terol depends upon the age of the animal, with a considerably greater synthesis in the younger rat.

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Metabolism and Urinary Excretion of Several Flavonoid Compounds.* (19856)

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There is considerable disagreement among investigators regarding metabolism and urinary excretion of flavonoid compounds. Fukuda(1) claimed that orally administered rutin appeared unchanged in the urine of rabbits within 20 hours after administration. Garino (2) made similar claims for the dog, but Field and Rekers(3) could not confirm his findings. Porter *et al.*(4) found only traces of rutin in the urine of humans given oral doses of 60 to 2250 mg daily. Clark and MacKay(5) confirmed these findings in humans and also in the rat. Furthermore, they showed that rutin had little effect on urinary total phenol and glucuronide excretion in the rat. It was also pointed out that intestinal destruction of orally administered flavonoids made it improbable that very much of the administered dose could be absorbed and excreted in the urine. Kirtley and Peck(6) reported that hesperidin methyl chalcone was not excreted in the urine in the free form after large oral doses in man. From the above evidence it appears that the oral route of administration results in almost complete destruction of the flavonoid compounds before absorption can take place. However, it is possible that if the compounds were administered by another route, information could be derived concerning the manner in which the body metabolizes and excretes such compounds.

Experimental. Male CFW strain rats weighing an average of 294 g were caged separately in Norwich type metabolism cages. Six animals were used for each compound. Twenty-four hour urine samples were collected daily for 2 days prior to and 5 days after the subcutaneous injection of 100 mg/rat of the following compounds: rutin, hesperidin, hesperidin methyl chalcone, naringin and phloroacetophenone. Chemical analyses were made for the following: free rutin by the aluminum complex method of Porter, *et al.*(4), free hesperidin and naringin by the cyanidin test (5); free hesperidin methyl chalcone by borocitrate reaction(7); free phenol by O'Leary's method(8); ethereal sulfate by the method of Treon and Crutchfield(9); and glucuronide by Dische's method(10).

Results. The results obtained in the anlyses are given in Table I, in which the excretion as degradation products and conjugates is based upon differences between pre- and post-injection values. These are average excretion figures and represent only the first 24 hours

* This article is based on work performed under contract between the Atomic Energy Commission and the University of California at Los Angeles.

TABLE I. Urinary Excretion of Flavonoids after Subcutaneous Injection in Rats.

Compound	Chemical name	% excreted as: Unchanged	Free phenol	Ethereal sulfate	Glucuronide	Total % excreted*
Rutin	3-rhamnoglycoside of 3,5,7,3',4'-pentahydroxyflavone	15.89	2.57	10.90	11.15	40.51
Hesperidin	7-rhamnoglycoside of 4'-methoxy,3,5,7,3',4'-pentahydroxyflavone	ND	4.85	11.37	9.82	26.04
Hesperidin methyl chalcone	7-rhamnoglycoside of the methyl chalcone of 4'-methoxy,3,5,7,3',4'-pentahydroxyflavone	ND	0	3.95	4.29	8.24
Naringin	5-rhamnoglycoside of 5,7,4'-trihydroxyflavone	ND	2.82	2.87	5.12	10.81
Phloroacetophenone	2,4,6-trihydroxy acetophenone	X	22.91	33.24	22.40	78.55

* This percentage refers only to the four components measured.
ND—None detected with tests used.
X—Unchanged and free phenol were equivalent with the chemical methods used.

