different bile acids, the results from this small group agree with those of Wootton and Wiggins(5), Isaksson(6) and Sjövall(7,8). Deoxycholic acid seemed to disappear in obstructive jaundice. In hepatic bile obtained from patients without jaundice only very small amounts of deoxycholic acid were found. Similar results have been reported by Sjövall(7) and Rudman and Kendall(9).

An interesting finding is the very small amount of deoxycholic acid present in a patient with familial hypercholesterolemia. This patient had been on a general mixed diet without the consumption of highly unsaturated fats or any other treatment of the hypercholesterolemia. Sjövall(7) has reported a similar bile acid pattern in a patient with hypercholesterolemia but on a formula diet. Further studies are in progress on the bile acid pattern in diseases with an altered cholesterol metabolism.

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Effect of Citrus Bioflavonoids on Metabolism of Hydrocortisone in Man.* (26556)

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It has been recognized for some time that the class of compounds generally termed bioflavonoids may be of physiological importance(1,2). Our previous observations of the relatively transient rises in plasma 17-hydroxycorticosteroid levels following major elective surgery(3) have interested us in possible pharmacological agents that would decrease the rate of metabolic inactivation, *i.e.*, Δ^4-3-keto reduction, or, due to other factors, impede removal from plasma of hydrocortisone and possibly other adrenocortical steroids. Masri and DeEds(4) observed that administration of certain bioflavonoids to rats produces involution of the thymus gland in the presence of the adrenal glands, but *not* after their removal, suggesting mediation of the physiological effects of bioflavonoids through an action on the pituitary-adrenal

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axis. It occurred to us that these findings might be in part explained on the basis of prolongation of the biological half-life of the rat's endogenously produced corticosterone and that the bioflavonoids may have acted in a similar manner to salicylic acid(5,6) and possibly N-acetyl-p-aminophenol (NAPA) as suggested by Corte and Johnson(7) in connection with the observed prolongation of the half-life of hydrocortisone in rabbits following administration of NAPA.

We believe that a non-toxic pharmacological agent which could prolong the biological half-life, and thereby activity, of endogenous and exogenous hydrocortisone might be of clinical importance. This report concerns the effects of various bioflavonoids on the metabolism of hydrocortisone in normal man.

Materials and methods. The patients who participated in these studies were volunteers of both sexes. All had been hospitalized for at least 3 weeks following surgical repair of fractures of long bones or recovery from knife wounds not involving the abdominal cavity. They were in an adequate nutritional status with no laboratory evidence of metabolic disorder. To 19 such subjects we have administered parenterally 50 and 100 mg doses of Hydrocortone®—Merck,†, an intravenous infusion concentrate of hydrocortisone which was diluted with 250 ml of 5% Dextrose in water and infused over a period of 15 minutes. Fasting venous blood samples (8:00 a.m.) were obtained on several days before the infusion, and 15, 30, 60, 120, 180, 240 and 360 minutes following the end of infusion of hydrocortisone. This procedure was repeated after several days with the inclusion of certain bioflavonoids in the infusion mixture, or in identical fashion following a period of 6 or 7 days of oral administration of the bioflavonoids.

In several instances no hydrocortisone was administered but endogenous hydrocortisone levels in plasma were measured once daily during oral administration of the bioflavonoids. Tracer amounts of $4C^{14}$-hydrocortisone were similarly employed in several observations. Bioflavonoids used were Hesperetin, Eriodictyol glycoside and Hesperidin methyl chalcone. Hesperetin‡ was formulated as a suspension in glucose syrup slightly flavored with oil of orange, N.F., so that 15 ml of suspension contained 2 g of hesperetin. The Eriodictyol glycoside was administered as a sterile solution added to 250 ml of 5% Dextrose in water. Hesperidin methyl chalcone was also formulated as a solution in glucose syrup flavored with natural oil of lemon and limes, so that 25 ml of solution contained 1.5 g of hesperidin methyl chalcone. N-acetyl-p-aminophenol (NAPA)§ was administered by mouth in tablet form. "Free" and conjugated 17-hydroxycorticosteroids were measured by a modified procedure based on the authors' own methods(8). Following administration of $4C^{14}$-hydrocortisone, total radioactivity in the free and conjugated plasma fractions were determined by counting in an alcohol-toluene, DPO (diphenyloxyzol) and POPOP (1,4-bis-2(5-phenyloxazolyl)-benzene) phosphor in a Packard liquid scintillation spectrometer.

Results. The logarithmic values of the plasma "free" 17-OH corticosteroid concentrations, measured as Porter-Silber chromogens, are plotted against time and a linear relationship is obtained one hour after the end of infusion of hydrocortisone. From such curves the biological half-life of the infused steroid has been calculated(9). In all cases herein presented an increase of this half-life has been consistently observed following oral or parenteral administration of various bioflavonoids and of NAPA (Table I). Maximal concentrations of "free" 17-OHCS following hydrocortisone infusion are not affected by administration of these compounds. Rates of disappearance (Fig. 1) from the plasma of normal subjects before and after administration of bioflavonoids and NAPA

† Hydrocortone® was generously supplied by Merck, Sharp & Dohme, Research Laboratories, West Point, Pa.

‡ Hesperetin, preparations 59-S-1 and C-7090, Eriodictyol glycoside from lemon, preparations P-56 and P-57 and Hesperidin methyl chalcone were supplied by Sunkist Growers, Inc., Ontario, Calif.