because no further excretion could be detected on the following 4 days. Average pre-injection control values in mg/ml of urine were: free phenol, 0.9; ethereal sulfate, 0.66 and glucuronide, 0.55. On the second post-injection day the excretion values for sulfate and glucuronide were 0.6 mg/ml and 0.3 mg/ml of urine respectively, indicating that the animals may have reached their maximum capacity for conjugation and excretion in the first 24 hours. Determinations made on the last 3 post-injection days were essentially the same as the pre-injection control values. Rutin was the only compound detected in the urine by the method of Porter, *et al.*(4). The more sensitive cyanidin reaction gave negative results with hesperidin and naringin indicating that these compounds were not excreted unchanged in the urine. Similar results were obtained with the boro-citrate reaction and hesperidin methyl chalcone. However, it is possible that if more sensitive and more highly specific reactions were available, it might be possible to detect the unchanged compounds in the urine. On the other hand, the urine from animals given rutin was highly colored, whereas that obtained from the animals receiving the other compounds was a normal straw color. None of the injected compounds could be detected when the site of injection and the surrounding tissue was excised and extracted 24 hours after administration, showing that the compounds were completely absorbed after subcutaneous injection. The free phenol values for all compounds probably represent degradation products of the flavonoids because all unchanged compounds are precipitated with and tightly bound to the gelatinous precipitate formed in the first step of the reaction. This step, which is used to clarify the urine, results in a clear filtrate which contains the free and combined phenols. Such co-precipitation was observed with urine containing rutin and it was not possible to elute the rutin from the complex precipitate. On the other hand, the free phenol values for phloroacetophenone were found to correspond to the unchanged compound and thus it appears that this compound is partially degraded by the rat into compound(s) which could not be detected with the phenol methods used.

Discussion. The results herein presented indicate that after parenteral administration, flavonoid compounds can be excreted in the urine in conjugated form and that they and/or their breakdown products are conjugated with sulfate and glucuronide in the same manner as other phenolic compounds(11). It appears that the absence of a glycoside linkage as in phloroacetophenone results in a greater excretion as a free phenol and a greater conjugation as sulfate and glucuronide. The increase in glucuronide excretion would confirm the observation of Scarborough(12) that oral vitamin P (flavonoids) increases glucur-

onic acid excretion. The detection of free rutin in the urine of rats makes it appear that Fukuda's(1) observations on rabbits and Garino's(2) observations on dogs are correct although the routes of administration were different. Our inability to detect hesperidin methyl chalcone in the urine indicates that Kirtley and Peck(6) were correct in assuming that the compound was not excreted in the urine in a free state. Furthermore, the small amount of the compound detected in conjugated form indicates that the compound is either stored or completely destroyed. Our results are contrary to those of Clark and MacKay(5) but the methods of administration of rutin differed. They showed that most of the rutin remained unabsorbed in the intestine after oral administration, whereas we were able to show complete absorption after subcutaneous administration. Such absorption leads to either excretion or biological destruction, and our results indicate that the latter process is the most important in the handling of hesperidin, hesperidin methyl chalcone and naringin and equivalent to excretion in the handling of rutin.

Summary. A study has been made of the metabolism and urinary excretion of rutin, hesperidin. hesperidin methyl chalcone, naringin and phloroacetophenone. Whereas the latter compound is to a large extent excreted either free or combined, the flavonoids, with the exception of rutin, are probably destroyed in the body or converted into other compounds which were not detected by the methods used. Rutin is partially destroyed in the body and partially handled by the usual phenol excretion pathways of sulfate and glucuronide.

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Chronic Toxicity of Ammonia Fumes by Inhalation.* (19857)

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The frequent exposure of industrial workers to ammonia fumes for long periods of time appears to have led to the impression that if the concentration is not sufficient to cause acute damage, then no damage need be anticipated. However, the author has been unable to find in the literature any substantiating evidence from controlled experimentation. In view of the fact that Fazekas(1,2) found that the administration of 50-80 cc of 0.5% ammonium hydroxide orally to rabbits either daily or on alternate days for periods of several months caused a marked increase in size of the suprarenal glands, it would appear desirable to investigate the potential toxicity of ammonia by inhalation. Accordingly, this was done.

Experimental methods. Twelve male guinea pigs were used for experimentation and 6 for controls. All animals were fed Purina rabbit chow supplemented with greens. The con-

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