§ Kindly supplied to us by F. W. Horner, Ltd., Montreal, Canada under the trade name of "Atasol".

TABLE I. Effect of Various Bioflavonoids and NAPA* on Half-Life of Intravenously Administered Hydrocortisone.

No. of subjects	Ref. letters (Fig. 1)	Hydrocortisone, mg	Compound administered: Compound	Amount	Duration	Max conc. "free" 17-OHCS in plasma, μg %	$t_{1/2}$(θ), min.	"t" test P
2		3 μc $4C^{14}$					94†	
2		3 μc $4C^{14}$	Hesperetin	2 g t.i.d.	7 days		148	
3		50				724	99	
3		"	"	2 g t.i.d.	7 days	97	207	
14	A	100				165 ± 8‡	98 ± 2.6‡	
2	B	"	Eriodictyol	50 mg I.V.	15 min.	170	135	
2	C	"	Hesperidin methyl chalcone	100 mg I.V.	15 min.	185	150	
3	D	"	Hesperetin	2 g t.i.d.	7 days	160 ± 7.1	162 ± 6.3	$.01 < P < .05$
2	E	"	NAPA*	2 g t.i.d.	6 days	145	168	
5	F	"	Hesperidin methyl chalcone	1.5 g	6 days	178 ± 7.4	184 ± 19.5	$.01 < P < .05$

* N-Acetyl-p-Aminophenol (Atasol, Horner). † Mean values. ‡ S.E.

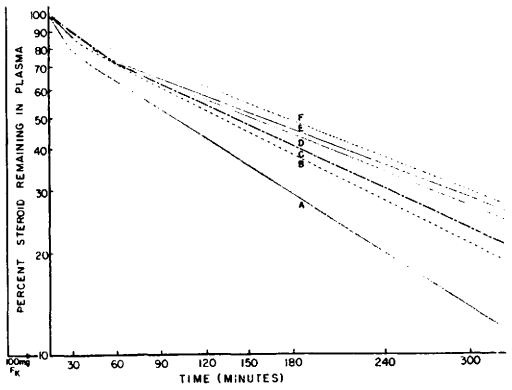

FIG. 1. Disappearance of hydrocortisone from plasma following intravenous administration. (See Table I for explanation of letters A through F.)

are presented in a manner similar to Peterson(10). The delayed disappearance of hydrocortisone following administration of these compounds together with the resulting half-life (t ½) values suggests that an altered metabolic rate of the infused hydrocortisone results from the bioflavonoids and NAPA or their respective metabolites. Examples of the effects of bioflavonoid administration on the endogenous 17-OHCS levels in plasma (Table II) indicate a similar alteration of the metabolic rate of endogenously produced 17-OHCS. This is confirmed by the results obtained with the tracer amounts of $4C^{14}$-hydrocortisone (Table I).

The values in Tables I and II are mean values obtained from the indicated number of observations. Whenever possible, standard errors of the mean were calculated and the data subjected to a "t" test of significance of the difference in the half-life of hydrocortisone obtained before and after administration of the various compounds. The resulting P values are given.

Discussion. These studies indicate that steroid degradation, judging from rates of disappearance of the 17-OHCS in plasma, is significantly decreased following administration of certain non-toxic phenolic compounds, such as the bioflavonoids hesperetin, eriodictyol and hesperidin methyl chalcone, and the drug N-acetyl-p-aminophenol. More data are needed to explain the mechanisms involved in these effects. Corte and Johnson(7) have suggested that NAPA is a competitive "glucuronylation" inhibitor of steroids. Our preliminary studies of the glucuronic acid conjugated 17-OHCS in plasma, following infusion of hydrocortisone discount this hypothesis because there are no significant differences between the conjugated 17-OHCS levels before and after administration of these

TABLE II. Effect of Two Bioflavonoids and NAPA on Fasting (8 A.M.) "Free" 17-OH CS Levels in Plasma.

No. of subjects	Compound admin. (orally) Compound	Amount	Control determinations (*i.e.* before admin. of drug) No. of det.	μg % 17-OH CS	μg % 17-OH CS (mean values) Day of admin. 1	2	3	4	5	6	7
4	Hesperetin	2 g t.i.d.	12	11 ± .44‡	12	12	14	17	19	20*	19
4	Hesperedin methyl chalcone	1.5 g q.i.d.	12	14 ± .48	17	21	24†	23	23	22	—
2	N-Acetyl-p-Aminophenol (NAPA)	2 g t.i.d.	6	13 ± .67	15	15	18	18	21	22	20

* Maximum value on 6th day: D.F. = 3; t = 3.70; .01 < P < .05.
† " " " 3rd " : D.F. = 3; t = 3.36; .01 < P < .05.
‡ Mean ± S.E.

compounds. Similarly a comparison of urinary 17-OHCS excretion during these 3 control days and the days of drug administration has not shown significant differences, in either "free" or conjugated fractions. The delayed disappearance of 17-OHCS from plasma would seem to be due to either a delayed reduction of the steroid, or other hitherto unknown factors impeding its removal. Use of $4C^{14}$– and $1,2H^{3}$– hydrocortisone and actual paper-chromatographic isolation of free labeled materials is contemplated in order to show definitively the prolongation of the half-life of unmetabolized hydrocortisone.

Summary. Administration to normal humans of certain phenolic compounds, such as citrus bioflavonoids and N-acetyl-p-aminophenol (NAPA) significantly decreased the rate of disappearance from the plasma of parenterally administered hydrocortisone.

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